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HealthMED

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Published by DRUNPP, Sarajevo
Volume 6 Number 2, 2012
ISSN 1840-2291

HealthMED journal with impact factor indexed in:

- Thomson Reuters ISI web of Science,
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Measurement of intracranial, cerebral, cerebellar, and ventricular volumes of Korean people in their 20s and 40s

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Abstract

This study measured intracranial volume (ICV) and normalized cerebral, cerebellar, and ventricular volumes of Korean people in their 20s and 40s, to identify differences in brain volume according to gender and age group and also the relationship between each brain volume and the physical indices. We recorded magnetic resonance brain images for 80 people in their 20s and 82 in their 40s. To investigate the effects of gender and age on ICV and normalized cerebral, cerebellar, and ventricular volumes, we employed a two-way analysis of variance. We analyzed the relationship between each brain volume and physical index using the Pearson correlation method. ICV, cerebral and cerebellar volumes of males and the 20s group were significantly greater than those of females and the 40s group. Ventricular volumes for males and the 40s group were significantly greater than those of females and the 20s group. For all subjects there was a positive relationship between ICV, cerebral and cerebellar volume and weight and height. There was a brain volume difference correlated with both gender and age; however, there was a close correlation between brain volume and height, as well as weight. ICV and cerebral and cerebellar volumes of Korean people were similar to, or slightly smaller than, those of Western people. Differences in brain volume due to gender and age group were similar to previously published results. There was a brain volume difference correlated with both gender and age; however, there was a close correlation between brain volume and height, as well as weight.

Key words: MRI, Intracranial volume and cerebral, cerebellar, and ventricular volume, Gender, Age, Physical index

Introduction

Each region of the human brain has different functions and structures. Currently, analyses of the brain's structure and volume mainly utilize Magnetic Resonance Imaging (MRI), and these results aid researchers in understanding the brain's structure and function and in diagnosing its diseases. There have been numerous studies on changes in brain volume due to aging, examining people from youth through old age (1, 2, 3, 4, 5, 6, 7). Likewise, researchers have studied changes in brain volume in connection with gender (4, 6, 7, 8, 9, 10, 11). With regard to Korean people, researchers have examined differences in cerebellar and ventricular volume that are correlated with gender, age, and also physical indices (12, 13).

Chung et al. (2005, 2006) reported a relationship between cerebellar and ventricular raw volumes and age, gender, and physical indices. Since the raw brain-volume data might vary greatly among participants, the raw data needs to be normalized, to reduce this variance (7, 14). There have not been any previously published reports on the relationship between normalized brain volume and gender, age, and physical indices for Korean people. Daniel et al. (2008) and Free et al. (1995), who examined Western people, used several approaches to study the relationship between the volumes of several brain regions and both gender and age, as follows. First, they used correlation analysis to examine the relationship between (non-normalized) brain volume and the independent variables of age and gender. Second, after setting Intracranial Volume (ICV) as the covariant, they analyzed the relationships between the independent variables (age and gender) and normalized brain volume, using regression equations (i.e.,

analysis of covariance or ANCOVA). Third, they analyzed the relationships after obtaining the ratios between ICV and several brain volumes (ratio analysis). They reported that ANCOVA was the optimal method for reducing variances. Therefore, to do an accurate brain volume study on Korean people, we also needed to use ANCOVA.

Studies have reported that males and younger people have larger brain volumes than do females and the elderly (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11). This study's first objective was to measure ICV and cerebral, cerebellar, and ventricular volumes in Korean people (males and females, in their 20s and in their 40s). Furthermore, after normalization of the raw data, we aimed to identify the differences in brain volume that correlated with gender and age and to compare the results with the published data based on Western people.

Most previous studies reported a relationship between brain volume and height (15, 16, 17) but no relationship between brain volume and weight (15). However, our studies using raw data showed a relationship between ventricular volume and height and between cerebellar volume and weight (12, 13). Therefore the study's second objective was to accurately determine whether there is a relationship between brain volume and height or weight, by using normalized data regarding gender and age.

This study measured intracranial volume (ICV) and normalized cerebral, cerebellar, and ventricular volumes of Korean people in either their 20s or their 40s. Also, after normalizing the cerebral, cerebellar, and ventricular volumes based on ICV and using covariance analysis, the study aimed to identify differences in brain volume correlated with gender and age and the precise relationship between each brain volume and the physical indices of height and weight.

Materials and methods

Subjects

We selected for this study 162 normal Korean participants (in either their 20s or their 40s) who had no previous brain damage or head injuries and did not have any medical problems, as confirmed by neurologists. Table 1 shows the basic information on the participants. For the individual groups of males, females, and participants in their twenties or in their forties, independent t-tests on age, weight, and height showed that there was a significant difference between males and females with regard to both weight and height and a significant difference between the 2 age groups with regard to both age (as would be expected) and height.

Image acquisition

We conducted MRIs using a 3.0-T FORTE machine (ISOL Technology, Korea), equipped with whole-body gradients and a quadrature head coil, and obtained T1-weighted brain images with a three-dimensional, magnetization-prepared, rapid-gradient echo sequence (TR/TE/TI=10/4/100 ms; slice thickness, 1.5 mm; field of view, 220mm×192mm×192 mm; number of slices, 128; slice gap, 0; matrix size, 256×224×128; and number of excitations, 2).

Volumetry

Using Brain Voyager 2000 (Brain Innovation BV, Germany) software, we separated the regions of the cerebrum, cerebellum, and ventricle and measured their volumes. After MRI acquisition, we reconstructed each image and performed the post-processing using a Brain Voyager 2000. Note that if there are any irregularities in image brightness along each slice, errors may occur during the automatic segmentation procedure based on image brightness. To prevent this problem, we carried out inhomogeneity corrections, based on the brightness of the white matter, for all axial, sagittal, and coro-

Table 1. Basic information on participants

	Male	Female	Sig.	20s	40s	Sig.
Number	70	92		80	82	
Age (year)	34.29±13.46	35.02±13.28	.729	21.60±2.36	47.49±3.50	.000
Body Weight (kg)	70.07±9.95	55.64±6.35	.000	60.99±11.45	62.74±10.12	.302
Body Height (cm)	172.96±5.74	159.12±4.78	.000	167.96±8.58	162.29±7.73	.000

nal planes. We used sigma filtering, which is similar to the standard Gaussian smoothing filter and can remove impulse noise, to increase image contrast. After these two pre-processing routines, we performed a region-growing algorithm, based on image brightness, for automatic segmentation. Finally, one of the authors, who has sufficient neuroanatomical knowledge to process boundary and detailed regions properly, performed manual segmentation. After measuring the regions of interest, we calculated the total volume of each region by multiplying the area by the slice thickness to get the volume of each slice and then summing each region's slice volumes.

Finally, we calculated Intracranial Volume (ICV) based on the Talairach Atlas and using Eq. 1 below and the length, width, and height of the cerebrum (10, 18).

$$ICV(cc)=4/3*\pi*length/2*width/2*height/2 \quad \text{..... (1)}$$

We measured the cerebrum by excluding the cerebellum and brainstem. It was easy to divide the left and right parts of cerebellum in the axial image, and we extracted the cerebellum by excluding the cerebellar peduncles, brainstem, and medullary vela regions (12). The ventricular volume was the total volume contained in the lateral ventricle, 3rd ventricle, and 4th ventricle (13).

Data analysis

To apply the ANCOVA, which is the data normalization method, it is necessary to analyze the relationships among the brain region volumes. When there is a significant relationship among ICV, cerebral, cerebellar, and ventricular raw volumes, ANCOVA can be used. Since there was a significant relationship between the ICV and the other brain volumes, as shown in the results, we could carry out the normalization based on the ICV. In this study, we divided each brain volume into 4 groups (20s age group males, 20s females, 40s males, and 40s females), calculated each gradient based on ICV, and used Eq. 2 to normalize the data (14, 19).

$$\text{Normalized volume} = \text{original volume} - \text{grad}(ICV - ICV\ \text{mean}) \quad \text{..... (2)}$$

(grad: the gradient of the regression line between the original volume and the intracranial volume)

To investigate the effect of gender and age on ICV and normalized cerebral, cerebellar, and ventricular volumes, we employed a two-way analysis of variance (ANOVA), which used gender (2 levels) and age (2 levels) as independent variables, using SPSS software (ver. 12.0). We analyzed the relationship between each brain volume and the physical indices using the Pearson correlation method.

Results

The mean ICV for 20s males was 1489.81 ± 107.99cm^3; for 20s females, 1337.74 ± 93.49cm^3; for 40s males, 1435.39 ± 83.69cm^3; and for 40s females, 1319.44 ± 76.89cm^3 (Table 2). The relationships between ICV and cerebral, cerebellar, and ventricular volumes were .665, .488, and .206, respectively, which are all positive (Table 3). Since there was a relationship between ICV and these volumes, we give the results, which were normalized by covariance methods, in Table 2. The normalized cerebral volume for 20s males was 1362.44 ± 69.54cm^3; for 20s females, 1220.74 ± 73.46cm^3; for 40s males, 1233.32 ± 73.85cm^3; and for 40s females, 1103.14 ± 75.86cm^3. The normalized cerebellar volume for 20s males was 140.42 ± 15.06cm^3; for 20s females, 125.80 ± 10.88cm^3; for 40s males, 127.15 ± 9.25cm^3; and for 40s females, 116.71 ± 18.42cm^3. The normalized ventricular volume for 20s males was 18.35 ± 9.42cm^3; for 20s females, 18.75 ± 12.79cm^3; for 40s males, 30.57 ± 12.60cm^3; and 40s females, 21.10 ± 6.62cm^3.

Males' ICV, cerebral, and cerebellar volumes were significantly greater than those of females, and those of the 20s group was significantly greater than those of the 40s group (Table 4). However, there was no interactive effect between age and gender (Fig.1 (a, b, and c)). Males' ventricular volumes were significantly greater than those of females, those of the 40s group were significantly greater than those of the 20s group, and there was an interactive effect between age and gender (Table 4). There was a sizable increase in ventricular volume as males became older, as compared to females (Fig.1 (d)).

Table 2. The means, the minima, and the maxima of the intracranial and normalized cerebral, cerebellar, and ventricular volumes (unit: cm^3), by gender and age.

	Age	Male			Female			Male + Female		
		Mean ± S.D.	Min.	Max.	Mean ± S.D.	Min.	Max.	Mean ± S.D.	Min.	Max.
Intracranial Volume	20s	1489.81±107.99	1277.5	1737.3	1337.74±93.49	1178.1	1627.4	1406.26±125.42	1178.1	1737.3
	40s	1435.39±83.69	1262.7	1590.3	1319.44±76.89	1110.1	1487.1	1364.60±97.46	1110.1	1590.3
	Total	1463.99±100.41	1262.7	1737.3	1327.91±85.06	1110.1	1627.4	1384.98±113.64	1110.1	1737.3
Cerebral Volume	20s	1362.44±69.54	1235.61	1552.74	1220.74±73.46	1050.19	1413.52	1283.72±100.51	1050.19	1552.74
	40s	1233.32±73.85	1100.78	1390.54	1103.14±75.86	914.55	1283.35	1153.68±98.21	1283.35	1390.54
	Total	1304.07±96.07	1100.78	1552.74	1160.79±94.94	914.55	1413.52	1220.56±118.63	1050.19	1552.74
Cerebellar Volume	20s	140.42±15.06	105.10	172.15	125.80±10.88	96.12	148.39	132.39±14.79	96.12	172.15
	40s	127.15±9.25	103.72	153.35	116.71±18.42	97.37	153.08	120.82±16.24	97.37	153.35
	Total	134.12±14.22	103.72	172.15	120.96±15.9	96.12	153.08	126.51±16.55	96.12	172.15
Ventricular Volume	20s	18.35±9.42	10.98	35.92	18.75±12.79	9.32	45.56	18.56±11.27	9.32	45.56
	40s	30.57±12.60	7.75	61.93	21.10±6.62	7.74	39.30	24.82±10.46	7.74	61.93
	Total	24.13±12.56	7.75	61.93	20.04±9.89	7.74	45.56	21.80±11.27	7.74	61.93

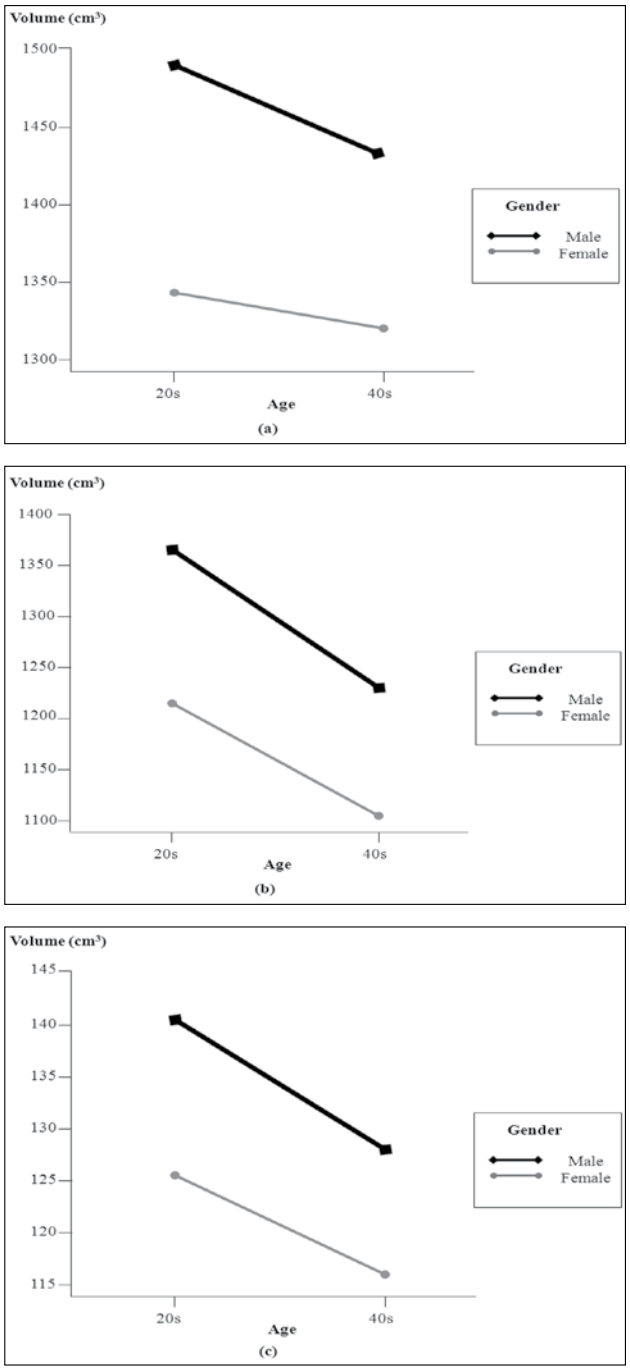
Table 3. The relationships among ICV and cerebral, cerebellar, and ventricular volumes.

	Intracranial Volume	Cerebral Volume	Cerebellar Volume	Ventricular Volume
Intracranial Volume	1	.665 (**)	.488 (**)	.206 (**)
Cerebral Volume		1	.649 (**)	-.052
Cerebellar Volume			1	-.056
Ventricular Volume				1

Table 4. Results of intracranial, cerebral, cerebellar, and ventricular volumes from two-way ANOVA using age and gender as independent variables.

	Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intracranial Volume	**Corrected Model**	905348.826[(a)]	3	301782.942	37.014	.000
	Age	59661.183	1	59661.183	7.318	.007
	Gender	810267.056	1	810267.056	99.381	.000
	Age´Gender	14712.767	1	14712.767	1.805	.181
Cerebral Volume	**Corrected Model**	1527508.523[(b)]	3	509169.508	94.536	.000
	Age	643864.025	1	643864.025	119.544	.000
	Gender	781957.480	1	781957.480	145.184	.000
	Age´Gender	1402.085	1	1402.085	.260	.611
Cerebellar Volume	**Corrected Model**	13445.757[(c)]	3	4481.919	21.955	.000
	Age	5619.876	1	5619.876	27.529	.000
	Gender	7057.687	1	7057.687	34.572	.000
	Age´Gender	196.703	1	196.703	.964	.328
Ventricular Volume	**Corrected Model**	3596.279[(d)]	3	1198.760	11.116	.000
	Age	2224.988	1	2224.988	20.632	.000
	Gender	863.659	1	863.659	8.009	.005
	Age´Gender	1022.512	1	1022.512	9.482	.002

- *R Squared = .379 (Adjusted R Squared = .369)*
- *R Squared = .624 (Adjusted R Squared = .617)*
- *R Squared = .267 (Adjusted R Squared = .255)*
- *R Squared = .359 (Adjusted R Squared = .213)*



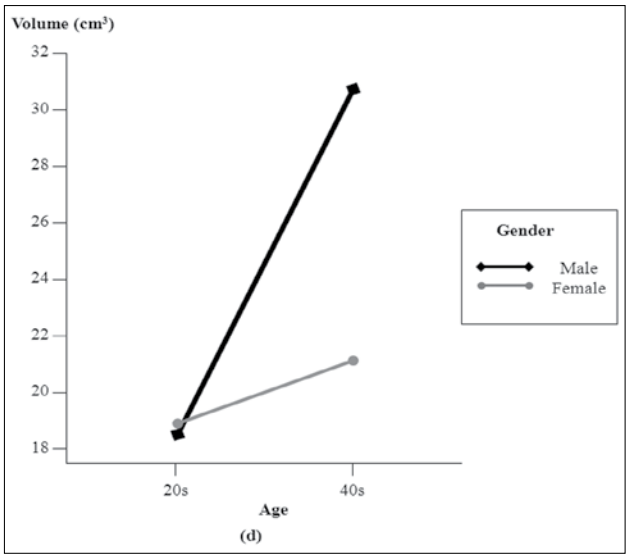


Figure 1. Interactive effects between age and gender on (a) ICV (b) cerebral volume (c) cerebellar volume (d) ventricular volume

Table 5 shows the relationship between the physical indices (weight and height) and ICV and normalized cerebral, cerebellar, and ventricular volumes. For all subjects there was a positive relationship between both the physical indices and ICV and cerebral and cerebellar volume. For males, there was a positive relationship between height and both ICV and cerebral volume. For females, there was a positive relationship between height and cerebral volume. For the 20s group, there was a positive relationship between both the physical indices and ICV and cerebral and cerebellar volumes. For the 40s group, there was a positive relationship between both the physical indices and ICV and cerebral and cerebellar volume, as well as a positive relationship between height and ventricular volume.

Table 5. The relationship between the physical indices (weight and height), ICV, and normalized cerebral, cerebellar, and ventricular volumes and age and gender

		All	Male	Female	20s	40s
Intracranial Volume	**Weight**	.421(**)	.121	-.079	.540(**)	.337(**)
	Height	.546(**)	.309(**)	-.004	.547(**)	.484(**)
Cerebral Volume	**Weight**	.432(**)	.125	-.042	.650(**)	.498(**)
	Height	.661(**)	.426(**)	.338(**)	.634(**)	.593(**)
Cerebellar Volume	**Weight**	.244(**)	.119	-.175	.380(**)	.223(*)
	Height	.406(**)	.189	.158	.433(**)	.234(*)
Ventricular Volume	**Weight**	.061	-.067	-.075	-.106	.201
	Height	.074	-.205	-.012	-.031	.420(**)

** *Correlation is significant at the 0.01 level (2-tailed).*

* *Correlation is significant at the 0.05 level (2-tailed).*

Table 6. Typical published results of the measurements of intracranial, cerebral, cerebellar, and ventricular volumes

		Age	Gender (number) : Volume (cm^3)
Intracranial Volume	Duane et al., 1995	16-65 years	male (89) : 1548.91~1594.10 female (105) : 1335.49~1400.33
	Wilkinson et al., 1997	30s	male (32) : 1596±136 female (19) : 1388±103
	Nopoulos et al., 2000	20s	male (42) : 1490.4±116.7 female (42) : 1351.5±112.8
	Edith et al., 2001	40s	male (51) : 1449 female (41) : 1234.8
	Wolf et al., 2003	70s	male (42) : 1555±110 female (42) : 1408±131
	Kruggel 2006	20s	male (145) : 1616.3±91.1 female (145) : 1494.9±96.3
Cerebral Volume	Ruben et al., 1991	18~80 years	male (34) : 1137.36±100.51 Female (35) : 1045.79±109.86
	Duane et al., 1995	16-65 years	male (89) : 1188.14~1310.99 female (105) : 1357.84~1508.29
	Wilkinson et al., 1997	30s	male (32) : 1403±117 female (19) : 1228±97
	Nopoulos et al., 2000	20s	male : 1254.1±98.0 female : 1130.3±93.0
	Goldstein et al., 2001	30s	male (27) : 1113.1±92.5 female (21) : 1021.8±89.5
	Eileen et al., 2002	20s	male (50) : 1510±0.4 female (50) : 1320±0.1
	Kovalev et al., 2003	18-70 years	male (76) : 1282±93 female (76) : 1161±87
	Kruggel 2006	18-70 years	male (145) : R 625.8±39.3 / L 627.3±39.0 female (145) : R 575.5±40.8 / L 575.4±38.9
Cerebellar Volume	Nopoulos et al., 2000	20s	male (42) : 148.9±19.6 female (42) : 139.3±14.4
	Hutchinson et al., 2003	20s	male (30) : 143.28±12.70 female (30) : 137.15±11.28
	MacLullich et al., 2004	65-70 years	male + female (50) : 144.1±15.3
	Kruggel 2006	18-70 years	male (145) : R 69.9±9.9 / L 74.2±8.2 female (145) : R 64.7±8.7 / L 66.7±7.4
Ventricular Volume	Nicholas et al.., 1997	4–20 years (lateral ventricular)	male (89) : 10.7 female (105) : 9.3
	Nopoulos et al., 2000	20s (internal CSF)	male (89) : 35.3 female (105) : 34.6
	Good et al., 2001	17-79 years (CSF including ventricular and surface sulci)	male (265) : 397 female (200) : 401
	Steven et al., 2003	70 s (lateral ventricular)	male (42) : 35.3±10.5 female (42) : 34.6±12.2

R: Right, L: Left

Discussion

This study measured ICV and normalized cerebral, cerebellar, and ventricular volumes for Korean males and females in their 20s and 40s and analyzed the differences in brain volume according to age, gender, and the physical indices of height and weight.

Table 6 shows typical results of ICV and cerebral, cerebellar, and ventricular volumes for Western people (1, 2, 4, 5, 6, 8, 10, 16, 17, 20, 21, 22, 23, 24, 25). For younger subjects (20s-30s), ICV for males ranged between 1490cm^3 and 1616cm^3, for females, between 1351cm^3 and 1494cm^3. Cerebral volume for males ranged between 1113cm^3 and 1510cm^3, for females, between 1021cm^3 and 1657cm^3. Cerebellar volume for males ranged between 143 cm^3 and 148cm^3, for females, between 137 cm^3 and 139 cm^3. Internal CSF volume for males was 35cm^3, for females, 34cm^3. For older subjects (40s-70s), ICV for males ranged between 1449cm^3 and 1555cm^3, for females, between 1234cm^3 and 1408cm^3. Cerebral volume for males ranged between 1137cm^3 and 1282cm^3, for females, between 1045cm^3 and 1161cm^3. Cerebellar volume for males was 143cm^3, for females, 130cm^3. Lateral ventricular volume for males was 35cm^3, for females, 34cm^3. The normalized volumes for this study are shown in Table 2. By comparing published results with the result of this study, based on age and gender (i.e., for people in their 20s and 40s and for males and females), we found our participants' ICV and cerebral and cerebellar volumes were similar to or smaller than those of Western people. However, the comparison of ventricular volumes was difficult, since ventricular volume showed lots of variation due to measured range. We believe that further study is necessary to analyze differences in brain volume with regard to race.

Published results show that ICV and cerebral, cerebellar, and ventricular volumes are greater for males than for females and that ICV and cerebral and cerebellar volumes decrease and ventricular volume increases with age (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 20, 21, 22, 23, 24, 25, 26, 27). The corresponding results of this study on Korean people were identical to the published results of age and gender effects for Western people. In this study, there was no interactive age and gender effect on ICV or on cerebral and cerebellar volumes, but there was an interactive age and gender effect on ventricular volume. This means that, for males, ventricular volume increased with aging more than it did for females. This result agrees with the results of Coffey et al. (1998) and Ruben et al. (1991) but disagrees with the results of Good et al. (2008) and Guttmann et al. (1998). The reason for these gender differences with regard to aging is still unclear and may be due to internal factors (such as sex hormones) and/or external factors (family circumstances, education, and habits such as smoking and drinking) (13, 28). This issue needs to be explored further.

Studies have reported that age and gender, as well as physical indices such as height, correlate with differences in brain volume (15, 16, 17, 29). Koh et al. (2005) reported that, for males, there was a positive relationship between height and cerebral volume and, for females, there was a positive relationship between height and cerebellar volume. After analyzing the relationship between height and cerebral and cerebellar volumes among 120 males and females in their 20s, Hutchinson et al. (2003) reported a positive relationship between cerebellar volume alone and height. Egan et al. (1995) reported a positive relationship between height and brain volume but not between weight and brain volume. Nopoulos et al. (2000) reported that, for females, there was a positive relationship between cerebral volume and height. As shown here, most of the previous results reported that, even though there was a difference between the genders, there was a significant relationship overall between height and brain volume. Published results by our team showed that, for males, there was a positive relationship between ventricular volume and height and a positive relationship between cerebellar volume and weight (12, 13). However, such previous results determined these relationships using un-normalized brain volumes, since the studies used raw data (12, 13). After normalizing the previous data, we found that, for males, there was a significant relationship between both ICV and cerebral volume and height, while, for females, there was a significant relationship between just cerebral volume and height. This study analyzed the relationship between brain volumes and physical indices by separating out gender as well as the age groups. For both the

20s group and the 40s group, there was a positive relationship between ICV and cerebral and cerebellar volumes and both weight and height. For the 40s group, there was a positive relationship between height and ventricular volume. There was no significant difference between the two age groups with regard to the relationship between brain volume and the physical indices. To find the relationship between brain volumes and the physical indices of height and weight, we used the stepwise approach in regression analysis. This showed that, for males, there was a significant relationship between height and ICV; for females, between weight and cerebral volume; and for the 20s group, between weight and cerebral volume. We can conclude there is a close relationship between brain volume and height, as well as weight. These findings suggest that overall body weight and height are closely related to, or regulated by, the factors that determine brain volume (30, 31).

Conclusively, this study found ICV and cerebral and cerebellar volumes of Korean people were similar to, or smaller than, those of Western people. Differences in brain volume due to age and gender were similar to published results. Even though there was a difference due to age and gender, there was a close relationship between brain volume and height, as well as weight. This study provides exact brain volume information for Korean males and females in their 20s and 40s and might give basic information on how age, gender, and physical indices influence on brain volume in Korean people.

Acknowledgements

This work was supported by Konkuk University in 2011.

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Patients Satisfaction Level Before and After HACCP/ISO 22000 Implementations to Food and Food service in University Hospital, Ankara, Turkey

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Abstract

The primary purpose of this study was to determine differences of patients' satisfaction after HACCP/ISO 22000 implementation in hospital food service. The subjects were 466 patients consisting of 191 males, 275 females from different clinics in one of university hospital. The questionnaire of food and food service satisfaction of patients were filled by intern dietician.Before HACCP/ISO 22000 implementation overall score of patients' satisfaction was 74.7, after HACCP/ISO 22000 implementation it was increase to 81.3 p=0.000. As like as this result approximately all of individual scores (organoleptic, menu and service) were increase after HACCP/ISO 22000 implementation p<0.05. HACCP and ISO 22000 implementation is not only for food quality but also improves organoleptic, menu and service specifications and increase overall satisfaction scores of patients.

Key words: HACCP, ISO 22000, patient satisfaction, Food Service

Introduction

The hazard analysis and critical control points (HACCP) is a food safety system which is accepted worldwide and developed by Codex Alimentarus Commission. HACCP is a preventive approach and it also has some standards and procedures for food services[1]. After all International Organization for Standardization (ISO) was bridging a gap between ISO 9001:2000 and HACCP. This called ISO 22000 and it integrates all procedures of HACCP, good manufacturing practices (GMP) and good hygiene practices (GHP). This standard can be applied with or without independent certification of conformity. There are lots of benefits for users. Some of them are better using of resources, more effective hazard analyses and at the end better satisfaction of customers [2].

Patient satisfaction is become a critical point in past two decades by which the quality of health care service is evaluated [3-5]. In overall patient satisfaction food service satisfaction sometimes looks like negligible. But many researchers stressed that food service satisfaction has very important role in overall score of patient satisfaction [6-9]. Food-quality components that patients are most concerned with are freshness, taste, temperature, variety, and aroma [10-13].

The studies on satisfaction with food and food service stressed that quality of food service is a common problem around the world. In general patients say food is not acceptable or they don't like food service [11, 14]. In this case patients are undernourished and maybe recovery duration of them increased. For this reason food service should be seen as an inseparable part of treatment[15]. Main purpose of study is to determine the factors affecting satisfaction level of food and food service.

Material Methods

The study is carried in a university hospital which has HACCP and ISO 22000 certificate for food service department. The main criteria for the sample selection are the ability of the patients on evaluating food service quality. For this reason we choose patients from different clinics in hospital. After choosing patients we inform them about study and questionnaire filled by last class students of Department of Nutrition and Dieteti-

cs at same university. This study was held in two stages. First of all there were 226 participants (78 male, 148 female) before HACCP and ISO 22000 implementation, and there were 240 participants (113 male, 127 female), after implementation who were voluntarily for study.

The questionnaire: consists of 3 parts. In the first section the general characteristics of individuals, the second part the satisfaction state of the service, the third section, the satisfaction state of food were investigated. The second and the third section, on the status of satisfaction of patients to the service and food were asked to score between 1 and 5 points. 1 point very bad, 2 bad points, 3 points, moderate, good 4 points, 5 points were assessed as very good. Bulk Nutrition Services component of 3 points or more in question was interpreted as satisfied.

The data were analyzed by using Statistical Package for Social Science (SPSS for Windows, version 15.0). The descriptive statistics were used to summarize socio-demographic characteristics of the patients, satisfaction levels, and quality of food services. Bivariate analyses (primarily chi-square test) were used to determine the relationship between each satisfaction scores before and after HACCP/ISO 22000 implementation.

Results

Table 1 represents the socio-demographic characteristics of the patients. According to table % 34.5 and % 47.1 patients were male before and after HACCP/ISO 22000 implementation respectively. Females were % 65.5 and % 52.9 respectively for before and after HACCP/ISO 22000 implementation. Most of total patients (%77) were have high school or less educational level. More than % 60 patients were stay in hospital 1 to 7 days in both genders.

Table 2 shows the patients' views on hospital food and service before and after HACCP/ISO 22000 implementation. Except viscosity, hygiene, cleanness of fork, spoon, dishes and attitude and behaviors of the serving staff scores there is statically significant differences between other scores.

Table 3 indicate that overall score of patients satisfaction. Although before HACCP/ISO 22000 implementation it is % 74.7, after HACCP/ISO 22000 implementation increase to % 81.3 p=0.000. Also minimum score of patients satisfaction is also increase % 10 to % 20.

Table 1. The socio-demographic characteristics of the patients in the sample of study

	Before HACCP (n=226)		After HACCP (n=240)		Total
	n	%	n	%	
Gender					
Male	78	34.5	113	47.1	191
Female	148	65.5	127	52.9	275
Age					
<18	39	17.3	13	5.4	52
18-30	43	19.0	30	12.5	73
31-50	72	31.9	77	32.1	149
>51	72	31.9	120	50.0	192
Marriage Status					
Married	139	61.5	161	67.1	300
Single	87	38.5	79	32.9	166
Education Level					
High school or less	176	77.9	183	76.2	359
Higher education	50	22.1	57	23.8	107
Length of Stay					
1-7	153	67.7	146	60.8	299
8-14	42	18.6	46	19.2	88
>15	31	13.7	48	20.0	79

Table 2. The patients' views on hospital foods and food services

	Before HACCP/ISO 22000						After HACCP/ISO 22000						p
	Yes		No		Uncertain		Yes		No		Uncertain		
Aspects	n	%	n	%	n	%	n	%	n	%	n	%	
Organoleptic Specifications													
Appearance of food*	179	79.2	33	14.6	14	6.2	220	91.7	7	2.9	13	5.4	0.000
Taste of food*	111	49.1	90	39.8	25	11.1	178	74.2	21	8.8	66	14.2	0.000
Viscosity of food	189	83.6	15	6.6	22	9.7	198	82.5	13	5.4	29	12.1	0.640
Warmth of food	183	81.0	23	10.2	20	8.8	195	81.3	13	5.4	32	13.3	0.064
Hygiene of food	219	96.9	3	1.3	4	1.8	236	98.3	2	0.8	2	0.8	0.582
Freshness of Salads*	161	71.2	59	26.1	6	2.7	187	77.9	18	7.5	35	14.6	0.000
Freshness of Fruit*	217	96.0	6	2.7	3	1.3	217	90.4	9	3.8	14	5.8	0.026
Menu Specifications													
Amount of food*	162	71.7	51	22.6	13	5.8	219	91.3	14	5.8	7	2.9	0.000
Variability of food*	196	86.7	24	10.6	6	2.7	412	88.4	33	7.1	21	4.5	0.040
Frequency of food*	156	69.0	41	18.1	29	12.8	216	90.0	8	3.3	16	6.7	0.000
Service Specifications													
Service Shape*	217	96.0	9	4.0	0	0.0	232	96.7	3	1.3	5	2.1	0.018
Cleanness of fork, spoon, and dishes	214	94.7	1	0.4	11	4.9	229	95.4	6	2.5	5	2.1	0.052
Cleanness of staff	226	100.0	0	0.0	0	0.0	240	100.0	0	0.0	0	0.0	
Cleanness of wear of staff	226	100.0	0	0.0	0	0.0	240	100.0	0	0.0	0	0.0	
Attitude and behaviors of the serving staff*	210	92.9	6	2.7	10	4.4	233	97.1	1	0.4	6	2.5	0.069
*p<0.05													

Table 3. Overall score of patient's satisfaction before and after HACCP/ISO 22000 implementation

	Overall score of satisfaction				
	Mean	Std. Dev.	Min	Max	n
Before HACCP/ISO 22000	74.7	21.73	10	100	156
After HACCP/ISO 22000	81.3	14.87	20	100	236
P=0.000					

Discussion

In this study we evaluate patients' satisfaction before and after HACCP/ISO 22000 implementation in hospital food service. According to this study before and after HACCP/ISO 22000 implementation % 49.1 patients were satisfied with taste of food like as Sahin's study [11]. In contrast more than 60 percent patients satisfied with taste of food according to Simmons research [16]. Approximately % 80 of patients were satisfied with appearance of food before HACCP/ISO 22000 implementation in contrast only % 60 of patients satisfied in Sahin's study [11]. According to this study more than three quarter patients were satisfied with variety of food, in contrast approximately % 60 of patients satisfied in some researches [11, 16].

Some results of this study show that there was a negative relationship with between length of stay and satisfaction score of food and food service. This supported by others [7, 11]. According to this study mean of overall score of patients satisfaction is approximately 78 and there is statistically differences between before and after implementation of HACCP/ISO 22000 p<0.01. These results supported by others [13, 17, 18]. In contrast Sahin [11], Aytar [19] and Akoijam [20] found the overall score only 48.7, 50.2 and 24.

Before and after HACCP/ISO 22000 implementation all of satisfaction scores are increased and most of them is statistically significant p<0.05. Maybe the reason for this, these systems makes food service in same standards. Starting from pur-

chasing raw materials to the end of service all of stages for food service is being standard quality. Healthy and adequate nutrition is very important in treatment of patients and decreases the length of stay in hospitals. For this reason hospital food and food service must be satisfied patients. According to this research HACCP and ISO 22000 implementation is not only for food quality but also improves organoleptic, menu and service specifications and increase overall satisfaction scores of patients.

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The Research of Lighting's Influence on the Psychological State of Employees in Working Environment

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Abstract

Having in mind often neglecting and insufficient paying attention to organizational measures concerning providing of proper lighting in work environment, i.e. at workplaces, and in order to understand how and in which way the measures for providing proper lighting in the work environment can be carried out, this paper deals with assessment of working conditions in relation to the parameter- level of lighting based on the research in an industrial environment , i.e. the places where the workers were during their work. Level of lighting was measured by an instrument- "Lux" at three work places: cutting-pressing works section, carpentry works section and the boiler room.

Key words: lighting, work environment, instrument-"Lux"

1. Introduction

Day light is the best lighting for all conditions of one's life and work due to its diffusion. When the artificial light is being used it should be screened, not glared and as diffused as possible. Light is a part of energy from electromagnetic spectrum radiation which one can register through eyesight.[2]

Since our eyesight is very important in all working activities, lighting is a significant factor of working environment as well as a necessary condition for conducting work. But from eyesight, inadequate lighting influences also the psychological state, work productivity and injuries at work. Day light is the best condition for working, but since it is not always achievable it is essential to use an adequate artificial light.

While organizing working places, space positioning in a room it is important to consider the penetrating of direct sunlight into the rooms or work space, preventing the shading of work field by objects or workers as well as preventing the glaring of light and smooth surfaces, prevent the setting of glared shiny surface under the working space except in the case when the working place demands it.[3]

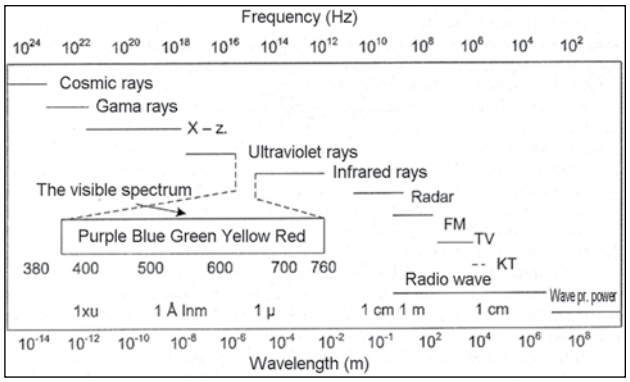
All working positions must have the greatest amount of day light as possible and should be equipped with artificial light which must provide adequate lighting in order to accomplish safe and healthy working conditions.[9]

2. Lighting

All professional activities involve eyesight. The majority of industrial jobs are done in factories where the use of artificial light is necessary to be used due to limited duration and intensity of day light. According to the definition of the Engineering Lighting Society (ELS) the light is "the energy of radiation which is able to awaken the retina and enable the sense of sight".

On the picture No. 1is presented the relation of color spectrum (and the colors within it) and other spectrums. The picture shows that the wave length of the light spectrum is from 380 to 760 nanometers, while the frequency is $(3, 5 - 8)\ 10^{14}$ Hz.

The recommendations and standards regarding the necessary lighting level differ among each other on the group of experts who gave the recommendation or the country where the standard is applied.



Picture 1. Electromagnetic spectrum (McCormick)

Table 1. The compared model of two standards which are used for determination of the necessary illumination level[8]

Type	German DIN (lx)	ELS (lx)
Accurate prefabricated work	1000	3000
Highly accurate work on tool machines	1000	7500
General office work	500	750

While determining the necessary illumination level the following issues are taken into consideration:

1. Employee's work activity;
2. Employee's age;
3. Demands regarding the speed and accuracy in conducting tasks; and
4. The reflection of the surface.

When the details which need to be differed are smaller, the higher level of lightening is needed as well as when it comes to older workers whose eyesight ability is reduced. More light is needed also when high speed and accuracy are needed and in case of reduced reflection as well.

2.1. The Quality of Light

A good quality light implies the light sources which contribute to one's eyesight performances, providing the easiness of watching and work security. In order to fully understand the need for good quality illumination of the work space it is necessary to know that inadequate illumination affects not only the eyesight but also the psychological state of a person which can be manifested through injuries at work and productivity. If it is possible the work should be conducted under day light, but since it is not always possible to enable these conditions the need for the uses of artificial light arouses.

The constant and equaled light contributes to good quality lighting. The brightness of light depends on the activity and work action which is being realized at work places. It also depends on the number of "lux" (the "lux" corresponds the illumination of a certain field that the light falls on –a candle strong and a meter distant). The illumination (the size which is the relation between the light flux and the illuminated surface) of the work place should vary between 40 and 500 "lux" (lx) and up to 2000 lx for conducting a very precise jobs.

The recommended light color is yellow (produced by sodium light bulbs-the middle wave length) because the absorption of yellow is smaller than of the blue or purple.[1]

The influence of the light color on the recognition speed, sharpness and precision of eyesight and its influence on work quality are determined by the conducted experiments and the following arrangement has been done: [4]

1. Yellow; 2. Yellow-green; 3. Orange; 4. Green; 5. Red; 6. Blue-green; and 7. Blue.

When choosing the light color it is necessary to do the following:

1. To avoid the use (monochromatic light) at ordinary work places;[1]
2. To use white or mixed light for illumination;[4]
3. To be aware that fluorescent light bulbs are convenient for illumination, since they provide the light which is similar to day light.[4]

While making the technical documentation in order to provide the necessary level, quality and characteristics of illumination based on domestic regulations and international HASAP standards as well, the technical documentation should contain:

1. Light calculation;
2. The choice of light bulbs which satisfies the demands for illumination and standards for industrial drive (depending on the production process which is being conducted);
3. The positions of light bulbs (i.e. joinery work department) and appropriate switches and single pole scheme form connecting selected light bulbs and cabinets;

4. The calculation for the selection of appropriate cables and fuses;
5. The calculation of the voltage break down;
6. The security efficiency check from dangerous voltage touch; and
7. A detailed specificatin with cable lenghts, as wel as the type and quantity of the rest of the needed equipment.

The role of management is to provide the order and consistency in organization (constituting the order and stability), and of the management system to cause changes and transmissibility (a search for adaptive and constructive changes).[7]

Considering the management which has enabled the employees the quality of life QL (Quality of life) all the necessary conditions for safe and efficient production are created. By high quality illumination efficient work ability among employees and quality of life are provided.

3. Illumination of the work place

An important element of the work place is illumination which is a necessary condition for successful conducting of the working process. The appropriate illumination of the work place should provide:

1. The protection of the eyesight;
2. Smaller level of eyesight tiredness and nerve-muscle intensity;
3. A higher precision at work and reduced number of mistakes;
4. A higher productivity level in all work fields; and
5. Less possibility of being injured.

While illuminating the work places with day light it is necessary to follow certain rules in order to use it as much as possible:

1. Day light illuminates the work place through window holes. The amount of day light does not depend only on the entire surface of the windows but of the relation between the surface and dimension of the room, as well as of the distance between the window and work place;
2. The day light which illuminates the study room should be adjusted to the light which is being reflected from the surrounding walls;
3. With one floor high halls the best results are achieved by using the day light through so called “saw tooth roofs”;
4. With multi-storey halls the window holes are put on the outside. In this case the best result are achieved by using the day light when the ground halls are projected so that the higher edge of the window holes reaches the ceiling of the study room; the lover level of the window should not be lower than the level of the work place surface; the effect of the illumination by day light will be greater if diffuse glass is used and if it is possible the day light should come from left side.[1]

The difference in illumination of a work space occurs due to several reasons:

- The variations in illumination of the work place occur due to variations of the voltage and in electro energetic system which manifests in the change of the light flux, depending on the light bulb type which is used in the room (±3,8% light bulbs with glowing filament; ±3% fluorescent light bulbs of the high pressure and ±1,7% fluorescent light bulbs).[5]

The color of surrounding influences on the illumination and the sense of pleasantness. In order to avoid reflection of the light which mostly comes from above, machines and the lower part of the walls should be dyed in dark colors. It is recommended to use light blue and green for the machines and equipment, as they do not tire the eyes. The intensity of light that colors reflect are white 84%, yellow 60.5%, green 54.1%, blue 53.6%, red 14.4% and black 1.12%.

The color which is recommended for walls in the study room is yellow-green with the addition of white.

Table 2. The harmony of colors[2]

The color of the working object	Matches the background color
Red	White and red
Green	White and yellow
Red	White
Blue	Red, green, blue
White	Black

4. Experimental part

For the evaluation of the working environment, the illumination has been analyzed as a characteristic parameter which influences the working conditions. In order to explain this parameter in the most successful way, the measurement have been conducted in three different industrial runs which are specific by their characteristics and disposition of the equipment.[6]

The first location where the measurements have been conducted is the run of scissor-cutting works. This department is located in the unique building object (industrial factory hall). The separation of the electrical energy has been conducted by setting the cable on the panel plate, set on the metal posts about 2.5 m high and protected from mechanical damaging. On this location the illumination is being conducted by lightings set on the roof construction of the building object designed to work and by introducing day light through transparent cupola on the roof.

The department of the carpenter works is places in the building department which is isolated from the industrial object. The building consists of one industrial hall where the mechanization for the operating and processing of the wood is placed. The walls of the object are made of the ceramic blocks and lined with mortar, dyed in light color on the inside. The illumination of the workshop (natural and artificial) is projected due to the technological process, i.e. it corresponds to the work type.

4.1. Results

On the picture No.2 there is a scheme of the work place placement within scissor-cutting drive for cutting panels.

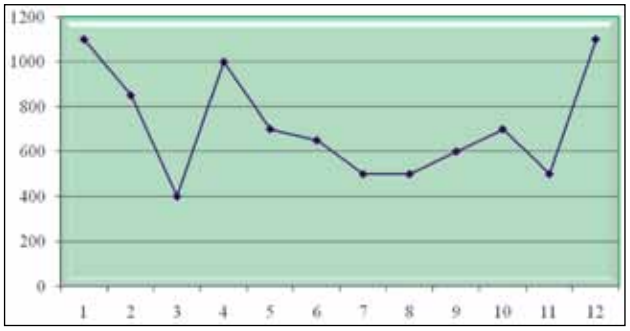
Picture 2. Schematic display of the scissor-cutting works

The measurement of the illumination in the scissor-cutting department works has been conducted in twelve work places. In the table No 3 there are the results gained by constant illumination measurement in the scissor-cutting drive.

Based on the results gained by the measurement of the illumination intensity in twelve work places, it is easy to come to a conclusion that the illumination intensity corresponds to the demands of the conditions for work places where the measurement has been done.

Table 3. The display of gained results from the measurement in the department of the scissor-cutting works

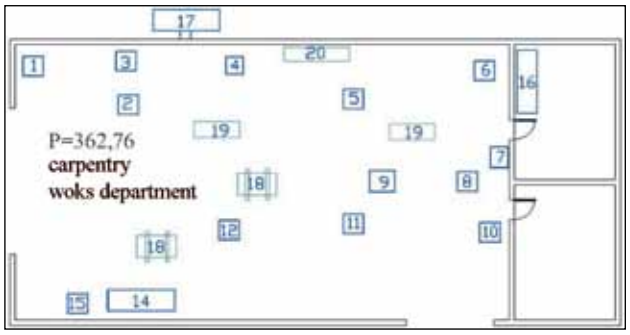
The ordinal number of the work place	Illumination (lx)	
	Work place	Controlling space
1	1100	-
2	850	-
3	400	-
4	1000	-
5	700	-
6	650	-
7	500	-
8	500	-
9	600	-
10	700	-
11	500	-
12	1100	-



Graph 1. Graph display of the gained results from the measurement (table 3)

On the picture No. 3 there is a scheme of the work places placement in the department of the carpenter works.

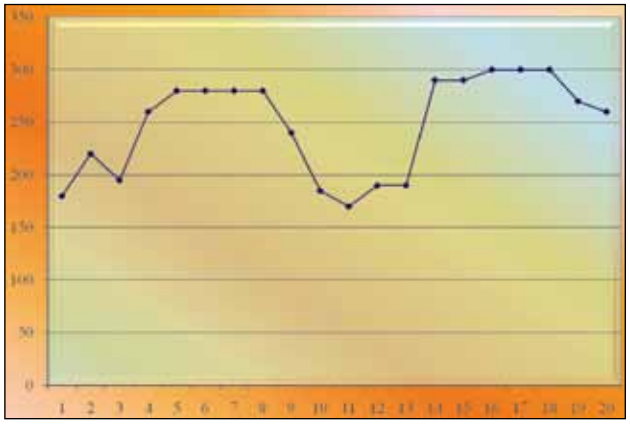
On the second measurement place which differs from the previous one by the placement of the equipment and geometry of the object, the level of illumination which is measured is shown in the table No.4. It has been taken into consideration 20 different positions in the carpenter department.



Picture 3. Schematic display of the carpentry woks department

Table 4. The display of gained results

The ordinal number of the work place	Illumination (lx)
	Work space
1	180
2	220
3	195
4	260
5	280
6	280
7	280
8	280
9	240
10	185
11	170
12	190
13	190
14	290
15	290
16	300
17	300
18	300
19	270
20	260



Graph 2. Graph display of the gained results from the measurement (table 4)

Based on the gathered results, by the measurements of illumination for every work place in the department of the carpenter drive, the intensity of illumination is on the normal level of necessary illumination in order to conduct certain work tasks.

In order to do the tasks safely, the review of necessary illumination has been given in relation to specific work places of the carpenter department:

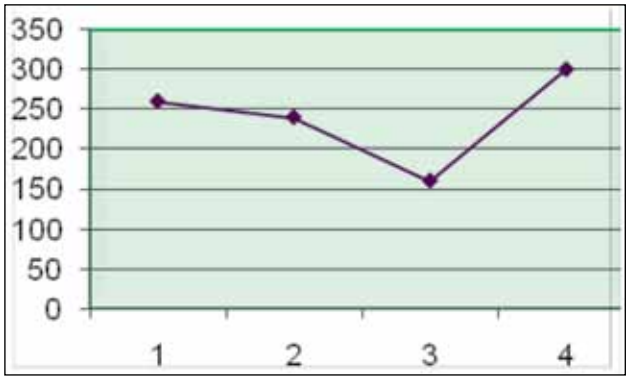
1. **Work desk:** the light should fall from above, especially above the place where the vice are**;**
2. **Table circular:** the light should fall from above, from left and behind the saw;
3. **Strip saw:** the light should fall from above and left from the saw;
4. **Table for milling machine:** it is necessary that the whole surface is illuminated;
5. **Carpentry lathe:** the light should fall from above focused on the process;
6. **Pillar drill:** the light should fall from above, directly on the part with the drill;
7. **Abrihter:** the light should come from the back side and left from the knife;
8. **Diht:** the illumination should be diffuse from all sides of the machine;
9. **Table grinder:** the illumination should be diffuse;
10. **Finishing table:** if it is possible the work should be done in day light, but if the work is being done in artificial light it should be white and diffuse (without any shades).

On the picture No.4 there is a display of the work place in the boiler-room.

Picture 4. A display of the work place in the boiler-room

Table 5. The display of gained results

The ordinal number of the work place	Illumination Work place (lx)
1	260
2	240
3	160
4	300



Graph 3. Graph display of the gained results from the measurement (table 5)

The characteristic which is common for all of these three places where the measurements have been conducted is the fact that the intensity of illumination corresponds to the demanded conditions of the work place. The system of illumination is direct or semi-direct, with additional illumination. The lightning is usually the wide tin reflector which can be applied to all types of light bulbs. When choosing the height of illumination and color of light one analyses the type of work which is being done.

For measuring natural and artificial illumination on the mentioned work places there has been used a photometric method and the instrument which has been used for the measurement of light intensity is a lux-meter, therefore this method is also called lux-metric method. The measured values are given in lux.

Lux-meter consists from photocells, head meter with a scale for reading the illumination and filters with the coefficient of absorption of the light energy marked on the filter. The lux- meter scale has two kinds of scales. For the illumination from 0 to 100 lx there is one scale that is used, and for the illumination from 100 to 1000 lx there is another. When the measurement is being conducted the lux-meter is held horizontally, and the photocell is horizontal in a certain place of the surface where the intensity is measured.

Measuring the brightness by lux-meter is based on the principle of converting light energy into electricity using a photoelectric cell. Exposure of selenium and cilium photo-elements to light creates an electric current. The intensity of the resulting electrical current measured by ammeter is proportional to the amount of light that reaches the photo-elements.

Periodic measurements of light intensity and measures for ensuring quality in the workplace lighting provide:

- adequate lighting for safe and efficient job performance;
- avoid overshadowing affecting the safety and efficiency;
- that the sources of lighting and illumination area does not adversely affect the eyesight of employees;
- avoid sharp contrasts and the blinding reflection of the visual field staff, which affected their eyesight;
- that the work environment is not an intense spark, blinking lights and vibrations resulting from visual stress;

Labor productivity, waste reduce and reduce of the number of injuries, reduce the fatigue of workers which depend on the good light factory halls. The main task of quality lighting is to enable the employee to work with the same accuracy and productivity as well as in daylight.

5. Conclusion

Due to the frequent neglect and giving insufficient attention to organizational measures-concerning the security of proper lighting in the workplace, the paper emphasized the importance of the concept of quality lighting. **Lighting** was discussed in terms of characteristic parameters affecting the work environment and a necessary condition for the safe implementation of work tasks. Subject is approached from two perspectives: theoretical, where the characteristics and importance of proper lighting, work space and how to measure the intensity of light, carried out in three different positions with each other and to their specific characteristics. Workplaces where they made measurements show that the intensity of li-

ght varies depending on the workplace and work processes, and the need for periodic measurement of the intensity lighting in workplaces.

The influence of working environment for a worker is manifested by the work environment significantly affects the productivity of workers. Under the working environment we mean the material factors in which employees have a work activity. Material factors include technical and physical conditions of the working environment. One of the physical conditions that affect the **senses of workers** is lighting. **Lighting,** if not adequately act only on vision but also the **psychological state** of workers, what implies **a significant impact on the movement of labor productivity, work capacity, degree of fatigue and injury**.

Identification of hazards and potential harmful effects in the working environment has a fundamental importance in the management of quality of working environment. Management and impact assessment in the workplace is achieved by a two-fold objective**: to reduce production costs and thereby optimize our processes, making both entrepreneurial capacities more competitive in the market, while the other improves the quality of life and work of employees.**

Methods of analysis and impact assessment in the workplace are the research process that must be professionally and scientifically based on a multidisciplinary approach. Well-done environmental impact assessment is a prerequisite for adequate **planning** of **prevention, preparation, response to the accident (which is the final option) to repair the consequences.** Also, the assessment provides relevant data for further optimization of the process of governing a technological production system and its environment.

Methods of impact assessment work environment are quite extensive and demanding (analysis of more than one place), but this is offset by the lack of reliability to the conclusions obtained from a larger quantity of information collected. Assessment and analysis of the impact of working environment can be observed effect of the measures taken to increase safety and quality of working environment conditions in production processes. Also, the conservation status can be expressed through different parameters, resulting in a more complete overview of possible impacts of working environment.

One of the possible alternatives to improve business operations, especially in countries in transition is the process of implementation according to some views, improve the business can be achieved only by the analysis of international best practice foreign experience and advancement of knowledge in development of quality management and achieving competitive ability.

Accountable management of each organization must identify the highest possibility to influence the domain of probability impact hazard to employees, and therefore take the necessary measures (organizational, technical and highly educational) that evaluated the risk decreases and it successfully managed.

On the other hand observed, a development expert is a prerequisite for successful participation in globe and European integration. It is believed that the young professionals, especially those that are directed towards the industrial sector, should have knowledge in the field of organizational management, strategic and operational management, marketing, quality management and technological development in order to enable them to solve managerial and technical problems.

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Respiratory Viral Infections in Patients With COPD Exacerbation

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Abstract

Background: There is growing evidence that viral infections play a role as a risk factor for COPD exacerbation. This study aimed at epidemiological evaluation of viral respiratory infections in patients COPD exacerbation.

Materials and Methods: In this descriptive cross-sectional study respiratory syncytial viruses (RSV), influenza viruses A, B and C, Parainfluenza viruses 1, 2 and 3, picornaviruses, corona viruses, adenoviruses, HMPV (human metapneumovirus) and human bocavirus (HBOV) were screened using nested PCR from 111 sputum, nasal lavage, and throat wash samples of patients with COPD exacerbation.

Results: A total of 43.24% of samples were positive for under study viruses. Influenza viruses, RSV, parainfluenza, picornaviruses, corona viruses, adenoviruses, HMPV and HBOV were detected in 3.6% (2 cases had type A and 2 cases had type C), 2.7% (all had type B), 1.8% (all had type 1), 21.6% (12 had rhinovirus and 12 had enterovirus), 17.12% (8 cases had OC43, 10 cases had 229E, and 1 case had NL63), 5.4%, 0.9% and 0.9% of under study patients, respectively. A total of 13 cases had co-infections.

Conclusion: Our study showed that respiratory viral infections were associated with COPD exacerbations. Picornaviruses and corona viruses were detected to be the most common cause of infection.

Key words: COPD exacerbation, Viral infection, COPD

Introduction

COPD is a type of airway obstruction which is progressive and irreversible and is associated with inflammatory response of the lungs to extrinsic harmful particles (1).Exacerbation of symptoms is defined as increased discomfort in breathing, and increased sputum volume. This exacerbation is usually in direct correlation with increased inflammation of the airways (2). Patients with COPD exacerbation have recurrent symptoms (3). Respiratory viruses are among the main factors responsible for exacerbation of COPD (4). Primary studies estimated the presence of such viruses in exacerbation phases to be 30% by using serologic methods and viral culture for detection of respiratory viruses (5). More sensitive molecular techniques showed that viral agents were present in 40 to 50% of exacerbated cases (6, 7, 8). Picornaviruses, corona viruses, influenza, parainfluenza, and RSV were among the most common respiratory viruses detected in these patients (8).

The most frequent viral infections evaluated in the year 2000 by Seemungal et al. were due to rhinoviruses which were detected in 58% of virus-induced COPD exacerbations and 23% of all COPD exacerbations (8). Rohde et al. used PCR for detection of viruses in patients COPD exacerbation and found respiratory viruses in 56% of cases with COPD exacerbation incomparison to 19% of controls with stable COPD (9). Researchers believe that the majority of COPD exacerbations are associated with upper or lower respiratory tract viral infections. However, the prevalence of these virus infections has yet to be determined in Iran. This study aimed at determining the role of these infectious agents in COPD exacerbations using PCR on respiratory samples and evaluating their correlation with clinical parameters.

Materials and Methods

This descriptive cross-sectional study evaluated the presence of respiratory viruses in patients with COPD exacerbation who presented to Masih Daneshvari Hospital in Tehran, Iran during 2007-2008. A total of 111 samples were collected from adult patients (sputum, nasal lavage, throat wash) during 4 seasons in year. At first, exacerbation of COPD was diagnosed by the pulmonologist in change of the study and after that samples were obtained and evaluated by the a virologist. The exclusion criteria were asthma and dyspnea related to causes other than COPD. This study was approved by the ethics committee of Masih Daneshvari Hospital. An informed written consent was obtained from all patients through questionnaires prior to the conduction of this study.

Data collection

Data regarding patients' age, sex, season of the year, viral infections, tobacco consumption, clinical symptoms and signs of viral infection, exacerbation of symptoms, FEV1 (Forced Expiratory Volume in 1 second) and number of exacerbations in the previous year were collected and recorded upon admission. A few hours after admission, nasal lavage, sputum and throat wash samples were collected from patients.

Samples

Sputum sample was collected either naturally or after using nebulised hypertonic saline. If the sputum sample was thin, it would be divided into 2 tubes. If it was thick, 500 microliter of phosphate saline buffer would be added, it would be vortexed for 3 minutes in order to separate the tissues and then the solution would be divided into 2 tubes.

In order to obtain the nasal lavage, patient was seated on a chair flexing the head towards the chest. A total of 6 ml of the insoluble isotonic saline buffer (0.9% salt) was injected into the nostrils. Nasal discharge was collected using a special adapter . This procedure was repeated 5 times. Samples were collected in a sputum tray and transferred to the laboratory. Samples were prepared within 20 minutes and divided into 2 tubes.

Throat wash sample was obtained by gargling phosphate buffered saline or transferase solution (Sinagen Co.) and after 1 minute of vortexing, it was transferred into 2 tubes.

All the divided samples were stored at -70°C freezer until DNA or RNA extraction.

Nucleic acid purification (DNA and RNA)

DNA was extracted from the clinical samples using the classic method of phenol, chloroform (Sinagen Co.). RNA was extracted using RNX solution (Sinagen Co.). The extracted DNA was stored at -20°C until PCR. The extracted RNA was stored at -70°C until the manufacturing of cDNA.

Reverse transcription (cDNA)

Total reaction volume was 20 µl. At first, 1 µl random hexamer with 100 micromole concentration and 11 µl RNA were extracted and added to a 0.5 ml sterile tube without RNase- DNase- Pyrogen (equal to the number of understudy samples). This mixture was heated for 5 minutes at 65°C which resulted in RNA helix to uncoil. Then the mixture was quickly transferred on ice. At the same time, the mother mixture for cDNA reaction was prepared which included 4 µl of RT buffer (5X), 2 µl of dNTP (10 mM), 1 µl Mmulv (100 unit/µl) and 1 µl RNase inhibitor (40 unit/µl)(Fermentas Co.). After completion of heating, 8 µl of the mother mixture was added to the tube. The tube was briefly centrifuged and incubated at 25°C for 10 minutes, 42°C for 60 minutes, and 70°C for 5 minutes. All the cDNAs were stored at - 70°C until further examinations.

Screening for respiratory viruses using polymerase chain reaction (PCR)

First, the quality of the extracted RNA (by amplification of some segments of human GAPDH gene) and DNA (through amplification of some segments of human Beta globin gene) was confirmed and then we searched for the respiratory virus genomes using RT-nested PCR for rhinoviruses, enteroviruses, coronaviruses (OC43, 229E, NL63), parainfluenza I, II and III, influenza A, B and C, RSV A and B and HMPV and nested PCR for ade-

Table 1. Primer sequences, genomic localisation, cycle profiles, the expected length of the PCR product and MgCL2

Virus	Primer	Primer sequence 5¢- 3¢ direction	Genomic position	Cycle profile	Length of PCR product (bp)	MgCL2
Adeno	HEX1deg	GCCSCARTGGKCWTACATGCACATC	Hegxon gene	94°C/40S-55°C/40S-72°C/45S(33X)	259	4
	HEX2deg	CAGCACSCCGCGRATGTCAAA		94°C/40S-55°C/40S-72°C/45S(33X)		
	neHEX3deg	GCCCGYGCMACCGACACSTACTTC		94°C/40S-55°C/40S-72°C/45S(33X)		
	neHEX4deg	CCYACRGCCAGCGTRWAGCGMRCYTTGTA		94°C/40S-55°C/40S-72°C/45S(31X)		
Entrovirus *	ENTRO1	ATTGTCACCATAAGCAGCCA	Polyprotein gene	94°C/40S-55°C/40S-72°C/45S(31X)	117	4
	ENTRO2	MGGTACTTTGTACGCCTGT		94°C/40S-55°C/40S-72°C/45S(31X)		
	ENTRO3	TCCGGCCCCTGAATGCGGCTA		94°C/40S-55°C/40S-72°C/45S(31X)		
	ENTRO4	GAAACACGGACACCCAAAGTA		94°C/40S-55°C/40S-72°C/45S(31X)		
Rhinovirus	NCRO1	CGGTAATYTTGTACGCCAGTTT	5¢LTR	94°C/40S-55°C/40S-72°C/45S(33X)	300	5
	NCRO2	GAAACACGGACACCCAAAGTAGT		94°C/40S-55°C/40S-72°C/45S(33X)		
	NCRin1	CAAGCACTTCTGTTTCCCCGG		94°C/40S-55°C/40S-72°C/45S(33X)		
	NCRin2	CTATCAGGGGCCGGAGGA		94°C/40S-55°C/40S-72°C/45S(33X)		
Influenza A/B*	A EXTL	CCGTCAGGCCCCCTCAAAGC	INF A: 7-segment matrix INF B: Glatonin	94°C/40S-50°C/40S-72°C/45S(33X)	INF A:311 INF B: 767	5
	A EXTR	GACCAGCACTGGAGCTAGGA		94°C/40S-50°C/40S-72°C/45S(33X)		
	B EXTR	GTGACTGGTGTGATACCACT		94°C/40S-55°C/40S-72°C/45S(33X)		
	B EXTR	TGTTTTCACCCATATTGGGC		94°C/40S-55°C/40S-72°C/45S(33X)		
	A INTL	CCGAGATCGCACAGAGACTTGAAGAT		94°C/40S-55°C/40S-72°C/45S(33X)		
	A INTR	GGCAAGTGCACCAGCAGAATAACT		94°C/40S-55°C/40S-72°C/45S(33X)		
	B INTL	CATTTTGCAAATCTCAAAGG		94°C/40S-55°C/40S-72°C/45S(33X)		
	B INTR	TGGAGGCAATCTGCTTCWGC		94°C/40S-55°C/40S-72°C/45S(33X)		
	CHAAS	ACACTTCCAACCCAATTTGG		94°C/40S-55°C/40S-72°C/45S(33X)		
Influenza C	CMIcis	GAGGATGTGRCAACTACTTTTCAAG	HE	94°C/40S-55°C/40S-72°C/45S(33X)	390	4
	CMIcis	GAGGATGTGRCAACTACTTTTCAAG		94°C/40S-55°C/40S-72°C/45S(33X)		
	CHADas	CCTGACAGCAACTCCCTCAT		94°C/40S-55°C/40S-72°C/45S(33X)		
ParaInfluenza*	PIIR	CCTTAAATTCAGATATGTAT	Hemogolubin Neuraminidase gene	94°C/40S-50°C/40S-72°C/45S(33X)	PAR INF I:317 PAR INFII:204 PARINFIII:103	5
	PI1F	GATAAATAATTATTGATACG		94°C/40S-50°C/40S-72°C/45S(33X)		
	PI2R	ATGTCAGACAATGGGCAAAT		94°C/40S-50°C/40S-72°C/45S(33X)		
	PI2F	AACAATCTGCTGCAGCATTT		94°C/40S-55°C/40S-72°C/45S(33X)		
	PI3R	TTTAAGCCCTTGTCAACAAC		94°C/40S-55°C/40S-72°C/45S(33X)		
	PI3F	CTGTAAACTCAGACTTGGTA		94°C/40S-55°C/40S-72°C/45S(33X)		
	Pine1R	CTTTGGAGCGGAGTTGTTAAG		94°C/40S-55°C/40S-72°C/45S(33X)		
	Pine1F	CCGGTAATTTCTCATACCTATG		94°C/40S-55°C/40S-72°C/45S(33X)		
	Pine2R	GCCCTGTTGTATTTGGAAGAGA		94°C/40S-55°C/40S-72°C/45S(33X)		
	Pine2F	CCATTTACCTAAGTGATGGAAT		94°C/40S-55°C/40S-72°C/45S(33X)		
	Pine3R	TAAATCTTGTTGTTGAGATTG		94°C/40S-55°C/40S-72°C/45S(33X)		
	Pine3F	ACTCCCAAAGTTGATGAAAGAT		94°C/40S-55°C/40S-72°C/45S(33X)		
RSV A/B*	AB R	GGGCTTTCTTTGGTTACTTC	Nucleocapsid protein gene	94°C/40S-50°C/40S-72°C/45S(33X)	RSV A:334 RSV B:183	5
	AB F	GTCTTACAGCCGTGATTAGG		94°C/40S-50°C/40S-72°C/45S(33X)		
	A R	GATGTTACGGGTGGGGAGTCT		94°C/40S-50°C/40S-72°C/45S(33X)		
	A F	GTACACTGTAGTTAATCACA		94°C/40S-50°C/40S-72°C/45S(33X)		
	B R	GAAATTGAGTTAATGACAG		94°C/40S-50°C/40S-72°C/45S(33X)		
	B F	AATGCTAAGATGGGGAGTTC		94°C/40S-50°C/40S-72°C/45S(33X)		

** The Influenza A/B, ParaInfluenza,RSV A/B of primers was used as a multi-plex*

Virus	Primer	Primer sequence 5¢- 3¢ direction	Genomic position	Cycle profile	Length of PCR product (bp)	MgCL2
Hmpv	PF	ACGGGGTAGAGAAGACTGG	Nucleocapsid protein gene	94°C/40S-60°C/40S-72°C/45S(31X)	259	4
	PR	GCAAAGTTGGGACAGTTGGC				
	NF	GCATCAACCATAGAAGTGGGAC		94°C/40S-60°C/40S-72°C/45S(31X)		
	NR	GCATTGTTTGACCGGCCCCA				
OC43	O1s	CCCAAGCAAACTGCTACCTCTCAG	Nucleocapsid protein gene	94°C/40S-55°C/40S-72°C/45S(33X)	304	5
	O3ias	GTAGACTCCGTCAATATCGGTGCC				
	O1s	CCCAAGCAAACTGCTACCTCTCAG		94°C/40S-55°C/40S-72°C/45S(33X)		
	O2as	GAATTAGGAGCAGACCTTCCT				
229E	E1s	AGGCGCAAGAATTCAGAACCAGAG	Nucleocapsid protein gene	94°C/40S-55°C/40S-72°C/45S(33X)	293	4
	3as	MGCAGGACTCTGATTACGAGAAGG				
	E1s	AGGCGCAAGAATTCAGAACCAGAG		94°C/40S-55°C/40S-72°C/45S(33X)		
	3as	CGAGAAGGCTTAGGAGTACC				
NL63	1s	GTGATGCATATGCTAATTTG	Nucleocapsid protein gene	94°C/40S-50°C/40S-72°C/45S(33X)	169	6
	1as	CTCTTGCAGGTATAATCCTA				
	2s	TTGGTAAACAAAAGATAACT		94°C/40S-50°C/40S-72°C/45S(33X)		
	2as	TCAATGCTATAAACAGTCAT				
Bocavirus	NS-3	CCCAAGATACACTTTGCWKGTTCCACCC	NS-1	94°C/40S-55°C/40S-72°C/45S(33X)	196	4
	NS-1	TATGGGTGTGTTAATCATTTGAAYA				
	NS-3	CCCAAGATACACTTTGCWKGTTCCACCC		94°C/40S-55°C/40S-72°C/45S(33X)		
	NS-2	AACAAAGGATTTGTWTTYAATGAYTG				

noviruses and HBOV and application of specific primers for each virus. For detection of coronaviruses OC43 and 229E, influenza C and HBOV heminested PCR primers were employed. For the first phase of amplification, 5µl of 10x PCR buffer, 5 µl (10 mM each)dNTPase, 3 µl (10µM) primer, 3 µl cDNA or DNA and 2 units (5u/ µl) Taq polymerase (hot star Fermentas) were added to the PCR reaction mixture. The amount of Mgcl2 (50mM) used was different for each virus. The final volume of the mother mixture in PCR reaction had to be 50 µl and water was added to the mixture to reach this volume. Finally, 2 drops of mineral oil was added to the mixture and it was placed in the PCR device. In nested PCR, the internal primer and 3 µl product of the first amplification step were substituted to make the same mother mixture. Table 1 shows the sequence of primers, genomic position, cycle profile, the expected length of the PCR product, and amount of MgCL2 for each virus.

PCR products were obtained through electrophoresis in ethidium bromide soaked in agarose gel 2% and the photograph was interpreted under UV light. One negative control (pure water) and one positive control were included in each reaction. On the agarose gel, DNA and cDNA segments are separated based on their size and move on the gel. Percentage of gel used was determined based on the size of DNA. In this study, considering the length of obtained segments, 2% agarose gel was used.

Data analysis

The obtained data were analyzed using mean± standard deviation test. T test was used for comparison of the distribution of continuous variables. For other variables, Mann Whitney u test was employed. Chi square test was used for discrete variables, frequency and percentage. $P<0.05$ was considered statistically significant. SPSS (Chicago) version 11.5 software was used for statistical analysis.

Results

Subjects

A total of 111 patients suffering from COPD exacerbation were enrolled in this study. Table 2 demonstrates the demographic characteristics of

patients. Patients were evaluated in terms of age, sex, tobacco use, and FEV1. Number of male and female patients was not equal in our study. We had 2 female patients and we were not able to obtain any result based on gender. Therefore, we considered all of our patients as males.

Table 2. Patient demographics and results of investigations

Measurement	Patient group Exacerbated COPD
No. patients	111
Sex(M/F)	109 M & 2 F
Age	66 ± 10.7
FEV1,litres(%Pred)	56 ± 23.09
Tobacco(packs/year)	40 ± 26.7

Patients' symptoms

Patients with COPD exacerbation usually experience increased severity of symptoms like wheezing, muscular pain, rhinorrhea, sore throat, fever, sputum purulence, nasal congestion, hoarseness, and headache (Table 3). A significant association was observed between rhinorrhea and hoarseness with COPD exacerbation ($P<0.041$ and $P<0.027$, respectively). Most patients suffering from COPD exacerbation in this medical center reported that they experience exacerbations 3.3 times a year.

Table 3. Patient symptoms during exacerbations

	During Exacerbation COPD
Wheezing/rhonchi (n, %)	82/111 (73.9%)
Nasal congestion (n, %)	34/111(30.6%)
Rhinorrhea (n, %)[a]	21/111(18.9%)
Sore throat (n, %)	27/111(24.3%)
Purulent sputum (n, %)	69/111(62.2%)
Fever (n, %)	18/111(16.2%)
Headache (n, %)[a]	21/111(18.9%)
Muscle pain (n, %)	32/111(28.8%)
Hoarseness (n. %)	44/111(39.6%)

[a].Chi squared test, Rhinorrhea and respiratory virus detection, Headache and respiratory virus detection

Detection of respiratory viruses

Respiratory viruses were isolated from 3 types of clinical samples including sputum, nasal lavage and throat wash. The highest rates of respiratory viruses during COPD exacerbation were detected in the sputum (68.18%) followed by the throat wash (27.27%), and nasal lavage (4.54%). In a total of 111 patients with exacerbation of COPD, 48 viruses were detected as follows (Table 4): rhinoviruses (10.8%), enteroviruses (10.8%), influenza A (1.8%), influenza C(1.8%), parainfluenza I (1.8%), RSVB (2.7%), adenoviruses (5.4%), HMPV (0.9%), corona viruses OC43 (7.2%), NL63 (0.9%), 229E (9%) and HBOV (0.9%). It should be mentioned that influenza B viruses, parainfluenza II and RSVA were not found in any patient. Co-infection with 2 viruses was found in 12 and with 3 viruses was found in 1 case (Table 4). The highest frequency of respiratory viruses were detected in patients with moderate to severe airway disease suffering from COPD exacerbation ($P>0.417$) (Table 5). A correlation was observed between viral infections and season ($P>0.001$). Also, significant correlations were observed between viral infections and some clinical symptoms. No correlation was detected between viral infection and patient's age ($P>0.490$). In general, no association was found between the isolated viruses and presence/lack of clinical symptoms ($P>0.669$).

Discussion

This study evaluated the epidemiology of respiratory viral infections in patients suffering from COPD exacerbation presenting to Masih Daneshvari Hospital. This study was conducted in one year period to avoid any bias related to the season of the year. In this study, respiratory viruses were detected in 43.24% of cases with COPD exacerbations which is comparable with the results of McManus et al. (37%)(7), Seemungal et al. (39.2%) (6)and Beckham et al. (41.8%) (10). However, our obtained figure was lower than that of Rohde et al. which reported a 56% rate (9).

To our knowledge, this study was the first descriptive cross-sectional study conducted in Iran to evaluate respiratory viruses (16 types of viruses) in patients with COPD exacerbations. Also, this is the first time in the world that a wide range of viruses (16 types) are evaluated in such study. The main preference of this study over others is that we detected influenza C, enteroviruses and HBOV and evaluated the co-infection with coronaviruses and parainfluenza. Since our understudy patients had been referred to this hospital from all over the

Table 4. Respiratory viral infection

Variable	Patient group Exacerbated COPD
No. patients	**111**
Respiratory viral screen positive (%)	**48(43.24%)**
Rhinovirus [a]	**12**
Entrovirus [a]	**12**
Influenza A	**2**
Influenza B	**0**
Influenza C	**2**
Parainfluenza I	**2**
Parainfluenza II	**0**
Parainfluenza III	**0**
Adenovirus	**6**
Metapneumovirus	**1**
RSV A	**0**
RSV B	**3**
Coronavirus 229E	**10**
Coronavirus OC43	**8**
Coronavirus NL63	**1**
Human bocavirus	**1**
Dual infections	
Entro and Rhino	**4**
RSV B and OC43	**2**
Rhino and OC43	**1**
Inf A and Entro	**1**
Adeno and 229E	**1**
Para I and Adeno	**1**
Inf C and 229E	**1**
HBOV and Entro	**1**
Trine infections	
Para I and Adeno and Inf C	**1**

.a Rhinoviruses and enteroviruses are members of the picomavirus family.

Table 5. Respiratory viral infection and COPD stage

GOLD Stage	Respiratory virus screen status Respiratory virus positive (%)
1	7(23.3) a
2	11(36.7)
3	11(36.7)
4	1(3.3)

a Chi squared test, comparison of groups 1e3 with group 4, $p < 0.05$.

country, this study might have shown the administration of these infections in our country as well.

Rhinoviruses were the most frequent respiratory viruses detected by Seemungal et al. (6). However, we found a high incidence of infection with picornaviruses (21.6%) and coronaviruses (17.12%) which had not been detected before altogether. In some other studies conducted by Johnson et al. and Nicholson et al. rhinoviruses and corona viruses were the most common viruses detected (11). McManus et al. their study demonstrated that detection of adenoviruses by PCR technique is more prevalent than through other conventional methods like florescent and culture techniques (7). In present study, we used nested PCR for detection of adenoviruses and the detection rate for this virus was 5.4%. However, McManus et al. and Coyle et al. reported a high rate of infection with adenovirus in their study (7). This difference may be due the fact that they evaluated smoker patients or may be attributed to the epidemiology of the under study area (Ireland)(7).

In our study, detection rate for influenza was 3.6% out of which 1.8% belonged to influenza type A and 1.8% belonged to influenza type C. One of the main findings of our study was detection of influenza type C in 1.8% of patients with COPD exacerbation. Whereas, in other studies like Hogg, only influenza B was detected in 3% of cases (12). McManus et al. detected RSV in 11.4% of hospitalized patients with COPD (7). In However, RSV B was detected only in 2.7% of patients with COPD exacerbations and RSV A was not detected in any patient in our study.

In a study conducted by Mohan in 2010, parainfluenza virus was detected in 2.6% of patients with COPD exacerbations (13). In another study conducted by camargo et al. in 2008, 2 cases of parainfluenza III and 1 case of parainfluenza I were detected (14). In our study, we only detected parainfluenza I in 1.8% of cases with COPD exacerbation presenting to Masih Daneshvari Hospital and no cases with parainfluenza II and III were detected.

Rohde et al. found HMPV in 2.3% of patients with acute COPD exacerbation. However,This virus was not detected in cases with stable COPD (15). In our study, HMPV was detected in 0.9% of the understudy cases.

HBOV was first found in 2005 and is responsible for acute respiratory infection in children. In

a study conducted in 2009 by Ringshausen et al., adult patients suffering from COPD exacerbation were evaluated for HBOV infection and 3 cases of HBOV were detected in these patients out of which 2 cases were isolated from sputum and 1 from nasal lavage (16). we only found one case(0.9%) HBOV of the under study case.

We had the highest incidence of co-infection in our study and found 12 cases of co-infection with 2 viruses (10.8%) and one case of co-infection with 3 viruses (0.9%). In McManus et al. study in 2008, co-infection with more than 1 virus was detected in 6 cases with COPD exacerbations (7).

Patients suffering from COPD exacerbations usually underestimate the role of respiratory viral infections in these exacerbations. Seasonal respiratory infections also have an impact on COPD patients (7). Respiratory viral infections may occur throughout the year. However, rate of infection in each season may vary from year to year. Since most studies on respiratory viruses (especially influenza and RSV) have been conducted in winter, we cannot reach a definite conclusion about the seasonal rate of the virus. However, in most studies conducted in areas with 4 seasons, the highest prevalence was detected in winter and fall (17). Although some studies could not explain the seasonal distribution of viral infections, they could show a difference when comparing the infection rate in various years (7, 18).

The main weakness of descriptive studies is that such studies are mostly done in winter which has a high prevalence of viral infections and consequently the exacerbation of COPD will occur most frequently (17, 19). Greenberg et al. reported that respiratory viral infections were detected in 23% of hospitalized patients out of which 45% occurred in COPD patients who had been hospitalized in fall and winter (4).

In order to avoid seasonal bios, we tried to collect samples from patients throughout the year in all 4 seasons. However, despite our efforts, most of our samples were collected in fall and winter. The majority of the detected viruses were isolated from samples collected in fall (59.1%). There was a significant correlation between the season and presence of respiratory viruses in patients ($P<0.001$). However, no significant correlation was detected between each type of virus and season of the year.

In a study by camargo et al. on patients with COPD exacerbation, about 68% of patients were males (14). We only had 2 female patients and therefore we could not evaluate the role of gender in this regard. Usually elderly people develop COPD exacerbation due to respiratory viral infections (20). Wedzicha in his study conducted in 2004 suggested that patients' old age was the most common cause of COPD (4).

Our understudy patients suffering from COPD exacerbation were in the age range of 18-109 years. However, no significant association was detected between age and presence of understudy viruses ($P>0.490$). Number and percentage of viruses were the highest in the age range of 60-80 years (61.36%).

In a study conducted by Hogg it was found that COPD exacerbation occurs more commonly in patients with infectious symptoms like dyspnea, sputum volume, sputum purulence (major symptoms) and nasal congestion, sore throat, cough and wheezing (minor symptoms)(12). A significant correlation was detected between all respiratory viruses and some symptoms especially rhinorrhea and hoarseness ($P<0.041$ and $P<0.027$, respectively). Examinations showed that clinical symptoms are due to some viruses that cause COPD exacerbation in patients. These symptoms include wheezing, nasal congestion, fever, rhinorrhea and hoarseness. This finding is in accord with the results of previous studies.

Wedzicha et al. in their study found that COPD exacerbations usually occur 2.5 to 3 times per year. However, we should note that about 50% of exacerbations are not reported by patients because COPD patients are accustomed to frequent symptom changes and this may explain the tendency of COPD patients to underreport the exacerbations (21). In a one year prospective study conducted in the year 2000 on 101 patients suffering from moderate to severe COPD in London, the mean number of exacerbations was reported to be 2.4 exacerbations per year (22). In our study, this figure was reported to be 3.3 exacerbations per year.

Conclusion

Our study results showed that respiratory viral infections were associated with COPD exacerbations. Picornaviruses and corona viruses were de-

tected to be the most common cause of infection. Our study found the highest rate of co-infection in COPD patients. The highest number of viruses was detected in the sputum samples (68.18%). This study was the first to evaluate the role of respiratory viral infections in patients with COPD exacerbation in Iran and is the first to evaluate such wide range of viruses worldwide (16 types of viruses). Since patients presenting to Masih Daneshvari Hospital are from all over the country, this study shows the administration of viruses nationwide. This study has the preference of detecting influenza C and enteroviruses along with simultaneous evaluation of coronaviruses and parainfluenza viruses. Enteroviruses were detected in 10.8%, influenza C in 1.8%, HBOV in 0.9%, coronaviruses in 17.1% (OC43 in 7.2%, NL63 in 0.9%, 229E in 9%) and parainfluenza I in 1.8% of patients. However, parainfluenza type II and III were not detected in any patient.

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Bioethics and Mental Health: The Rights of Patients With a Disorder

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Abstract

Bioethics can be included in the context of psychiatric patients' recovery of dignity and rights, creating a therapeutic interface of social rehabilitation, respect and closeness between professionals and the treated persons. The completion of the analysis of the facts occurred by reviewing 22 articles dated between 1999-2011, from a prior reading, selecting the ones that mention bioethics and mental health. Before the Psychiatric Reform, often, a lack of commitment to the mentally ill was noticed, isolating the patient from a social life. Before the Psychiatric Reform, often, a lack of commitment to the mentally ill was noticed by isolating the patient from a social life. After the Reform, the person with mental illness began to receive a more attentive care. It can be concluded that the Reform tried to bring life to those who had no respect and attention, these patients who needed to be included in a social and urban interaction.

Key words: Bioethics, Mental Health, Disorder.

Introduction

According to what the World Health Organization (WHO) proposed in 1948, health is a state of balance, unstable and dynamic faced in a continuity ranging from a complete well-being to disease; it is a state of physical, mental and social well-being related to the absence of disease.(1) In addition to these concepts, mental health encompasses the perceived self-efficacy, autonomy, competence and the individual's self-realization of the intellectual and emotional potencial.(2)

It is estimated that about 340 million people worldwide are affected by depression, schizophrenia, and 45 million from 29 million for dementia. Mental disorders are responsible for a high proportion of all years of quality of life lost due to a disability or disorder, and this burden is expected to grow significantly in the future.(3)

In July 2011, in Brazil, there were 23,272 admissions for mental and behavioral disorders according to ICD-10 classification, 4,318 of these occurred in Brazilian's Northeast. The most common specific cause was schizophrenia and schizotypal and delusional disorder. There is a slight increase, since in July 2010, in Brazil, the number of hospitalizations for these causes was 23,164, and 4,262 in Brazilian Northeast. In Ceará, for the same period, 1,127 admissions were reported for the same causes.(4)

Throughout human history, the concept of insanity was understood in different manners. This has been presented as a will of the gods, a demonic possession and, still, according to Hippocrates, with an organismic conceptualization, in which these mental disorders would be based on the human brain and the balance between the humors and the environment. With the intensification of scientific rationalism, these concepts have changed and large asylums have emerged to shelter and to isolate the insane and other marginalized society groups. In these institutions, treatment and rehabilitation of inmates were not aimed, but the imprisonment of these individuals. Violent punishments and erroneous and exaggerated medicalization were commonplace, allowing a serious disregard environment for individual rights.(5)

In the twentieth century, there was a strong tendency of hospitalizing the psychiatric patient as the only means of healing, as well as the distan-

ce between the treated and care providers. It was created an anti-therapeutic environment, focused on the internal alienation and depersonification. Reformist proposals have emerged, aiming to abolish the isolationism and to encourage multidisciplinary, besides horizontalizing the relationship between affected and care providers. In the 60's and 70's, it was aimed to promote the care network outside the hospital and the triad prevention/treatment/rehabilitation.[5]

The mark for the restructuring of Mental Health Policy was the Brazilian Psychiatric Reform in 1980. Guided by the Italian reform of 1960, it proposed the dehospitalization, the effective assistance to the egress of hospitals and to defend the rights of patients with mental disorders. So, the process of paradigm shift in mental health care was based on the premise of restoring the mentally ill to live in society, in order to reinsert him or her as a participatory element in citizenship, making the family and the community responsible for this process of rescuing patients with mental affections[1] These proposed changes were followed in the decades of 1990 and 2000 in Brazil for new resolutions and ordinances of the Ministry of Health and laws, especially, the 10,216/2001 created by Paul Delgado[6], about the assistance model in mental health and the protection of the users of these services' rights.[5]

Bioethics can be included in this context of psychiatric patients' recovery of dignity and rights, creating a therapeutic interface of social rehabilitation, respect and closeness between professionals and the treated persons.[5]

By worrying about the individuals' integrality and quality of life, Bioethics is deeply related to Mental Health. First mentioned by the oncologist Potter in 1971, Bioethics is defined as the postmodern ethics of the new humanism, focusing on defending and promoting human dignity and respect for life in all its stages of manifestation.[7] It is seen as the systematic study of human behavior within the health and life sciences, examined focusing on the values and morals. It implies, in fact, in a crosscutting and multidimensional living conditions and health.[2]

In the context of mental illness, Bioethics assumes an importance in the evaluation the posture of professionals and institutions rooted in the monitoring and treating of patients with psychopathology[3]. Besides, Bioethics is mobilized in order to strengthen the dignity of patients and their families, aiming to minimize weaknesses and deleterious effects. It can also be extended to the discussions and deliberations on the rights of people in mental distress, including the political and community instances, in which users, professionals and families are organized.[1]

Within the debate of Bioethics, it is clear to consider the current tendency to encourage active and responsible attitude of the patient during the course of treatment, respecting and valuing his or her autonomy. The mentally ill cannot simply be care and medicines' consumers. They are co-responsible by their treatment[3]. However, it is necessary to understand people with mental disorders they require a more considered approach, seeking to identify vulnerabilities that could compromise the exercise of rational autonomy.

In an attempt to protect and to improve the citizens' lives and welfare, mental health legislation was instituted and has undergone changes, seeking to seal the provisions of human rights. However, the legal framework is still imperfect on the psychiatric patient's approach. Transgressions of these individual's protecting laws are not unusual, once the stigma of insanity renders objections to the perception of patients with psychopathology as citizens and human beings. Nevertheless, it is important to understand that the mentally ill, despite the limitations, are subjects of potential, freedom and developing responsibility[8].

Considering the current scenario of rising of psychiatric diseases, it is important to understand the contribution of Bioethics in the conduct of mental health care, correlating the professional-patient and patient-society relations and legal framework, which bases the inter-relationships and the people with mental disorders' rights.

Methods

We performed a literature review, based on a manual lifting of books and indexed publications and access to databases: VHL (Virtual Health Library), SciELO, HIS (Hospital Information System), provided by the Department of the Unified Health System (DATASUS), at the electronic ad-

dress (http://www.datasus.gov.br). The legislative information exposed is in the Brazilian Civil Code, at the electronic address (http://www.planalto.gov.br/ccivil_03/leis/leis_2001/l10216.htm).

The descriptors used were: "bioethics, mental health." Most references date from the period 1999-2011.

The including criteria were: means of publication (books, journals); publication language (Portuguese); references that focus on the approach of the relation between Bioethics and Mental Health, giving preference to those with information regarding Bioethics history and the mechanisms used in the treatment of mental disorders. Studies addressing patient-family and family-health professionals relationships were also used as reference for this work. We excluded studies that did not date from the mentioned period.

A general reading was performed in order to obtain an overview of scientific issues involving bioethics and mental health.

Results

12 articles discourse about how it was treatment for the mentally ill in the past, which were abandoned in psychiatric clinics, characterizing the asylum healing model. These articles emphasize the mistreatment and depersonalization which these patients were submitted to. 11 articles mention the Psychiatric Reform, since it has been an important milestone in the evolution of the assistance of patients with disorders. Despite the Third National Conference on Mental Health also have been a significant event, it was mentioned by only one article. Law No. 10.216/2001 was cited by 8 articles, providing legal foundation to the text. The issue of social exclusion and stigmatization of the mentally ill and the right to citizenship were addressed in 15 works. In order to adhere to the new politics of care to people with mental disorder, the Centers for Psychosocial Care (CAPS) arise, and given their importance in this context of transformation, they were mentioned in 7 articles. 8 researches discoursed about the inclusion of their public in the labor market, suggesting a productive life. The ethical conduct that the mental health professionals should take before their patients was mentioned in 12 studies. Focusing on this relationship, a special feature was analyzed by 16 articles, given its relevance in the present moment: autonomy, as an alternative to a path marked by dependency and discrimination. 5 articles discoursed about the criminal law and the rights of the mentally ill while being subjected to scientific research was a theme found in 6 publications.

Civil and criminal right of people eith mental disorders

The previous constitutions were recognized by the lack of attention to the fundamental rights of the mental sick persons, who had their citizenship unrecognized during a long period of time.[9] Thereby, Brazilian, as well as worldwide psychiatry history, is focused on the reclusion of the ones who have metal disorders in places where violence, intolerance and individual depersonalization prevail, transforming the person into an object with no needs or rights, losing the citizen characterization.[10] It represented what, in psychiatry is called the "psychiatric patient's stigma", in which the largest limitations are the disqualification, the reification and the consequent return to the asylum environment.[11]

After the promulgation of the Federal Constitution of 1934, the first legal provision related to the mental sick person was approved on the Decree No 24.559/34, which, while discoursing about individual protection and assistance, as well as the psychopath's assets, considered insane the drug abusers, the usual intoxicated, individuals who are incapable of doing personally acts of the civil life and the different people.[12]

After this Decree, other federal laws related to metal health had been created, but only in the decade of 1980, significant inclusion, citizenship and ethics reforms became noticeable in the legislation, sustained by the Brazilian Psychiatric Reform[5]. Law No. 10.216/2001 was promulgated, in Brazil, because of movements conducted by the affected people. This law recognized right to equality to patients with mental disorders, imposing respect, besides focusing on issues that involve inclusion and assistance[6]. Similarly, since the decade of 1970, several countries are investing in efforts to introduce new alternatives on the treatment of those who have mental disorders[13].

With this Law, a new model of care in mental health has been developed, aiming to obtain the citizenship rights of people with psychic suffering. In 2001, the III National Conference in Mental Health took place, with wide participation of social movements, users and families, and it was responsible for the political and the theoretical substrates of mental health in Brazil[14]. This conference reaffirmed health as a citizen right and a duty of the Government, ensuring the principles of the Health System –SUS guide the mental health public politics, as free and universal access, equity, social participation and control of the three layers of the Government[15].

Society imposes segregation in "healthy" and "sick", which leads to reduced self-esteem and individual autonomy, besides exclusion and alienation. Thus, it is important to evaluate that citizenship rights of people with mental illness must provide them participation in society, ensuring their possibility to benefit from appropriate and quality care, and not to be in private care or psychiatric hospitals, where part of their rights is violated[14], as can be seen in the Article 2, single paragraph, of this Law.

In this context, the Psychosocial Care Centers (CAPS) have outstand in the health care policy, representing a significant progress of the Psychiatric Reform, by presenting, as a fundamental concern, "the subject and his or her singularity, history, culture and daily life", allied with other programs and actions, as the Ministry of Health's main strategy of reversing the centralized model in psychiatric hospital care. These units have the purpose of offering, to the mental sick person, clinical accompaniment, besides the patient's social reinsertion, by the opportunity of working, of having leisure time, and exercising the civil rights, thus helping the construction or reconstruction of family and social relationships[16].

Therapeutic residential and CAPS need to be created and expanded, in order to give opportunity to the citizens who have mental disorders, and, thus, to provide an alternative in the pursuit of the autonomy and the acceptance of this subjects as members of society, reducing the pejorative image of disability and dependence. In this context, the participation of health professionals, along with the users and their families, is indispensable in the fight to change this tendency of society to stigmatize the mentally ill, proposing redirecting the focus to the insanity and to obtaining these users' the exercise of citizen rights[14].

Even before all the progress, paradigm shifts and practices implemented in order to psychosocially rehabilitate, one of the major challenges that involve the inclusion of the mentally ill in society is their difficulty to access a productive life, which, when possible, results in both income and self-assertion and better quality of life and more concrete conditions of social inclusion[17]. Frequently, the fragility and the untying of social ties are present in cases of mental illness, and are represented by exclusion from the labor market, family, culture and politics, besides limiting the exercise of social role, which overrides the individual's singularity[18].

It is known that the value of work, as an activity that socially complements and legitimizes people, is recognized in different social classes, communities and groups. Thereby, while providing learning opportunities and workshop experiences to the users, it is also surveyed to expand these positive aspects of the family and community[17].

With regard to the criminal rights of those who have mental disorders, in the Code of Civil Procedure, the graduation of legal capacity would be of full potential (individuals over 21 years old with no mental disorders), relative inability (individuals over 16 years but under 21 years, the lavish and the forestry uncivilized) and absolute inability (individuals younger than 16 years old, the insane, the deaf-mute)[19]. The insane, because of this graduation, are not considered responsible for damages caused to others in the civil plan (independently if it is a result of commission or omission, lawful or unlawful – Code of Criminal Procedure – Brazil, 1973), and this responsibility is on their legal representatives, family members or trustees[20].

Bioethics and the relationship between mentally ill and health professional

In recent years, changes in the relationship between mental health professionals and their patients are clearly significant, and can be related to the appreciation of the autonomy of the mentally

ill. This changing requires the adoption of new psychotherapeutic approaches, new drugs, diagnosis and evaluation methods, as well as reflections in the Bioethics fields[21].

One of the Bioethics objectives is to outline a systematic study about how human conduct should be in science that involve life and health care, diluting possible questions about how to handle and what attitude to adopt towards the patient with mental disorder, justifying these practices in moral values and principles[21].

Thus, Bioethics should be viewed as the communication link between the biomedical sciences and the ethical and philosophical fundamentals, which should govern the conduct of those who are committed to health care. In this regard, it is worth to discuss some ethics considerations of the doctor-patient relation[21].

The first of these considerations would be privacy and confidentially, an individual right, not only of psychiatric patients, but any patient. Thereby, all the information collected in medical history, physical examination, investigation, diagnosis, prognosis and treatment is confidential, consolidating the professional-patient relationship on a basis of trust and loyalty, as stated in Article 2, single paragraph, of Law 10.216/2001 the Ministry of Health[6].

There are occasions in which the breach of confidentiality is justified, especially when the situation involves psychiatric disorders that represent a potential threat to the patient's life, as well as to other people's life, besides compulsory notification of some transmissible diseases, violence and suspected child abuse. In the last case, every citizen has the duty to communicate the Guardian Council, so that necessary measures can be taken.

In general, there are two ethic fundaments for breaking the sigil: the patient's and other's security and public and social welfare. In some situations, the patient's behavior can be so dangerous that a judicial interdiction is required. It may mean the patient, for example, is unable to decide about his ou her wells destiny, financial life, and even a place and conditions to live[22].

Another principle to be considered is veracity, highlighting the therapist must make a commitment to the truth, as long as that disclosure does not bring any injury to the patient. Besides, some professional positions in relation to the patient should not be adopted for being ethically incorrect, such as sexual involvement and obtaining personal gratification at the expense of the patient. It is also important that neutrality and anonymity of the patient's personal life are maintained, so there is no reversal of roles, culminating in a negative effect on the patient.

And finally, the professional-patient relationship must be permeated by the respect for the patient with mental disorder's autonomy, since the symptoms do not offer any risk for life and since he or she does not have the capacity to understand, to logically analyze a situation and to choose between various hypotheses[21]. Thereby, in clinical care of patients with mental or neuropsychiatric disorders, the most modern position, of counting on the patient's active and responsible attitude, is much less emphatic, given the specific conditions of these patients regarding the cognitive and emotional vulnerability. Thus, the most valued ethic parameter in the practices of health is, now, the patient's autonomy, although this always involves the consideration of vulnerability[22].

When there is no full psychic capacity, the autonomy, as well as auto conscience, freedom, and respect must be reacquired by the patient with the professional support of psychiatrists, juridical psychologists, social workers, for example[23].

Treatment must include the patients' participation in the construction of therapeutic projects and the configuration of their daily lives. The hospital beds must be progressively reduced and substituted by other ways of treatment, surveying psychosocial rehabilitation, without excluding the possibility of hospitalization[24].

According to this, there are some situations in which hospitalization is the most prudent decision to be made by the doctor. In some moments, this decision is even imperative, and the doctor must advice it by the consent of the patient, if it is possible, or determine it compulsorily. In this sense, Article 6 of Resolution 1.598/2000 of the Federal Council of Medicine specifies the following: *No treatment should be administered to psychiatric patients without their consent, unless the clinical conditions do not permit obtaining such consent, and in emergency situations, characterized and justified on medical records, to*

prevent immediate or imminent harm to the patient or to others[25].

Vulnerable subjects should be analyzed, especially, when dealing with the ethical analysis of human research because they are unable to decide or to express their decision, once cultural, ethnic, social, political, economic, educational and health conditions complicate such expression. Resolution No. 196/96 of the National Health Council, defines vulnerability in section II. 15 as a"state of individuals or groups who, for any reason or motive, have reduced their ability to self-determination, especially in regard to informed consent", and recommends in III. 1 that the observance of ethical principles in research implies that "for protecting vulnerable groups […] research must always treat them in their dignity, respect them in their autonomy and defend them in their vulnerability"[26].

In the codes of ethics and research guidelines, the mentally ill, but not only them, have their autonomy limited, being unable to provide after-information consent. However, explaining what is aimed and receiving the patient's agreement is indispensable. Ethically, no one is allowed to consent for somebody who has full autonomy. When it happens, the decision is ethically incorrect and invalid. However, it is important to highlight that in cases of reduced autonomy, the responsible or legal guardian is in charge of making decisions for the person, and these decisions must be respected even when a neutral observer understands them as wrong[26].

Conclusions

It is important to consider that, before a mental disorder, the patient has difficulties to expose his intellectual capacity and autonomy. Until the twentieth century, the mentally ill had to be isolated from society, by being considered in a complete disequilibrium with the environment in which he was inserted, and in order not to cause any harm. Thus, treatment has become difficult and the possibility of healing, distant. Giving the patients medication made it arduous for them to notice reality, which, in fact, is in full state of social exclusion, as well as an impediment of living in their environment. The breakdown of family ties makes it even more difficult to compare the mentally ill to the considered normal people.

National Congress reshapes the health system and the psychiatric reform brings a paradigm of the existing rules and with the following advances: removing patients from isolation, restoring them to social life, renewing their chance of reintegration of life and decreasing the deleterious effects of drugs.

Since then, those with psychiatric disorders will participate in their own recovery process, noticing, gradually, the severity of their dementia and becoming responsible for their recovery. In a changing humanization process, Bioethics provides new approaches and behaviors considering the individual who is caring and one who is being cared. In other words, the Reform suggests a friendly relationship between professional and patient, patient and family, and trust in those who are acting in the patient's healing process, strengthening family and affective ties.

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Comparison of artificial neural network and regression analysis for prediction of mortality in spontaneous intracerebral hemorrhage based on initial clinical parameters

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Abstract

Introduction: In recent years, outcome predictions using artificial neural networks (ANN) have been developed in many areas of health care research, but few studies considered ANN models for outcome prediction in intracerebral haemorrhage (ICH).

Objective: To compare the results of an ANN analysis with results of regression analysis, using the same data set based on initial clinical data, for prediction of mortality in ICH.

Methods: Analyses were conducted on data collected on 200 patients with spontaneous ICH, 100 patients who died and other 100 patients who survived. Initial clinical parameters used to predict hospital mortality, and then an ANN analysis was applied to the same data set. Input variables were age, gender, pulse and mean arterial pressure, parameters of the Glasgow Coma Scale and level of consciousness. For each of one the pairs of ANN and clinical predictors, the area under the receiver operating characteristic (ROC) curves with test accuracy parameters were calculated and compared.

Results: In the observed dataset ANN model overall correctly classified 83.3% of patients as alive or dead, compared with 73.5% correct classification for the logistic regression model. ANN present some advantages for correct classification of patients who died (100% vs. 75% of corrected classification for ANN and logistic regression model respectively) in this data sets.

Conclusions: ANN models are comparable with traditional predictive models, with some potential advantages in corrected classification of patients who died. A well-validated ANN may have a role in the clinical management of ICH.

Key words: intracerebral hemorrhage, mortality, clinical predictor, artificial neural network, regression

Introduction

Intracerebral hemorrhage (ICH) accounts for 10–15% of all strokes and is one of the leading causes of stroke related morbidity, mortality, and disability worldwide[1]. Many observational and epidemiological studies have identified a wide range of factors that are predictive of outcome after acute ICH. From these studies numerous outcome prediction models have been developed for mortality and functional outcome[2-7]. Factors found in most of these prediction models include clinical parameters, such as the score on the Glasgow Coma Scale (GCS) or National Institutes of Health Stroke Scale (NIHSS), age, blood pressure (BP), and/or neuroradiology data such as hematoma volume and location, and the presence and amount of intraventricular haemorrhage. We recently introduced a new parameter which considers and associates two important prognostic parameters, GCS score and mean BP value. This MG index is determined as ratio between mean BP value and GCS score. We supposed that MG index might reflect the influence of BP and brain injury degree on stroke outcome in patients with ICH[8].

Most predictive studies have relied on the use of logistic regression or discriminate function analyses that sort individuals into select groups based on linear combinations of the independent variables that increase the probability of maximal

distinction of subjects into groups[9]. In clinical studies many of the variables may be interconnected and the selection process used in logistic regression and discriminate function analyses often forces some variables out of the equation. This process may compromise the predictive power of the analysis and lead to less accurate case classification. To date, none of the published models have achieved the high degree of accuracy necessary for this purpose. Thus, the multivariate statistical models often lack the specificity needed for application in clinical decision-making.

Artificial neural networks (ANN) have been used as an alternative methodology to linear statistical approaches for more than a decade. Neural networks are parallel, distributed, adaptive information-processing systems that develop their functionality in response to exposure to information. ANN models use computerized artificial intelligence processes for classification, pattern recognition, and prediction. ANN is an information processing tool that is inspired by the structure and function of the human brain. The brain learns by adjusting the number and strength of synapse connections. A neural network is composed of a series of interconnecting parallel nonlinear processing elements (nodes) resembling biological neurons, that allow signals to travel through the network. The weights associated with the connections define the nature and the strength of the signal. McCulloch and Pitts[10] first described ANNs as a method of information processing using a network of binary decision elements or "neurons." The computational power in an ANN is derived from the density and complexity of the interconnections of neurons[11]. In recent years, outcome predictions using artificial neural networks have been developed in many areas of health care research, especially in critical care and trauma. To date, few articles considered ANN for outcome prediction in ICH[12,13]. In only such study in adults, Edwards et al.[12] developed ANN model with great potentials, but further studies with larger and independent datasets are warranted. Almost all of the published articles indicate that the performance of ANN models and logistic regression models have been compared only once in a dataset and the essential issue of internal validity (reproducibility) of the models has not been addressed. According to Wyatt and Altman[14], to be useful, a predictive model must be simple to calculate, have an apparent structure and be tested in independent data sets with evidence of generalization.

The present study was conducted to compare the results of an ANN analysis with results of regression analysis, using the same data set, based on initial clinical data. We hypothesized that ANN analysis could improve the classification of patients.

Methods and materials

A full description of the study methods regarding subjects and clinical examinations has previously been given[8]. In brief, we analyzed two groups of patients with ICH at admission to hospital: one group consisted of 100 patients (58 males and 42 females), average age 55.65 years (55.65±9.66, range 26 to 75 years) who died during hospital treatment and second group consisted of 100 patients (61 males and 39 females), average age 55.66 years (55.66±9.43, range 28 to 75 years) who survived and were discharged from the hospital after treatment. For all 200 patients at admission to tertian referral university clinic of neurology, besides other procedures, we determined Glasgow Coma Scale according to original instructions (eye opening on scale 1-4, verbal response on scale 1-5, and best motor response on scale 1 to 6)[15], we determined level of consciousness (1-alert, 2-drowsy, 3-stupor, 4-coma) and finally measured arterial BP values (mm Hg) by sphygmomanometer in a supine position (we determined systolic and diastolic BP, pulse and mean BP values). Pulse BP value is defined as difference between systolic BP and diastolic BP value, while mean BP (in mm Hg) is calculated by formula: 1/3 systolic BP + 2/3 diastolic BP. The upper normal value of BP is $\leq$ 139 mm Hg for systolic BP and $\leq$ 89 mm Hg for diastolic BP. Patients with no measurable BP (BP=0 mm Hg) were excluded from this study. Diagnosis of spontaneous supratentorial ICH was confirmed at all analyzed patients with neuroradiology findings (computed tomography or/ and magnetic resonance of endocranium).

Logistic regression

Logistic regression was employed for analyzing survival as outcome measured as dichotomous variable, which can be interpreted as death probability (0-survival, 1-death). Variables were

included in the multivariate analysis if the p value was ≤0.05. A backward selection procedure was used to select the way how independent variables are entered into the model. A probability cut point of 0.50 was used to classify observations as events or nonevents. The overall accuracy of the final model was determined by comparing the predicted values with the actual events.

ANN model

The ANN used in this study was a standard feed-forward, back propagation neural network with three layers: an input layer, a hidden layer and an output layer. Selected input network variables were age, gender, pulse and mean arterial pressure, three parameters of the Glasgow Coma Scale and level of consciousness. The single network output was the same patient survival outcome, expressed as death probability (0-survival, 1-death).

The ANN model, with the input network layer consisted of 8 input neurons, and the output layer consisted of one output neuron while the single hidden layer consisted of 20 hidden neurons (Figure 1) was established using Matlab (The MathWorks, Inc, USA). The number of the hidden network layers and the number of neurons in the hidden layer were determined through trial-and-errors process[16].

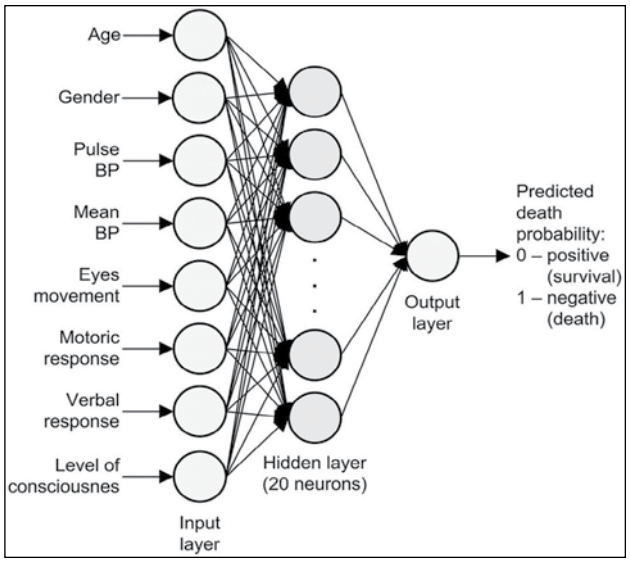

Figure 1. Artificial neural network with 8 input variables, 1 hidden layer with 20 nodes, and 1 binomial output variable

For training, the back propagation scaled conjugate gradient algorithm[17] was used, while the mean squared error was used as performance measure during training. The network training, validation and testing datasets were randomly selected from the same dataset of 200 patients used with testing clinical parameters, to provide fair comparison of artificial neural network and regression analysis for prediction of mortality in spontaneous intracerebral hemorrhage based on initial clinical parameters. Training sample (140 patients) was presented to the network during training, and the network was adjusted according to its error; validation sample (30 patients) was used to measure network generalization, and to halt training when generalization stopped improving; finally, the testing sample (30 patients) had no effect on training and so provided an independent measure of network performance during and after training.

Statistical analysis

Discrimination refers to the ability of a model to distinguish those who die from those who survive. A perfectly discriminating model would assign a higher probability of death to all cases that died than to any case that survived. The discriminatory power of the models was analyzed using the area under the receiver operating characteristic (ROC) curves. ROC curves were constructed by plotting true positives (patients who died and whom the model predicted as dying [sensitivity]) versus the false positive fraction (fraction of the patients who lived and were incorrectly classified as dying [(1 – specificity)]). Area under an ROC curve (AUC) with value of one corresponds to a test that perfectly separates two populations, whereas a AUC value of 0.5 corresponds to a perfectly useless test that performs no better than chance. Comparison of ROC curves are performed for two-tailed testing with $p < 0.05$.

Results

Of the 200 patients enrolled in the study, 100 died while they were hospitalized (Table 1). There was no difference in age and gender distribution between the groups. The patients who died were more likely to have a lower GCS score, and level of consciousness at admission ($p<0.001$). There was no difference between groups regarding blood pressure parameters (systolic, diastolic. pulse and mean BP) but the patients who died were more likely to have a lower MG index (mean BP score/GCS score)[8].

GCS score and level of consciousness were included in the final backward regression equation. The results of this analysis are presented in Table 2. The final model correctly classified 72% of the patients who survived and 75% of the patients who died (Table 3a).

The ANN training was performed on set of 140 patients, validation on 30 patients as well as testing on 30 patients. In testing sample ANN correctly predicted corrected outcome in 75% alive patients, and in 100% of died patient (Table 3b).

Table 1. Clinical characteristics of patients

Variable	All subjects (n = 200)	Survived (n = 100)	Died (n = 100)
Age (years)			
Mean ± SD	55.66 ± 9.52	55.66 ± 9.43	55.65 ± 9.66
Range	26–75	28–75	26–75
Gender (n)			
Male	119	61	58
Female	81	39	42
GCS score (3-15)*			
Mean ± SD	8.92 ± 4.54	11.4 ± 3.96	6.43 ± 3.65
Range	3–15	3–15	3–15
Level of consciousness (n)*			
Alert	95	70	25
Drowsy	40	20	20
Stupor	16	5	11
Coma	49	5	44
Systolic BP (mm Hg)			
Mean ± SD	176.125 ± 44.3	171.8±39.69	180.45±48.29
Range	80-300	100-300	80-300
Dyastolic BP (mm Hg)			
Mean ± SD	100.4 ± 22.21	100.10±20.28	100.7±24.09
Range	50-180	60-160	50-180
Pulse BP (mm Hg)			
Mean ± SD	75.73 ± 28.12	71.7±25.5	79.95±30.07
Range	20-160	30-150	20-160
Mean BP (mm Hg)			
Mean ± SD	125.64 ± 28.4	124±25.57	127.28±31.04
Range	60-220	73-200	60-220
Mean BP/GCS score *			
Mean ± SD	19.83 ± 13.8	13.51±9.8	26.15±14.35
Range	4.89 – 66.67	4.89- 66.7	5.89- 55.56

** $p < 0.001$.*
SD = standard deviation; GCS = Glasgow Coma Scale; BP = blood pressure.

Table 2. Comparison of odds ratios from logistic regression

Variable	Regression analysis			
	β	SEβ	$p <$	OR
GCS score	-0.192	0.06	0.0001	0.83 (0.74–0.92)
Level of consciousness	0.52	0.21	0.05	1.68 (1.11–2.55)

OR = odds ratio with 95% confidence interval;
SE = standard error; β = beta weight;
GCS = Glasgow Coma Scale.

Table 3. Classification table for logistic regression and ANN models (cut-off value p=0.5)
a) Logistic regression model

Outcome	Predicted		Percent correct
	Alive	Dead	
Alive	72	28	72%
Dead	25	75	75%
Percent of cases correctly classified			73.5%

b) ANN model (test dataset)

Outcome	Predicted		Percent correct
	Alive	Dead	
Alive	10	5	75%
Dead	0	15	100%
Percent of cases correctly classified			83.3%

A graphical comparison of the two models, using ROC plots, is shown in Figure 2. The curve for the ANN lies slightly above the curve for the logistic regression model. The area under the ROC curve for the logistic regression model is 0.819 (0.76 to 0.88, 95% CI), whereas the AUC associated with the ANN model is 0.883 (0.79 to 0.98, 95% CI). The area under the ROC curve reflects the predictive power of a model. Comparison of ROC curves indicate no statistical difference between them (z=1.1, p>0.1), hence ANN model is comparable to the logistic regression model for prediction or classification of hospital mortality in this sample.

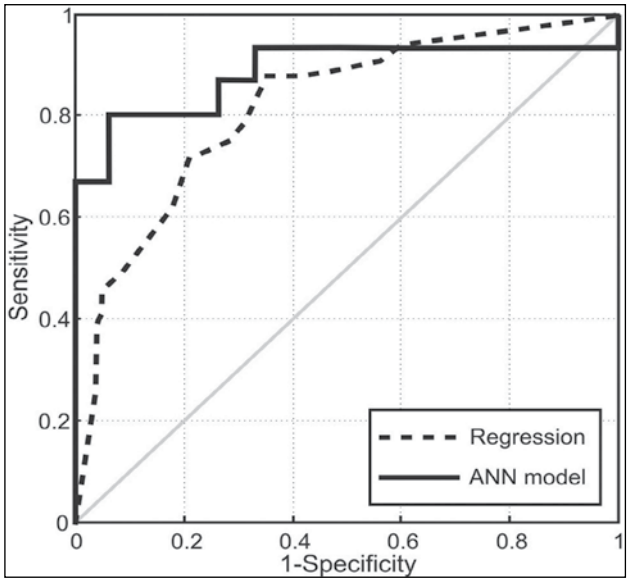

Figure 2. Receiver-operating characteristic (ROC) curves for the artificial neural network and logistic regression models

Discussion

In the present study, employed ANN presents overall comparable performance with logistic regression model, and advantages for correct classification of patients who died (100% vs. 75% of corrected classification for ANN and logistic regression model respectively) in this data sets.

These results suggest potential utility of ANN analysis for improvements in predicting outcome in patients who died from spontaneous ICH, what might offer benefits for clinical decision– making. Improved prognostic systems may result in more effective treatment through earlier and more efficient identification of patients in need of more intense treatment or those patients most likely to respond to a particular therapy. Predictive models may have particular interest with regard to new evidences and guidelines that aggressive care and goal-directed treatment could impact outcome of the patient with spontaneous ICH[18].

Also, these results support hypothesis that ANN model would generate a more accurate classification of subjects than a logistic regression analysis using the same input variables. Such superiority probably arise from different methodology approaches: logistic regression analysis exclude from final model some insufficiently independent variables in order to find the fewest number of variables that can accurately predict the dependent variable, while ANN is capable to use all information in the data set.

While our study suggest better classification of died patients from ICH, using the ANN model, interpretation of the data should take into account the relatively small size of test set and the resulting limitations in terms of statistical power. Although our study used significantly larger set compared to previous study[12], which especially holds true for comparison of sizes of testing subsets, datasets sample size was still insufficient to detect a true difference (β error). Such concerns regarding the sample size are already point out for models predicting hospital mortality of patients in the intensive care unit[19].

Several other potential limitations need to be considered. First, patient cohort is not controlled for some important clinical variables such as time from onset of symptoms, used therapy or level of functional (in)dependence on onset and/or on outcome. Such variable could influence initial clinical

presentation and/or prognostic outcome in ICH. In the recent review, Ariesen et al.[20] found that limited relevance in triage is the common drawback of all predictive models, and such issue should be addressed in future studies. Second, the external validity of our findings is limited by the study population (tertiary level hospital referral patients) and by the type of ICH (spontaneous, supratentorial), and results may not necessarily be extended to patients treated at primary care level or other types of ICH. Third, long-term effects could not be explored because follow-up rarely exceeded period of hospitalization. Perhaps the most important limitation, however, relates to the fact that our purpose in this study was to evaluate ANN model using only initial clinical parameters while paraclinic data were not considered. Although exception of neuroradiology factors weakens our model significantly, the idea of developing the prognostic models based solely on initial clinical data at admission is still worth trying and seems to be of practical value in some situations at the emergency department. Once an ANN is trained and validated, model requires simple PC equipment and it is quick and easy to perform, both in non-hospital environments and in underdeveloped countries, where time and specific recourses are limited. Also, some studies find no radiographic factors as independently predictor of outcome in the cohort and developed grading scales based only on clinical data[21]. Interesting, our ANN model based only on initial clinical information, correctly classified patients who died as well as the ANN model with several input neuroradiology data[12]. This may reflect differences in applied neural network structure or (in)dependent dataset for validation and testing, but we believe also that there is potential for further improvement of model based on initial clinical data.

In conclusion, the present study suggests that ANN model are comparable with traditional predictive models, with some potential advantages in corrected classification of patients who died. Further studies of the strengths and limitations of this method are needed with larger prospective samples of persons with ICH necessary before an ANN could be applied in a clinical setting.

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Polymorphisms in transforming growth factor-β1 have effects on asthma risk in a Chinese Han population via gene-gene interactions with CD14

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Abstract

Background and Objectives: Transforming growth factor-β1 (TGF-β1) and CD14 play pivotal roles in the pathogenesis of asthma. This study aimed to evaluate the effects of *TGF-β1* single nucleotide polymorphisms (SNPs) on asthma risk and asthma-related phenotypes and gene-gene interactions with *CD14* in a Chinese Han Population.

Methods: We consecutively recruited 318 unrelated adult asthmatic patients and 352 healthy volunteers. Genotyping of each selected SNP in *TGF-β1* and *CD14* was performed using SNPstream and TaqMan SNP genotyping technology. We conducted case-control and case-only association studies between the selected SNPs and asthma or asthma-related phenotypes. Multivariate logistic regression analysis was applied to detect the gene-gene interactions.

Results: Neither the alleles nor the genotypes of the 3 *TGF-β1* SNPs (i.e. rs1800469, rs1982073 and rs12983047) and *CD14* SNP rs2563298 were found to be associated with asthma risk separately. However, the frequency of *TGF-β1* rs1800469 TT genotype was significantly lower in asthmatic patients with *CD14* rs2563298 CA or AA genotype (adjusted OR = 0.19, 95%CI = 0.07-0.55, $P<0.001$), which indicated significant gene-gene interactions between the *TGF-β1* rs1800469 and the *CD14* rs2563298 (adjusted OR = 8.23, 95%CI = 2.68-25.30, $P<0.001$). Such interactions were also found between rs1982073 and rs2563298. An evidently positive association was observed between the *TGF-β1* SNPs and the percentage of $CD4^+CD25^{high}LAP^+$ T cells in asthmatic peripheral blood.

Conclusion: Polymorphisms in *TGF-β1* have an effect on the risk of asthma in a Chinese Han population via gene-gene interactions with *CD14*.

Key words: Asthma; CD14; Gene-gene interaction; Polymorphism; TGF-β1

Introduction

Asthma is a complex disorder of airway inflammation and airway remodeling with an imbalance in T helper (Th) type 1/Th2 immune response (1), and is influenced by genetic and environmental factors (2). As a pleiotropic cytokine, transforming growth factor-β1 (TGF-β1) plays pivotal roles in the inflammatory response of the airway, because of its pro- and anti-inflammatory and pro-fibrotic properties depending on microenvironmental and cellular conditions (3,4).

There are three mammalian isoforms of TGF-β, TGF-β1-3, which play important roles in regulating inflammation, cell growth and differentiation, including of extracellular matrix metabolism. Among these three isoforms, TGF-β1 is predominantly expressed in the immune system and is the most intensively studied cytokine with potent immunoregulatory properties (5,6). TGF-β1 is synt-

hesized in an inactive form, the pre-pro-TGF-β1 precursor, which constitutes mature TGF-β1 and the latency-associated peptide (LAP). Additional stimuli, such as low pH, proteolysis, and binding to the cell surface proteins are required to liberate active TGF-β1 (7). Accumulating evidence indicates a potential role of TGF-β1 in the pathogenesis of asthma, especially promoting airway remodeling. Blocking TGF-β activity inhibits epithelial shedding, mucus hyper-secretion, angiogenesis, arterial smooth muscle cell hypertrophy and hyperplasia in an asthmatic mouse model (4). TGF-β1 modulates the development and functions of various immune cells, such as dendric cell (DC), macrophage, NK cell, T cell, structural cells in the airways, and so on (8). Recent investigations have demonstrated that TGF-β1 is critical in the expansion and generation of regulatory T cells (Tregs) (9,10), a vital cell subset involved in regulating the immunologic derangement of asthma (11).

Toll-like receptors (TLRs) are pattern-recognition receptors (PRRs) that play an essential role in activation of the innate and adaptive immune system (12). Following binding to diverse conserved motifs in pathogens termed pathogen-associated molecular patterns (PAMPs), TLRs signaling activate antigen-presenting cell (APC), influence T cell polarization and development (13), and modulate the function of Tregs (14), all of which are key events involved in the pathogenesis of asthma. CD14 was characterized as a receptor for bacterial lipopolysaccharide (LPS) in 1990, almost a decade before the discovery and characterization of TLRs, and can be regarded as the first described PRR. CD14 is critical to LPS/TLR4-induced airway disease and that macrophage CD14 is sufficient to initiate neutrophil recruitment into the airways but that CD14 may need to interact with other cell types as well for the development of airways hyperresponsiveness and for cytokine production (15). In addition, CD14 also amplifies a number of other TLR-dependent responses, including TLR2 and TLR3 (16,17).

The above data suggest that both *TGF-β_1* and *CD14* are potential candidate genes of asthma. The *TGF-β_1* (gene ID 7040) and *CD14* gene (gene ID 929) are located at chromosome 19q13.1 and 5q31.1, respectively. Many genetic association studies have been performed in diverse populations to examine the role of *TGF-β_1* (18-23) and *CD14* (24-30) single-nucleotide polymorphisms (SNPs) on asthma and atopy. However, the results are contradictory, and data on the simultaneous action of several polymorphisms are not well understood. Small effects of genotypes on complex traits have proven difficult to detect. It is likely that multiple genes contribute to the development of asthma through main effects and through gene-gene interactions (31).

Considering the crosstalk of TGF-β_1 and CD14 signaling (32,33), the aim of this study was to investigate the effect of *TGF-β_1* gene-*CD14* gene interactions on asthma risk in a Chinese Han population

Patients and Methods

Subjects

In total of 318 asthmatic patients (aged 14 to 75 y) and 352 nonasthmatic controls (aged 16 to 74 y) were enrolled from March 2006 to May 2007 (Table 1). All subjects were unrelated Han Chinese residing in Nanjing city and the surrounding regions. The Asthmatics were consecutively recruited from the outpatient department at the First Affiliated Hospital of Nanjing Medical University. Each patient underwent a detailed clinical investigation, including medical history, family history, smoking habit, occupation, general physical examination, spirometric assessment, medication, skin prick test (SPT), blood count, and extended laboratory tests. Asthma diagnosis and severity were verified by an experienced pulmonary specialist according to the Global Initiative for Asthma (GINA) guidelines (34). The patients were categorized into 4 groups based on their clinical features (intermittent, mild-persistent, moderate-persistent, and severe-persistent). The healthy volunteers were recruited from spouses of the patients and the general population, and had to meet the following criteria: *(a)* good health status and matched with the cases for age, gender, and area of residence; *(b)* no positive result for SPT; and *(c)* normal levels of total serum immunoglobulin (Ig) E. The study protocol was approved by the institutional ethics committee. Written informed consent was obtained from all subjects.

Table 1. Demographic characteristics of control subjects and asthmatic patients

	Controls (n = 352)	Patients (n = 318)	P value
Age (years)*	38.26±13.31	39.80±14.23	NS
Gender *n* (%)			
Male	152 (43.18)	135 (42.45)	NS
Female	200 (56.82)	183 (57.55)	
Smoking *n* (%)			
Non-smoker	242 (82.88)	280 (88.05)	NS
Smoker	50 (17.12)	38 (11.95)	
TGF-β1 (ng/mL)	8.54±6.78	5.33±3.90	<0.001
Eos ($\times 10^6$/mL)*	0.14±0.11	0.46±0.71	<0.001
$\log_{10}$IgE (IU/mL)*	1.19±0.60	1.82±0.50	<0.001
FEV_1% (%)*	94.75±18.07	74.11±23.66	<0.001
FEV_1/FVC (%)*	88.66±9.43	76.18±12.78	<0.001
Atopy *n* (%)	0	237 (75.00)	–
Severity *n* (%)			
Intermittent	–	70 (22.01)	–
Mild persistent	–	50 (15.72)	–
Moderate persistent	–	87 (27.36)	–
Severe persistent	–	111 (34.91)	–
ICS treatment *n* (%)	–	175 (55.03)	–

Abbreviations: Eos, eosinophil; FEV_1%, percent of predicted forced expiratory volume in 1 second; FEV_1/FVC: FEV_1 as percentage of forced vital capacity; hsCRP, high-sensitivity C reactive protein; ICS, inhaled corticosteroid; $\log_{10}$IgE, $\log_{10}$-transformed immunoglobulin E levels; NS, nonsignificant; TGF-β1, transforming growth factor-β1.
**Data are expressed as mean ± standard deviation (SD). t test or χ^2 test is used appropriately.*

Assessment of Clinical Data

Atopy was defined by at least 1 positive response to a SPT of 13 common aeroallergens, including *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Felis domesticus*, *Canis familiaris*, cockroach, pollen, ragweed, mugwort, mold (*Cladosporium* and *Alternaria*) and animal allergens (cat, dog, and horse). The level of serum TGF-β1 was measured using a multispecies TGF-β1 ELISA kit (BioSourse, CA, USA) according to the manufacturer's instructions. The minimum detection level of serum TGF-β1 was 15.6 pg/mL. Peripheral $CD4^+CD25^{high}LAP^+$ T cells were detected by Four-color flow cytometry with FACSCalibur (Becton Dickinson, Mountain View, CA, USA) and CellQuest software, as described in detail in our previous study (11). SPT, total serum IgE levels, serum levels of high-sensitivity C reactive protein (hsCRP) and pulmonary function assessments have been described in detail in previous studies by our group (11, 35).

SNPs Selection and Genotyping

Three *TGF-β1* SNPs, rs1800469 (C-509T), rs1982073 (T869C) and rs12983047, and one *CD14* SNP, rs2563298, were selected (Table 3). Genomic DNA was extracted from EDTA-anticoagulated peripheral blood with the QIAamp DNA Blood Mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. We genotyped rs1800469, rs12983047 and rs2563298 using GenomeLab SNPstream genotyping platform (Beckman Coulter, Fullerton, CA, USA), and rs1982073 using TaqMans SNP Genotyping Assays (ABI PRISM 7900HT Sequence Detection System; Applied Biosystems, Foster City, CA, USA) at the Chinese National Human Genome Center in Shanghai, China. The PCR primers and extension probes with the tag sequence were designed using the web-based Autoprimer design tool (http://www.autoprimer.com), and were synthesized by SBSgene (SBS Genetech Technology, Shanghai, China), or were purchased from Applied Biosystems (Applied Biosystems, Foster City, CA), respectively (Table 2). SNPstream genoty-

Table 2. PCR primers and extension probes

SNPs	Primer	Sequence 5'-3'
rs1800469 (C/T)	Forward	TAAAGGAGAGCAATTCTTACAG
	Reverse	TCCGGAGGGTGTCAGTGG
	Probe	CGACTGTAGGTGCGTAACTCTGTCTGCCTCCTGACCCTTCCATCC
rs12983047 (A/G)	Forward	AGCTGCTGCCAAATGACA
	Reverse	ACACAAATATTCACCTCTCAGAGC
	Probe	GGCTATGATTCGCAATGCTTCAGAGCCGCCGTGGACACACAGAGA
rs2563298 (C/A)	Forward	CCTAAGTATGATGAATTCCCCA
	Reverse	AATTCAACCCTTTGCCCC
	Probe	AGAGCGAGTGACGCATACTAAGGTCTGTTAAATGAATGACACGGA
rs1982073 (C/T)	TaqMans	

Abbreviations: SNPs, single nucleotide polymorphisms.

Table 3. Candidate Genes and Variants

Gene	SNPs (NCBI rs Number)	Base Change	HWE	Genenotyped Call Rate (%)	MAF(%)	
					Control	Case
TGF-β1	rs1800469	T>C	0.71	100.00	47.16	48.27
	rs1982073	C>T	0.81	98.36	46.66	47.62
	rs12983047	A>G	0.36	99.85	35.04	32.86
CD14	rs2563298	C>A	0.04	100.00	11.79	11.32

Abbreviations: SNPs, single nucleotide polymorphisms; HWE, Hardy-Weinberg equilibrium; MAF, minor allele frequency; TGF-β1, transforming growth factor-β1.

ping and TaqMans SNP Genotyping were performed as previously described (36,37). We duplicated 10% samples to confirm the concordance and accuracy of genotyping. A sample call rate >98% was observed with 100% matching for quality control samples and blind replicates.

Statistical Analysis

Differences in the distribution of demographic characteristics, clinical data and genotypes of the *TGF-β1* and *CD14* variants between controls and cases were evaluated using the *t* test, χ^2 test or the Fisher exact test as appropriate. The Hardy-Weinberg equilibrium (HWE) was tested using the χ^2 goodness-of-fit test to compare the observed genotype frequencies with the expected frequencies among the controls. Logistic regression was used to estimate crude and adjusted odds ratios (ORs) and 95% confidence intervals (CIs), adjusted for age, sex and smoking status, as a measure of association with the risk of asthma. The effect of gene-gene interactions on asthma risk was assessed using multivariate logistic regression analysis with adjustment for age, sex and smoking status. The relationship between clinical phenotypes and genotypes was tested using the linear regression and logistic regression analysis, adjusted for age, gender, smoking status, inhaled corticosteroid treatment, and atopy. The EM algorithm in SAS 9.1.3 PROC HAPLOTYPE was used to infer haplotype frequencies based on the observed genotypes. A 2-sided *P* value <0.05 was considered significant and all calculations were carried out with SAS 9.1.3 (SAS Institute, Cary, NC, USA).

Results

Demographic and Clinical Characteristics

The demographic and clinical characteristics of the study population were summarized in Table 1. No significant differences were observed between the controls and cases for age, sex, smoking habit, and serum hsCRP. However, differences in peripheral eosinophil counts, serum IgE, serum TGF-β1, percent of predicted forced expiratory volume in 1 second (FEV_1%) and FEV_1 as percentage of forced vital capacity (FEV_1/FVC) were pronounced between the controls and the cases (all *P*<0.001).

TGF-β1 and CD14 Gene Polymorphisms Allele/Genotype Distributions

There was high linkage between the two *TGF-β1* SNPs, rs1800469 and rs1982073 (D' = 0.99). The distribution of each genetic variant met the conditions of the HWE (Table 3). There seemed to be similar allele distributions of each SNP between controls and cases (P>0.05, Table 3). The genotype distributions of the polymorphisms in the controls and cases were shown in Table 4. We compared the frequencies of each SNP between the controls and cases by using three genetic models (i.e., recessive, co-dominant, and dominant genetic model). Regrettably, no difference of the genotype distributions of each SNP was observed between cases and controls (P>0.05). Also, when asthmatic subjects were stratified using the sorting criterion of atopy, no positive results were found (data not shown).

Haplotype Analysis

We analyzed the distributions of haplotypes in *TGF-β1* and their effects on asthma. Similarly, none of the haplotypes showed a significant association with asthma (data not shown).

Gene-Gene Interactions of TGF-β1 with CD14

In a further step, we analyzed the combined effect of *TGF-β1* and *CD14* polymorphisms on risk of asthma. As shown in Table 5, subjects who carried both the *TGF-β1* rs1800469 TT genotype and heterozygote CA or homozygote AA for the *CD14* rs2563298, had a significant lower risk of asthma, when compared with those who simultaneously carried *TGF-β1* rs1800469 TT and *CD14* rs2563298 CC (adjusted OR = 0.19, 95%CI = 0.07-0.55, P<0.001). Thus, blazing interactions between these two polymorphisms were found by multivariate logistic regression analysis (adjusted

Table 4. Genotype frequencies of TGF-β1 and CD14 polymorphisms in controls and patient

SNPs	Genotype	Controls† *n* (%)	Patients† *n* (%)	Adjusted OR (95%CI)*	*P* value
rs1800469	TT	100 (28.41)	85 (26.73)	1	
	TC	172 (48.86)	159 (50.00)	1.12 (0.77-1.63)	NS
	CC	80 (22.73)	74 (23.27)	1.14 (0.73-1.77)	NS
	TC/CC	252 (71.59)	233 (73.27)	1.13 (0.79-1.60)	NS
	TT/TC	272 (77.27)	244 (76.73)	1	
	CC	80 (22.73)	74 (23.27)	1.06 (0.73-1.53)	NS
rs1982073	CC	99 (28.78)	86 (27.30)	1	
	CT	169 (49.13)	158 (50.16)	1.14 (0.78-1.65)	NS
	TT	76 (22.09)	71 (22.54)	1.16 (0.74-1.82)	NS
	CT/TT	245 (71.22)	229 (72.70)	1.14 (0.80-1.63)	NS
	CC/CT	268 (77.91)	244 (77.46)	1	
	TT	76 (22.09)	71 (22.54)	1.07 (0.73-1.56)	NS
rs12983047	AA	152 (43.30)	146 (45.91)	1	
	AG	152 (43.30)	135 (42.45)	0.93 (0.66-1.30)	NS
	GG	47 (13.39)	37 (11.64)	0.73 (0.44-1.21)	NS
	AG/GG	199 (56.70)	172 (54.09)	0.88 (0.64-1.20)	NS
	AA/AG	304 (86.61)	281 (88.36)	1	
	GG	47 (13.39)	37 (11.64)	0.76 (0.47-1.22)	NS
rs2563298	CC	278 (78.98)	249 (78.30)	1	
	CA	65 (18.47)	66(20.75)	1.19 (0.80-1.77)	NS
	AA	9 (2.56)	3 (0.94)	0.33 (0.08-1.27)	NS
	CA/AA	74 (21.02)	69 (21.70)	1.08 (0.74-1.58)	NS
	CC/CA	343 (97.44)	315 (99.06)	1	
	AA	9 (2.56)	3 (0.94)	0.31 (0.08-1.23)	NS

Abbreviations: CI, confidence interval; NS, nonsignificant; OR, odds ratio; SNPs, single nucleotide polymorphisms.

** Logistic regression is used, adjusted for age, sex, smoking.*

†Because of genotyping failure, the total case and control numbers for each SNP may be less than 318 and 352 respectively.

Table 5. Gene-gene interactions of TGF-β1 and CD14

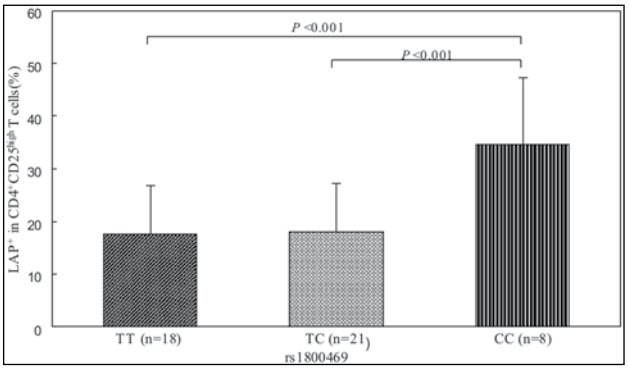
CD14	TGF-β1	Controls† *n* (%)	Patients† *n* (%)	Adjusted OR (95%CI)*	*P* value
rs2563298	rs1800469				
CC	TT	76 (21.59)	80 (25.16)	1	
CA/AA	TT	24 (6.82)	5 (1.57)	**0.19 (0.07-0.55)**	< 0.001
CC	TC/CC	202 (57.39)	169 (53.14)	0.81 (0.55-1.20)	NS
CA/AA	TC/CC	50 (14.20)	64 (20.13)	1.31 (0.79-2.15)	NS
Gene-gene interaction				**8.23 (2.68-25.30)**	< 0.001
rs2563298	rs1982073				
CC	CC	76 (22.09)	81 (25.71)	1	
CA/AA	CC	23 (6.69)	5 (1.59)	**0.20 (0.07-0.57)**	< 0.001
CC	CT/TT	196 (56.98)	165 (52.38)	0.83 (0.56-1.22)	NS
CA/AA	CT/TT	49 (14.24)	64 (20.32)	1.36 (0.82-2.25)	NS
Gene-gene interaction				**8.23 (2.65-25.53)**	< 0.001
rs2563298	rs12983047				
CC	AA	115 (32.76)	116 (36.48)	1	
CA/AA	AA	37 (10.54)	30 (9.43)	0.82 (0.47-1.44)	NS
CC	AG/GG	162 (46.15)	133 (41.82)	0.79 (0.55-1.13)	NS
CA/AA	AG/GG	37 (10.54)	39 (12.26)	1.07 (0.62-1.82)	NS
Gene-gene interaction				1.64 (0.76-3.55)	NS

Abbreviations: CI, confidence interval; NS, nonsignificant; OR, odds ratio.
** Multivariate logistic regression analysis is used, adjusted for age, sex, smoking.*
†Because of genotyping failure, the total case and control numbers for each SNP may be less than 318 and 352 respectively.

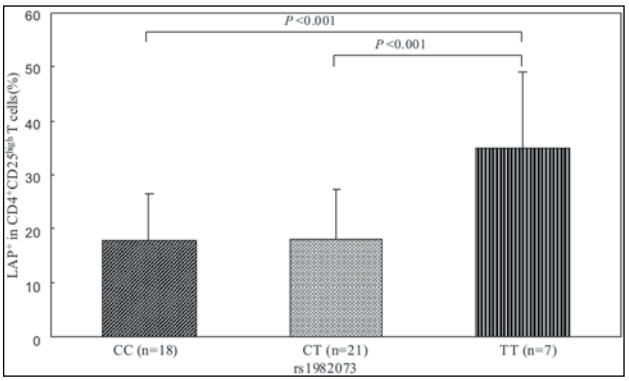
OR = 8.23, 95%CI = 2.68-25.30, *P*<0.001). As high linkage between rs1800469 and rs1982073, such interactions were also found between *TGF-β1* rs1982073 and *CD14* rs2563298 (adjusted OR = 8.23, 95%CI = 2.65-25.53, *P*<0.001).

Genotypes of TGF-β1 and CD14 and Asthma-related Phenotypes

Subsequently, we focused on the associations of genotypes with asthma-related phenotypes in a case-only study. As shown in Fig. 1A, patients who carried the TT homozygote or the TC heterozygote of *TGF-β1* rs1800469 had significantly lower percentage of $CD4^+CD25^{high}LAP^+$ T cells than those who carried the CC genotype ((17.72 ± 8.83)% vs. (34.57 ± 12.90)% and (18.11 ± 9.15) vs. (34.57 ± 12.90)% respectively, all *P*<0.001, adjusted for age, sex, smoking status, inhaled corticosteroid treatment, and atopy). Such association was also seen in rs1982073 but not in rs12983047 (Fig. 1B and 1C). However, no association was found between these SNPs and other asthma-related phenotypes, such as eosinophil counts, serum hsCP, serum TGF-β1, total serum IgE levels, lung function, and asthma severity (data not shown).



A



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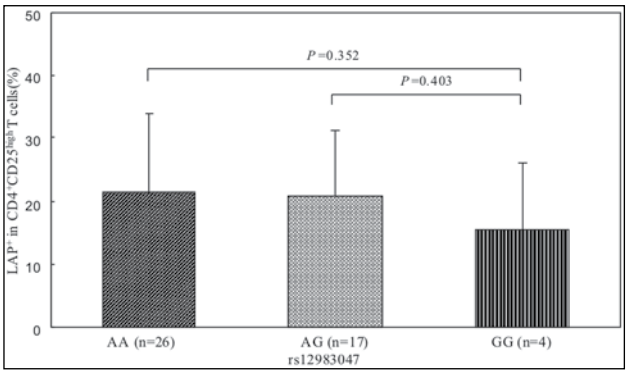

C

Figure 1. Association between TGF-β1 polymorphisms and CD4$^+$CD25^{high}LAP$^+$ T cells in asthmatic peripheral blood
A: rs1800469; B: rs1982073; C: rs12983047.
Linear regression is used, adjusted for age, sex, smoking status, inhaled corticosteroid treatment, and atopy.

Discussion

This study represents a novel combined effect (gene-gene interactions) of *TGF-β1* and *CD14* polymorphisms on the risk of asthma in a Chinese ethnic population. We found no evidence to support a significant association between individual *TGF-β1* or *CD14* polymorphisms and asthma susceptibility. However, our results indicated that the frequency of *TGF-β1* rs1800469 TT genotype was significantly lower in asthmatic patients with *CD14* rs2563298 CA or AA genotype. Significant gene-gene interactions were also observed between the *TGF-β1* rs1982073 and the *CD14* rs2563298. An evidently positive association was found between the *TGF-β1* polymorphisms and the percentage of CD4$^+$CD25^{high}LAP$^+$ T cells in peripheral CD4$^+$CD25^{high} T cells, which was *TGF-β1* rs1800469 CC and rs1982073 TT had higher CD4$^+$CD25^{high}LAP$^+$ T cells percentage in asthmatic patients.

Some studies have indicated that TGF-β1, a pleiotropic cytokine regulating inflammatory reactions and airway remodeling, and CD14, a PRR bridging innate and adaptive immunity, play pivotal roles in the pathogenesis of asthma. Although the potential role of TGF-β1 and CD14 as regulators in immune responses have been received considerable attention in recent years, the results of published association studies on *TGF-β1* and *CD14* SNPs and asthma risk are inconsistent. Hobbs et al (38) were the first to report that *TGF-β1* rs1800469 linked to elevated total IgE, which was confirmed by Acevedo et al (20). This SNP was also reported to associate with asthma susceptibility in Mexicans and Chinese (19,39), but not in Polishs and Caucasians (22,23). As for *CD14*, most (24-27), but not all (29,30), studies indicated an association of *CD14* polymorphisms with asthma. In the present study, we found no significant association between *TGF-β1* and *CD14* SNPs and asthma susceptibility in a Chinese population, despite the atopic status. However, our interesting results underlined a synergistic effect of interactions of *TGF-β1* with *CD14* on asthma risk. This seems to be biologically plausible because a crosstalk of TGF-β1 and CD14 signaling pathway has been suggested. Several studies provided evidence that TGF-β1 negatively regulated TLR signaling, in which CD14 is critical (40,41). Mou et al (32) observed that TGF-β1 suppressed LPS-induced activation of nuclear factor-κB, extracellular signal-related kinases (ERK)-1/2, and p38 in DCs, which identified suppressive effects of TGF-β1 on TLR4 signal transduction. Conversely, TLR4-ERK1/2 signaling cascade antagonized the anti-inflammatory activity of TGF-β1 (33). In support of our results, interaction effects on asthma of *TGF-β1* or *CD14* with other genes have been reported (42-44). It has been pointed out that individual genetic effects of complex diseases, such as asthma, are mostly moderate or small (45). The present study uncovered the combined effect of *TGF-β1* and *CD14* gene polymorphisms with a significant decrease in asthma risk. The observation of gene-gene interactions provides a better understanding of the genetic mechanisms of asthma.

Although no association was found between each individual *TGF-β1* SNP and asthma susceptibility, our results revealed an evidently positive association between the *TGF-β1* SNPs and the percentage of CD4$^+$CD25^{high}LAP$^+$ T cells/ CD4$^+$CD25^{high} T cells in asthmatic peripheral blood. Nakamura et al (6) reported that murine and human CD4$^+$CD25$^+$ Tregs expressed high levels of membrane-bound LAP/TGF-β1, mediating cell contact-dependent immunosuppression. Furthermore, our previous study (11) observed that asthmatic patients who suffered from an acute exacerbation had less CD4$^+$CD25^{high}LAP$^+$ T cells than those with stable asthma and healthy controls,

and these patients had a significant increase of $CD4^+CD25^{high}LAP^+$ T cells in parallel with amelioration of clinical symptoms after 4 weeks of treatment with inhaled corticosteroid. The percentages of $CD4^+CD25^{high}LAP^+$ T cells were inversely correlated with total serum IgE and asthma severity, and positively correlated with FEV_1%. Thus, we hypothesize that genetic variants of *TGF-β1* may influence the function of Tregs via modulating the expression of membrane-bound LAP/TGF-β1 on Tregs, and in turn, play a potential role on asthma phenotype. Functional assays are necessary to support this hypothesis.

A number of limitations of our study need to be discussed. First, environmental influences were not considered in our analyses. Several studies have shown that environmental influences can be of great importance in the development of asthma and we recommend considering these in future research. Second, sample size was relatively small for some genotype distributions, which may be prone to false negatives due to low statistical power or fortuitous false positive results. Third, rigid adherence to an empirical significance level of $P<0.05$ may be too conservative and obscure true-positive associations.

In summary, our study suggests that polymorphisms in *TGF-β1* have an effect on asthma risk in a Chinese Han population via gene-gene interactions with *CD14*. The genetic interactions we observed occur in a biologically plausible way. Further functional and larger better-designed prospective studies considering effects of multiple interacting loci in one gene as well as in multiple genes are warranted to explore the exact genetic mechanisms of asthma in diverse populations.

Acknowledgments

This study was supported by the grants from the Opened Project for Respiratory Medicine of Jiangsu Province People's Hospital (KF200920 to Q.Z.), the Changzhou Principal Innovational Investigator of Medicine (to Q.Z.), the Key Project of Changzhou Municipal Health Administration (ZD201002 to Q.Z.) and the Youth Foundation of Changzhou Municipal Health Administration (QN200904 to Q.Z.).

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Myelin basic protein AB-1 immunolocalization in rat nervous system

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Abstract

Histology and immunohistochemistry (IHC) are rather effective methods for the evaluation of neuropathological changes. In spite of the widespread use of immunohistochemistry and cell type specific markers, the myelin basic protein Ab-1 immunolocalization has not been systemically described in rat nervous system. The myelin basic protein Ab-1 only ever been tested on human tissues not rat tissues. Therefore the myelin basic protein Ab-1 immunolocalization were examined in this study.

In conclusion, the protocol will be applicable in studies dealing with pathological aspects in nervous system.

Key words: MBP Ab-1, brain, cerebellum, sciatic nerve, spinal cord, rat

Introduction

Histology and immunohistochemistry (IHC) are the methods of choice for evaluation of pathological changes in nervous tissue (Unver Saraydin et al., 2011). The rat model is used often in basic experimental studies of human neurological diseases. In spite of the widespread use of immunohistochemistry and cell type specific markers, the myelin basic protein Ab-1 immunolocalization has not been systemically described in rat nervous system. The myelin basic protein Ab-1 only ever been tested on human tissues not rat tissues. Myelin basic protein is expressed in oligodendrocytes, myelin of white matter in the brain, in the spinal cord and in peripheral nerves (Landry et al., 1996). Myelin basic protein accounts for about 30 per cent of the proteins of myelin in the central nervous system. A number of related distinctive diseases are characterized by the active degradation of central nervous system myelin, an axonal sheath comprised essentially of proteins and lipids (Deber et al., 1991). In the peripheral nervous system, MBP is referred to as the P1 protein. Certain MBPs also play a regulatory role in oligodendrocyte maturation prior to compact myelin formation (Achiron and Miron, 2007).

As an introduction to working with the rat model, the present study was performed to establish specific, sensitive and robust immunohistochemical protocols applying a panel of antibodies directed aganist cell type specific markers relevant for analyses of the myelin basic protein Ab-1 immunolocalization.

Materials and Methods

Five adult male Wistar albino rats were maintained in accordance with the recommendations of "The Guide for the Care and Use of Laboratory Animals", and the experiments were approved by the Cumhuriyet University-Medical Faculty, Animal Care Ethics Committee. The rats were sacrificied by a lethal dose of Pentobarbital. The tissue samples (brain, cerebellum, sciatic nerve and spinal cord) were removed immediately and immersion fixed in 10% buffered neutral formaline.

For immunohistochemical staining, the deparaffinized and redehydrated tissue sections were inactivated the endogenous peroxidase by an incubation with 3% H2O2 for 10 minutes. To recover antigen, these sections were put into EDTA solution (pH 8.5) and heated in the microwave oven twice. The slides were then washed with PBS (pH7.2-7.6) twice. Non-specific binding sites were blocked with Ultra V Block (Lab Vision, USA) for 20 minutes. After the redundant liquid was discarded, the sections were incubated in primary antibody Myelin Basic Protein Ab-1 (MBP Ab-1, Lab Vision USA) at room temperature for 1,5 hour and washed in PBS. Then slides were incubated in biotinylated secondary antibody (Lab Vision, USA) for 20 minutes and washed in PBS, followed by incubation in

streptavidin-HRP (Lab Vision, USA) for 20 minutes and washed in PBS. The antibody binding sites were visualized by incubation in a AEC kromogen (Lab Vision, USA) solution. The slides were counterstained for 1 minute with hematoxylin and then dehydrated with sequential ethanol for sealing and microscope observation.

Results

MBP Ab-1 expressions were examined by immunohistochemistry in normal adult rat brain, cerebellum, sciatic nerve and spinal cord (Figure 1-8). The fibres and the cell bodies were well detected in brain, cerebellum and spinal cord sections (Figure 1-4, 7, 8). In white matter, the fibrebundles had very intense staining. The significant staining was also noted in sciatic nerve (Figure 5, 6). The myelinated fibres were thoroughly identified by the application of a polyclonal antibody MBP Ab-1.

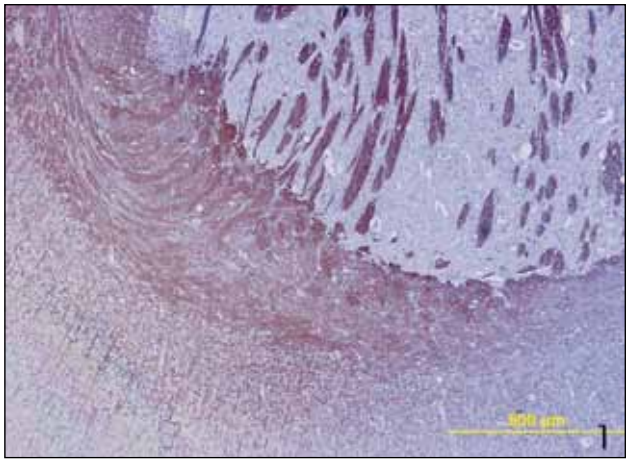

Figure 1. Fibers in white matter were intense immunostained. MBP Ab-1

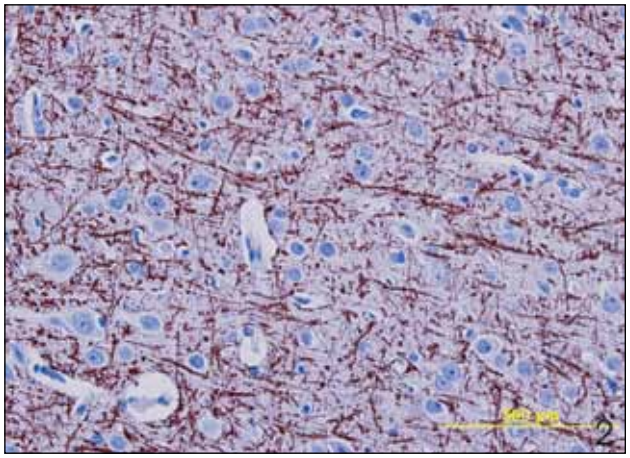

Figure 2. The cell body and fibres in brain gray matter. MBP Ab-1

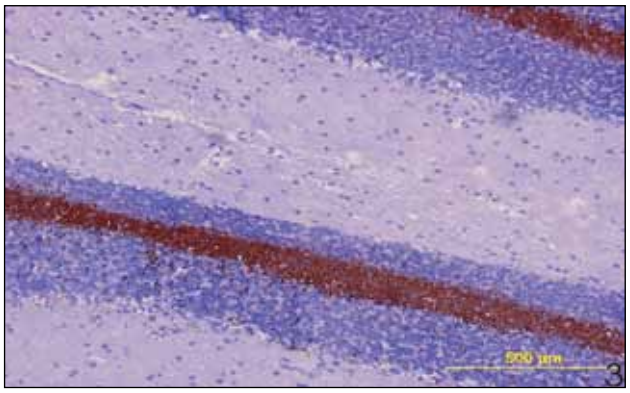

Figure 3. MBP Ab-1 expression in cerebellum

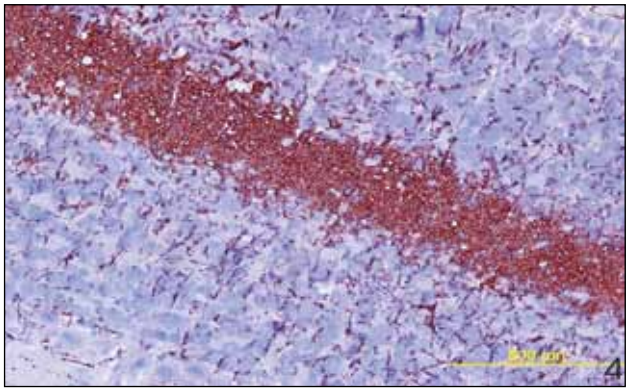

Figure 4. Micrograph showing MBP Ab-1 immunoreactivity in cerebellum is magnification of figure 3

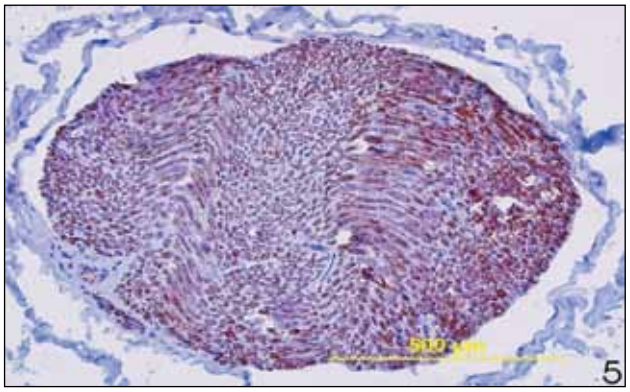

Figure 5. Intense immunoreactivity of MBP Ab-1 in sciatic nerve

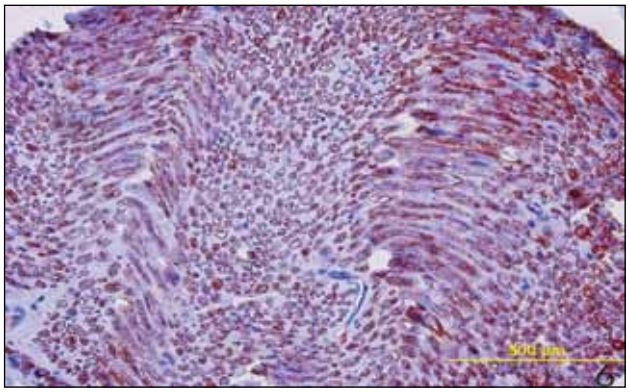

Figure 6. High magnification micrograph from sciatic nerve

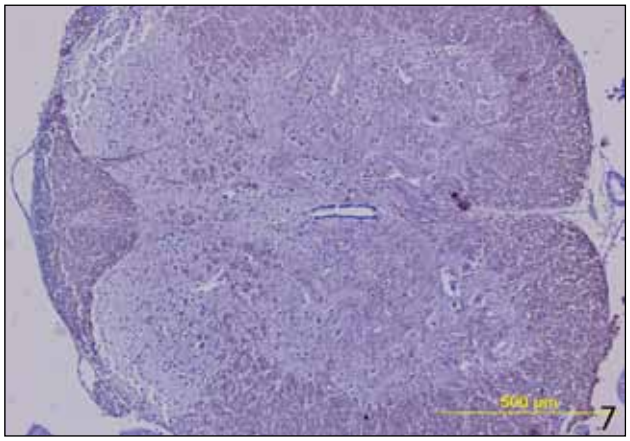

Figure 7. MBP Ab-1 expression in spinal cord

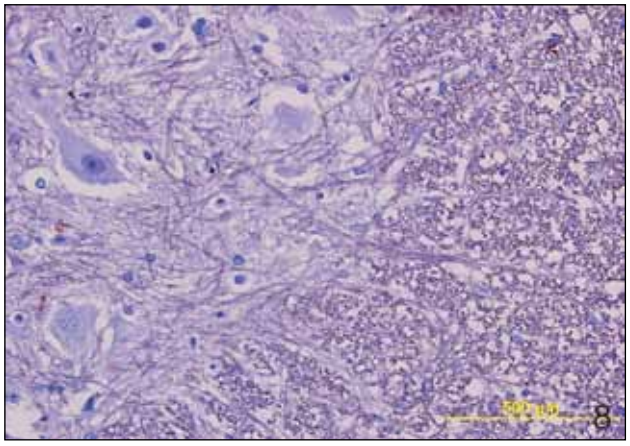

Figure 8. High magnification micrograph from spinal cord

Discussion

The myelin sheath is a multilamellar, spirally wrapped extension of the plasma membrane of oligodenrocytes in the central nervous system and of Schwann cells in the peripheral nervous systems (Seiwa et al., 2000).

MBP Ab-1 has been used as a marker for myelin sheath. Myelin basic protein accounts for about 30 per cent of the proteins in myelin from the central nervous system. Myelin may serve various functions associated with signaling, support and regulation of nerve activity under normal and pathological conditions such as demyelination or myelinopathy (El-Badry et al., 2007).

In this study, all staining tecniques were performed in respect to data sheet of MBP Ab-1 for human tissue. We were aware that other useful labelling techniques are available for discrimination of the different cell populations of the nervous tissue, such as silver staining techniques or Luxol Fast Blue for the visualization of myelinated fibres and oligodendroglia. However, we have concentrated on immunohistochemical labelling techniques only, because it is both very robust and very versatile.

This study reveals that the polyclonal MBP Ab-1 antibody is valuable for the demonstration of the immunolocalization of myelin basic protein in nervous tissue of rat. Thus, the present study might provide a basis for immunohistochemical identification of the myelinated fibres in the rat nervous tissues.

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Comparison of hospital balanced Scorecard (BSC) performance among seven hospitals in Taiwan using the Multi-faceted Rasch Model: diagnosis and prediction

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Abstract

Objective: To diagnose, compare, and predict the balanced scorecard (BSC) performances within and between hospitals each year.

Methods: We used a multi-faceted Rasch Model (MFRM) to analyze BSC data from seven government-run hospitals in Taiwan for 2004 and 2005, and compared the results with traditional parametric and nonparametric methods.

Results: Comparison of the results of various statistical tests suggests that the MFRM can be robust enough to predict BSC performance in hospitals. Its distribution-free estimation of latent independent and dependent variable interactions are suitable for small sample sizes, and repeated measurements at various points in time.

Conclusions: This study provides examples of the rescaling of hospital performance data through the MFRM. Its findings suggest that hospital executives and researchers should routinely consider rescaling ordinal data by using the Rasch measurement technique to evaluate and predict the BSC responses in hospitals.

Key words: balanced scorecard, multi-faceted Rasch Model, Rasch measurement, nonparametric methods

Introduction

The balanced scorecard (BSC[1]) is a performance measurement framework that has been used extensively in business, industry, government, and nonprofit organizations (e.g., hospitals). The BSC aligns business activities to the vision and strategy of the organization, improves internal and external communications, and monitors organizational performance against strategic goals. Many hospitals have successfully implemented the BSC[2], including the Mayo Clinic[3], the North Carolina Duke Children's Hospital[4], the University of Colorado Medical Center[5], the Summa Health System[6], the Ontario Hospital System in Canada[4], and Taiwan's St. Martin De Porres Hospital[7]. The major task for the hospital executives who are engaged in implementation of the BSC is to routinely report the discrepancies and improvements of the four BSC perspectives of finance (F), customer (C), business process (P), and learning and growth (LG) based on analyses of longitudinal data from their hospitals.

Multilevel structure

BSC data has multiple levels, as illustrated in the left-hand portion of Figure 1. The occasions are nested in hospitals, which in turn are nested in groups. Using traditional uni-level modeling, analysis of variance (ANOVA) or other nonparametric methods may be used to verify the main effects of "occasion," but these methods fail to consider the fact that the measurements obtained from the same hospital are generally better correlated than measurements obtained between hospitals [8].

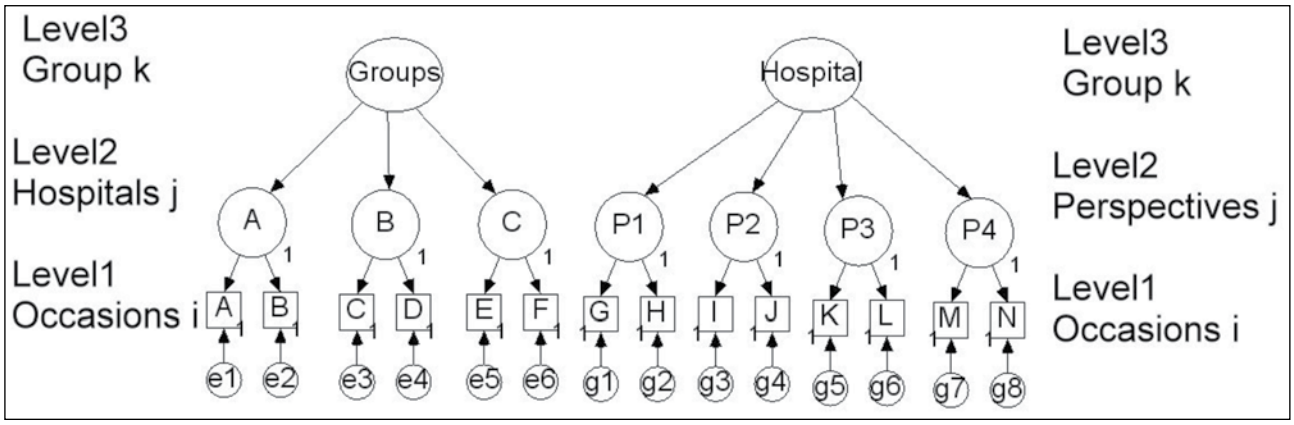

Figure 1. Path diagrams of two models (left panel: between hospitals; right panel: within hospital)

Multi-faceted Rasch model

The multi-faceted Rasch Model (MFRM[9,10]) can be used to analyze multi-level BSC data. In the MFRM, each response is associated with a probability such that test users (e.g., hospital executives) can diagnose aberrant responses and predict future performances through the use of item-fit and person-fit statistics [11-13]. For example, a value of a fit statistic greater than 2.0 together with a significant *p*-value indicates the involvement of too much unexpected noise [14]. In contrast, traditional parametric methods [15] (e.g., general linear models or ANOVA) and nonparametric methods (e.g., the Kruskal-Wallis analysis, the Wilcoxon matched pairs test) could not provide the same level of diagnostic or predictive information as the MFRM [16].

Methods

1. Subjects and Data

The revised BSC (RBSC) data of six perspectives, which were obtained from seven of Taiwan's government-run hospitals (in three groups) on two separate occasions of year 2004 and 2005, were previously analyzed by conventional statistical methods [17].

In addition to the traditional BSC four perspectives of F, C, P, and LG mentioned above, the remaining two data perspectives were patient safety (PF) and social accountability and contributions (AC). Altogether, there were four facets in the MRFM model used in this study: group type (3 levels), hospital (7 levels), occasion (2 levels), and perspective (6 levels). These seven hospitals were classified as one of the three group types: regional (2 hospitals), district (3 hospitals) and specialty (2 hospitals).

The data were collected and evaluated by the staff of each individual hospital over two successive years (i.e., occasion 1: the end of year 2004 and occasion 2: the end of year 2005) according to the six perspectives. The raw scores were categorized into a 3-point (1 = under average 10%, 2= up to average level and 3 = over average 10%) rating scale on the six perspectives.

2. Models and data analyses

For the two facets of hospital (person) and perspective (item), the MFRM is defined as:

$$\log it_{nij} \equiv \ln\left(\frac{P_{nij}}{P_{ni(j-1)}}\right) = \theta_n - \left(\delta_i - \tau_j\right),$$

..... (Model 1)

where P_{nij} and $P_{ni(j\text{-}1)}$ are the probability of scoring j and j-1 in perspective i for hospital n, respectively; θ_n is the proficiency of hospital n, δ_i is the overall difficulty of perspective i, τ_j is the j-th threshold.

For the three facets of hospital, perspective, and occasion, the MFRM is defined as:

$$\log it_{nijk} \equiv \ln\left(\frac{p_{nijk}}{p_{ni(j-1)k}}\right) = \theta_n - \left(\delta_i - \tau_j\right) - \gamma_k,$$

..... (Model 2)

where P_{nijk} and $P_{ni(j\text{-}1)k}$ are the probability of scoring j and j-1 in perspective i at occasion k for

hospital *n,* respectively; γ_k is the threshold of occasion *k*, and the others are defined as above.

For the four facets of hospital, perspective, occasion, and group type, the MFRM is defined as:

$$\log it_{mhkl} \equiv \ln\left(\frac{p_{nijkl}}{p_{ni(j-1)kl}}\right) = \vartheta_n - \left(\delta_i - \tau_j\right) - \gamma_k - \lambda_l ,$$

..... (Model 3)

where P_{nijkl} and $P_{ni(j-1)kl}$ are the probability of scoring *j* and *j-1* in perspective *i* at occasion *k* for hospital *n* of hospital type *l,* respectively; λ_l is the threshold of hospital group type *l*, and the others are defined as above.

3. Separation index and reliability

The computer software Facets 3.54[18] was used to perform MFRM analysis. Facets software uses the joint maximum likelihood estimation method (also called unconditional MLE) for parameter estimation. It also reports separation indices for person and item, which can be transformed to strata and sample separation reliability (similar to Cronbach's α)[19].

4. Conventional parametric and nonparametric methods

The raw scores of the six perspectives were combined into a single dependent variable. The data collected from the two occasions at the same hospital were treated as if they were from different occasions at different hospitals. One-way ANOVA was used to determine the significance of differences among hospital types. Due to the small sample size, nonparametric methods of the Kruskal-Wallis and Wilcoxon matched paired test were also used.

Results

1. Model 3: four facets of hospital, perspective, occasion, and group type

When Model 3 was fit to the data, as shown in Figure 2, the hospital type was shown to have a small effect. For example, the thresholds of the three hospital types were 0.24 logits (*SE* = 0.25) for district, 0.00 logits (*SE* = 0.30) for specialty, and -0.24 logits (*SE* = 0.31) for regional. Although regional hospitals with less difficult threshold outperformed the other two hospital types, this effect was not significant due to bigger standard errors resulted from the small sample size of this study. The occasion for year 2004 and 2005 in the third column had a significant effect. The threshold was 0.26 logits (*SE* = 0.23) for occasion 1 and -0.26 logits (*SE* = 0.23) for occasion 2.

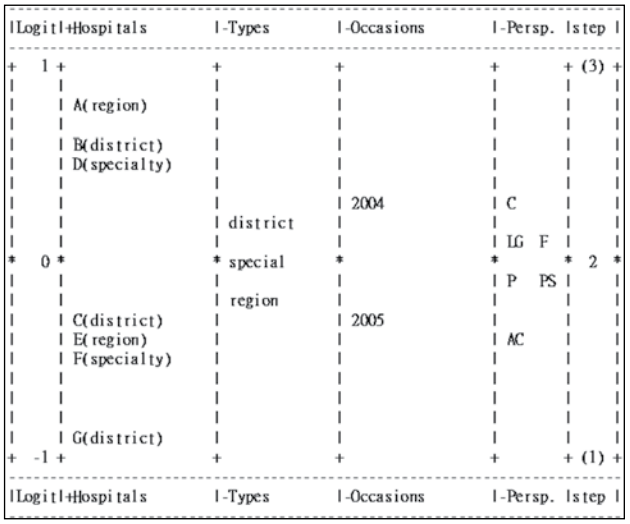

Figure 2. Graphic representation of the four facets under Model 3
Note. No significant difference in hospital types

2. Model 2: three facets of hospital, perspective, and occasion

Hospital type was not associated with a significant effect, so further analysis of this facet became unnecessary, and Model 2 was fit to the data. The seven hospitals had significant differences in performance, with the best performance at 1.11 logits (*SE*= 0.47) and worst performance at -1.06 logits (*SE* = 0.47). As found in the analysis of Model 3, occasion was also had a significant effect. Table 1 lists the overall difficulties, standard errors, and fit statistics for the six perspectives. Financial perspective had a significant misfit (with a mean square error smaller than 0.5), indicating that this financial perspective overlapped with the other perspectives and could be removed for simplicity for further comparison. The easiest perspective was obtained for social accountability and contribution (-0.37 logits; *SE* =0.40), whereas the most difficult perspective was for the customer (0.26 logits; *SE*=0.40).

Table 1. Measures and fit statistics of the six perspectives under Model 2

Perspective	Measure	SE	MNSQ	Z
Customer	0.26	0.4	1.18	0.60
Finance	0.10	0.4	0.46	-2.10
Learning	0.10	0.4	1.42	1.30
Patient safety	-0.05	0.4	0.70	-1.00
Process	-0.05	0.4	1.49	1.50
Accountability	-0.37	0.4	0.78	-0.60

Note. The finance prospective has a misfit; the six perspectives do not differ in measures.

3. Model 1: two facets of hospital (person) and perspective

Assuming that the variables of hospital groups and time points were independent in unilevel tests as shown in Table 2 with the symbol of ※I in both sets of two rows, we confirmed our results showing no significant difference among (a) the MFRM, (b) raw score p-values in parentheses, and (c) parametric and nonparametric methods. On the contrary, as shown in Figure 3, only two hospitals' (B and G) had RBSC performances that were worse than in the previous year, and Table 3 shows many more outfit MNSQ values, meaning many unexpected and aberrant responses endorsed for these hospitals (e.g., 1.4 and 2.0) than for the others.

Assuming that the variables of hospital groups and time points were mutually dependent in unilevel tests as shown in Table 2 with the symbol of ※D in both sets of two rows of hospital groups and time points, the inference results from the MFRM were the same for the time points as raw scores with p-values in parentheses, as the inference results from the original combined raw scores. However, the results were different from those obtained using the Wilcoxon signed-rank test or paired t-test in the row of time points and hospital groups. This is attributable to the failure of the univariate approach to take into account measurement errors and interactions between occasions and groups.

4. Diagnosing and predicting RBSC performance

Table 4 shows the six perspective measures, the mean raw ratings of the hospitals on a Likert scale, the corresponding hospital measures, the measures of time, and the probabilities that were calculated according to Model 3. These probabilities developed in this study allow us to diagnose and predict RBSC performance. Suppose that at occasion 1 (threshold = 0.26 logits), Hospital A with an overall mean rating of 2.5 (hospital measure = 1.14) is rated as category 3 ("over average 10%") on perspective

Table 2. Comparison of the MFRM with conventional parametric and nonparametric methods

Independent Covariates	MFRM Models 2 & 3	Nonparametric method Four approaches for tests		Parametric method Model 1 combined with various tests		
Dependent or not	*p*-value					
Hospital Group ※I	>.05	.358[a](.358[c])		.304[b](.342)	>.05(>.05)	>.05(>.05)
Hospital Group ※D			.001*(.000*)			
Time-point ※I	<.05*	.273(.273)		.309(.298)	.309(.293)	.309(.293)
Time-point ※D			.123(.001*)	.112(.000*)		
Tests[d]						
K samples	Rasch model	K-W	F-T	ANOVA	L-R	GLM
Two samples	Rasch model	M-W	W-S	Paired t-test	L-R	GLM

Note 1. a: p-value derived from nonparametric test with K-W (M-W) or F-T (W-S) for independent or dependent groups, respectively.

b: p-value derived from Rasch transformed logit scores for tests of ANOVA(paired t-test) , L-R and GLM.

c: p-value for raw scores are shown in parentheses at the right side of above none and parametric methods

** :statistically significant difference (p <.05)*

※I: Assume differences between independent groups

※D: Assume differences between dependent groups

d: Tests comprises K-W (Kruskal-Wallis test), W-S (Wilcoxon signed-rank test), F-T (Friedman test), L-R (linear regression) and GLM (general linear model)

Note 2. The MFRM is similar to nonparametric statistics mandating the raw scores on which types of hospitals (Group) as independent and Occasion as dependent samples.

6 (leaning and growth perspective; threshold = 0.10 logits), then the combined measure is 1.14 + (0.10 + 0.26) = 1.50. This rating of the perspective has to be considered as quite normal because its predicted probability of occurrence on the RBSC variable is rather high (Prob. = 0.69). But the same cannot be said when Hospital G is rated 3 on perspective 6 because this has a low probability (Prob. = .20) at a mean raw score of 1.5 (-1.04 logits); however, Hospital G earned an unexpectedly high performance on perspective 6.

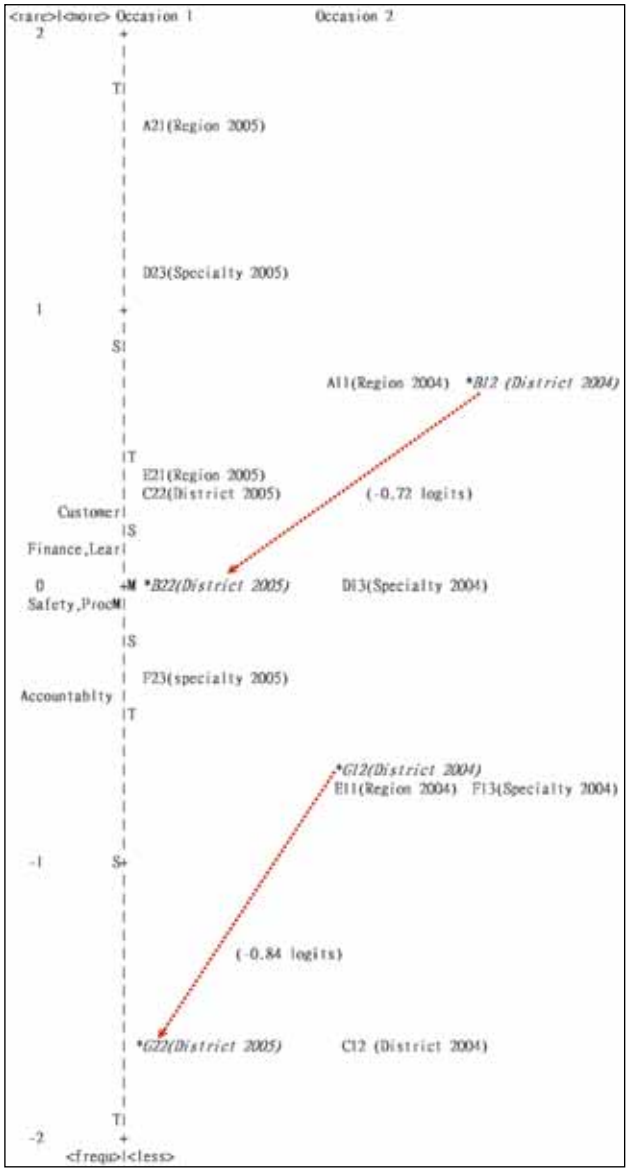

Figure 3. Wright map of the six perspectives and seven hospitals on occasions under Model 1
Note. Hospitals B and G performed worse than in the previous year

The probability of a higher relative rating was always greater at occasion 2 than at occasion 1, which is in accordance with the difference between the time-point measures of 0.52 logits. The combination of Rasch analysis and time-point calibrations allows the examination and prediction of the performance diagnosis of the RBSC. Further, the inclusion of an occasion facet enables the longitudinal investigation and diagnosis of hospital performance, which is helpful in detecting or predicting the RSBC performance.

Table 3 provides diagnostic information about aberrant responses. An outfit mean square error (MNSQ) equal to or greater than 2.0 together with statistical significance indicates a misfit [14]. Our results showed that Hospital G had a misfit at occasion 1 on perspective 6, which is worthy further investigation according to the index of outfit MNSQ.

Discussion

Key findings

It is accurate putting omitted-SE bias in a model

As shown in Table 2, the MFRM inferences are similar to those of the other two statistics that mandate the raw scores on both independent and dependent samples, thus indicating that the MFRM possesses the property of specific objectivity [19, 20] and involves the interactions of standard errors for independent variables in the model.

Measurement error in the latent trait should not be ignored in order to produce an unbiased estimate of the regression coefficient, which is sometimes referred to as an omitted-SE bias. The MFRM considers multiple facets (e.g., occasion and perspective) simultaneously and yields interval measures of hospital type, perspective, and occasion, which is not possible for conventional univariate methods. The visual representation of MFRM analysis (Figure 2) enables us quickly examine differences in performances within and between hospitals each year.

What this adds to what was already known

It is reasonable using Rasch transformed scores to obtain a useful measure

Many statistical methods require that dependent variables follow a normal distribution and that residuals have equal variance, thus implying an interval scale of measurement [21-23]. This means that data with an ordinal scale as in tra-

Table 3. Standardized residuals of the MFRM for the seven hospitals

Perspective	C	F	LG	AC	P	PS	Outfit
Hospital	4	1	6	3	5	2	MNSQ
Criteria	Within +/-2.0						Below 1.5
Occasion 1							
A	1.04	-0.53	-0.53	0.89	-0.65	-0.91	0.61
B*	1.54	0.02	1.42	-1.49	1.31	-0.32	1.40
C	-0.83	0.65	-0.89	-0.97	0.52	-1.14	0.74
D	-1.39	-0.1	-0.1	1.2	-0.22	-0.45	0.61
E	0.5	-1.06	-1.06	0.27	-1.15	1.44	0.99
F	-0.83	-0.89	-0.89	0.52	2.02	0.29	1.12
G*	-0.62	1.14	***2.95***	-0.72	-0.72	0.74	***2.00****
Occasion 2							
A	0.8	0.74	-0.96	0.68	0.68	-1.4	0.83
B	-0.24	-0.36	-0.36	-0.47	-1.95	0.86	0.85
C	1.81	0.25	-1.17	0.14	1.54	-0.09	1.18
D	-1.81	0.99	0.99	0.92	-0.61	0.78	1.18
E	0.12	0.01	1.4	-0.11	-1.5	1.1	0.91
F	-1.08	0.25	0.25	-1.26	1.54	-0.09	0.88
G	0.83	-0.87	-0.87	0.57	-0.94	-1.1	0.77

Note: Hospital G had a misfit at occasion 1 on perspective 6 (LG); Hospital B and G performed worse than the previous year

Table 4. Probabilities (colored from black to gray) of the six perspectives at two occasions for the seven hospitals using MFRM Model 2

Hospital average rating		Occasion 1 (0.26 logits)							Occasion 2 (-0.26 logits)					
		Perspective measure							Perspective measure					
		Hospital Measure	C	F	LG	AC	P	PS	C	F	LG	AC	P	PS
			4	1	6	3	5	2	4	1	6	3	5	2
		logits	0.26	0.1	0.1	-0.05	-0.05	-0.37	0.26	0.1	0.1	-0.05	-0.05	-0.37
A	2.5	1.14	0.65	0.69	0.69	0.72	0.72	0.78	0.76	0.79	0.79	0.81	0.81	0.85
D	2.3	0.56	0.51	0.55	0.55	0.59	0.59	0.66	0.64	0.67	0.67	0.70	0.70	0.77
B	2.2	0.38	0.47	0.50	0.50	0.54	0.54	0.62	0.59	0.63	0.63	0.67	0.67	0.73
E	1.9	-0.12	0.35	0.38	0.38	0.42	0.42	0.50	0.47	0.51	0.51	0.55	0.55	0.62
C	1.8	-0.46	0.27	0.31	0.31	0.34	0.34	0.41	0.39	0.43	0.43	0.46	0.46	0.54
F	1.8	-0.46	0.27	0.31	0.31	0.34	0.34	0.41	0.39	0.43	0.43	0.46	0.46	0.54
G	1.5	-1.04	0.17	0.20	0.20	0.22	0.22	0.28	0.26	0.29	0.29	0.33	0.33	0.40

Note. Hospital's average rating (on 6 perspectives at 2 occasions) Range (1-3) composed different predicted probabilities from lower at left bottom (colored with dark black) to higher at right top (colored with light gray color)

ditional calculations for the BSC will not satisfy the assumption of normality needed by many statistical methods and may produce misleading results that threaten the validity of inferences, such published reports as evaluation in quality of life [24-26], judgment in performance [27-30], and attitude in assessment [31-34].

This paper shows how the Rasch measurement technique can be used to rescale ordinal data to interval data[13] and also how the MFRM as in Figure 2 can generate visual diagrams which are easy to interpret for diagnosis and prediction. The use of ordinal raw scores that are not interval Rasch transformed logit additive scores would also violate the assumption of normality. If it is necessary to measure something, an interval scale might as well be used in order to obtain a useful measure [35].

What is the implication and what should be changed

It is necessary applying MFRM to compare BSC performance among hospitals

Even with a small sample size, the MFRM analysis yields similar results to those achieved with traditional nonparametric methods. Under the MFRM, raw scores are also used to produce linear measures that are test-free and sample-free. The Rasch model based MFRM using joint maximum likelihood estimation approach also has strength to deal with sparse data, as shown in this study and other references [12,13,19,36,37].

It is applicable using visual information for detecting aberrant responses

Report cards can complement and enhance regulatory and training activities as part of an overarching strategy to improve quality [15]. Visual report cards produced from the MFRM such as shown in Figures 2 and 3 allow easy identification of core issues. The use of standardized residual analysis [38-42], as shown in Table 3, also helps swiftly probe into the aberrant responses for further detailed investigation.

As for the issue of BSC raised in public healthcare [43], it is worth considering the utility of Model 2, as Model 1 is simpler in terms of model parsimony. As the occasion effect in Model 2 is significant, Model 2 provides a statistically better model-data fit than Model 1.

Conclusion

Since the phrase "balanced scorecard" was coined in the early 1990s, it has evolved from its early use as a simple performance measurement framework to a full strategic planning and management system. Incorporated with Rasch analysis, the BSC can provide a framework to measure the performance of hospitals, to help planners identify problems in need of further examination, and to predict future performance. In order to continuously improve the quality of healthcare, further studies are needed to compare individual perspectives between and within hospitals across time periods, and to evaluate BSC performance on the Internet.

Abbreviations

BSC – Balanced scorecard;
MFRM - multi-faceted Rasch Model

Authors' contributions

TW conceived of the idea for the paper. WC worked out the statistical issues and drafted the manuscript. TS drafted this manuscript and ND, SB and WC reviewed it and offered comments and their final approval. All authors contributed to discussions and critical comments on the manuscript, and read and approved the final version.

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Multidrug-resistant P. aeruginosa strains in Burn Center Hospital, Tehran, Iran

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Abstract

Pseudomonas aeruginosa is one of the most common causes of burn wound infections. The aim of this study was to determine the frequency of antibiotic resistance burn morbidity during September. 2005 to October 2007.

Background and objective: The incidence of multi-drug resistant P. aeruginosa infection was indicated in 9238 patients admitted to our Burn Hospital. Specimens were collected after admitting the patients in the hospital wards. Susceptibility test were performed for all isolate.

Materials and Methods: Specimens were collected based on hospital policy and cultures were performed on each patient. Isolated strains were identified by using standard bacteriological protocols.

Results: P. aeruginosa was identified in 3012 (70.5%), Staphylococcus aureus in 581 (13.6%), Acinetobacter 426 (9.9%), the rest organisms were 279 (6%). Frequencies of antibiotic resistant degree to P. aeruginosa on Tobramycin, Ceftazidim, Ceftizoxime, Ciprofloxacin, Amikacin, Gentamycin, Tetracyclin, Teazabactam, and Cotrimocxazol were 82, 78, 82, 72, 73, 80, 60, 81, and 98% respectively, 98%, 82% and 80% were resistant to one, three and five antibiotics respectively.

Conclusion: High multi resistant rate in pseudomonas infection, needs to immediately revise the hospital policy in control of nosocomial infection and treatment strategy such as more efficient antiseptic agents or new antibiotic drugs may reduced the rate of multidrug resistant

Key words: Pseudomonas aeruginosa, multidrug resistant, Antibiotic Resistance, Burn Hospital

Introduction

Resistance to antimicrobial is a global problem with the particular level of resistance. Yet, the problems posed by antimicrobial resistance in the burn population are not limited to a few microorganisms (Khosravani 2008). Besides the Gram-positive microorganisms, a number of gram negative bacteria are losing their susceptibility to mainstay antibiotics, as well (Simor 2002, Rastegar1998). Ceftazidime resistant klebsiella pneumonia increased from 0% to 16.6%. Cefoperazone and Ceftazidime resistant Enterobacteriacea were up to 22%. Also 13% of the P. aeruginosa were resistant to Cefoperazone (Lee 2006, Obritsch 2005). As resistance develops, out breaks occur. Burn victims are obviously at high risk for nosocomial infection due to the nature of the burn injury itself. Bacterial infections in burn patients are widely known. The time related changes in the predominant flora of the burn wound from gram-positive to gram-negative recapitulate the history of burn wound infection. Selection and dissemination of intrinsic and acquired resistance mechanisms increase the probability of burn wound colonization by resistant species such as Pseudomonas aeruginosa (Bonomo 2006, Lister 2009, El'Garch 2007).

However, everything from minor out-breaks to major epidemics of antibiotic resistant, P. aeruginosa remains is frequently reported as a multi-resistant organisms (Hajia 2008, CLSI 2006) with major cause of burn injury colonization and serious wound infections, which have been reported in our Burn referee Hospital as well as Burn Centers around the world (Hussein 1989). The purpose of this study was to assess the activity of commonly used antibiotics, in Tehran Burn Reference (TBR) Hospital.

Material and Methods

Studied group: A retrospective study was conducted during September 2005 to October 2007 on burn patients who were admitted to the Tehran Burn Reference (TBR) Hospital. In the present

study, a total 9238 admitted patients were examined for bacteriological investigation during study period. Those patients with P. aeruginosa positive culture were entered in the study.

TBR Hospital policy for admitting patients: The policy of our Burn Hospital is to admit male patients ≥20% total body surface area (TBSA) burns. These patients are mainly burned by different kinds of burning agents such as electrical and chemical contacts, or building firing.

Silver Sulphadiazine was used topically and the dressings were changed, daily. Cephalothin and Amikacin were administered as prophylactic antibiotics from the first day of admission in-patients with ≥ 20% TBSA burns (II and III degrees). The wound was inspected daily during the dressing changes. The data gathered in the study was stored and processed by a computer database.

Specimens and sampling procedure: All wound and blood specimens were collected from those patients suspected to scar infection or septicemia after admitting at hospital wards. These samples were collected by sterile swabs when early and rapid scar disorientation bleeding into the subcutaneous tissues, and increasing edema in surrounding areas observed.

Culture and isolation: All samples were cultured on sheep blood agar and Eosin methylene blue. All isolated organisms were identified by using standard bacteriological protocols (Frobes 1998).

Antimicrobial susceptibility test: The antimicrobial activity against P. aeruginosa and other organisms was tested with the disk diffusion method of Kirby-Bauer recommended by clinical laboratory standard institute (CLSI 2006) using available paper disc (Hi-Media). Applied standard organisms: Following organisms were used as quality control strains. Organisms for quality control of disk diffusion method were E.coli (ATCC 25922), S.aureus (ATCC 25923), and P. aeruginosa (ATCC 27853). Standard organisms for the checking the quality of the Mueller-Hinton medium were the above mentioned organisms plus E. faecalis (ATCC 29212). The used antibiotics were as follow: Amikacin, Gentamicin, Tobramycin, Ceftazidime, Ceftizoxime, Trimethoprim/Sulfamethoxazole, Ciprofloxacin, Tetracycline, and Tazabactam.

Results

Among 9238 microbiologic samples, which were taken during the study period, bacterial strains were isolated and the frequency of Pseudomonas aeruginosa (3012 strains) was found to be 70.5%. This was followed by Staphylococcus aureus (581 strains) 13.6%, Acinetobacter (426 strains) 9.9%, with other microorganisms (279 strains) 6%, were responsible for the remaining.

P. aeruginosa were isolated from either wound or blood samples of all these patients. These patients were aged up to 72 years old with the mean 34.2 ± 20.18.

Quality control of disk diffusion were performed each time of runing the test to ensure of relibility of the test. Results of the susceptibility test were performed for all P. aeroginosa isolates.

The frequency of resistant P. aeruginosa strains was 60% to 98% for the examined antibiotics in 3012 isolates. 82% of the isolates were resistant to tree and 80% was resistant to 5 or more antibiotics.

The lowest and highest frequency rate was observed in Tetracycline (60%) and Cotrimoxazol respectively (98%) (Figure 1).

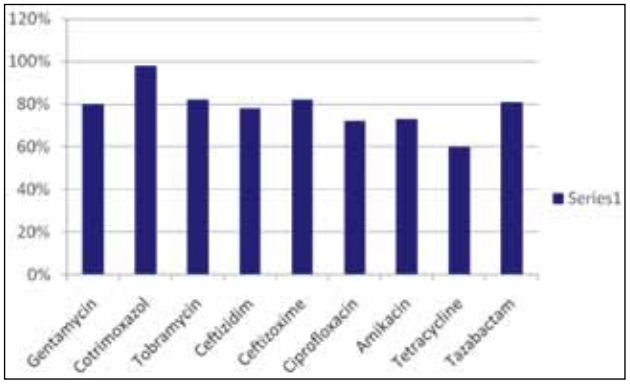

Figure 1. Frequencies and antibiotic resistant of P. aeruginosa

Discussion

Pseudomonas aeruginosa was the most common pathogen causing wound infection compatible with other reports, especially from developing countries (Rahbar 2010, Hajia 2008). There was a significantly higher mortality rate in the Pseudomonas group (33% vs. 8%, $p < 0.001$) compared with other isolated organisms. Amount of blood products used, length of stay, number of surgical procedures

and the cost of care were all significantly higher in the Pseudomonas (Armour 2008). In our burn hospital the high incidence of P. aeruginosa infections and the widespread of high resistance to antibiotics, may be one of the most important influences on the mortality rate in burned patients.

Comparison with Shahid (et al. 2005) report`s that studied in India, the multi-resistant rate was higher than this research. Owlia (et al 2006) has studied similar study in Tehran. He reported the resistant rates for various antibiotics in burned patients that were slightly higher than our data.

Burns continue to be a major environmental factor responsible for significant morbidity and mortality in developing countries (Weber 1999). The key to control antibiotic – resistant pathogens in the Burn hospital is rigorous adherence to infection control guidelines and prevention of antibiotic misuse. Antibiotic restriction policies clearly result in reduced drug costs. Prevention strategies are based on developing a program to prevent or reduce antimicrobial resistance. It indicates the necessity for urgent measures to be taken to restrict the spread of the species in the units and to limit administration of antimicrobial agents including; complete isolation of the contaminated patients, applying highly effective of disinfections and hygienic procedures.

The SHEA/ IDSA guidelines have reviewed specific methods to implement antibiotic control policies. The goal is to have all patients receive the most effective, least toxic, and least costly antibiotic for the precise period needed to cure or prevent an infection (Weber 1999).

Factors affecting the increase and dissemination of antimicrobial resistance can be divided into transfer of resistance genes from one microbe to another, and mutation of existing genes to more resistant variants by the over–use and misuse of antimicrobial, increase infection control measures. Recently researchers have noted to Molecular typing methods to find out about resources of the nosocomial infections. Pulse field gel electrophoresis has been reported successfully for this purpose (Lambiase 2009).

Besides hospital might consider monitoring antibiotic use and resistance patterns and then decreasing the use of specific antimicrobial (Wang 2003, Forbes 1998). The careless health care workers are often the main vectors to promote the dissemination of nosocomial infections.−

Conclusion

High multi resistant rate in pseudomonas infection, needs to immediately revise the hospital policy in control of nosocomial infection and treatment strategy such as more efficient antiseptic agents or new antibiotic drugs may reduced the rate of multidrug resistant

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Clinical importance of cellular responses to stress

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Abstract

The organisms and their cells are exposed to many internal and external stimuli, some of which induce stresses. These trigger responses which may change cellular responses to subsequent environmental signals or even cause cell death. There are four main types of cellular responses: induction of cell repair mechanisms, temporary adaptation to some stressors, autophagy, and cell death. As the consequences of the above mentioned processes may change the responses of cells and organisms to subsequent environmental signals or even cause cell death, the cellular stress responses must be taken into account when studying the degenerative disorders, diseases associated with aging, tumorigenesis and when the cells are used for therapies and in regenerative medicine.

Key words: Cell stress, apoptosis, autophagy, adaptation, repair, graft

Types of cellular stress responses

Cells are exposed to internal and external stimuli, when they are a part of a normal tissue or when they grow in a culture. At least some of the stimuli induce stresses, which trigger responses that modify the cellular responses to subsequent environmental signals or even result in cell death. Exposure to stress over time may cause the accumulation of damage to DNA, proteins and lipids. If not repaired, this will enhance susceptibility to aging associated illnesses, like neurodegenerative diseases, diabetes, heart diseases, etc., and to cancer.

The cellular stress responses can be viewed as a reaction to changes or fluctuations of extracellular conditions, which damage the structure and function of macromolecules (Kültz, 2003). Depending on the severity and duration of stress encountered, cells either re-establish cellular homeostasis to the former state or adopt an altered state in the new environment. Therefore, different stressors and different intensities of stresses result in different cellular responses: (1) induce cell repair mechanisms; these use considerable amounts of available resources and often result in recovery of normal cells, (2) induce cell responses that result in temporary adaptation to some stressors, (3) induce autophagy or (4) trigger cell death (Figure 1). Mechanisms of cell adaptation to stress and their possible roles will be described below.

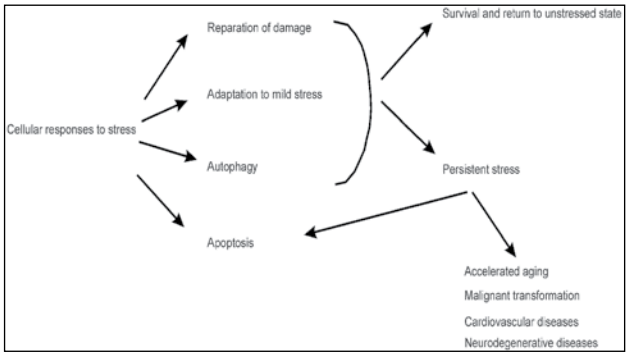

Figure 1. Cellular responses to stress and their consequences for the organism.

Induction of cell repair

Stressors can damage intracellular macromolecules, including proteins, DNA, RNAs and lipids. The repair mechanisms include induction of molecular chaperones (Buchberger et al., 2010), clearance of damaged macromolecules (Kroemer et al., 2010), growth arrest and changed gene expression patterns (Spriggs et al., 2010), etc. Among best studied are the changes in gene expression, either altered transcription or translational regulation.

Modulation of transcription

The changes in gene expression are often mediated by micro ribonucleic acids (miRNAs, reviewed in Leung and Sharp, 2010), which are short noncoding RNAs. The miRNAs known by April 2010 could target about 60% of mammalian mRNAs. miRNAs bind to mRNAs and either accelerate their degradation or inhibit the translation.

The stress responses modify the synthesis of miRNAs and the activities of miRNA-protein complexes. The level of miRNA-mediated repression depends on the relative concentrations of target genes and miRNAs and on the amount of expressed other mRNAs of the transcriptome, which are targeted by the same miRNA. The outcome of miRNA repression depends also on interactions with other stress proteins that can modulate the activity of miRNA protein complexes, e.g. by inhibiting the access to target mRNA.

Sometimes, miRNA can time the stress response. Timing is important in acute stress responses, such as during inflammation. Then the transcription factor nuclear factor-κB (NF-κB) upregulates the transcription of miRNAs together with other inflammatory responsive genes through a cascade of reactions in macrophages. NF-κB has been detected in many cell types, which express cytokines, chemokines, growth factors, cell adhesion molecules, and some acute phase proteins in health and in various disease states. It is activated by different stimuli, like cytokines, free radicals, ultraviolet irradiation, and bacterial or viral products. Inappropriate activation of NF-κB leeds to inflammatory events associated with autoimmune arthritis, asthma, septic shock, lung fibrosis, glomerulonephritis, atherosclerosis, and AIDS, while its persistent inhibition may result in apoptosis, inappropriate immune cell development, and delayed cell growth (Chen et al., 1999).

While NF-κB responds to extrinsic stress, another transcription factor, transformation-related protein 53 (p53) responds to the intrinsic stress. p53 and NF-κB cannot function at the same time in the same cell. On activation of one, the other is inactivated. p53 regulates the expression of miRNA at the levels of transcription and processing. Most p53 mutations found in cancers are in a domain required for miRNA processing and transcription (Leung & Sharp, 2010). p53 enhances the processing of a population of pre-miRNA in cancer cells. Therefore loss of p53 function in transcription and processing may contribute to tumor progression.

p53 responds to many types of cellular stress, such as DNA damage, hypoxia and oncogene activation. It regulates target genes that induce cell cycle arrest, apoptosis, senescence, DNA repair or changes in metabolism (Speidel, 2010). In addition to its functions as a transcription factor in the nuclei, p53 has transcription independent activities in the cytoplasm. Both, transcription dependent and transcription independent activities of p53 may result in apoptosis. Sometimes its transcription independent mechanisms are essential for the full apoptotic response (Speidel, 2010). p53 interact with some regulators of apoptosis, the members of BCL2 family. Additionally, the transcription targets of p53 are apoptotic regulators, like Puma, Mdm2, IGFBP1. The apoptotic stimuli will induce transcription dependent and transcription independent activities of p53 in most cases, as the two complement each other (Speidel, 2010). p53 is inactive in unstressed cells. The concentration and activity of p53 are regulated mostly on the posttranslational level. The half life of p53 protein in normal, unstressed cells, is about up to 20 minutes, while it can be hours under stress (Ak & Levine, 2010). This is achieved by the selective post-translational modifications, which are activated by different stress signals.

Regulation of translation

Nutrient stress, temperature shock, DNA damage and hypoxia can lead to changes in gene expression patterns caused by shutdown and reprogramming of protein synthesis through selective recruitment of ribosomes to mRNAs (Spriggs et al., 2010). This is regulated by elements in 5' and 3' untranslated regions of mRNAs, like internal ribosome entry sites, upstream open reading frames and miRNA target sites. mRNAs for stress response proteins evade global repression of translation by several mechanisms. For example, an otherwise common cap-dependent recruitment of mRNA is bypassed, by using the internal ribosome entry sites (IRES, Spriggs et al., 2010). This involves cofactors. Changes in the abundance or activity of these cofactors influence the degree of IRES mediated translation. The translation from IRES is common during stress conditions.

Heat shock factors (HSF) are inducible transcriptional regulators of genes encoding stress proteins, like molecular chaperones heat shock proteins (Hsp) and others (Akerfelt et al., 2010). Hsp are molecular chaperones and among others

assist proteins in folding or prevent and reverse protein missfolding and aggregation. The activity of HSF is regulated also by different interactions with Hsp during normal and stress conditions.

Upon stress-induced transcription of Hsp genes, HSF accumulate into nuclear stress bodies, which participate in rapid, transient, and global reprogramming of gene expression (Biamonti & Vourc'h, 2010).

Protein quality control and repair

Proteins are often damaged as a result of stress. Often they have exposed hydrophobic regions, which may facilitate aggregation (Buchberger et al., 2010). Failure to clear aggregated proteins leads to cell stress common to many disorders, especially to neurodegenerative diseases. The environmental stress triggers can induce nonnative posttraslational modifications and damage proteins in other ways and consequently induce cell stress as well.

Some protein damage occurs normally in every cell, as translation is an error-prone process. The extent of protein damage increases by adverse intrinsic and environmental conditions, like unbalanced protein synthesis, oxidative stress, metabolic stress, some environmental toxins and pollutants, elevated temperature, high-energy radiation, etc. To cope with considerable extent of protein damage, the damaged proteins are either repaired by molecular chaperones or degraded by the ubiquitin proteasome system or autophagy (Buchberger et al., 2010).

Adaptation to stress

Organisms and the cells can adapt to environmental stress to a certain degree and this can make them more resilient to environmental stresses. In 2010 our group has shown that a mild stress can inhibit triggering of apoptosis through one of the two main pathways (the intrinsic pathway) in the liver cells (Nipic et al., 2010). This is the first evidence of apoptotic pathway shut down caused as a consequence of mild stress. The state of the cells after encountering the mild stress was named preapoptotic cell stress response. This state is temporary, since the cells revert to a normal state in a few days in the absence of further stressors (Nipic et al., 2010, Banic et al., 2011). Despite the inactivation of apoptosis triggering by caspase-9 through the intrinsic pathway, apoptosis can be triggered when the inducer is strong enough, however, the apoptosis executing enzymes caspase-3 and -7 are activated to a lesser degree than when the cells are in the normal state. This is the first report of molecular mechanism of acclimation and physiological adjustment to stress.

Adaptations to sublethal stress, which result in greater stress tolerance, were observed also in mouse blastocysts, where applying hydrostatic pressure improved their survival after freezing and in suboptimal culture conditions (Pribenszky et al., 2005). Similar results were obtained also in bovine blastocysts (Pribenszky & Vajta, 2011). Also, signals from DNA damage can facilitate osmotic stress adaptation (Kültz, 2005).

The phenomenon that a sublethal stress induces a resistance to mild stress was observed from bacteria to multicellular organisms and humans. Some cells tolerate large changes in the environment because of their role in the organism. For example, the cells in papilla of mammalian kidney have to tolerate varying degrees of hyperosmotic stress during urine concentration. There were differences in protein expression during antidiuresis, and during diuresis relatively to normally hydrated controls on the expression profiles (Gabert & Kültz, 2011). Protein expression altered significantly, mainly that of metabolic enzymes, molecular chaperones, proteins involved in redox balance, transport and transcription.

Discovering the mechanisms of adaptation to stress may prove tremendously important, as the cells, during the process of malignant transformation and the resulting tumor cells, undergo adaptations, which enable them to resist the signals that would trigger cell death in their normal state. Persistent stress may also contribute significantly to neurodegenerative processes.

Autophagy

Autophagy is an intracellular lysosomal degradation process characterized by the formation of double-membrane vesicles, autophagosomes, which sequester cytoplasm. Autophagy is a term

used for several processes: (1) macroautophagy, (2) microautophagy and (3) chaperone-mediated autophagy (Funderburk et al., 2010). (1) In macroautophagy, the cytoplasmic material is engulfed by a double-membrane, which fuses subsequently to the lysosome. Sequestered material is indiscriminately removed. (2) Lysosomes engulf a portion of cytosol in microautophagy. (3) Chaperone-mediated autophagy is a way for removal of selected proteins. These proteins can be modified, e.g. ubiquinated. As macroautophagy is the most prevalent form, it is often referred to as autophagy; this term shall be used in such manner below. Autophagy starts by formation of an isolation membrane (phagophore) around the portion of cytosol. The membrane elongates and seals on itself to form a double membrane vacuoles autophagosomes. These then fuse with lysosomes where the entrapped components are degraded.

During cell stress, autophagy is a process through which the cells can reuse the resources. For example, the starvation-induced autophagy helps to recycle the amino acids for protein synthesis and produce the substrates for oxidative phosphorylation when the supplies of nutrients are limited. Degradation of whole regions of cytoplasm therefore generates free amino acids, which can be metabolized to meet energy demand during periods of stress. Autophagy may be the last attempt to rescue the cells from dying. On the other hand, autophagy may have a role in some cases of cell death, too. This is currently under intensive investigation.

Cell death

There are many pathways for triggering cell death; the best known are necrosis and apoptosis. Unlike when the cells dye through necrosis, there is no inflammation in tissue as the consequence of apoptosis (Savill & Fadok, 2000; Kurosaka et al., 2003).

Apoptosis

Apoptosis is a process through which the unwanted cells are orderly dismantled (Kerr et al. 1972). It is characterized by several typical morphological manifestations (Kroemer et al., 2009): rounding up of the cell, reduction of cellular volume, chromatin condensation, nuclear fragmentation, little or no ultrastructural modifications of organelles, plasma membrane blebbing, maintenance of plasma membrane integrity until the final stages of the process and phagocytosis of remains of the cells. Caspases are proteases central to triggering and executing apoptosis (Cohen 1997, Salvesen & Dixit 1997), of which caspase-9 is one of the main initiators through intrinsic pathway (Slee et al. 1999), while caspase-8 is the main initiator when the apoptotic signal originates from the cell surface (Denault & Salvesen, 2002). The two pathways converge to activate caspase-3, which is the best understood executioner caspase. The extrinsic pathway is important in immune responses. The intrinsic or mitochondrial pathway can be activated by many stress stimuli, including DNA damage or extensive perturbation of mitochondrial membrane potential. The proteins of BCL2 family (B cell lymphoma-2) are important regulators of apoptosis; some of them are pro-apoptotic, others are anti-apoptotic. They have several functions, among others anti-apoptotic BCL2 proteins control the integrity of the mitochondrial outer membrane by inhibiting some pro-apoptotic members (Chipuk et al., 2010). Upon activation, the effector pro-apoptotic BCL2 proteins homo-oligomerize into pores in the outer mitochondrial membrane and promote its permeabilization. This releases apoptosis regulators, and often results in apoptosis initiation through the internal pathway.

Consequences of cell stress

Any deviation from an ideal environment could be a stressor for cells, i.e. the stress is caused by too much or too little of an agent, stimulus or other environmental condition. Heat or cold, modification of pH, hyper- or hypo- osmolarity, the increased concentrations of reactive oxygen species, etc., all result in cellular stress. Encountering the stressors is the normal consequence of living in a fluctuating environment, therefore, the cells have developed mechanisms to ameliorate the stress or to adapt to it. This is achieved through the repair of damage, adaptation, reuse of resources and a limited cell death. The cells have to divert at least some of their resources from other pathways, to deal with stressors, as is described above. Our cells are well adapted to a mild stress for a short

time, however, there are potentially serious consequences of the long term stress.

One of the recently established hallmarks of cancer is the presence of stress phenotypes (Leung & Sharp, 2010). The expression of microRNA is often aberrant in cancer; also, microRNA expression patterns often correlate with clinically relevant tumor characteristics. Several microRNAs regulate genes that control proliferation, apoptosis, differentiation, tumor invasion or tumor metastases, and are therefore involved directly in cancer initiation and progression. MicroRNAs are also important in regulating the development of immune cells and in modulating innate and adaptive immune responses (Stern-Ginossar et al., 2008).

The connection of prolonged stress to the development or deterioration of various pathologies is clearly seen in the consequences of aggregated proteins accumulation, which is a hallmark of many neurodegenerative diseases (Petrozzi et al., 2007). Cell stress and stress proteins have a profound effect in triggering/developing the cardiovascular diseases, too. It was first observed in 1970's that the patients infected with *Mycobacterium tuberculosis* or *M. leprae* have antibodies to an antigen, which was later identified as one of the Hsp (Hsp60). It is now confirmed that some Hsp are strong imunogens and imunomodulators in experimental models of arthritis, diabetes and atherosclerosis (Shamaei-Tousi et al., 2007). Atherogenesis may be driven by crossreactive immunity to bacterial proteins. Accumulation of damaged macromolecular structures over time was long recognized to lead to aging. The rate of aging and the appearance of age-related signs are modulated by stress responses (Haigs & Yankner, 2010).

In conclusion, cellular stress can at least contribute to, or even trigger, many diseases and malignant transformations and has an important role in aging. To ameliorate or repair these processes the cells are being used in cell therapies and regenerative medicine. For maximal therapeutic success it is important to use the cells in the best condition possible or those adapted to stress. Therefore, discovering the detailed mechanisms of stress responses, is important basis for development of new diagnostic and treatment procedures and for better assessment of transplanted cells consequently improved transplant survival rates in regenerative medicine.

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The use of complementary and alternative medicine by older patients that have chronic diseases

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Abstract

Background: Many methods related with the health system or not are being used in disease treatment. The community's lack of knowledge leads the patients to use extra medical treatment methods. The majority of patients who use CAM use more than one method.

Objective: This study aimed to investigate the use of complementary and alternative medicine (CAM) by older patients with chronic diseases.

Methods and material: This study was conducted in the internal medicine, cardiology, chest diseases, and physical therapy units, between 10.04.08 and 31.07.08 in Ondokuz Mayıs University, Faculty of Medicine. 275 patients, who accepted an invitation to participate in the research, were taken into the scope of the study. A self administered descriptive questionnaire was used as the data collection instrument for this study. The questionnaires included descriptive clinic characteristics, CAM information, the implementation situation and CAM list. The collected data was evaluated by using the following tests: descriptive statistics, chi-square test, and the student t test.

Results: The most preferred methods were determined as self prayer, herbal treatment, relaxation techniques, diets, exercises, fast walking, multivitamins and music therapy. The satisfaction level of the patients regarding CAM methods was found to be mediocre (2.40±0.59). A total of 22 different types of herbal products were reported as being used by 238 older patients. Garlic, lemon, stinging nettle, blackberry, black mulberry, rosehip, garden thyme, flaxseed, ginger, mallow, sheep sorrel, and French lavender were the most frequently consumed herbal preparations.

Conclusion: In the study it was determined that although patients do not have enough knowledge about CAM methods, CAM prevalence and satisfaction levels are high.

Key words: older patients, chronic disease, complementary medicine, alternative therapy, satisfaction.

Introduction

Aging is a complex phenomenon that has biological, economic, social and physiological dimensions. The technological innovations, improvements in healthcare, and better living conditions have increased the expected life time. The population of the world is growing old and also the older population is enlarging day by day in Turkey. People who are 65 years and older comprise about 5.7% of the population in Turkey (2005), and by the year 2050 this figure is estimated to reach 17.6% [1].

Essential medical problems of old people are chronic and degenerative diseases and the probability of their occurrence is increasing while age is increasing. In Turkey, like in other countries, factors like increased life span, changes in family structure and social relations, lower activity levels depending on advanced technology and high stress are increasing the problems and chronic diseases relating to old age. It is reported that 90% of those over 65 years have at least one chronic health condition, 35% have two, 23% three and 15% four and more in Turkey [1].

Many methods related with the health system or not are being used in disease treatment. The community's lack of knowledge leads the patients to use extra medical treatment methods [2,3]. The majority of patients who use CAM use more than

one method. Recent investigations have demonstrated that CAM is commonly used in other countries as well as in Canada, Australia and the United States [4-7]. As the exchange of medical information and international communication continue to grow, it is important to learn the extent of use of alternative medical therapies among different countries, especially those with similarly aging populations [8].

The reasons for the present popularity of CAM are multifaceted and are closely related to the social, cultural, economic, and traditional structures of societies. Trends in the use of CAM may differ across countries, geographic locations, ethnic backgrounds, education levels, socioeconomic factors, religions, age groups and gender, and it is difficult to compare the studies in the literature because of the different definitions of CAM used [2].

Also, concerns have been raised about the quality and safety of complementary alternative medicine products, particularly because many herbal medicines, homeopathic remedies, vitamins, and nutritional supplements can be purchased directly from health food stores, mail and internet order catalogues and mainstream food markets and pharmacies without the proper information on interactions with prescriptions and over the counter medications [9].

In Turkey, there is inadequate information on whether older patients use CAM, the therapies used, the frequency of use of reported therapy, whether patients find these useful, and how they learned about these therapies. It is essential that accurate data is available to reflect the use of CAM in older patients that have chronic diseases, because these methods affect conventional treatment directly or indirectly. However, it is very important to know the usage status in terms of the unexplained effects and results. Exploring CAM use in clinical settings may help clinicians better understand the needs of their patients. Therefore, the use of CAM and the opinions of older patients should be determined.

The aims of this descriptive study were to determine the prevalence of CAM use among older patients with chronic diseases, the method of use of the reported therapy, the frequency of use of the reported therapy, the source of information about CAM therapies, and to compare the socio-demographic characteristics of users and non-users of CAM. We also report on the satisfaction of using CAM.

Method

Study design and Sampling Method

This study used a descriptive design. The population of this study was Turkish people who lived in Samsun, Turkey. This study was conducted in the internal medicine, cardiology, chest diseases, and physical therapy units between 10.04.08 and 31.07.08 in Ondokuz Mayıs University, Faculty of Medicine. The sample of the study consisted of 275 persons. 318 older patients were treated in these clinics between these dates. Patients who did not want to participate in the study and those unable to communicate or with concurrent mental illnesses were excluded from the study. 43 people did not complete the questionnaire because they did not want to participate in the study. The eligibility criteria were: aged 65 years or more, having a chronic disease, and able to read.

Procedures

A self administered descriptive questionnaire was used as the data collection instrument for this study. Nevertheless, a checklist about CAM implementations was given. The checklist included the statements of the patients about whether they knew the treatments or not, whether they have used them or not and if they have ever used CAM treatments, and whether they are satisfied with them or not. Patients answered these questions as 'I know/do not know the described methods', 'I used/did not use them', and if they used them, they answered that their satisfaction level was 'low', 'middle', or 'high'. The CAM users with a low satisfaction level scored 1 point, the ones with a middle satisfaction level scored 2 points, and the ones with a high satisfaction level scored 3 points. While evaluating the data, participants were categorized as ones able to use CAM and the ones not.

Instruments

The patients completed a self administered, 27-item questionnaire prepared by the researchers. The questionnaire included questions about the descriptive characteristics of patients (age, gender, education, socio-economic condition, marital status, family type, have children, place of residence, social assurance, socio-economic status); the satisfaction level regarding the medical treatment

they undergo for their disease; their knowledge and usage of another treatment method except for medical treatment; suggestions of a treatment method except for medical treatment and the person suggesting it; CAM usage/non-usage with medical treatment methods, considering if medical or CAM types are much more efficient, taking information about the methods except for medical methods, the sources from which this information is taken, and the CAM checklist.

The CAM checklist developed according to the literature [10-15], which consisted of 18 popular methods (herbal treatment, diets, multivitamins, self prayer, massage, psychotherapy, yoga, meditation, aromatherapy, acupuncture, fast walking, relaxation techniques, exercises, music therapy, support group, imagery, hypnosis, humour), was given to the patients. The questionnaire was tested in a group of 20 people making a pre-implementation; the questions not understood or not completed were determined and corrected. After the pilot study, the last draft was adapted and given to the research group. In this study, the alpha coefficient was.78.

Ethical approval

We received written confirmation of approval from the Ondokuz Mayis University Ethical Committee before we started the study. Also, we obtained the informed consent of every patient that approved participation in the study. Data was collected from the units in four months, simultaneously. The researchers assured patients that their identities and answers would be kept confidential. This process was carried out before or after the routine treatments of patients in a separate room by the researchers and it lasted about 8-10 minutes. This study was conducted by taking into account the principles of the Helsinki Declaration.

Statistical Analysis

The data was evaluated using the SPSS 15.0 program. In this evaluation, descriptive statistics, chi-square and student t tests were applied. Sociodemographic characteristics, the CAM usage situation, and satisfaction levels regarding CAM types were specified with descriptive statistics; the ones using and not using CAM were compared and an age interval was determined with the student t test.

Results

Sample Characteristics

The distribution of the demographic characteristics of the patients using and not using CAM is given in Table 1. The average age of the participants was 70.4± 4.3. In this study it was found out that there is a remarkable statistical difference between the ones using and not using CAM in terms of education level (x^2:10.789, p:0.029), and social assurance (x^2:5.164, p:0.023). There was no difference in age (x^2:6.819, p:0.146), gender (x^2:0.052, p:0.820), marital status (x^2:0.018, p:0.892), family type (x^2:0.819, p:0.366), residence (x^2:3.549, p:0.170), have children (x^2:0.334, p:0.563), living with person (x^2:1.190, p:0.552), and socioeconomic status (x^2:2.133, p:0.344) between these two groups (Table 1). Female patients (53.4%, n=127), literate (41.6%, n=99), married ones (89.9%, n=214), nuclear families (89.9%, n=214), the ones living in towns (42.0%, n=100), and the ones whose incomes and expenses are the same in terms of socio-economics (63.9%, n=152) are more likely to apply CAM (Table 1).

Clinic Characteristics

The clinic characteristics of older patients with chronic diseases and the treatment of the patients using and not using CAM are given in Table 2. In this study, it was determined that 37.5% (n=103) of the old patients perceived their medical status as good, 74.5% (n=205) of them stated that their chronic diseases affected their daily life activities, and 54.2% (n=149) of them thought that non-medical methods may be useful. The rates of CAM therapy users and non-users were higher among those with a fair and good perceived health status.

Use of CAM and Knowledge State

The knowledge level of patients is given in Table 3. In the study, average CAM usage prevalence was specified as 86.5% (n=238). In accordance with the findings gained it was determined that the patients knew more about herbal treatments, diets, fast walking, multivitamins, self prayer, exercises, relaxation techniques, massage, and psychotherapy (89.1%, n=245; 85.1%, n=234; 84.7%, n=233; 84.0%, n=231; 78.2%, n=215; 77.1%, n=212; 67.6%, n=186; 58.9%, n=162; 41.1%, n=113), and

Table 1. Demographic Characteristics of the Complementary Alternative Therapy Users, Nonusers, and Total Sample (N=275)

Characteristics	CAM users n(%) n=238	Nonusers n(%) n=37	Total Sample N(%) N=275	Significance
Age Group				
65-70	140 (58.8)	22 (59.5)	162 (58.9)	
71-75	64 (26.9)	10 (27.0)	74 (26.9)	
76-80	28 (11.8)	3 (8.1)	31 (11.3)	x²: 6.819 NS
81-85	6 (2.5)	1 (2.7)	7 (2.5)	
86 +	--- ----	1 (2.7)	1 (0.4)	
Gender				
Female	127 (53.4)	19 (51.4)	146 (53.1)	x²: 0.052 NS
Male	111 (46.6)	18 (48.6)	129 (46.9)	
Current Marital Status				
Married	214 (89.9)	33 (89.2)	247 (89.8)	x²: 0.018 NS
Not married	24 (10.1)	4 (10.8)	28 10.2	
Education				
Literate	99 (41.6)	22 (59.5)	121 (44.0)	
Elementary School	92 (38.7)	6 (16.2)	98 (35.6)	x²: 10.789
Secondary School	22 (9.2)	5 (13.5)	27 (9.8)	p: .029
High School	13 (5.5)	4 (10.8)	17 (6.2)	
University	12 (5.0)	--- -----	12 (4.4)	
Family Type				
Large	24 (10.1)	2 (5.4)	26 (9.5)	x²: 0.819 NS
Small, nuclear	214 (89.9)	35 (94.6)	249 (90.5)	
Place of residence*				
City	70 (29.4)	6 (16.2)	76 (27.6)	
Town	100 (42.0)	16 (43.2)	116 (42.2)	x²: 3.549 NS
Village	68 (28.6)	15 (40.6)	83 (30.2)	
Have Children				
Yes	219 (92.0)	33 (89.2)	252 (91.6)	x²: 0.334 NS
No	19 (8.0)	4 (10.8)	23 (8.4)	
Living with				
Spouses	94 (39.5)	17 (46.0)	111 (40.4)	
Family members	119 (50.0)	18 (48.6)	137 (49.8)	x²: 1.190 NS
Lonely	25 (10.5)	2 (5.4)	27 (9.8)	
Social assurance				
Present	231 (97.1)	33 (89.2)	264 (96.0)	x²: 5.164
Absent	7 (2.9)	4 (10.8)	11 (4.0)	p: .023
Socioeconomic status				
Income<expenditure	67 (28.1)	14 (37.8)	81 (29.5)	
Income=expenditure	152 (63.9)	19 (51.4)	171 (62.2)	x²: 2.133 NS
Income>expenditure	19 (8.0)	4 (10.8)	23 (8.4)	

NS, Statistically no significant difference between the group of CAM users and the group of nonusers (p>0,05)
P value, x^2 test (two-sided) between users and nonusers within cancer patients for all variables except mean age (student t test)
**City: urban area/the largest of area of residence. Town: Semi-urban area /approximately population between 5 and 10 000. Village: rural area /the smallest of area of residence, approximately population between 2 and 3000.*

Table 2. Clinical Characteristics of Older Patients (N=275)

Characteristics	CAM users n(%) n=238	Nonusers n(%) n=37	Total Sample N(%) N=275	Significance
Perceived health				
Very Good	14 (5.9)	2 (5.4)	16 (5.8)	
Good	86 (36.1)	17 (46.0)	103 (37.5)	x^2: 2.869 NS
Fair	91 (38.2)	9 (24.3)	100 (36.4)	
Poor	47 (19.8)	9 (24.3)	56 (20.4)	
***Have Choronic Ilness**				x^2: 1.484 NS
Hypertension	156 (65.5)	28 (75.7)	184 (66.9)	
Arthritis	5 (2.1)	---- ----	5 (1.8)	x^2: 0.792 NS
Diabetes	62 (26.1)	13 (35.1)	75 (27.3)	x^2: 1.332 NS
Coronary arterial disease	20 (8.4)	---- ----	20 (7.3)	x^2: 3.353 NS
Congestive heart failure	60 (25.2)	9 (24.3)	69 (25.1)	x^2: 0.013 NS
Osteoporosis	38 (16.0)	6 (16.2)	44 (16.0)	x^2: 0.001 NS
Asthma	31 (13.0)	1 (2.7)	32 (11.6)	x^2: 3.318 NS
Chronic Bronchitis	49 (20.6)	3 (8.1)	52 (18.9)	x^2: 3.253 NS
Chronic obstructive lung disease	14 (5.9)	1 (2.7)	15 (5.5)	x^2: 0.628 NS
Chronic renal failure	1 (0.4)	7 (18.9)	8 (2.9)	x^2: 38.797 p:.000
Effect on daily living activities of cronic disease				x^2: 1.097 NS
Effect	180 (75.6)	25 (67.6)	205 (74.5)	
Non effect	58 (24.4)	12 (32.4)	70 (25.5)	
Satisfaction about medical treatment				x^2: 3.173 NS
Satisfaction	219 (92.0)	37 (100.0)	256 (93.1)	
No satisfaction	19 (8.0)	---- -----	19 (6.9)	
Thinking to be useful non medical therapies				x^2: 0.527 NS
Useful	131 (55.0)	18 (48.7)	149 (54.2)	
Non Useful	107 (45.0)	19 (51.3)	126 (45.8)	

**It was given answer more one.*

they knew less about support groups, hypnosis, aromatherapy, meditation, humour, music therapy, yoga, acupuncture, and imagery (7.6%, n=21; 17.5%, n=48; 22.5%, n=62; 26.2%, n=72; 26.2%, n=72; 26.2%, n=72; 26.5%, n=73; 31.3%, n=86; 33.1%, n=91).

Frequency of CAM use and Perceived Satisfaction

The frequency of CAM use and perceived satisfaction are given Table 4. The most preferred methods were determined as self prayer, herbal treatments, relaxation techniques, diets, exercises, fast walking, multivitamins and music therapy (55.0%, n=131; 54.6%, n=130; 43.7%, n=104; 40.3%, n=96; 37.0%, n=88; 31.1%, n=74; 27.7%, n=66; 16.8%, n=40). The levels were evaluated with a scale varying between 1 and 3. In accordance with the findings gained, the average satisfaction level patients perceived regarding CAM types was specified as 2.40±0.59.

Herbal Therapies use in Chronic Diseases

A total of 22 different types of herbal products were reported as being used by 238 older patients. Table 5 shows the 12 most frequently reported herbs, along with the Turkish name, botanical name, and reported disease and frequency of use. Garlic, lemon, stinging nettle, blackberry, black mulberry, rosehip, garden thyme, flaxseed, ginger, mallow, sheep sorrel, and French lavender were the most frequently consumed herbal preparations.

Table 3. Knowledge of Patients CAM Methods (N=275)

Characteristics	Cam users		Non users		Total Sample		Significance
	Know n(%)	Not know n(%)	Know n(%)	Not know n(%)	Know N(%)	Not know N(%)	
Herbal treatment	219 (92.0)	19 (8.0)	26 (70.3)	11 (29.7)	245 (89.1)	30 (10.9)	x^2: 15.581 p: .000
Diets	206 (86.6)	32 (13.4)	28 (75.7)	9 (24.3)	234 (85.1)	41 (14.9)	x^2: 2.987 NS
Multivitamins	206 (86.6)	32 (13.4)	25 (67.6)	12 (32.4)	231 (84.0)	44 (16.0)	x^2: 8.589 p: .003
Self prayer	190 (79.9)	48 (20.1)	25 (67.6)	12 (32.4)	215 (78.2)	60 (21.8)	x^2: 2.824 NS
Massage	156 (65.5)	82 (34.5)	6 (16.2)	31 (83.8)	162 (58.9)	113 (41.1)	x^2: 32.191 p: .000
Psycotherapy	106 (44.5)	132 (55.5)	7 (18.9)	30 (81.1)	113 (41.1)	162 (58.9)	x^2: 8.682 p: .003
Yoga	66 (27.7)	172 (72.3)	7 (18.9)	30 (81.1)	73 (26.5)	202 (73.5)	x^2: 1.275 NS
Meditation	65 (27.3)	173 (72.7)	7 (18.9)	30 (81.1)	72 (26.2)	203 (73.8)	x^2: 1.167 NS
Aromatherapy	55 (23.1)	183 (76.9)	7 (18.9)	30 (81.1)	62 (22.5)	213 (77.5)	x^2: 0.322 NS
Acupuncture	79 (33.2)	159 (66.8)	7 (18.9)	30 (81.1)	86 (31.3)	189 (68.7)	x^2:3.036 NS
Fast walking	216 (90.8)	22 (9.2)	17 (46.0)	20 (54.0)	233 (84.7)	42 (15.3)	x^2: 49.689 p: .000
Relaxation techniques	170 (71.4)	68 (28.6)	16 (43.2)	21(56.8)	186 (67.6)	89 (32.4)	x^2: 11.621 p= .001
Exercises	192 (80.7)	46 (19.3)	20 (54.0)	17 (46.0)	212 (77.1)	63 (22.9)	x^2: 12.847 p: .000
Music therapy	70 (29.4)	168 (70.6)	2 (5.4)	35 (94.6)	72 (26.2)	203 (73.8)	x^2: 9.549 p: .002
Support group	19 (8.0)	219 (92.0)	2 (5.4)	35 (94.6)	21 (7.6)	254 (92.4)	x^2: 0.302 NS
Imagery	87 (36.6)	151 (63.4)	4 (10.8)	33 (89.2)	91 (33.1)	184 (66.9)	x^2: 9.585 p: .002
Hypnosis	43 (18.1)	195 (81.9)	5 (13.5)	32 (86.5)	48 (17.5)	227 (82.5)	x^2: 0.461 NS
Humor	70 (29.4)	168 (70.6)	2 (5.4)	35 (94.6)	72 (26.2)	203 (73.8)	x^2: 9.549 p= .002

Table 4. Frequency of CAM Use and Satisfaction of CAM Users (N=238)

Characteristics	Cam users n (%)	Satisfactory levels*
Herbal treatment	130 (54.6)	2.32±0.62
Diets	96 (40.3)	2.21±0.50
Multivitamins	66 (27.7)	2.30±0.65
Self prayer	131(55.0)	2.75±0.43
Massage	30 (12.6)	2.46±0.62
Psycotherapy	15(6.3)	2.40±0.73
Acupuncture	1 (0.4)	3.00
Fast walking	74 (31.1)	2.10±0.71
Relaxation techniques	104 (43.7)	2.19±0.48
Exercises	88 (37.0)	2.20±0.59
Music therapy	40 (16.8)	2.46±0.67
Support group	1 (0.4)	3.00
Imagery	29 (12.2)	1.65±0.61
Humor	17 (7.1)	2.58±0.50

**Satisfactory levels: 1=least satisfied, 2=middle satisfied, 3=most satisfied*

Table 5. Herbal Therapies Most Frequently Used By Older Patients (N:130)

English Name	Turkish Name	Botanical Name	Reported Disease and Frequency of Uses
Garlic	Sarmısak	Allium sativum	Hypertension (75.4%)
Blackberry	Böğürtlen	Rubus caesius	Diabetes (12.3%)
Rosehip	Kuşburnu	Rosa canina	Diabetes (5.4%)
Black mulberry	Karadut	Morus nigra	Diabetes (12.3%)
Lemon	Limon	Citrus limon	Hypertension (20.8%)
Garden thyme	Kekik	Thymus vulgaris	Hypertension (1.5%), Diabetes (0.8%), Coronary arterial disease (7.7%)
Flaxseed	Keten tohumu	Linum usitatissimum	Coronary arterial disease (6.9%)
Ginger	Zencefil	Zingiber officinale	Chronic bronchitis (3.8%), chronic obstructive lung disease (0.8%),
Mallow	Ebegümeci	Malva sylvestris	Diabetes (7.7%)
Sheep sorrel	Kuzukulağı (evelik)	Rumex crispus	Arthritis (3.1%)
French lavender	Karabaşotu	Lavandula stoechas	Chronic bronchitis (5.4%)
Stinging nettle	Isırgan otu	Urtica dioica	Hypertensin (3.1%), Arthritis (3.8%), Diabetes (10.8%), Coronary arterial disease (1.5%), Congestive heart failure (0.8%), Asthma (0.8%), Chronic bronchitis (0.8%) Chronic obstructive lung disease (0.8%)

Discussion

To our knowledge, this is the first survey to determine the prevalence of the use of CAM by older patients with chronic diseases in Turkey. Today, among people, the CAM usage rate is pretty high [4-7, 16-17]. Even though there are some studies about CAM use in older people [8,18-23], there are very few studies about CAM use in older patients with chronic diseases [24-28] and there are limited studies on this matter in Turkey, which restricts the debate of study findings.

In the study it was specified that although most of the patients (93.1%) are satisfied with medical treatment, average CAM usage prevalence is 86.5%. It was determined in the researches conducted for determining the status relating to the CAM use of old people and covering various countries that CAM use prevalence ranges between 7.8% and 88.0% and the average is 53.4% [8,9,18-20,22,24-26,29,30]. In this study, CAM use prevalence was found to be high compared to the results of the studies conducted in other countries.

In this study, the CAM usage rate of females (53.4%) was found to be higher, and this result is similar to other study results [8-9,19,23,25,28]. In accordance with the findings gained from this study, the CAM usage rate is higher in the literate for older people (41.6%). Although this result is similar to other study results gained in some countries [9,29], in the studies conducted in foreign countries it was stated that this rate is higher in educated people compared to the ones with a low education level [16-19,21,23,28]. The reason why literate older people use CAM types much more may be because the research groups consisted of mostly literate people. Besides, married people (89.9%) and the ones (63.9%) whose income is equal to their expenses in terms of the socio-economic factor benefit much more from CAM types, although this result is similar to other studies' results [29].

In terms of the selection of CAM types, family, relatives, medical staff, and neighbours are an important knowledge source [22,29,31]. In this study, the same thing was found. It was found out that these methods were suggested to patients by their relatives (6.7%), medical staff (6.7%), families (5.5%), their children (3.4%), neighbours (2.1%), and their spouses (0.8%). The fact that family, medical staff and relatives are the most important social support structures for patient individuals can explain this situation. Also, media reports such as on TV and radio (90.5%), medical

staff (47.6%), newspapers and journals (42.9%), and medical books and articles (9.5%) were other sources of information.

The most preferred methods were determined as herbal treatments, relaxation techniques, diets, exercises, fast walking, multivitamins and music therapy. Even though CAM types vary according to the social, cultural, economic and traditional characteristics of a society, the relevant studies showed that the most common treatments applied by older patients are herbal therapy, reflexology, massage, acupressure, acupuncture, relaxation techniques, diets, vitamins/minerals, chiropractic services, exercises, homeopathic treatments, meditation, music and humour [4,8,16-18,20,21-28].

In this study, it was specified that patients did not apply acupuncture (0.4%), support groups (0.4%), psychotherapy (6.3%), humour (7.1%), imagery (12.2%), and massage (12.6%) so much. This is due to the fact that they did not have enough knowledge about these methods and their implementation. In addition, these methods are not popular in Turkey, probably as a result of different social, cultural, religious and economic motives.

In this study, the perceived satisfaction level of the patients was about 2.40±0.59, which is above the mediocre satisfaction level. The reasons for the present satisfaction level of CAM are multifaceted and are closely related to the social, cultural, economic and traditional structures of societies. Trends in the satisfaction level of CAM may differ across countries, geographic locations, ethnic backgrounds, education levels, socioeconomic factors, religions, age groups and genders.

The chronic diseases for which they choose to use CAM therapies, such as hypertension (65.5%), diabetes (26.1%), congestive heart failure (25.2%), bronchitis (20.6%), osteoporosis (16.0%), and asthma (13.0%), were common among older people in this study. In other studies relating to CAM usage, people who were suffering from diabetes, cancer, arthritis, hypertension, chronic renal failure, chronic heart failure, musculoskeletal disorders, strokes and asthma diseases used CAM methods more compared with others who had no chronic diseases [4,9,18,19,26,31-32]. Also, it was reported that there was no statistically significant variance between people who are suffering from ischemic heart diseases, chronic obstructive lung diseases, and those who are not suffering from such diseases, in terms of CAM usage, and the rate of benefiting from CAM methods is higher in diabetics aged 65 or older [32].

Within the scope of the study it was determined that 54.6% of the older patients with chronic diseases used herbs. Garlic, lemon, stinging nettles, blackberry, black mulberry, rosehip, garden thyme, flaxseed, ginger, mallow, sheep sorrel, and French lavender were the most frequently consumed herbal preparations. The main CAM therapies among Turkish patients are herbal and folk remedies. The reason they are more popular might also be because they are more readily available and inexpensive. Also, this excessive use of herbal therapies in Turkey can be explained by the fact that plants and grasses play a great part in the diets of individuals. A positive attitude towards herbal therapies may also cause a delay in medical diagnoses and treatments, and some applications of CAM may be harmful. Therefore, healthcare professionals must be aware of the effectiveness of dosages, side effects, toxicities, and potential drug interactions of herbal remedies.

Through various data analyses, the patterns of use of complementary therapies by older patients with chronic diseases, the types of therapies most commonly used, and the frequency of use of various therapies, were evaluated. This information can provide critical information for the development of practice guidelines. Healthcare providers must be aware of the use, efficacy, adverse affects, and scientific evidence concerning CAM in order to provide safe and effective practice as well as appropriate and accurate information to their clients.

Limitations

The sample in this study reflects only one area of Turkey. The number of patients within the scope of the study was low. The findings, therefore, cannot be generalized to the entire Turkish population. The study included only older people in one Turkish region. Further work would be needed to investigate the findings' generalizability to other regions.

Conclusions

Complementary and alternative therapy use is common among older people, particularly among those with chronic medical conditions. In the study it was determined that although patients do not have enough knowledge about CAM methods, CAM prevalence and satisfaction levels are high. Healthcare professionals should elicit and document information on CAM use from older adult patients. They must be aware of older patients' attitudes towards CAM, because CAM may also cause a delay in medical diagnoses and treatments, and some applications of CAM may be harmful. Thus, health professionals need to be more effective in providing individuals with research based information on these therapies.

Acknowledgements

The authors wish to thank the participants of the research groups for giving their time generously.

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Phase II clinical study of an association for the treatment of interstitial cystitis (Cystex®)

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Abstract

Painful bladder syndrome associated with interstitial cystitis (PBS/IC) is a clinical condition characterized pelvic pain, urinary urgency, and urinary frequency. In this study, 22 patients were assigned to make two visits over a three weeks period. The patients were randomly, double-blinded assigned in two groups. The first group received Cystex® capsules. The second group received placebo capsules. Two capsules were taken three times a day away from meals. The change from baseline in the O'Leary-Sant IC symptom and problem index was the primary outcome parameter. Changes in functional bladder capacity and intensity of pain and urgency have been chosen as secondary outcome parameters. Mood as well as physical and sexual activity were rated by 10 questions on a scale 0 to 6. The ratings were analyzed and the average for each patient in both groups Cystex® and placebo was determined as the quality of life index. For the primary outcome there was a statistically significant difference between the groups. Mean symptom score-sum decreased from 28.4 to 20.5 in the Cystex® group compared with 29.5 to 26.8 in the placebo group ($p<0.05$). For the secondary end points, pain and urgency intensity improved statistically significantly in the Cystex® group compared with the placebo group ($p<0.05$). The frequency and functional bladder capacity improved to greater degree in the Cystex® group. The differences were statistically significant for comparison of frequency ($p<0.05$) and not for functional bladder capacity ($p>0.05$). In our study, Cystex® enhanced quality of life over the placebo showing a statistically significant. This trial have shown that the efficacy and safety of therapy with Cystex® in the treatment of interstitial cystitis and is an alternative for patients suffering from this pathology. Therefore, it can be concluded that the composition of Cystex®, increased the quality of life in treated patients.

Key words: Interstitial cystitis, clinical trial, painful bladder, quality of life.

Introduction

Painful bladder syndrome/ interstitial cystitis (PBS/IC) is a clinical condition characterized by 3 symptoms: pelvic pain, urinary urgency, and urinary frequency (1).

Interstitial cystitis (IC) is a chronic and debilitating bladder syndrome, affecting primarily women, characterized by pelvic/ bladder pain associated with urinary urgency, frequency and sterile, cytologically normal urine (2).

In individuals with a normal bladder, the epithelial layer is relatively impermeable and does not allow potassium ions to be absorbed. The glycosaminoglycans (GAG) layer lining the bladder urothelium has been implicated in the pathogenesis of IC/PBS (3). Relevant components of the GAG layer include hyaluronic acid, sulfated forms of heparin, dermatan, keratin and chondroitin. A deficiency in the GAG layer changes the permeability of the urothelium to urinary components, principally to potassium ions. In this sense, an excess

of potassium ions in the urothelial layer may be responsible for the intense urgency and/or pain of painful bladder syndrome (4). It has been diagnostic that this layer may be impaired, abnormal, or disrupted in patients with IC/PBS (5). This compromised epithelium becomes permeable to urinary solutes, resulting in local inflammation and neural upregulation and subsequent pain, frequency, and urgency. In the event the epithelium has a lesion, potassium ions will be absorbed, generating symptoms of pain and urgency through the stimulation of the chemical sensitive component of type C nerve fibres (6,7). GAGS are an important structural part of bladder mucosal integrity and therapeutic GAG or GAG-like agents, such as sodium hyaluronate (8), heparin (9) and pentosan-polysulfate (10) have been previously examined with some variable results.

Systemic treatment options for patients with painful bladder syndrome associated with interstitial cystitis are limited. The first report Hanno and Wein (1987) on the use of the tricyclic antidepressant amitriptyline was in 1987 (11) and, although amitriptyline has subsequently become the most frequently prescribed oral drug for IC (2).

Many treatments are available for IC, but it is generally accepted that the results are disappointing, and few have been subjected to rigorous clinical evaluation (2).

The aim is to verify the safety and effectiveness of treatment with Cystex® in patients with painful bladder syndrome.

Pacients and Methods

Females and males with the clinical diagnosis of IC/PBS and no medical condition or therapy that would exclude safe concomitant use of Cystex® were evaluated in this clinical trial. The diagnosis of IC/PBS used clinical definitions, including the diagnostic criteria described in the IC Data Base Study (12). IC/PBS was diagnosed on the basis of pelvic pain, pressure, or discomfort perceived to be related to the urinary bladder accompanied by at least one urinary symptom, such as urgency or frequency. Urine must have been sterile at time of diagnosis and assessment and urinary cytology negative. All other diseases that could cause pelvic symptoms were excluded.

The following inclusion criteria for this study were: female or male; negative bacterial urine culture; a patient-reported average urinary frequency of at least 11 times per 24-hour period during the screening period.

In this study, 22 patients, aging between 50 to 60 years, were assigned to make two visits over a three week period. The patients were randomly, double-blinded assigned in two groups to complete the assessment of the questionnaire (13, 14, 15).

The patients enrolled were both male and female and known to be subjected to physical and mental stress and/or to present fatigue symptoms not related to any exclusion criteria. An informed written consent was obtained from each patient after a lecture about the study to make it clear to them. Prior to conducting this study, we trained four medical doctors (MDs) to establish consistency in research methods. All MDs attended initial training and orientation in Alfenas, MG at the beginning of the study. In addition, each of them coded sample cases and received feedback from the project senior researcher prior to their first case. The research was conducted at Alzira Velano Hospital. Data collection began on March 6, 2000 and ended on March 27, 2000. The patients were randomly divided in two groups. Randomization is being performed by computer-generated random allocation sequence. The first group (group A) had received Cystex® capsule containing Acriflavin hydrochloride 15.00 mg; Methenamine 250.00 mg; Methylene blue 20.00 mg; Beladona extract 15.00 mg. The second group (group B) had received placebo capsules, identical in form, color and taste to the ones of the active formulation.

Both treatments were provided by EMS Sigma Pharma, based in Hortolândia (SP), Brazil, and had the same characteristics. Two capsules were taken three times a day away from meals, for a 3 weeks period. Mood as well as physical and sexual activity were rated by 10 questions on a scale 0 to 6. The ratings were analyzed and the average for each patient in both groups Cystex® and placebo was determined as the quality of life index. The level of quality of life was assessed by a questionnaire (13, 14, 15) approved by the Ethical Committee for Human Research of The University of Alfenas, Alfenas, MG. This project was registered under number 685/99.

From the 22 patients enrolled, 11 were assigned to group Cystex® and 11 to group placebo. The physiological parameters (pulse rate, blood pressure and clinical history) were assessed with relation to the demographic characteristics (Table 1) and they were found to be similar in the groups enrolled. Also, there were no significant intergroup differences for obesity, arterial hypertension, renal and liver diseases or diabetes mellitus. No worsening of these pathologies was detected during the study. Adverse effects were recorded in the case report form and, if severe, were added to the discontinuation criteria, including pregnancy, interaction with others medications not allowed, as well as voluntary withdrawals or protocol violation.

This study used a randomized, placebo controlled, and double blind design and was conducted at one institution (University of Alfenas – Alzira Velano Hospital). The study protocol was approved by the Institutional Review Board of the Alzira Velano University Hospital and written informed consent was obtained from all patients.

The initiation of treatment with Cystex® occurs in the first day of one group while another takes the placebo. Patients eligible for the study were randomized into two groups in a 1:1 ratio. One group will start treatment with placebo and the other group will receive Cystex® for 21 days.

Patients were subsequently treated prospectively for 3 weeks with a selftitration protocol. The change from baseline in the O'Leary-Sant IC symptom and problem index was the primary outcome parameter for this study. This validated, self-administered index comprises 8 questions assessing major pain and voiding symptoms. The maximum index score-sum of 36 reflects maximum symptom and problem severity and the lowest possible score-sum is 0.5. Secondary outcome measures included patient reported symptoms of pain and urgency (visual analog scales, VAS), and changes in functional bladder capacity and frequency (48-hour voiding log). Additionally, patients were requested to rate satisfaction with the therapeutic outcome as excellent, good, fair or poor (Patient Global Assessment Form). Outcomes were evaluated at baseline and in 3-week intervals after randomization. The study sample size of 22 patients (11 per group) was powered for a difference of approximately 1 standard deviation between the Cystex® and placebo group with a 95% confidence interval ($\alpha = 0.05$) and a statistical power of 85% ($\beta = 0.20$). Baseline factors were compared between the 2 groups using Fisher's exact and Mann-Whitney-U tests. Statistical comparisons were made using the Mann-Whitney-U test for changes in symptom score (primary outcome parameter), changes in pain and urgency intensity, and changes in voiding patterns (frequency, functional bladder volume). Chisquare statistics were calculated to compare proportion of responders between treatment groups, with $p < 0.05$ considered significant. All statistical tests were 2- tailed, and calculations were performed using Graph Pad Instat®.

Results

No patients dropped out of the study because of adverse side effects. The data on 22 patients (11 patients in each group) were available for evaluation. Selected baseline characteristics are shown in table 1. For the primary outcome there was a highly statistically significant difference between the study groups. Mean symptom score-sum decreased from 28.4 to 20.5 in the Cystex® group compared with 29.5 to 26.8 in the placebo group ($p < 0.05$).

Table 1. Baseline patient demographic and clinical characteristics by treatment group

Characteristic	Cystex	Placebo
No. pts randomized	11	11
No. females (%)	9	9
No. males (%)	2	2
Mean age ± SD (yrs)	54.9 ± 5.1	55.3± 5.8
Mean symptom duration before treatment ± SD (yrs)	4.7 ± 2.6	5.2 ± 3.1
Mean age at onset of symptoms ± SD (yrs)	49.3 ± 3.4	47.8 ±4.3
Mean pain intensity ± SD (mm on VAS)	58 ± 26	55 ± 29
Mean urgency intensity ± SD (mm on VAS)	64 ± 22	68 ± 17
Mean 24-hr frequency ± SD	14.2 ± 6.4	1.5 ± 6.3
Mean functional bladder volume ± SD (ml)	134 ± 68	126 ± 71
Mean score-sum ± SD	28.4 ± 6	29.5 ± 5

For the secondary end points, it can be noticed several outcome parameters statistically significant difference between the study groups (table 2). Pain

and urgency intensity improved statistically significantly in the Cystex® group compared with the placebo group ($p<0.05$). The frequency and functional bladder capacity improved to a much greater degree in the Cystex® group. The differences were statistically significant for comparison of frequency ($p<0.05$) and not for functional bladder capacity ($p>0.05$). Of the 11 patients receiving Cystex®, 4 (36%) rated satisfaction with the therapeutic outcome as excellent, 5 (45%) rated as good. Anticholinergic side effects were noted by all except 1 patient in the Cystex® group (91%) and by 9 patients in the placebo group (81%). A dry mouth was the most frequent side effect in the Cystex® group.

The treatment with Cystex® increased the quality of life compared to placebo treatment. At baseline, there was no significant difference between treatments groups. After 21 days of study, in visit 1, a significant difference between the treatments was observed, showing an increase in the group treated with Cystex® average scoring from 1.64 to 4.88 points. The statistical comparison found that every item the group treated with Cystex® had been better than with placebo (Table 3).

Discussion

Normal bladder function is the organ's competence to hold a sufficient volume of urine, a painless urge to void and finally almost complete emptying. This ability, which is impaired in patients with IC, is based on a mechanism of activation and maintenance of reflex mechanism involving parasympathetic, sympathetic and somatic control of the lower urinary tract (16 ,17).

It was observed a significant change in the symptoms that accompany interstitial cystitis, prior and subsequent to treatment with Cystex®. It has also been noted a good tolerance of the treatment. It has been demonstrated that every single symptom was improved after 3 weeks of treatment by means of an analogue visual scale (18). The clinical and urodynamic benefit that we found may be explained by the property of the product composition with urinary antiseptics and parasympathetic drugs. Briefly, the patients with typical symptoms and urodynamic parameters of painful bladder syndrome associated with interstitial cystitis have been benefited from treatment with Cystex®. Likewise, we found evi-

Table 2. Changes in symptoms from baseline and within 21 days

Characteristic	Cystex	Placebo	P value
Mean score-sum $\pm$ SD	- 7.9 $\pm$ 7.2	- 2.7 $\pm$ 5.8	< 0.05
Mean pain intensity $\pm$ SD (mm on VAS)	- 25.2 $\pm$ 23.3	2.4 $\pm$ 13.9	<0.05
Mean urgency intensity $\pm$ SD (mm on VAS)	- 47.8 $\pm$ 21.5	1.4 $\pm$ 3.2	<0.05
Mean 24-hr frequency $\pm$ SD	- 5.8 $\pm$ 6.1	1.1 $\pm$ 5.4	<0.05
Mean functional bladder volume $\pm$ SD (ml)	27.8 $\pm$ 49.3	17.8$\pm$ 47.6	>0.05

Table 3. Mean ratings in each of the 10 items of the questionnaire in groups receiving Cystex® capsules or placebo at visit 0 (baseline) and at visit 1 (after 21 days from visit 1)

	Cystex®		Placebo	
Item	Baseline	Visit 1	Baseline	Visit 1
A	1.64	4.88*	1.59	1.92
B	1.58	4.73*	1.63	1.68
C	1.88	4.26*	1.90	2.01
D	2.07	4.32*	2.01	2.22
E	1.87	4.48*	1.86	2.05
F	1.96	5.16*	1.87	2.11
G	1.69	3.86*	1.93	1.96
H	1.34	3.95*	1.59	1.74
I	1.48	3.68*	1.63	1.99
J	1.78	5.42*	1.72	2.01

*Compared to baseline. *P < 0.05.*

dence that the said improvement is noticeable both in subjective parameters of the disease and in the objective parameters of urodynamics. The clinical use of intravesical hyaluronic acid in patients with painful bladder syndrome possibly associated with interstitial cystitis has demonstrated that the clinical improvement was related to a bladder's capacity and sensitivity increase (19).

In our study, Cystex® enhanced quality of life over the placebo showing a statistically significant improvement of any among ten items comprised in the assessment questionnaire. Therefore, it can be concluded that the composition of Cystex®, increased the quality of life in treated patients.

No weight gain and increased physical activity were also observed in treated patients. The present study has shown that the daily use of Cystex®, taking two capsules daily three times a day away from meals, has been effective in improving quality of life in patients suffering from interstitial cystitis. Moreover, its use has shown an undesired effects incidence similar to placebo among individuals in the groups. This trial have shown that the efficacy and safety of therapy with Cystex® in the treatment of interstitial cystitis and is an alternative for patients suffering from this pathology.

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Evaluation of Neurogenic Appendicopathy in negative appendectomy in pediatric patients

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Abstract

Introduction: Neurogenic appendicopathy (NA) still represents a not well known pathological entity. Clinically, NA couldn't yet be differentiated from acute appendicitis. It is characterized by nerve proliferation and an increased number of endocrine cells in the appendix.

AIM: The goal of study was to establish the presence of neurogenic appendicopathy in the negative appendectomy in pediatric patients, to evaluate a correlation of anamnestic, clinical, psychological, laboratory and ultrasound diagnostic data to the existence of neurogenic appendicopathy. Testing the validity of standard hematoxylin-eosin (HE) staining and immunohistochemical methods with S-100 protein antibodies in pathohystological verification was performed. The ultimate goal of research was an attempt to confirm the presence of this entity, which would be part of the negative appendectomy done therapeutically justified.

Material and methods: A case-control study included 209 consecutive patients, aged 4-19 years, underwent appendectomy for clinical sindication of acute appendicitis. All patients were examined clinically, laboratory and by ultrasound. In patients with negative appendectomy was carried out histological examination of hematoxylin-eosin staining and immunohistochemical staining with S-100 protein antibodies, to verify the presence of neurogenic appendicopathy. Data obtained from anamnesis, clinical and ultrasound examination, psychological evaluation and postoperative follow-up were analyzed and correlation with the presence of neurogenic appendicopathy is made.

Results: Negative appendectomy rate was 23.92%, neurogenic appendicopathy was confirmed in 19 patients (9.09%) clinicalally presented as acute appendicitis and in 19 patients (38%) with negative appendectomy. The most important clinical signs for diagnosis of neurogenic appendicopathy were antalgic position of patients, ileocecal pain and presence of previous similar problems. The most important ultrasound criterias in the diagnosis of neurogenic appendicopathy were low US grade (I or II), thickness of appendiceal wall < 2mm, without the presence of echogenic and anehogenic content. Hematoxylin-eosin staining confirmed the neurogenic appendicopathy in 18 cases, while immunohistochemical staining with S-100 protein antibodies confirmed the neurogenic appendicopathy in 19 patients. Neurogenic apendicopathy is a histopathological entity that can be detected by standard HE staining with an accuracy of 94.74% in the negative appendectomy group. Immunohistochemical staining with S-100 protein antibodies increases the safety of diagnosis. Histological and clinical confirmation of the existence of neurogenic appendicopathy in negative appendectomy group requires revision of indication for appendectomy in pediatric patients.

Conclusion: Neurogenic appendicopathy, as the clinical and histological entity, is the indication for appendectomy in pediatric patients. On this way the part of negative appendectomy declared surgically and therapeutically justified, thus the negative appendectomy rate in the present study was 14.83%.

Key words: Neurogenic appendicopathy; Appendicitis; Diagnosis, Negative appendectomy

Introduction

Acute appendicitis is the most common surgical condition in pediatric patients [1,2]. Despite the use of modern diagnostic algorithm, acute appendicitis can't be proven histologically in 10 to 40% [3,4,5]. The rate of "normal" histological findings, identifies as negative apendectomy, have an incidence in different series from 10 to 30% [6,7,8,9,10]. Discrepancy between the clini-

cal presentation and the absence of morphological changes of appendix is still a dilemma in pediatric surgery. Results of many studies suggest that neurogenic appendicopathy could be a possible reason for the patient's complaints [11].

Neurogenic appendicopathy is a condition of neurogenic hyperplasia, nerve proliferation and increased number of endocrine cells in the appendiceal wall. Histologically it is present as increasing the number of sympathetic nerve fibers, schwannoma ganglia cells, thickening of the submucosa and outer muscle layer and occurs in 40% "normal" appendix [12]. The first description of neurogenic appendicopathy gave Maresch (Austria) and Masson (France), who 90 years ago noted specific neuromatous histological lesions of the appendix without signs of acute inflammation [13]. They were the first to recognize the close relationship of nerves proliferations and clinical signs of acute appendicitis [14,15]. Etiopathogenesis of neurogenic appendicopathy still remains controversial. Recent studies have confirmed that the immune and enteric nervous system regulate physiological processes in the gastrointestinal system, including the epithel and smooth muscles. Inflammatory response could damage the proper function of the local endocrine cells leading to hyperplasia of enteric nervous system components. Expression of neuropeptides is changed in chronic inflammation of the gastrointestinal sysetem. Studies in animal models indicate a possible role of neuroimmune reactions in chronic and painful inflammation. Inflammatory response includes local endocrine cells and neuroproliferation and can cause repeated attacks of acute pain [16].

Objective

The aim of study was to confirm the presence of neurogenic appendicopathy in patients with negative appendectomy, to check the validity of hematoxylin-eosin staining and immunohistochemical methods with S-100 protein antibodies in diagnosis of neurogenic apendicopathy, to correlate the anamnestic, clinical, psychological, laboratory and ultrasound data with presence of neurogenic appendicopathy. The ultimate goal of research is to confirm the neurogenic appendicopathy as a clinical and histological entity.

Material and methods

A case-control study included 209 consecutive patients, aged 4 to 19 years, underwent appendectomy for suspected acute appendicitis. All patients were examined clinically, with laboratory and ultrasound tests performed. The study was designed and conducted at tertiary care Children hospital.

Experimental group consist 50 patients with clinical suspicion of acute appendicitis underwent appendectomy in whom macroscopic and histopathological findings didn't confirm the inflammation. Thus, the negative appendectomy is defined as an independent variable of research, and then conducted further histological examination using hematoxylin-eosin (HE) staining and immunohistochemical methods to S-100 protein antibodies to confirm the presence of neurogenic appendicopathy. In the group of patients with negative appendectomy mark off two subgroups of patients: with neurogenic apendicopathy (NA) and without neurogenic appendicopathy(NNA).

Control group consists of 159 surgically treated patients in whom acute appendicitis was confirmed histopathologicallly (AA). Data obtained by medical history, physical examination, psychological evaluation and results of diagnostic procedures were analyzed and the correlation between the presence of neurogenic apendicopathy, clinical findings and diagnostic results has made.

The results of a diagnostic algorithm carried out according the standard surgical diagnostic protocol for appendiceal disease were analyzed. Primary variables of interest were divided into categories of demographic data (sex, age, body weight), anamnestic data –abdominal pain duration and localisation (RLQ, periumbilical, epigastrium, diffuse), previous similar symptoms, loss of appetite, nausea, vomiting, diarrhea, urinary discomfort, change in stool, fever. Clinical variables- - antalgic position, coated tongue, palpation tenderness, guarding RLQ, muscles defance. Laboratory analysis included the number of white blood cells (WBC). The ultrasound examinations were performed with Siemens sonoline sienna ultrasound color Doppler with 7.5MHz linear transducer. We previously described a new ultrasound signs of acute appendicitis in pediatric patients [17]. Ultrasound grade emerged as new criteria in diagnosis of acu-

te appendicitis. Attempts to correlate ultrasound and histological findings are reported [18]. We analyzed the prognostic value of each clinical, laboratory and ultrasound parameters in relation to the further clinical course.

Data obtained from the preoperative psychological assessment of patients and their parents were correlated with the presence of neurogenic appendicopathy.

Histopathological review of appendix included:

1. Hematoxylin-eosin (HE) staining. Minimum diagnostic criteria for acute appendicitis is the initial focal mucosal erosion with polymorphonuclear infiltrate, followed by submucosal and transmural inflammation. Neurogenic appendicopathy was confirmed by hematoxylin-eosin staining if:
   - focal collection of pale spindle cells in the lamina propria mucosae is present (mucosal type NA);
   - focal collections of cells in a pale shaft obliterated appendix (axial neuroma type NA).
2. Immunohistochemical staining for S-100 protein antibodies to present perineural schwannoma cells thus confirming the neurogenic nature of the lesion[19].

Standard paraffin clips are stained and deparaffinizared with anti-serum S-100 peroxidase - antiperoxidase technique described by Sternberger before.

Clinical course and length of hospital stay, wound healing, the concomitant pathology and reoperation are observed. Follow up was conducted for 3 months after discharge, and included the presence of pain in the scar in the right lower quadrant of the abdomen, digestive symptoms and re-operation.

Statistical analysis

The data were statistically analyzed using the software package Statistica for Windows ver. 9.1 (StatSoft, 2010) [20]. The data of comparative studies examined groups were analyzed by methods of multivariate statistics. For analysis the data is encoded first, and for characters with more information formed the ranks (age, weight, white blood cell count). The correspondent canonical analysis was used in order to present the impact of the condition, anamnestic, clinical, laboratory and ultrasound parameters to define the difference between the studied groups as well as the mutual influence of the subgroups. The collected data were grouped and presented in tables and graphs. Statistical analysis was performed at the Department of Biology and Ecology, Faculty of Natural Sciences, University of Novi Sad.

Results

The demographic structure of the sample of patients

The study included 209 patients aged 4 - 19 years underwent appendectomy. Distribution of patient groups is shown in **Table 1** and **Figure 1**. Acute appendicitis is intraoperative and histologically confirmed in 159 patients (76%). The rate of normal histological findings - negative appendectomy, initially was confirmed intraoperatively in 50 (24%) patients. After further testing using hematoxylin-eosin staining and imunohistochemical with S-100 protein antibodies neurogenic appendicopathy was confirmed in 19 patients (9%), while the appendix was without pathological changes in 31 patients (15%). Looking at the distribution within the group of negative appendectomy, neurogenic appendicopathy is present in 38% of macroscopically "normal" appendix.

Table 1. Distribution of patients

Experimental group	Neurogenic appendicopathy (NA)- 19 No neurogenic appendicopathy (NNA) - 31
Control group	Acute appendicitis (AA) – 159
Total	**209**

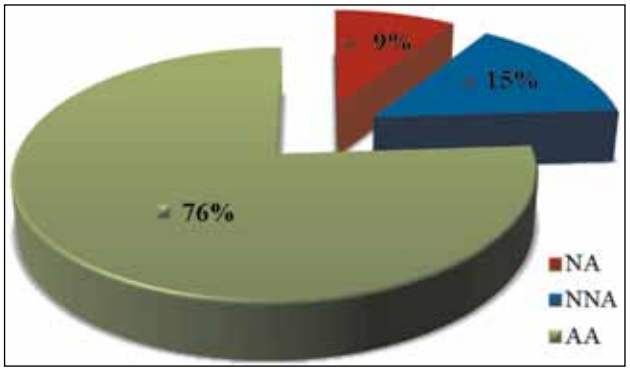


Figure 1. Distribution of analysed groups

Sex ratio of the sample was 120 girls (57.42%) and 89 boys (42.58%). The average age of operated patients was 11.52 years (12.42, 12.73 NNA, AA 11.19 years). Acute appendicitis is the most common at the age of 12 to 15 years, while the neurogenic appendicopathy usually occurs in at the age of 16 to 19 years **(Figure 2)**. Correspondent canonical analysis is used to correlate demographic characteristics (sex and age) in the examined groups. NA group is determined by age gradient of the elderly population (16-19 years), mostly female. The NNA subgroup is isolated and there is no effect of connecting with age and sex. **(Figure 3).**

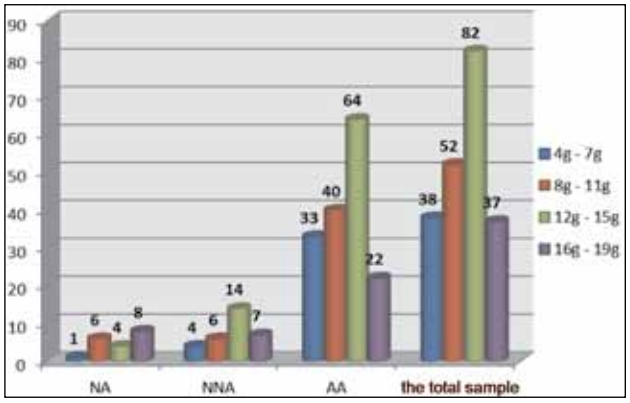


Figure 2. Age distribution of patients

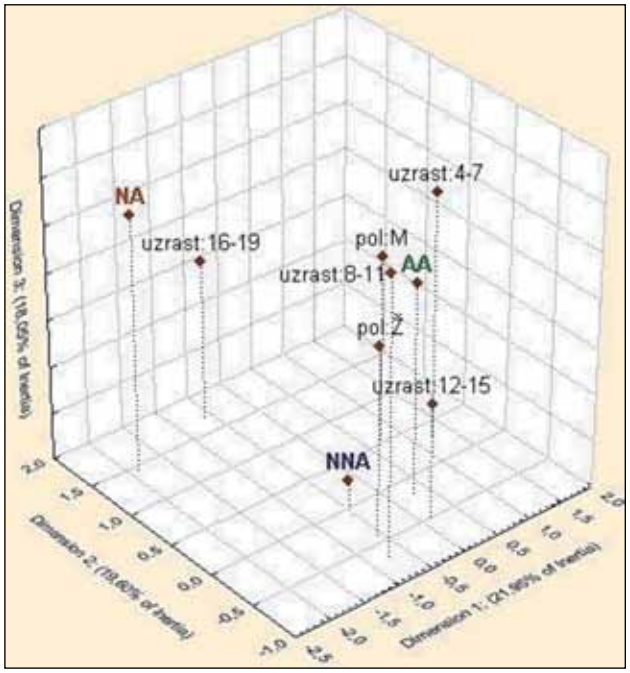


Figure 3. Demographics centroid positions of the analyzed group of patients

Clinical data includes eight symptoms showed a different frequency and diagnostic value. **Table 2** is clearly apparent low value of most of the anamnestic data. The effect of psychological evaluation does not show a clear determination to the appropriate study group. The psychological assessment unobtrusive is feature of the patients who effectively cooperate during diagnostic and anamnestic procedures, while striking is a psychological profile of the characteristics of patients who are not willing to cooperate and oriented towards the NNA group. Girls aged 16-19 years and 8-11 years were mostly psychologically with the striking and absence of cooperation.

Table 2. Potential diagnostic significance of the analyzed parameters

Symptom	Sensitivity	Negative predictive value
abdominal pain	0.893	0.620
pain migration	0.932	0.522
previous similar problems	0.737	0.500
loss of appetite	0.936	0.565
nausea	0.918	0.500
vomiting	0.939	0.500
disuria	0.903	0.500
fever	0.900	0.450

Results of clinical signs in all groups are shown in **Table 3**. Laboratory tests included theWBC count and have significant value in the diagnosis of acute appendicitis **(Figure 4).** Ileocoecal pain as the central character in groups NA and AA from NNA group separation. It has a set of properties, among them the muscle defance. Diffuse muscle defance could indicate the presence of acute appendicitis, and it's absence is uncertain nature of the intermediary position depending on the load level corresponding axis**.** Neurogenic appendicopathy (NA) was highly correlated with the presence of previous similar symptoms, which included the correspondent system with clinical parameters. The character of the previous problems successfully separated on the NNA group is reflected in a central position in relation to the second correspondence axis. Patients with neurogenic appendicopathy (NA group) determines the set of characteristics: the antalgic position, ileocoecal pain, without coated tongue, and low or normal WBC, and previous similar problems. Characteristics of NNA groups: antalgic position absence, eleva-

ted WBC and absence of ileocoecal pain which exclude acute inflammation of the appendix. The control group (AA group) defines: antalgic position-coated tongue-abdominal pain with percussion - moderately elevated WBC (Class 3-5) ileocoecal or periumbilical pain.

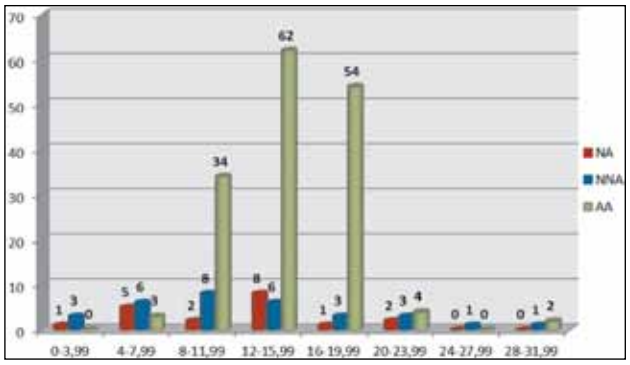


Figure 4. WBS count distribution in group of patients

Results of ultrasound examination

Ultrasound examination of the abdomen and the appendix has a high diagnostic value in diagnosis of appendiceal diseases in pediatric patients. The potential diagnostic significance of individual elements using ultrasonographic examination is given in **Table 4.** Advanced ultrasound grade (III and IV) was absolutely dominant feature for the acute appendicitis diagnosis. Especially important factors are the diameter of the appendix> 7 mm, wall thickness > 2.2mm. Most of the features correlate with the AA group and advanced US degrees. The NA group is characterized by: US grade I or II - appendix wall thickness <2.2 mm - without hiperechogenic or anechogenic content-appendix peristalts preserved. Characters related to the AA group: advanced US grade III or IV - appendix wall thickness> 2.2 mm, hiperechogenic and anechogenic content presence - appendix peristalts damaged- a positive test of compressibility. Set of common characteristics for groups of AA and NA are absence of free fluids - with or without the presence appendicolith. Characters related to the NNA group: US grade I - appendix diameter <7 mm - wall thickness <2.2 mm - negative compressibility test-anteromedial appendix position.

The results of operative treatment and histological examination

The most frequent position of the appendix founfd intraoperatively are medial, lateral and re-

Table 3. Representation of the clinical examination elements

Clinical signs	NA		NNA		AA	
	No.	%	No.	%	No.	%
antalgic position	18	94.74	22	70.97	132	83.02
coated tongue	9	47.37	13	41.94	119	74.84
muscle defance	6	31.58	11	35.48	104	65.41
ileocoecal pain	17	89.47	25	80.65	149	93.71

Table 4. Diagnostic significance of US examination

US criteria	Sensitivity	Negative predictive value
positive test compressible	0.927	0.520
outer diameter of appendix >7 mm	0.949	0.600
appendiceal wall thickness >2mm	0.953	0.600
hyperechogenic and anechogenic contents around the appendix	0.935	0.636
appendicolith	0.926	0.600
aperistalts	0.842	0.500
fluid-filled abscess	1.000	0.000
free fluid noted in the RLQ of the abdomen	0.879	0.533
ultrasound grade		
grade I	0.750	0.826
grade II	0.796	0.500
grade III	0.926	0.200
grade IIV	1.000	0.000

trocoecal. Concomitant pathology was verified in 46 patients. The most common mesenterial lymph node - at 28 (-5 NA, NNA-10, AA -13), ovarian pathology - in 9 (NA 3, NNA 3, AA 3), adhesions in six (NA 3, NNA 3), and Meckel diverticulum in three patients. Appendicitis grade was determined macroscopically during the operation, and the diagnosis was confirmed by histological examination. **Table 5**

Table 5. Patohistological results

PH analysis	No	%
Negative appendectomy	50	23,92
Appendicitis catarrhalis	18	8,61
Appendicitis phlegmonosa	52	24,88
Appendicitis gangraenosa	43	20,57
Appendicitis perforativa	39	18,66
Abscessus periappendicularis	7	3,34
Total	209	100

In surgical patients with negative appendectomy, further histological examination was performed of the direction of proving the presence of neurogenic appendicopathy using HE methods and immunohistochemical methods with S-100 protein antibodies. Hematoxylin-eosin staining confirmed the presence of neurogenic appendicopathy in 18 patients. Immunohistochemical staining to S-100 protein confirmed the presence of neurogenic appendicopathy in 19 patients. Success rate of HE staining in the group was 94.74% **(Figure 5)**. Hematoxylin-eosin staining is observed emphatically rolled muscle wall of the appendix with lymphocite infiltration. In the lamina propria mucosae hyperplastic lymph follicles are present with an increased germinative centers between which nerve fibers are altered by the type of degenerative neurinoma. Superficial appendicitis typical cylindrical epithelium **.** There is a marked hyperplasia of nerve fibers in the center of obliterited appendix**,** as well as in the lamina propria mucosae **(Picture 1).** Cross-section of the appendix stained with antibodies to S-100 protein revealed that the nerve bundles and neurons are arranged between the circular and longitudinal muscle layers as well as inside it. There is a clear layer of muscle interposition between the ganglia, which proves that the ganglia are located at different levels of muscle layer **(Picture 2).**

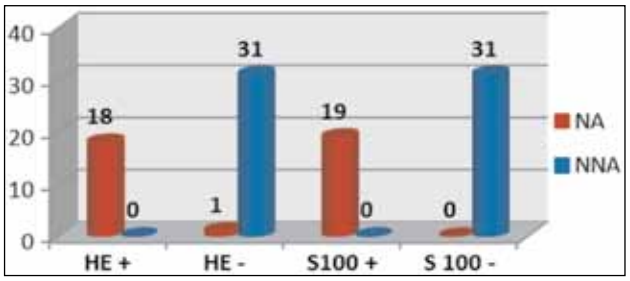
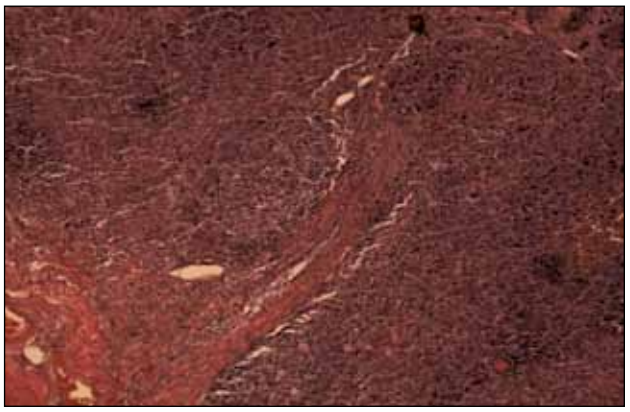
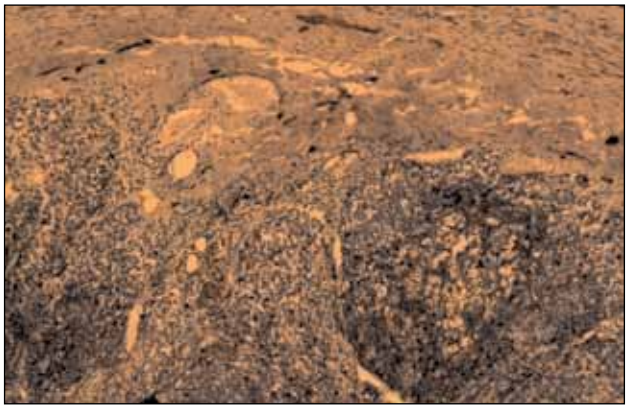


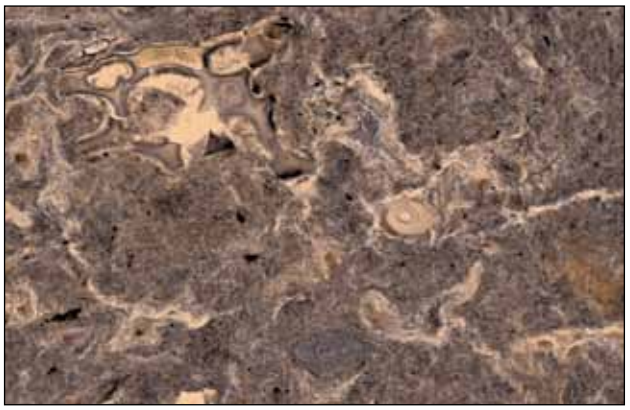
Figure 5. Patohistological results of HE staining and immunohistochemical methods with antibodies to S-100 protein



Picture 1. Hematoxylin-eosin staining



Picture 2. Immunochistochemical staining with S-100 protein antibodies



Picture 3. Immunochistochemical staining with S-100 protein antibodies

Confirmation that the HE staining is suficient for evidence of neurogenic apendicopathy illustrates the introduction of additional variables in the correspondent area (US wall thickness and diameter of the appendix). Inflammatory process changed the morphology, accompanied by increased lumen diameter and wall thickness of the appendix. This feature is not related to neurogenic appendicopathy distance, which proves the value of increased diameter and wall thickness of the appendix to the groups. Thus it was confirmed that it is sufficient to use only one of the techniques of examination to prove neurogenic appendicopathy because both are directly related to the group.

The clinical course results

The operative wound healing, length of hospitalization and correlation with associated pathology were followed postoperatively. There is a predominance of primary healing in all tested groups (94.7%, NNA 90.32%, 91.82% AA). Length of hospitalization for patients to groups was short. Girls are definitely the main carrier of the NA and NNA groups, followed by juvenile and adolescent ovarian pathology. Adhesions are more common in patients on groups, while more common is mesenterial lymph node in NNA group. Body weight influences directly on the wound healing process, while the older age in girls with the elements directly linked ovarian pathology, most often in combination with the previous problems. This fact is supported by an increased incidence of ovarian juvenile physiological and pathological conditions in the study group patients.

Patients were followed up during the three months postoperatively. Particularly are noted the presence of pain in the right lower abdominal quadrant, pain in the scar, the presence of digestive disorders and reoperation. Summary of the above parameters is shown in **Table 6**. Increased associate is observed: the appearance of digestive complaints - weight of 30 to 50 kg at the age 8 - 11 years, the need for reoperation in young children with body weight to 30 kg, pain in the scar and the lower right quadrant more frequently in boys, which is potentially associated with previous traumas**.** We analyzed postoperative parameters of patients' behavior in correlation with previous health problems and psychological assessment. Patients with neurogenic appendicopathy (NA group) have a set of characteristics: psychologically unobtrusive, less conspicuous patient - female - present previous symptoms - without pain in the scar - no digestive disorders - age 16-19 years. Patients with negative appendectomy (NNA group): the patient psychologically striking - half female, less male - present previous symptoms - with pain in the scar - present digestive disorders - age 12-15 years. Position AA group corresponds with a number of characters but not sexually classified - younger patients with no previous symptoms, psychological inconspicuous, rarely with postoperative problems.

Discussion

The clinical presentation of acute appendicitis in children is often different from the usual description and varies in a wide range from minimally discomfort to the intestinal obstruction and septic shock. Respecting the risk of complications from delayed diagnosis and treatment, early surgical exploration inevitably leads to a rise of negative, unnecessary appendectomy. It is undisputed that the surgical treatment relieves the symptoms of patients despite "negative" standard histological findings. Since appendectomy deprives the patient of pain, increased interest of researchers for the role of local endocrine cells of the appendix as a part of diffuse endocrine system and the neuroproliferation in the development of recurrent pain attacks [11].

Masson and Maresch described the group of diseases named "neuroappendicopathy" [14,15]. The original name of "neurogenic appendicitis" [15] was criticized by a Knoflach [21], who stated that an inflammatory process is not a part of this histological entity. Chiari introduced the term "neurogenic ap-

Table 6. Postoperative follow-up data

Symptom	NA		NNA		AA	
	No.	%	No.	%	No.	%
pain in the scar	2	10.52	5	16.12	18	11.32
RLQ pain	1	5.26	3	9.67	4	2.51
digestive disorders	1	5.26	4	12.9	9	5.66
reoperation	0	0.00	2	6.45	6	3.77

pendicopathy", which has accepted in the literature[22]. Nemeth, Reen and Puri confirm a elevated expression of inflammatory mediators as indicators of the presence of inflammatory response in patients with neurogenic appendicopathy[23]. Singh studied the role of eosinophils, mast cells and neural hyperplasia in acute appendicitis. He found that the number of eosinophils and mast cells significantly increased in patients presented with clinical manifestations of acute appendicitis, due to normal histological findings of appendix and assumed that the pain in these patients caused by mediators released from degranulated mast cells. Number of mast cells, nerve fibers and ganglion cells in the submucosa is significantly increased in acute appendicitis [24]. The term "neuroimmune appendicitis" suggested Di Sebastiano showing that abnormal interactions between cells of the immune system and the intramural nerve fibers plays a key role in the pathogenesis of neurogenic appendicopathy. [25] on the subject of discussion on the disease entity that is separate from the inflammatory changes. Di Sebastiano and Buchler have confirmed that 15 to 25% for appendicitis in patients operated for suspected acute appendicitis on histological examination seems "normal", while the cause of pain in the right lower abdominal quadrant is unclear. The assumption of the modified contents of neuropeptides in chronic inflammation has led the authors to examine the possible change in peptidergic innervation of substance P (SP), vasoactive intestinal peptide (VIP) and growth associated protein 43 (GAP-43) using immunohistochemical methods. Neuroproliferation in theappendiceal wall in a state with elevated levels of SP and VIP may be involved in the pathophysiological mechanism of acute pain in the right lower quadrant in situations where is no histological confirmation of acute appendicitis. The same authors distinguish neuroimmune appendicitis as a separate pathological entity[25]. Coskun examined the relationship between the enteric nervous system and mast cells in normal and pathological appendiceal conditions [26]. Xiong and Puri investigated the neuronal hypertrophy and presence of mast cells in histologically verified acute appendicitis, as well as histological findings neat presented clinically as acute appendicitis. They found that increasing the number of nerve fibers, schwanoma cells and increased number of ganglia in the submucosa and in the outer muscle layer at 40% "normal" appendix. The number and size of ganglia, the number of nerve fibers, schwanoma and mast cells in each histological compartment was determined quantitatively. Granulocite infiltration laminae muscularis propriae a key moment in the development of acute appendicitis [27]. Guller described neurogenic appendicopathy as a condition that is clinically indistinguishable from acute appendicitis. Diagnosis is made histologically, with the proliferation of nerve and increase the number of endocrine cells in the submucosa of the appendix which is significant for this entity. In the preceding study neurogenic appendicopathy was present in 17.1% of negative appendectomy [28]. Several authors explain the presence of the disease form of chronic appendicitis. Despite repeated description and development of diagnostic methods, such as ommunohistochemistry, all attempts to establishe neurogenic appendicopathy (NA) as a new clinical entity has so far failed [13,29,30,31].

The study derived the rate of negative appendectomy was 23.92%, while the neurogenic appendicopathy was confirmed in 19 patients (38%) with negative appendectomy. Neurogenic appendicopathy occurs in the whole sample in 19 patients (9.9%) where the highest incidence in the oldest study group of 16 to 19 years where the same was confirmed in eight patients. The group is dominated by negative appendectomy girls aged 16-19 years, increased body weight (over 50 kg). This can be explained by increased frequency of symptoms in the right lower quadrant of the abdomen in girls due to juvenile gynecological physiological and pathological conditions. Increased body weight followed by thickening of the subcutaneous fat and set conditions for the difficult clinical ultrasound examination [32].

The study confirmed that more clinical and ultrasound criteria have to be used for the timely detection of patients with neurogenic appendicopathy. The most important clinical parameters in the diagnosis of neurogenic appendicopathy are antalgic position of patients with a pronounced ileocoecal pain, the absence of coated tongue and muscle defance, and low the normal WBC, with anamestic data of previous similar problems. The most important ultrasound parameters in the diagnosis of neurogenic appendicopathy are US grade 1 or 2, the wall thickness <2.2 mm, and the absence of hiperechogenic and anechogenic content while appendic peristalts is preserved. Hematoxylin-eosin staining confirmed

the presence of neurogenic appendicopathy in 18 patients. Immunohistochemical staining with S-100 protein antibodies confirmed the presence of neurogenic appendicopathy in 19 patients. Neurogenic appendicopathy a histological entity that can be detected by standard HE staining with an accuracy of 94.74% in the negative appendectomy. Application imunohistohemijskog staining with S-100 protein increases the security of diagnosis, but it is not necessary for its definitive set.

Franke preformed the first prospective, multicenter clinical study that examined differences between acute appendicitis, neurogenic appendicopathy and negative appendectomy. In some aspects of the history and clinical findings were observed significant differences in these three groups, but none was strong enough to clearly distinguish these conditions. Franke attempted to define the clinical signs that can extract preoperative neurogenic appendicopathy as a separate clinical entity and the validity of HE staining of histological confirmation of neurogenic apendikopatije [33,34]. In a previous multicenter study, the same author confirmed that the neurogenic appendicopathy is histological entity. Analysis of its presence in children and adolescents using the HE and / or imunohistochemical staining with antibody for S-100 protein, confirmed the neurogenic appendicopathy in only 4.8% in patients younger than 14 years, while in adolescents it is present in 24, 2% of patients. In particular, analyzing the group of negative appendectomy rate of this entity was 16.7% in the group to 14 years, while patients over 14 years the incidence of 56.6%. The result of these studies is that the neurogenic appendicopathy is very rare in children with the basis of anamnesis and clinical examination is not possible preoperatively to identify patients with this entity [28,35,36,37,38].

In our study for the evaluation of neurogenic appendicopathy patients were followed up postoperatively for three months. In doing so, are particularly noted the presence of pain in the right lower quadrant abdominal pain, in the scar, the presence of digestive disorders and reoperation. A comparative analysis of these parameters revealed the presence of pain in the scar at 10.52% in patients with neurogenic appendicopathy, 16.12% in the negative appendectomy and in 11.32% of patients with acute appendicitis. Right lower quadrant pain was present postoperatively in 5,26% in patients with neurogenic appendicopathy, 9,67% in the negative appendectomy and 2,51% in patients with acute appendicitis. Digestive disorders were present postoperatively in one (5,26%) patients with neurogenic appendicopathy, 12,9% in the negative appendectomy and 5,66% in patients with acute appendicitis. There were no re-operation in the group of patients with neurogenic appendicopathy, while in the negative appendectomy group was in two patients and acute appendicitis in six patients because of the development of intestinal obstruction.

Conclusion

This study confirmed the neurogenic appendicopathy is a clinical and histological entity and an indication for the apendectomy in pediatric patients. Histological and clinical confirmation of neurogenic appendicopathy requires revision of negative appendectomy and widening of indications for apendectomy. On that way, part of the negative appendectomy surgery declared justified, thus the proportion of negative appendectomy in the derived study is 14.83%. Confirming the presence of neurogenic appendicopathy, part of the of the negative appendectomy are surgical and therapeutically justified.

Abbreviations

NA - neurogenic appendicopathy
NNA - without neurogenic appendicopathy
AA - acute appendicitis
WBC - white blood cell
US – ultrasound
RLQ- right low quadrant
HE- hematoxylin-eosin

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Effect of Hemodynamic Changes During Percutaneous Mitral Balloon Valvuloplasty on Short and Long Term Clinical Outcome

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Abstract

Aims: We aim of this study is to determine whether hemodynamic changes that occur before and after percutaneous mitral balloon valvuloplasty (PMV) procedure is a predictor in determining the short- and long-term clinical results in patients with mitral stenosis (MS).

Methods: The study population consisted of 118 (26 males and 92 females) consecutive patients with symptomatic rheumatic MS, underwent successful PMV. Mean pulmonary artery pressure, pulmonary capillary wedge pressure (PCWP), mean transmitral gradient and mean left atrial was obtained before and immediately after valvuloplasty. Two separate groups were formed on the basis of these measured pressure differences. The first group pressure differences (PD) ≤ 15 mmHg and the second group PD > 15 mmHg.

R**esults:** There was no difference in immediate outcomes between two group. There was no significant difference in between groups in terms of primary endpoints. However, there was a difference in mean left atrial pressure difference (LAPD) between ≤ 15 mmHg group and > 15mmHg group. MVR and redo PMV rates were significantly lower in LAPD> 15mmHg group (p=0.027 and p=0.03, respectively), the difference between stroke and death rates was not statistically significant (p=0.6).

Conclusion: The significant risk factors in determining long-term outcomes in patients undergoing PMV are; mitral valve morphology, atrial fibrillation, advanced age, NYHA class, post-PMV, MR grade, post-PMV MVA, prior surgical commissurotomy, post-PMV pulmonary artery pressure and echocardiographic score. In addition to these parameters we found that LAPD could also be a predictor of long term outcomes patients undergoing PMV.

Key words: Mitral stenosis; Valvuloplasty; Left atrial pressure

Introduction

Mitral stenosis (MS), a valvular heart disease that develops as a late sequela of rheumatic fever, is still an important problem in developing countries, but it is increasingly being identified in an degenerative form in developed countries. In developing countries, patients tend to be young with a pliable valve, whereas in developed countries patients are of increased age with several comorbidities[1-4].

Percutaneous mitral balloon valvuloplasty (PMV) has been established as an alternative to surgical mitral commissurotomy in the treatment of most patients with symptomatic rheumatic MS. In 1982, Kanji Inoue, a Japanese cardiac surgeon, first developed the technique that has become the mainstay of treatment for MS[5]. Current guidelines of the European and American Cardiac Societies recommend PMV as the first-line therapy in patients with isolated mitral stenosis with a feasible valve morphology[6,7].

The aim of this study is to determine whether hemodynamic changes that occur before and after PMV procedure is a predictor in determining the short- and long-term clinical results in patients with mitral stenosis.

Methods

From 2001 to 2010, a total of 118 patients with rheumatic MS (26 male/92 female) were included in the study. Exclusion criteria were the presence

of significant mitral, tricuspid, aortic regurgitation, aortic stenosis, left ventricular dysfunction, balloon valvotomy, renal or hepatic failure, and coronary or pulmonary disease. None of the patients had left atrial thrombus or unfavorable valve morphology for PMV on transesophageal echocardiography.

Functional capacity was assessed on the basis of the New York Heart Association (NYHA) classification. Postprocedural evaluation of functional capacity was conducted, six month after PMV and last visit. The study was approved by the Local Ethics Committee and written informed consent was obtained from all patients included in this study.

Echocardiographic analysis

Comprehensive 2-dimensional and color Doppler echocardiographic evaluation was performed in all patients on the day before and within 24 h after PMV. All echocardiographic examinations were performed with an echocardiography machine (HDI-5000; ATL Bothell, Washington) with a 3.0-mhz transducer.

The morphological features of the mitral valve which were calcification, leaflet mobility, thickness, subvalvular structure and mitral regurgitation were categorized as described previously. Mitral valve area (MVA) was measured by direct planimetry at the parasternal short-axis view, and pulmonary artery systolic pressure was estimated by continuous wave

Doppler echocardiography using the modified Bernoulli method, with 10 mmHg added for the estimated right atrial pressure[8], mitral gradient and the peak pressure gradient of tricuspid regurgitation calculated by the continuous wave Doppler technique. Within 24 hours of PMV, again the same measurements were taken especially for mitral regurgitation and interatrial left to right shunt.

Transesophageal Echocardiography evaluation was performed in all patients; mitral valve, mitral regurgitation, left atrium and left atrial appendage just before PMV.

Technique of PMV

After right and left cardiac catheterization, all PMV procedures were performed by an antegrade transvenous approach using the Inoue balloon system. Balloon size was selected according to body surface area (26 mm if 1.5 m2, 28 mm if 1.5 to 1.7 m2, and 30 mm if 1.7 m2), modulated by anatomy (1 to 2 mm smaller in unfavorable cases). Left ventriculography was performed before and after the balloon inflation.

Hemodynamic Analysis

Right and left pressure measurements including mean pulmonary artery pressure, pulmonary capillary wedge pressure (PCWP), mean transmitral gradient and maximum transmitral gradient, mean left atrial and left ventricular pressures as well as the degree of mitral regurgitation (MR) was obtained before and immediately after valvuloplasty. Then the difference between the measurements before and after the procedure was taken. Two separate groups were formed on the basis of these measured pressure differences. The first group was those whose pulmonary artery mean pressure, PCWP, mean transmitral gradient, mean left atrial pressure differences were $\leq$ 15 mmHg and the second group was those whose pressure differences (PD) > 15 mmHg. MR was assessed during left ventriculography using the Sellers classification[9].

Clinical follow-up

In-hospital adverse clinical events, including pericardial tamponade, papillary muscle rupture, thromboembolic events, mitral valve surgery, complete heart block and death were collected prospectively. Follow-up times were first month and sixth month after PMV and then annually. Primary endpoints of follow-ups were all cause mortality, mitral valve replacement (MVR), redo PMV, stroke, change of patient's New York Heart Association (NYHA) functional class and the presence of echocardiographic restenosis (MVA< 1.5 cm2).

Statistical analysis

Statistical analysis was performed using SPSS for Windows, version 16.0 software (SPSS, Chicago, IL). All continuous variables were expressed as mean±SD and categorical data as percen-

tages. Student's *t* test and $\chi 2$ analysis were used to compare continuous and categorical variables, respectively. $P \leq 0.05$ was considered significant. Demographic, clinical, echocardiographic, procedural, and hemodynamic variables were tested. The variables included were age, sex, pre-PMV NYHA functional class, pre- and post-PMV MVA, pre-and post-PMV left atrial pressure, pre-and post-PMV PCWP pressure and pulmonary artery pressure (PAP).

Results

The study population was 118 patients (26 male and 92 female) with a mean age of 46±11,2 years. There were 70 (59,3 %) patients with pressure differences ≤15 mmHg and 48 (40,6 %) patients with pressure differences ≥15. Baseline characteristics and onset results are displayed in Table 1. Mean left atrial pressure, mean transmitral, mean pulmonary artery pressure and PCWP significantly reduced after PMV. Pre- and post-PMV basic haemodynamic parameters are summarized in Table 2.

Table 1. Clinical characteristics of the study population

	Total	LA PD≤15	LA PD>15	P
Patients	118	70(63.63)	48(40.67)	
Age (year)	46.7±11,2	46,7±12,1	46,7±9,1	0,99
Female	92(77,9)	60(50,8)	32(27,1)	0,5
Male	26(22,03)	20(16,9)	6(5,08)	0,37
Prior PMV	5(4,23)	3(2,54)	2(1,69)	NS
AF	16(13,5)	12(10,6)	4(3,38)	0,70
NYHA class				
I	0	0	0	NS
II	30(25.4)	18(12.2)	12(10.1)	0,04
III	82(69,4)	48(40.6)	34(28.8)	NS
IV	6(5.0)	4(3.3)	2(1.6)	
LA diameter(cm)	4,48±0,48	4,59±0,46	4,27±0,43	0,002
Prior commissurotomy	2(1,69)	1(0,84)	1(0,84)	NS
Echo score	8,03±1,58	8,19±1,62	7,88±1,50	0.9
Thickness	2,4±0,6	2,2±0,1	1,9±8,4	NS
Calcium	2,5±0,8	1,9±0,7	1,5±1,1	0,0001
Mobility	2,1±1,9	2,0±0,4	19,1±1,7	NS
Subvalvular	2,4±1,8	2,2±2,8	1,9±9,8	NS

LAPD; Left atrial pressure differences, PMV; Percutaneous mitral balloon valvuloplasty, AF; Atrial Fibrilation, NYHA; New York Heart Association

Table 2. Hemodynamic Variables Before and After Percutaneous mitral balloon valvuloplasty (PMV)

	before PMV	after PMV	P
Mean left atrial pressure (mm Hg)	26,5±8,8	12,4±6,1	< 0,0001
Pulmonary artery systolic pressure (mm Hg)	26,3±10,5	16,4±8,8	<0,0001
Mean pulmonary artery pressure (mm Hg)	26,7±3,6	21,4±5,3	<0,0001
Pulmonary capillary wedge pressure (mmHg)	27,4±8,0	13,5±4,5	< 0,0001
Mean transmitral gradient (mm Hg)	13±5,3	7,3±2,1	<0,0001
Maximal transmitral gradient (mm Hg)	17±2,1	9,3±3,7	<0,0001
Mitral regurgitation			
None	66(55,93)	25(21,18)	
Mild	43(36,44)	64 (54,23)	<0,0001
Moderate	9(7,62)	26 (22,03)	
Severe	0	3 (2,54)	

Immediate Outcomes

Patients with PD>15 mmHg had a larger increase in post-PMV MVA (2,39±2,20 versus 1,94±0,43 p=0.4). There was 1 (0.84 %) in-hospital death, and this was a procedure-related death. One patient with PD>15 mmHg underwent MVR due to papillary muscle rupture. One patient with PD≤15 mmHg underwent emergency MVR because of the development of haemodynamic instability immediately after PMV and also three patients (% 2,54) underwent emergency MVR due to significant MR (two patient with ≤15mmHg and one patient with >15mmHg).Thromboembolic events occurred in one patient (% 0,84) who had an ischemic attack just after PMV, treated with trombolytic teratment without any neurologic sequelae. There were no other clinically significant complications (tamponade, vascular injury or transient ischemic attack, complete atrioventricular block) observed either during or after the procedure.

Echocardiographic findings of left-to-right shunt at the interatrial level was observed in 7 patients after PMV (% 7,6); however, none of them were hemodynamically significant because all had pulmonary/systemic flow ratios. The echocardiographic findings of the study population before and after PMV and of patients with PD≤15 and >15 are shown in Table 3**.**

Atrial fibrillation(AF) was seen more frequently in patients with PD≤15 mmHg group (12 versus 4, p=0.7), also higher echocardiographic scores were observed in patients with PD≤15 mmHg (8.19±1.62 versus 7.88±1.50 p=0.46). In addition; severe mitral regurgitation were seen more in patients with PD≤15, but this was not statistically significant. Mitral valve opening was less in PD≤15 mmHg group, while was more in >15 mmHg group, but this was statistically insignificant.

Table 3. MVA Variables Before and After PMV

	MVA Prior PMV	MVA Post PMV	P
Total	1,13±0,16	2,20±1,76	<0,0001
PD≤15	1,14±0,17	1,94±0,43	<0,0001
PD>15	1,11±0,16	2,39±2,20	<0,0001

MVA; Mitral valve area PMV; Percutaneous mitral balloon valvuloplasty

Clinical Follow-Up

The mean follow-up time after PMV was 60,9±34,5 months, maximal 124 months. Pulmonary hypertension was occured in one patient (NYHA class III) within six months after the procedure and MVR was performed.

There was no significant difference in mean pulmonary artery pressure, mean transmitral gradient and PCWP between PD ≤ 15 mmHg group and PD > 15 mmHg group in terms of primary endpoints. However, there was a difference in mean left atrial pressure difference (LAPD) between ≤ 15 mmHg group and > 15mmHg group. MVR and redo PMV rates were significantly lower in LAPD> 15mmHg group , the difference between stroke and death rates was not statistically significant (p=0.6).

Follow-up events in patients with LAPD≤15mmHg were 4 deaths, 4 MVR, 14 redo PMV, and 1 ischemic attack. Six patients (% 5,0) died during the follow-up: two of the cardiac causes, one road accidents, two due to breast and ovarian neoplasm, and one of an unknown cause. Follow-up events occurred less frequently in patients with LAPD>15mmHg and they were 2 deaths, 1 MVR, 1 ischemic attack, and 3 redo PMV. Death, MVR, redo PMV, and ischemic attack results between LAPD≤15mmHg group and LAPD>15mmHg group are summarized Figure 1.

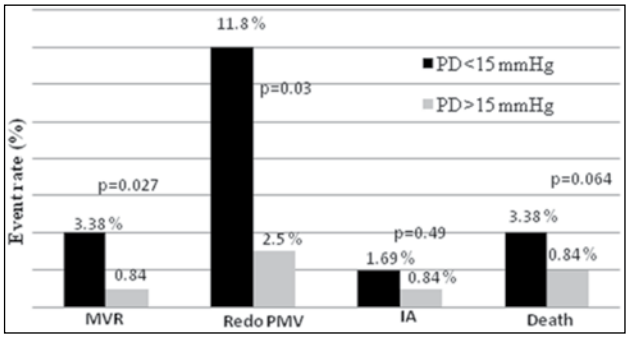

Figure 1. LA PD≤15mmHg and LA PD>15mmHg Death, MVR, Redo PMV, and IA results
LAPD*; Left atrial pressure differences,*
MVR; *Mitral valve area*
PMV*; Percutaneous mitral balloon valvuloplasty,*
IA*; ischemic attack*

While 14 of the 70 patients with LAPD≤15 mmHg underwent redo PMV, three of the 48 patients with LAPD>15mmHg underwent redo PMV (p=0.03). However, while MVR was done in 4 pa-

tients with LAPD≤15 mmHg, only 1 patient with LA PD>15mmHg underwent MVR (p=0.027). When LAPD ≤ 15 mmHg and >15 mm Hg groups compared in terms of increment of NYHA class after the procedure or decrement of NHYA class in follow-up period, there was no difference between both groups but in the group with LAPD>15mmHg the number of patient with NYHA class I were significantly higher (47% versus 15%).

NYHA functional classes of 118 patients before PMV, at 6 month and at the last clinical visit is summarized in Figure 2.

Discussion

PMV has been accepted as standard therapy today for patients with moderate-severe MS with symptomatic or significant pulmonary hypertension. It is an effective and safe treatment option if done in experienced hands.

Recent studies have shown that the significant risk factors in determining long-term outcomes and of event-free survival in patients undergoing PMV are; mitral valve morphology, age (old age), rhythm (having AF), NYHA class, post-PMV MR grade, post-PMV MVA, post-PMV pulmonary artery pressure, echo score and prior surgical commissurotomy10-16. Wilkins et al. and Palacios et al. found that the hemodynamic consequences were associated with echo-score17. In our data, echo score was higher in group in which the pressure difference was lower but this was not statistically significant (p = 0.9). The amount of calcium score in the first group group was higher (p <0.0001), this result was consistent with previous studies.

Pamir et al. found that in post-PMV patients although LA pressure decreased, early diastolic filling fraction of the left ventricle is unchanged, and the diastolic dysfunction did not recover at an early stage following PMV[18]. Bitigen et al. sug-

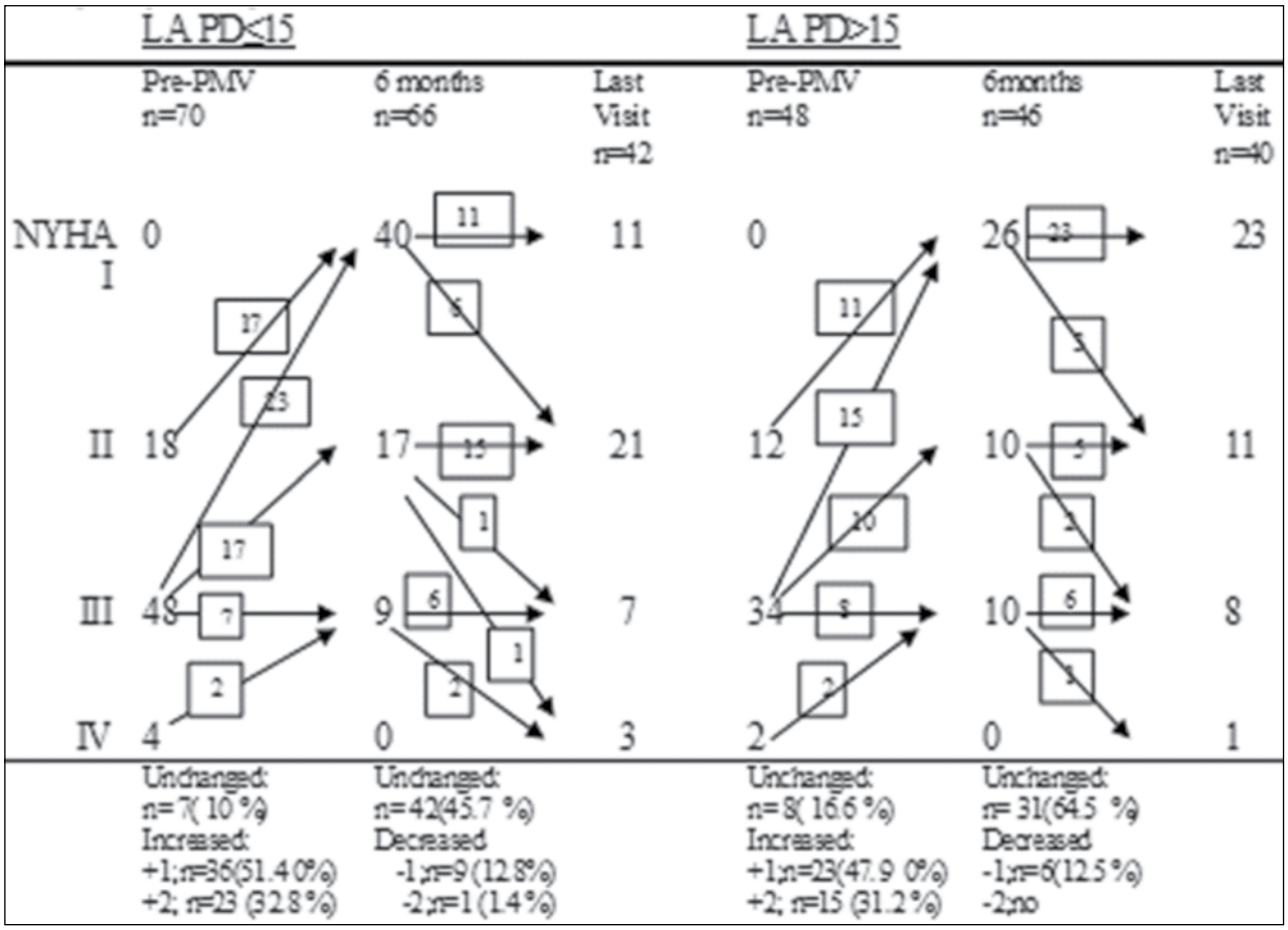

Figure 2. Left atrial pressure differences (LAPD)≤15mmHg and LAPD>15mmHg New York Heart Association (NYHA) functional class before percutaneous mitral valvuloplasty (PMV) at 6 and last visit clinical follow-up study in 118 patients

gested that despite the increase of the valve area and the decrease of the LA, which functions did not improve completely in the early stage after PMV[19]. In our data overlapping with these studies, there was no difference in immediate outcomes between LAPD≤15 mmHg and LAPD>15. However, long-term clinical results in patients with mitral stenosis such as MVR and redo PMV rates were significantly lower in mean left atrial pressure difference > 15mmHg group. Additionally, stroke and death rates were lower but were not statistically significant.

Atrial fibrosis and increased left atrial wall stress are the common pathophysiological changes associated with both MS, therefore we suggests that provided LAPD > 15 mm Hg group with a better mitral valve opening. This changes may contribute to cause remodeling of the left atrium (regression of fibrosis and decrease left atrial wall stress).

There was not a significant difference between the two groups in terms of NYHA class change after the procedure and in the follow-up period, but in the follow-up period LAPD> 15 mm Hg group's class was observed to stay more in NYHA class I. These results demonstrate the clinical success of PMV procedure and these results are important for the survival of patients with a better exercise capacity.

Study Limitations

Several limitations of this study should be emphasized. The most important limitation was that the measurement classification that we have created based on pressure differences taken before and after the procedure that was the cut-off value of 15 mmHg determined by our clinical experiences. The determined cut-off value for MVA was 1.8 cm2 in the previous studies, above this value was considered as optimal, below was considered as suboptimal. We have accepted the cut-off value as 15 mmHg, however it must be verified by clinical studies. One other limitation was some patients had received diüretic therapy before the procedure. Hemodynamic measurements may have been affected by diuretic therapy. We have tried to withdraw diuretic treatment three days ago in the majority of patients , but very few patients had to be given because of the persistent complaints.

Conclusion

The significant risk factors in determining long-term outcomes in patients undergoing PMV are; mitral valve morphology, rhythm (atrial fibrillation), advanced age, NYHA class, post-PMV MR grade, post-PMV MVA, prior surgical commissurotomy, post-PMV pulmonary artery pressure and echocardiographic score. In addition to these parameters we found that LAPD may also be a predictor of long term outcomes.

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Human embryo pancreatic stem cells differentiating into active insulin secreting islet-like structure

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Abstract

Background: Prevalence rate of diabetes is increasing dramatically these years and it has become one major disease threatening people's health. Pancreatic islet transplantation is the most effective treatment to cure the insulin dependent diabetes. While deficiency of the pancreas donators limit the clinical application of islet transplantation. Stem cells have the potency to self-renewal and differentiate into all kinds of tissue cells.

Objective: To establish a method that induced islet cells differentiated from pancreatic stem cells to form the islet-like structures in *vitro*.

Methods: Inducing human fetal pancreatic stem cells differentiating into the islet cells in *vitro*, and the islet cells were suspended in medium containing extra-cellular matrix (ECM) and cultured for 24 h to induce the formation of the islet-like structures. The morphology and size of the islet-like structures were detected under fluorescence stereomicroscope; the ECM components and cellular types and location in the islet-like structures were detected by immunofluorescence staining. The islet-like structures and the insulin release levels in the supernatant were detected by radioimmunoassay. Rat models of diabetes where established, and transplanting the islet-like structures into the liver of rats through the portal vein, detecting the glucose levels for 3 days through the tail vein of rats.

Results: 90% of islet cells differentiated from human fetal pancreatic were aggregated spontaneously into the islet-like structures, which displayed obvious integrity outer membrane and similar size with native human islets. Using the diameter of 150 μm as a standard, the relationship between cells concentration and the formation rates of the islet-like structures was analyzed, and the result showed that the formation rate of the islet-like structures in the 1×10^5/ml group (36.5% ± 4.0%, $P < 0.01$) was best compared to others. The insulin release levels of the islet-like structures induced by 30.0 mmol/L glucose (110 μIU/ml ± 12 μIU/ml, $P < 0.01$) increased significantly compared with 5.6 mmol/L glucose (59.5 μIU/ml ± 8.0 μIU/ml), and the glucose levels in diabetes rats decreased significantly after the islet-like structures transplantation ($P < 0.01$).

Conclusion: Islet cells differentiated from pancreatic stem cells could be induced to form functional islet-like structures largely and quickly by the established method in *vitro*.

Key words: Diabetes mellitus; Embryonic stem cells; Cell differentiation; Pancreas; Islets of Langerhans transplantation

Introduction

Prevalence rate of diabetes is increasing dramatically these years and it has become one of the major diseases threatening people's life. As a micro-organ, pancreatic islets possess their own glucose sensor, produce insulin, release insulin in response to glucose, and maintain normoglycemia eventually. Therefore, β-cell replacement by islet cell transplantation can reverse or prevent diabetes. Pancreatic islet transplantation is seemed to be the most effective treatment to cure the insulin dependent diabetes (Shapiro et al 2000, Merani et al. 2006). Especially after the publication of the Edmonton protocol in 2000, the number of clinical islet cell transplantation increased and islet transplantation outcome improved (Shapiro et al. 2003, Shapiro et al. 2006) While the Edmonton protocol required

two or more islet transplants, deficiency of the pancreas donators limit the clinical application of islet transplantation (Jahansouz et al. 2011). Scientists are working hard to find other sources of insulin releasing cells for many years (Hirofumi. 2010), Stem cells have the potency to self-renewal and differentiate into all kinds of tissue cells (Xu et al. 2001). It is possible for stem cells to be the source of islet transplantation if they can be induced into pancreatic islet cells (Suzuki et al. 2004).

Pancreatic islet is composed of four types of different cells, which include beta cell, alpha cell, delta cell and pp cell. Ratio and location of the cells in the islets are relatively constant and there are special cell-cell junctions and blood supply in the islets (Calne 2005). The endocrine hormones secreted by the different islet cells are also important to promote the survival of beta cells and to regulate insulin secretion (Pieleers et al 1982, Ling et al. 1994). Some data showed that αcell loss during islet digestion and isolation procedure might result in apoptosis and dysfunction of implanted islets (Korbutt et al. 1997, Paraskevas et al. 2000). As well, the islets with α cell loss were not so sensitive to glucose changes and released enough insulin to decrease blood glucose effectively after transplantation mainly because of loss of glucagon regulation effect (Wang et al. 2011). Therefore, it is possible to keep the normal function of islets only by making the islet cells differentiated from stem cells to form islet-like structures. In addition, islet cells differentiated from stem cells can be located in the sinus hepaticus after transplantation only by being induced to form the islet-like structures either.

Bonner-Weir (2005) reported that insulin secreting cells induced to be differentiated from stem cells can form islet-like structures spontaneously by being semi-dried cultured in media containing extracellular matrix (Bonner-Weir et al. 2000). However, the approach cost much time, formation rate of islet-like structures was rather low and the sizes of the islet-like structures were various, either. Especially it is not suitable to do it in large scale. We explored many times under the foundation of previous research and set up a rapid technique by which islet cells differentiated from stem dells can be induced to form islet-like structure rapidly, expecting to establish the foundation for islets induced from stem cells to treat diabetes.

Materials and Methods

Agents and equipments

M199 media, human fibronectin, human laminin, human type IV collagen, ATP, rabbit-anti-human insulin antibody, goat-anti-human glucagon antibody, rabbit-anti-human C-peptide antibody, FITC labeled rabbit-anti-goat IgG antibody, Cy3 labeled goat-anti-rabbit IgG antibody and STZ (50mg/bottle) are all purchased from Sigma corporation USA. Others include type P collagenase from Roche, German, rabbit-anti-human fibronectin antibody and rabbit-anti-human type IV collagen antibody from SANTA CRUZ, USA, rabbit-anti-human laminin antibody from CHEMICON, USA, human insulin radio immunity kit from MILLIPORE, USA, silicified 50ml centrifuge tubes from ORCUGEN, USA. Equipments include SMZ1500 fluorescent stereomicroscope from Nikon, Japan and IX81 laser confocal microscopy from OLYMPUS, Japan.

Sample source

Human embryonic pancreas obtained from fetus (fetal age 8 ~ 16 weeks) of induced abortion by mifepristone in the department of gynecology and obstetrics, Japan-China Friendship Hospital. All donators signed the informed consents. The program was given approval by ethic committee of Japan-China Friendship Hospital.

Experimental animals

Five SPF grade healthy, male Wister rats, aged 7 ~ 8 weeks body weight 250 ~ 300 g, obtained from Beijing Vital River Laboratories [certificate number: SCXK (jing) 2006-0009], kept in SPF animal lab in Institute of Clinical Medical Sciences, China-Japan Friendship Hospital.

Isolation of stem cells from pancreas tissue

Isolation of stem cells from human embryo pancreatic tissues was made according to (Ge et al. 2007). Identification of the isolated stem cells was made by detecting the expression of stem cells markers including PDX-1, nestin and neurogenin-3 by immunofluorescence staining, and flowcytometry.

Induction and differentiation of stem cells to pancreatic islet cells

Human embryonic pancreatic stem cells were cultured and amplified for 2 weeks in 25cm^2 culture

flask.15% fetal calf serum (FCS) , 10mM nicotinic amide, 42ng/ml glucagon like peptide-1 (GLP-1) , 30ng/ml altrans-retinoic acid, 50ng/ml activin A and 100U/ml penicillin were added into the M199 culture media. The media were changed every 2 days.

Induction of an islet –like structures formation

After 2 weeks of induction the differentiation, the cells were nearly 80% confluence in 25cm^2 culture flaks. Digestion was made by 0.1% trypsin. The cells were counted and prepared into 10ml single cell suspension in 1×10^4 /ml, 3×10^4 /ml, 1×10^5 /ml and 3×10^5 /ml concentrations, which were cultured in 50ml silicificated centrifuge tubes for 24 hours in 37C CO_2 incubator .The tubes were shacked every 8 hours. Three parallel tubes were set for the different concentration group. The inducing media was 20% FCS, 1.5mmol/L Ca^{2+}, 1 mmol/L ATP 2mmol/L glutamine, 100U/ml penicillin and 2μg/ml human laminin as extracellular matrix, 5μg/ml human type IV collagen and 3μg/ml human fibronectin. The tubes were shaked gently every 8 hours. After 24 hours culture, the formative islet-like-structures were collected, and their number and size were estimated by fluorescent stereomicroscope.

Analysis of islet-like-structure

Component analysis of extra-cellular matrix (EMC) of islet-like structure

The islet-like structures induced from stem cells and natural human islets were prepared as freezing histological sections. After being fixed by acetone for 10 minutes, 1% bovine serum albumin-PBS was added to block for 20 minutes, and then 1:100 rabbit-anti-human fibronectin, 1:100 rabbit-anti-human type IV collagen, 1:100 rabbit-anti-human laminin were added respectively. The sections were washed by PBS after overnight incubation under 4C^o moisture condition. Then 1:100 FITC-labeled goat-anti-rabbit IgG antibody were added and incubated for 2 hours at room temperature. After 5 times washing by PBS, the sections were examined under laser confocal microscopy and photos were taken.

Composition and location analysis of the islet-like-structure

To a fixed freezing histological sections of induced islet-like structures and natural human islets that prepared as described above, 1:100 rabbit-anti-human insulin antibody and 1:100 goat-anti-human glucagon were added respectively as the first antibody; 1:100 FITC-labeled goat-anti-rabbit IgG antibody and 1:100 FITC-labeled rabbit-anti-goat IgG antibody as the second antibody. Meanwhile, 1:100 goat-anti-human glucagon and 1:100 rabbit-anti-human C-peptide antibody were as the first antibody, while 1:100 FITC-labeled rabbit-anti-goat IgG antibody and 1:100 Cy3-labeled goat-anti-rabbit IgG antibody as the second antibody were added respectively. Insulin staining and glucagon staining positive positions exhibited green color, while C-peptide staining positive positions exhibited red color under laser confocal microscopy.

Functional evaluation of islet-like-structures

Insulin releasing induced by glucose

The formative islet-like-structures were cultured in 24 holes culture plates. Every hole contained 100 islet equivalents (IEQs) which were incubated at 37 C for 5 hours in 1 ml RPMI-1640 culture media containing 5.6 mmol/L glucose. The medium was changed into 1ml RPMI-1640 culture media containing 5.6 mmol/L and 30mmol/L glucose respectively and the islet-like structures were incubated at 37C for 2 hours. There were 3 parallel holes for each. The supernatants were collected and tthe insulin levels of every hole was detected with human insulin radio immunity kit.

Effect of islet-like structure in regulating blood glucose levels of diabetic rats

Wister rats were injected peritonealy with Streptozotocin (STZ) at 60 mg/kg (dissolved in 0.1% citrate buffer solution at pH 4.5). The models were defined diabetic when the vena caudalis blood glucose over 20 mmol/L for at least 3 days. The islet-like structures (800 IEQ/rat) were suspended in 200 μl RPMI-1640 culture media without serum and then transplanted into diabetic rat liver through portal vein. Determination of the rat vena caudalis blood glucose levels was done for the consecutive days after islet transplantation.

Statistics

All data were processed by SPSS statistics software version 11.0. All data were expressed by $x \pm$

s. The difference between groups were analyzed by Mann-Whitney test, and the statistical significances were determined when $P < 0.05$.

Results

Identification of human embryo pancreatic tissue stem cells:

The stem cells isolated from human embryo pancreatic tissues adhered to the bottom of the culture flask soon; they grew and proliferated rapidly (Figure 1). Identification was made through detecting the expression of the stem cells markers including PDX-1, nestin and neurogenin-3 not only in RT-PCR results but also with flow cytometry and immunofluerescence detection.

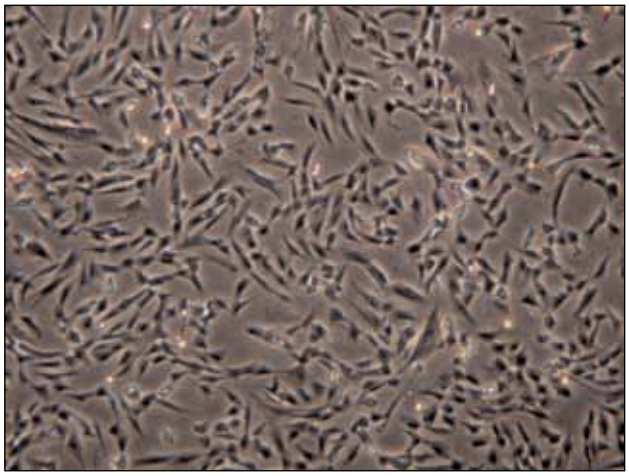
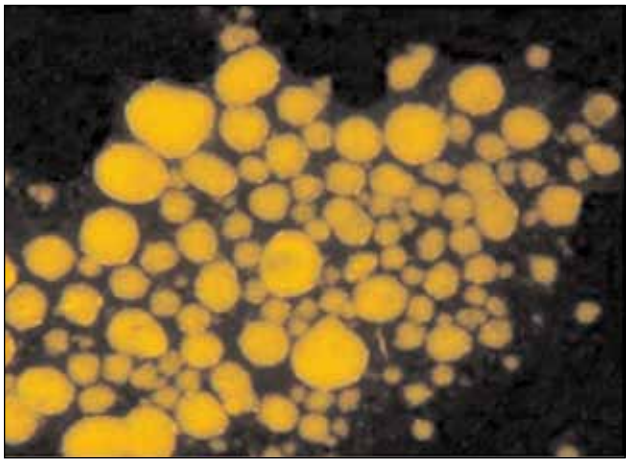


Figure 1. The observed morphology of human embryo pancreatic stem cells cultured in vitro (Inverted phase contrast microscope × 200)

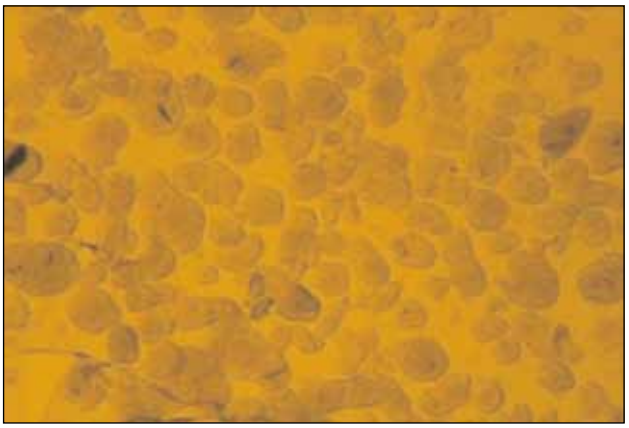
Formation rate of islet-like structures

After incubating the cells' suspension for 24 hours it was found that 90% of the islet cells differentiated from human embryo pancreatic stem cells formed islet-like structures, of diameters ranged from 50 – 400 μm. They were found similar to natural human islets. The outer membrane of the islet-like structures was observed fairly integrated under microscopy (Figure 2).

The relation between stem cell amount and concentrations and the formation rate of islet-like structures was studied. The results showed that the largest size and the highest formative rate of islet-like structures were in group 1×10^5/ml conc. (36.5% ± 4.0%) , followed by 1×10^4/ml (25.0% ±2.5%) , 3×10^4/ml (28.0% ± 3.0%) ,and the group of 3×10^5/ml conc. (21.5% ± 2.5%), respectively. The difference was statistically significant ($P<0.01$) (Figure 3).



A



B

Figure 2. The observed morphology of the islet-like structures formed by cells differentiated from human embryo pancreatic stem cells (Fluorescence stereomicroscope × 40). A: The islet-like structures formed by cells differentiated from human embryo pancreatic stem cells; B: Natural human pancreatic islets

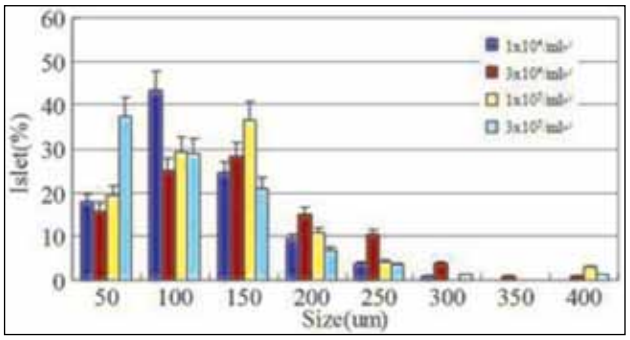


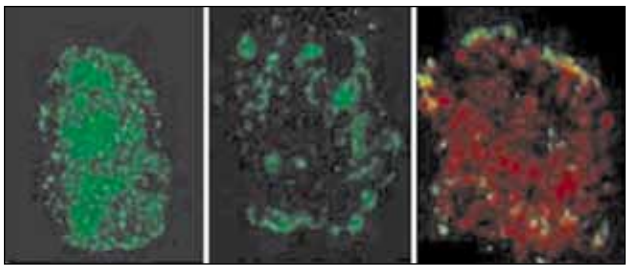
Figure 3. The relation between human embryo pancreatic stem cells concentration and the size and formation rates of the islet-like structures. Group 1 × 10^5/ml conc. Compared with the other 3 groups, P <0.01

Components of extra-cellular matrix of islet-like structures

Immunofluorescence detection showed that the outer membrane of islet-like structures contained human fibronectin, human laminin, and human type IV collagen, which were similar to natural human pancreatic islets. The ideal concentration of the three extra-cellular matrix added in the induction media were 2 μg/ml human laminin, 5 μg/ml human type IV collagen, 3 μg/ml human fibronectin(totally 10μg/ml), and the ideal induction time was 24 hours (Figure 4).

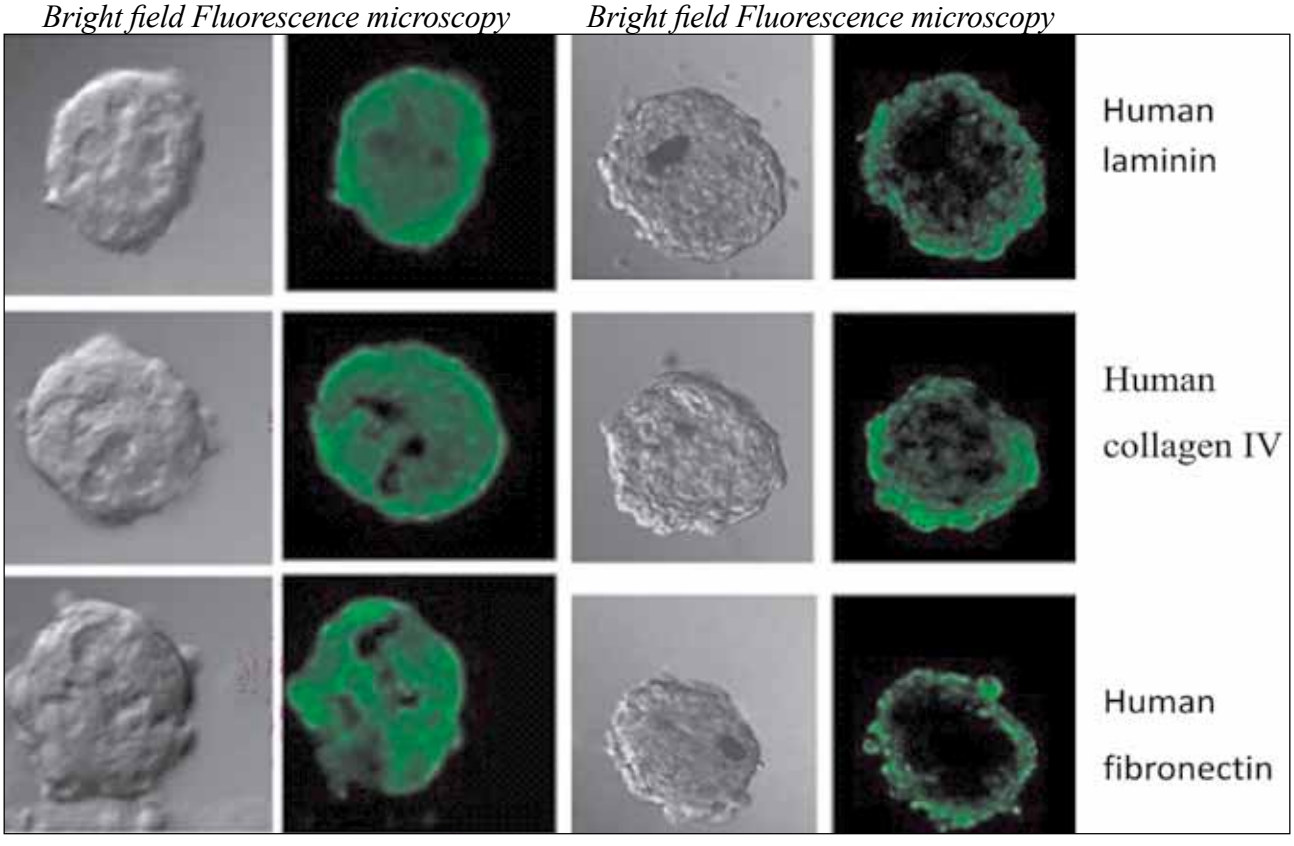
Components and location of cells in islet-like structures

Immunofluorescence detection showed that insulin-positive cells were the most in islet-like structures (about 50% ~ 60%); glucagon-positive cells accounted nearly 15% ~20%; after double labeling of C-peptide and glucagon, c-peptide positive cells located in the center of the islet-like structures, while glucagon-positive cells located at the outmost of the islet-like structures,which is similar to natural human islets (Figure 5).



A *B* *C*

Figure 5. Detection of cellular types and location in the islet-like structures formed by cells differentiated from human embryo pancreatic stem cells by immunofluorescence staining (Laser scanning confocal microscope × 200). **A:** *Insulin-positive cells (green);* **B:** *Glucagon-positive cells (green);* **C:** *C-peptide-positive cells (red) and glucagon-positive cells (green).*

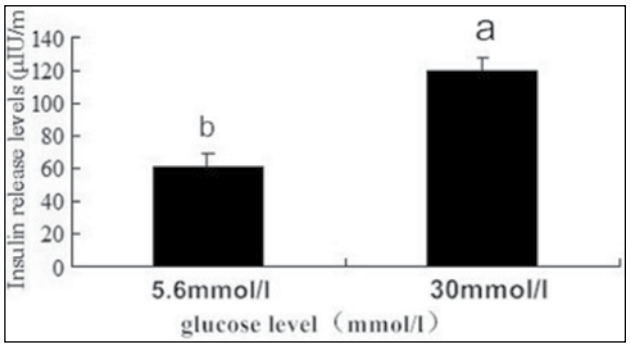


A *B*

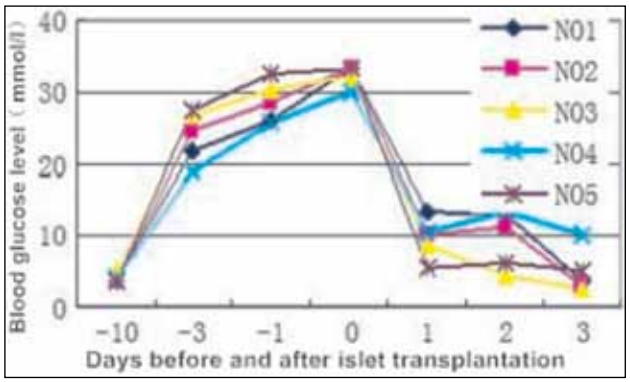
Figure 4. Detection of ECM components in the islet-like structures formed by cells differentiated from human fetal pancreatic stem cells by immunofluorescence staining (Laser scanning confocal microscope × 200). A: Natural human islets; B: The islet-like structures formed by cells differentiated from human fetal pancreatic stem cells

Functional evaluation of islet-like structures

Via radio immunity detection, the amount of insulin released- stimulated by 30mmol/l glucose, was (110 ± 12) μIU/ml which was much higher than that stimulated by 5.6mM glucose (59.5 ± 8.0) μIU/ml ($P < 0.01$) (Figure 6A). After transplantation of the islet-like structures into liver through the portal vein, the blood glucose levels of the diabetic rats decreased obviously and made differences of statistical significances compared to that before transplantation of the islet-like structure ($p<0.01$) (Figure 6B)



A



B

Figure 6. The insulin release induced by glucose and the effect on glycaemia level in the diabetic rats after the islet-like structures formed by cells differentiation from human fetal pancreatic stem cells transplantation. A: The insulin release level induced by glucose; B: Glycaemia regulation in the diabetes rats after the islet-like structures transplantation; a Insulin release level as compared with 5.6 mmol/L glucose, P < 0.01; b Insulin release level as compared with before transplantation, P < 0.01.

Discussion

The endocrine hormones secreted by the different islet cells are important to promote the survival of beta cells and to regulate insulin secretion (Pipeleers et al. 1982,Ling et al. 1994). Therefore, it is possible to keep the normal function of islets by making the islet cells differentiated from stem cells to form islet-like structures. In addition, islet cells differentiated from stem cells can be located in the sinus hepaticus after transplantation only by being induced to form the islet-like structures.

Pancreatic islets were transplanted into liver through portal vein and located in the sinus hepaticus. The main reasons were: islets may gain more blood supply and can respond to the changes of blood glucose sensitively; liver is the biggest target organ of insulin effect since insulin secreted from islets mainly react on hepatic cell and promote blood glucose to synthesize hepatic glycogen;and liver can secret many immune suppression substances (such as α-1 antitrypsin) which may inhibit transplantation-immunological rejection (Shapiro 2000).

The methods to induce stem cells to form islet-like-structures in previous studies were not very ideal for the reasons mentioned above (Soria et al. 2001). While in this study, islet endocrinal cells differentiated from embyo pancreatic stem cells were suspended in silicificated centrifuge tubes to avoid them being attached to the tube bottom and to induce them to form islet-like structures in the culture media containing extra-cellular matrix, ATP and Ca^{2+}. After 24 hours incubation, the cells formed islet-like structures having obvious capsule. It was found that the sizes of islet-like structures were well-distributed when the stem cells concentration was 1×10^5/ml and in which group the proportion of 150 μm diameter size islet-like structures was the most and just like natural human islets size.

Extracellular matrix is an ectocytic formed element or ground substance components, which are the important components of the microenvironment of the pancreatic islets and the supporting frame of islet cells. Extracellular matrix in human islets is mainly composed by laminin, type IV collagen and fibronectin. Extracellular matrix protein molecules adhere and combine with islet cells

mediated by integrin to regulate the endocellular signal pathway. The interactions of pancreatic islet cells and the extracellular matrix are essential for islet beta cells to survive and maintain their normal function. Extracellular matrix influence the proliferation, differentiation, adherence, metabolism, signal conduction ect. Laminin-1 promotes pancreatic precursor cells proliferation and differentiation *in vitro* (Jiang et al. 1999); lamin-5 increase insulin releasing *in vitro* (Parnaud et al. 2006); islet viability and expression of insulin increase when cultured in jelly containing collagen and laminin; interaction of type IV collagen an integrin α1β1 are important for islet beta cells adherence, mobility and insulin secretion; moreover, type IV collagen is the only important factor for beta cell migration(Kaido et al. 2004). Otherwise, the extracellular matrix protects the islet beta cells and resists apoptosis (Hammar et al. 2004). Reconstruction of proper extracellular matrix can decrease the mortality of isolated islet cells (Wang et al. 1999). Islet cell apoptosis and functional incapacitation post-transplantation are very common, while extracellular matrix can prevent antibodies and complements in blood to attack the islets, and decrease the immunological rejection to some extent after islet transplantation.

Investigations indicated that islet beta cells transplantation without alpha cells couldn't rectify the glucose metabolic disturbance in diabetic animals (Li et al. 2002) hence, it is possible to keep the integrity of pancreatic islet function via induction of the stem cells to differentiate into various kinds of islet endocrine cells and form islet-like structures according to certain cell proportion. In natural human islets, beta cells secreting insulin account about 60% to 65% and are distributed mainly in the center of islets; alpha cell secreting glucagon account about 20% to 25% and are distributed mainly at the perimeter zone of islets. This study found that there was similarity regarding the proportion of insulin and glucagon positive cells in islet-like structures and the distributions of the cells to natural islet.

Various sources of stem cells have the potential to differentiate into islet like structure and to regulate blood glucose (Sun et al. 2007, Kroon et al. 2008). Our investigation has confirmed that the islet-like structures differentiated from fetal stem cells can release insulin when stimulated by high glucose level. They could down-regulate the high blood glucose levels of diabetic rats after the islet-like structures implanted into the animals' livers. All those results indicated that islet-like structures differentiated from fetal pancreatic stem cells have function of insulin secretion. Our stem cells in this study came from human fetal pancreatic tissues containing large quantities of precursor cells of islet endocrine cells, therefore, the introduction efficacy was high and the functions of islet-like structures were good too.

Thus, our study here established a rapid, effective technology to induce the stem cells to differentiate into islet cells and to form great quantity of functional islet-like structures. This technology may have resolved a key problem for the use of stem cells in the treatment of diabetes.

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The first experience of intercourse in married women of Sabzevar city: a phenomenological study

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Abstract

Introduction: the first Intercourse experience has an important role in sexual health as well as increasing the satisfaction from marital life. The aim of this research is to understand the first intercourse experience of women of Sabzevar city

Methodologies: this is a descriptive phenomenological study which was done through the purposive sampling, including interviews with 25 women in Sabzevar city with less than 5 years since their first marriage. The researcher helped participants with profound and semi-structured interview to determine their first intercourse experience. Data were analyzed using Colaizzi's method and Atlas Ti Software.

Findings: Psychological changes (fear, psychological readiness of spouse, sense of relaxation after sex), vision and behavior changes (a sense of need for precise consultancy, gaining knowledge through modern means of communication, gaining knowledge through well experienced individuals, good manner of spouse), physical changes (pain, body irritation, the tearing of the hymen, lack of orgasm, the way of preparing, intercourse starter, the appearance of sexual partner, avoiding contraception tools)and cultural changes) are the focus of this study.

Conclusion: Training the couples and modifying their beliefs and attitudes can increase sexual health as well as improving this experience. Understanding this experience can help policymakers to promote training courses before marriage.

Key words: first intercourse, sexual health, phenomenology, women

Introduction

World Health Organization defines sexual health as follows: "Sexual health is a state of physical, emotional, mental and social well-being related to sexuality; it is not merely the absence of disease, dysfunction or infirmity. Sexual health requires a positive and respectful approach to sexuality and sexual relationships, as well as the possibility of having pleasurable and safe sexual experiences, free of coercion, discrimination and violence. For sexual health to be attained and maintained, the sexual rights of all persons must be respected, protected and fulfilled". (1)

Sexual issues in terms of importance are in the first-rate of marital life's problems and sexual adaptation is considered as an effective factors for felicity in life.(2) The results of studies in the family health care clinic of Shahed University of Iran in 2006 show that the most common women's problems is orgasmic disorder with frequency of %38.7. Vaginismus in women has been the second major group of sexual disorders. (3)Other sporadic and limited studies conducted in this regard have mentioned women's sexual disorders between %10 and %52. (4) and revealed that %10-40 of Iranian women have sexual disorders.(5)

Other studies have reported this value between %25 and %63.(4) This issue has even been reported among couples who state to be satisfied from their marital life up to %40.(5) It has been told that about %5 of women over 35 years old have never experienced orgasm.(6) The results of studies indicate that the first intercourse has a prominent role in the individual's life and increases the individuals' commitment to their partner. Also if the

first intercourse happens after reaching the higher degree of commitment between the two people, it will increase the affection and closeness between two people.(7)

In many countries, the sexual relationship before marriage is common and individuals usually begin their shared life with these experiences but in the teachings of Islam, sexual relationship before the official marriage is considered as contrary to chastity. (8)Therefore, in Islamic countries, like Iran, for most people, the first sexual relationship is the first experience of intercourse after the marriage, a marriage which often lasts for a lifetime; thus, most of the times, the consequences of this experience continues all through the individual's life. In some areas of Iran still , at the wedding night, the relatives of the bride wait for the bloody underwear of the woman, as the assurance of the fact that the sold product has been brand new and intact, otherwise the wedding will be called off and the groom will be reimbursed for all the costs that he has bare for the wedding ceremony as well as the amount he has paid to the father of the bride as the price of buying his daughter.(9)

Satisfactory sexual relationships are among the most important factors in the survival, happiness and health of a family.(10) Unpleasant sexual experience due to any reason would have negative consequences. The deficiencies in the sexual relations have a close correlation with the social problems, such as crimes, sexual assaults, psychological diseases and divorce. There are many couples who are suffering from sexual deficiencies and are themselves unaware about the negative impacts of those deficiencies on their relationships and low self confidence, Moreover, being nervous, experiencing lower abdomen and back pains, lack of mental concentration and even disability in performing daily tasks are among the consequences of unsuccessful satisfaction from sexual relations. On the other hand, pleasant sexual relation is an important factor in the reinforcement of the family and a good basis for achieving and maintaining a stable culture.(11)

Although sexual desire is considered as innate and non-self regulated, but still there is much to learn about the perspectives and sexual behaviors. Therefore similar sexual relations might deliver different meanings to various individuals or even change for the same person, from time to time.(6) unfortunately, sexual complexities among women are among the have been given less significance and attention due to the special social and cultural limitations and perspectives.(5)

The traditional customs and culture of families, especially for women, do not accept the right of declaring sexuality as a normal human need and a woman who expresses such intentions would be considered as outrageous . The expression of sexual expectations which is absent from the point of view of custom causes that the spouses do not pay attention to this need and consequently as a results ignoring such a need, lack of effort in resolving or treatment of the sexual deficiencies appears. And therefore, data in this regard is relatively rare, while this disorder is considered as a great alarming factor in individuals' public health.(12)

The number of researches conducted in Iran in the field of sexual problems in terms of sexual health is not significant, furthermore even in these few studies, the subject of fist sexual intercourse has not been given importance. Thus, the current research has been conducted with the aim of determining the first intercourse experience of married women of Sabzevar city.

Methodologies

The current arrempt is a qualitative study that is conducted as a Descriptive Phenomenological research.(13) The participants of this research were 25 women with less than 5 years since their first marriage, who were screened not to be addicted to drugs, diseases affecting sexual activity, severe spiritual crisis or other mental physical and psychological disorders. Targeted sampling was applied in this research and was continued up to the saturation levels.(14) Moreover, in cases where the researcher has faced cases overlapping and repeating previous confirmed issues while collecting data and reviewing the elicited information, the sampling was terminated.(15)

Data collection

The method of data collection was semi-structured and in-depth interview.(16)

First of all, the researcher introduces himself to the participants completely and then explains

objectives, importance and the method of data collection to them precisely. Then, the researcher took written consent of individuals who participated in research to interview. He assured the participants of recorded interview, about the confidentiality of the data and finally acknowledged them about the information elicited from the interviews.

Interviews were conducted at the houses or offices of the participants .(14) The age of participants ranged between 18 to30 years old.

The duration of each interview was 45 to 60 minutes (2 sessions). The interview was started with a general question on the subject of the study and the researcher helped the participants in explaining their own life experiences.

During listening, transferring issues and reviewing them by the researcher, the responses of some participants were extended to other related issues, in these cases the interviewer would listen to all the answers given by the participants so that he could completely understand the participants situations. This kind of interview gave the participants a chance to explain their experiences regarding the intended subjects entirely and talk about it freely.(14)

The researchers recorded the interviews but if the participants were not satisfied with it, they could belong the recorded tapes themselves or ask the researcher to delete them at the end of the study. In cases which information was not recordable or direct observation was necessary, like non-verbal gestures of the interviewee, observation and note taking techniques were applied.(15)

In each interview, the experiences of participants and the subsequent questions with regard to their answers were asked. After finishing the interview, the witten notes or recorded tape were reviewed by the researcher immidiately so that in case the answers were obscure or needed to be followed up, it was done quickly and necessary questions were asked from individuals.(15)

Data Analysis

Data analysis was conducted by colaizzi's seven-stage method: (15) To ensure the validity of information derived from the participants and to become certain that they had not provided inaccurate information, various participants were engaged.(15) Also regarding the data collection tools, the interviewer or observer in the process of data collection tried carefully to avoid any orientation and in the final stage, the validity of changing and interpreting information was acknowledged by the participants.(17)

The researcher referred to participated individuals in the study and took their confirmation regarding his description of their experiences and did adding and removing of any items with their notice. (17)

Findings

From the analysis of obtained data in this research, four major themes and a lot of codes from participants' viewpoint about the first intercourse experience were achieved that included: 1- psychological changes 2- changes in attitude and behavior 3- physical changes 4- cultural changes.

Theme 1: psychological changes (fear, psychological readiness of spouse, sense of relaxation after sex)

The most extensive extracted concept was the concept of psychological load which extracted codes for main concept and sub-concepts of that were as followed:

Subtheme 1: Fear (due to misconception, pain, bleeding, pregnancy)

Most of the participated women have mostly mentioned their fear of relationship due to their misconceptions, pain, bleeding and pregnancy. They stated the primary cause of their fears and being uncomfortable was due to what they had heard from others. They also considered lack of proper knowledge about the subject and their misconceptions as another reason for this matter.

Furthermore, they believed their lack of knowledge during the pre-marital life as one of the important factors in creating their fears. A change in their perception after experiencing their first intercourse was one of the emphasized issues and they declared that their bleeding and pain were not to the extent that they expected it to be and therefore feared from it.

One of the participants said: "Because of the misconception that I had formerly, I feared a lot

and when it was done, I recognized that it did not pain much and it was normal. There was no such a pain that others talked about."

Participants at the time of describing the fear about the pain expressed their feelings about the pain as a stage which they intended to pass it more quickly and to enter to the next level.

"I did not feel good about the tearing of the hymen and I was hoping that the time would pass quickly".

Subtheme 2: Husband's physical and psychological readiness

Most of the participated women mentioned that men generally have more physical and psychological readiness than women. They stated that their lack of readiness revealed in being upset and anxious. Also they considered more vulnerability for women due to the tearing of their hymen and having less knowledge about the subject in comparison to their husbands, they also related their fears to the misconceptions from what they had heard from unpleasant experiences and conversations of others. Moreover, the role of man in preparing woman was emphasized so that the majority of participants considered the readiness of husband as a significant factor in achieving calmness at the start of intercourse:

"The readiness of my husband was more and he was very relaxed without any anxiety and discomfort". I was ready but not to that extent, and feared because of what I had heard before. But my husband's calmness made me calm too.

Subtheme 3: The relaxation feeling after the intercourse

Another important reportable issue in this study was the feeling of relaxation after the intercourse in the participants. According to what participants said, their relaxation feeling after the intercourse happened because of getting rid of the fears and misconceptions.

"A feeling of relaxation and deep sleep, as if all of my fears was gone. As though I got rid of all of my disturbing thoughts and my husband reached orgasm too".

Theme 2: Change in attitudes and behaviors (a sense of need to a precise consultancy, gaining knowledge through modern means of communication, gaining knowledge through well experienced individuals, good manner of spouse)

The majority of participated women considered having the precise and accurate consultancy as an important factor to reduce their fears and concerns. And also they have considered satellite, pornography websites and friends as factors to get information and knowledge. They have considered little knowledge regarding the subject, fear, anxiety and misconceptions about intercourse among the reasons for their discomfort.

Furthermore, participants considered good moral of a man, and not his occupation and education as a more important factor in enjoying the intercourse. Individuals also have considered the good intercourse experience, providing training CDs for spouses during consultation before marriage, access to specified centers, providing sexual consultations as significant tools for preparing woman to have an enjoyable intercourse.

One of the women said: "the man's morality and his good temper, and not his occupation and education, during the first intercourse is very crucial so that he can make his spouse reach orgasm.

Subtheme 1: (pain, body irritation, the tearing of the hymen, lack of orgasm, preparation, intercourse starter, the appearance of sexual partner, avoiding contraception tools)

The majority of participated women reported physical changes such as pain, body irritation, tearing of hymen and lack of orgasm after the first intercourse, and almost all of them mentioned that because of their fear from intercourse, stress and anxiety, they could not achieve orgasm. On the other hand, they considered the physical and psychological readiness of their husbands as an important factor to help them reach orgasm. The majority of participated women have expressed their husbands as the intercourse starter and they related it to more readiness of their husbands for starting intercourse and reaching orgasm, as well as men's earlier stimulation as.

About the warm up, all emphasized the embracing, kissing, touching and decent appearance

of husband as significant factors in sexual relationship. One of the other behaviors that the majority of women liked it was gentle and soft behaviors, romantic and lovely language during intercourse. They said that such behaviors cause women to take more pleasure of having intercourse.

"My husband began to cuddle, kiss and touch me before the intercourse and prepared me for sexual relationship. He expressed romantic and erotic sentences. My husband did this calmly and gently, without anxiety and very easy".

None of the participated women had used contraceptive devices regardless of the level of their awareness. One the reasons that women expressed was that at this point they believed contraceptive devices prevent themselves and their husbands from having pleasure.

"I did not use condom and pills since condom does not give you a good feeling. It is a plastic and nothing is like direct contact because one gets more stimulated, has pleasure and will be in a good mood.

Theme 3: cultural changes (not to display the bloody tissue after the tearing of hymen, the intercourse during engagement period)

Subtheme 1: not to display the bloody tissue after the tearing of hymen

Some of the changes that participated women mentioned as cultural changes was the issue of the relationship privacy and relatives not interfering in the intercourse matters at the spousal night or some days after that. Participants considered this as one of the positive changes that has emerged in their culture.

Although the majority of participants were conventionally aware of the tradition of showing bloody tissue in their families, they did not show it to anyone and no one asked for it.

The tradition of bloody handkerchief was: the spousal night officially starts with the arrival of the bride and groom into the bride's room. Since the thirteenth century and until 50 years ago, in Tehran and many other cities, it was common that bride and groom would be alone in the room for three days and just would leave the room for essential needs. In the past, if groom could not be successful to remove the hymen, the bride's relatives and even the groom called him desperate and blame him. In the traditional form of this custom, one of the close relatives of bride was responsible to be near the bride's room on spousal night and to collect the bloody handkerchief after the intercourse and show it to some women such as the mother of the groom.

One of the participants said: "We had a tradition of showing bloody handkerchief, but we did not show it to anyone since nobody asked and elders told us that it is up to you and families do not interfere in this issue any more because culture has changed and these traditions are fading. Therefore, it is related to your husband, not to others, how sure he is about you".

Subtheme 2: the intercourse during engagement period

The participants did not consider having intercourse during engagement period as a disturbing and unpleasant issue. In contrast, they considered it as having a significant role in loving their husband. In Iran, according to the Islamic teachings, the sexual relationship before marriage is prohibited and there is a punishment for it. On the other hand, even after the legal engagement, some families with regard to customs and traditions which are common among them, prohibit sexual intercourse and postpone it to the spousal night.

The spousal night might happen a few days or even years after legal engagement and spouses have to be patient until the time when they can start their sexual relationship. Almost the majority of participants believed that after the legal engagement husband and wife are able to have sex with each other. However, they expressed to being ashamed during the early days of their relationship. The majority of women considered the lack of having sex during the early stages of marriage i.e. engagement as an important factor in misunderstanding and dissatisfaction in their relationship with their husbands. They considered it as a barrier to intimate talking and deciding on the improvement of the quality of their sexual relationship for the spousal night.

One of the participants said: "Our first intercourse was during the engagement period. We did not find any reasons to postpone our intercourse until the first night of our wedding. We were very

eager to be closer to each other through intercourse and to show our feelings. Because it is not like the old days anymore that it should only happen on the wedding night. Young people should be given more freedom because they are married to each other legally and there is no problem".

Discussion and conclusion

The current study is a descriptive phenomenological research that has been conducted in Sabzevar city and interviewed 15 women with less than 5 years from their first marriage. The main themes extracted from this study are: psychological changes 2- changes in attitudes and behaviors 3- physical changes, 4- Cultural changes

The expression of fear has been one of the main issues which women reported in this study. Fear has different aspects such as the fear due to their misconceptions, pain, bleeding, experiencing the first intercourse or the fear from pregnancy which have appeared in women in one form or a combination of different forms. The variety in types of fears indicated that this issue is very widespread and identifying its dimensions is a very important subject. But a very important fact which has been common among all these experiences is that the fear has been unreal and in reality the whole experience has been much less painful or bothering as they have imagined at first. The results of other studies also indicate a percent of women having the fear of intercourse (18). The remarkable point in the result of this study is that most women have declared that their anxiety and fear has been disappeared; a fact that reveals their fear has not been based on the real phenomenon on the first hand.

Fear inevitably has a great effect on this experience and probably for many it has made their first intercourse to change into an unfavorable experience. Hence, this issue can be alleviated and totally resolved by suitable training and transferring real experiences. The role of husband is very significant in decreasing this fear. Based on this research men were mostly the beginner of the sexual relationship and women declared that this can give them more comfort.(19)

Men are mostly the beginner of the sexual activities in comparison to women (2/3 of men in comparison to ½ of women).(19) Research has also reported very different data regarding the satisfaction from sexual relationships between men and women. These researches revealed that men were more facilitator than women in sexual issues and focused on gaining more pleasure. Other researches have reported that the men's ability to express their sexuality is more than women.(20-22) Using various psychological methods, trying to understand women and treating them well, and helping them to get rid of their misconceptions about intercourse have been very influential in decreasing their fears, increasing the sexual pleasure and giving them a pleasant feeling. Therefore, advising men and training them in this regard can be very effective. (23)

The feeling of need for proper counseling before marriage has been considered as a key factor for decreasing the fear, anxiety, misconceptions and in bringing calmness during the intercourse by the majority of participants. With regard to the key role of counseling before marriage, more attention should be paid to it and training before marriage should be delivered more patiently and accurately so that the majority of issues which cause mental concerns for women will be resolved before the marriage. It seems that being ashamed of talking about sexual issues, lack of proper and fundamental understanding and information regarding the sexual issues are among the most important factors for sexual problems in early stages of marriage. The issue of proper counseling is consistent with other studies that have been done.(24) With regard to the importance of family planning, it is better that spouses would be trained in this regard before deciding to marry and the period of high school education is suitable for this training. (25)

Regarding sexual relationships, there are two important training priorities. For women, the time and suitable condition as well as various healthy sexual relationships methods most be introduced, for men, the health of sexual relationships and intercourse disorders and their treatments must be considered.(26) Sexual health training in addition to its positive preventive role which is preventing unwanted pregnancy, sexually transmitted diseases, sexual abuses, sexual violence, and sexual frustrations and so on can bring positive results at the individual and interpersonal levels.

From among these positive results establishing an appropriate relationship between sexual par-

tners can be mentioned (enjoyment of intercourse, reinforcement of self-confidence and self-esteem, etc.). With regard to the social and cultural differences between Iran and western countries(27), it seems that considering the cultural and religious aspects and also dominant laws, norms and values in the society and families are necessary in arranging sexual health education plan in Iran. In order for any training program to become successful, media, proper messege, and the one who delivers the messege are considered as very important factors. Gaining knowledge through modern mass media devices is the other main concept that has been revealed from the findings of this research.

The other point that should be mentioned is when the attitude of target group towards sexual issues is positive and they consider this subject as a necessary issue in marriage life, but there is no appropriate training sources available to them for gaining this information, they probably approach to unsubstantiated and occasionally destructive sources such as obscene products, satellite programs and pornographic websites. This issue can be easily inferred from the descriptive results of the conducted studies which indicate that some families are using obscene films and programs and it shows that these couples probably approach unsubstantiated sources to get information about sexual issues.(27)

Sexual health training not only causes the increase in the individuals' awareness, but also by changing the behavior, helps them to reach the appropriate health level.(28) The majority of married women mentioned that the level of their sexual knowledge is low and sexual training before marriage is necessary. In current research, couples have described that an individual is appropriate for training if it is not embarrassed by saying the details. (29) another research indicated that trainings and sexual information related to sexual response cycle, human anatomy and sexual techniques are among the basic principles of effective behavioral approach for treatment of sexual disorders.(26)

The expectation of feeling pain before the intercourse has not been unrelated to the actual feeling of pain experienced from the intercourse and it had caused the feeling of severe pain and lower sexual passion. In a research, it was concluded that stimulation stage disorders in many women causes painful intercoutrse and lack of sexual desire.(5)

The issue of not reaching orgasm is another subject that is emphasized in this research which originates from insufficient awareness about the intercourse followed by the fears and misconceptions. Anxiety, feeling guilty, distress, mental preoccupation with other thoughts and lack of focus on sexual excitement are negative stimulants that can cause disorders on the way of achieving orgasm.(4) A painful intercourse can cause the lack of sexual stimulation, orgasm, decrease or elimination of sexual desire.(30) In a study in the United States of America, the concept of considering intercourse as a sin, modesty and shame of intercourse, painful intercourse and the history of sexual abuse were considered among factors in not reaching orgasm.(31)

The issue of warm up method (individual's behaviors in becoming prepared for intercourse) and the appearance of sexual partner are issues that have been emphasized by many participants in this research as well. This issue is consistent with the results of the other studies.(32) Kissing, touching and intercourse were among activities which the majority of samples have called them as sexual activity. (32)Women's beauty is more important than men in an effective sexual relation .(32)

The behavior of the sexual partner is a significant tool to make emotional deep connections with the spouse and sexual adequacy is a requisite for completing the experiencie of physical love. Sexual behavior that happens continously and regularly, will enable the couples to establish mutual love. (4) When an individual touches his/her spouse without intercourse intention, he/she loves his/her spouse and couples should be aware of the fact that the closeness is not necessarily with the intention of intercourse, but any displays of physical love is a kind of sexual activity.(33)

The majority of interviewees did not prefer using contraceptive devices, especially physical methods such as condom which prevents direct contact and declared that the reason as lack of awareness or decrease in sexual pleasure. During the past 15 years, many efforts have been made to extend condom as one of the three main tools to prevent AIDS during intercourse. In contrary, the use of condom among the married couples has gained less than 3% success in developing countries in family control plans. (34)

Conclusion

In the first experience of intercourse in women multiple changes might occur which are partly due to lack of sufficient awareness, deficiency in delivering necessary information and weak counseling services from the health staff. And it is worth to mention that the use of descriptive research is necessary to understand the complicated women's world and their attitudes, and in providing documented data to extend the knowledge about sexual issues before the marriage. Furthermore, proper training of the health staff, families and the women themselves are very important in enabling them to receive appropriate information regarding the sexual issues.

Acknowledgment

The Authors would like to extend their thanks to the women who participated in this study and to the Deputy of Research in Mashhad University of Medical Sciences for their support and cooperation.

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West nile virus surveillance in humans and mosquitoes and detection of cell fusing agent virus in Vojvodina province (Serbia)

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Abstract

After the large 1996 outbreak of West Nile virus (WNV) in Romania and spreading from East (1999) to the West Coast (2002) of USA at a remarkable speed, WNV has, in 2010, reemerged in Europe causing outbreaks in human populations reported from Greece, Hungary, Italy, Romania and Russia. Surveillance of WNV in humans and mosquitoes by enzyme-linked immunosorbent assay, rapid antigen panel assay, virus isolation in Vero cells and real time polymerase chain reaction assay was carried out from 2005 to 2010 in Vojvodina Province, northern Serbia. Human sera samples were collected from 27 persons with viral meningitis or encephalitis disease history or from 18 persons with acute viral meningitis or encephalitis and 406 randomly chosen healthy persons from Vojvodina Province. Mosquitoes were sampled in bird reservoirs and human settlements by dry ice-baited traps. IgG antibodies were found in 18 out of 451 (3.99%) human sera with yearly rates varying between 1.97% and 6.04%. Viral RNA was detected in 6 out of 841 pools made out of 56757 sampled, identified and pooled mosquito specimens. In three pools of *Aedes vexans* sampled in 2005, RNA sequences related to cell fusing agent virus (CFAV) were detected. Data obtained from serological surveillance in 2009 were used to indicate "hot spots" of possible WNV transmission in urban area of Novi Sad (capital of Vojvodina Province) where mosquito trapping was focused during the next year. In 2010, three out of 50 pools of *Culex pipiens* sampled in "hot spot" areas were positive for WNV RNA. Both CFAV and WNV detections in mosquitoes are the first records of such type in Serbia.

Key words: West Nile Virus (WNV), Cell Fusing Agent Virus (CFAV), mosquitoes, seroprevalence

Introduction

The West Nile virus (WNV), an RNA arbovirus (*Flaviviridae*, *Flavivirus*) is the most extensively distributed flavivirus of the Japanese Encephalitis Serocomplex group worldwide. The virus was first isolated in 1937 in West Nile district of Uganda in the blood of a woman with neurologic disorders (1). Over a hundred wild and domestic birds species may serve as reservoir of WNV from which is transmitted by mosquito vector species. Mosquitoes of the genus *Culex* are generally considered the principal vectors of WNV worldwide (2). A broad spectra of mammalian species including man, horse, cat and rabbit can be infected naturally or experimentally with WNV (3). Humans are dead end hosts, unsuitable for further transmission by mosquitoes because of low virus titer and short duration of viremia. Most human infections are asymptomatic. Clinical manifestations can range from uncomplicated febrile illness to fatal meningitis or encephalitis (4). Severe neurologic cases have been reported in around 1% of infected (5).

The presence of WNV is registered in the western Mediterranean and southern Russia in early sixties of the last century (6). The significance of

WNV is not only in the fact that there has been expansion into new areas but also in the changes of the virulence of the virus that has been registered in recent outbreaks. Mild cases of fever, dominant in previously described epidemics have been replaced by outbreaks of cases with severe neurological manifestations and deaths. In USA, the virus was first registered in 1999 when it led to an epidemic of fatal encephalitis in 12% of patients (7). Since then as of end of 2010, 30600 clinically manifested human infections, 12668 cases of meningitis/encephalitis and 1206 fatalities have been reported to CDC (8).

Among the outbreaks registered in recent decades in Europe, encephalitis epidemic in southeastern Romania in 1996 was the first large urban outbreak (9). The activity of the virus remained in Romania after the epidemic. From 1997 - 2000 there were 39 cases of human infection and 5 (13%) deaths among them (10). In 2010 a total of 57 cases of WNV infection were identified in Romania with the case fatality rate of 8.8% (11). The same year the outbreak of WNV infections in humans in Greece (12) is another timely reminder that WN fever is a reemerging vector borne disease in Europe.

The incidence of West Nile fever in Serbia is largely unknown. Only scarce historical data obtained by hemagglutination inhibition test indicate seroprevalence of WNV in republics of former Yugoslavia of 1-3% in Croatia, 1% in Bosnia and Herzegovina and Kosovo, 1% in Montenegro and 1-8% in Serbia (6). Only serological investigation in Vojvodina Province was conducted in 1972 and antibodies against West Nile virus were found in 2.6% - 4.7% of samples in different rural parts of province (13). To our knowledge, no West Nile virus RNA was detected in mosquitoes on the territory of both former Yugoslavia and Serbia.

The aim of this study was both to reestablish surveillance for WNV specific antibodies in the human population in Serbia applying enzyme-linked immunosorbent assay (ELISA) and to explore the presence of viral RNA in possible mosquito vectors by virus isolation in Vero cells, rapid antigen panel assay and real time polymerase chain reaction (RT-PCR) test.

Materials and methods

Vojvodina is a province in northern Serbia bordering with Croatia on the west, Hungary on the north and Romania on the east. It is a southern part of Panonian plane, rich in rivers (the Sava, the Danube and the Tisa) and covered typically by agricultural land. The climate is temperate, average yearly temperature 11°C, average monthly minimum January 1.3°C, maximum July 21.4°C, mean yearly rainfall 658 mm.

ELISA testing, calculation and interpretation of results, for IgG determination, were performed according the instructions of manufacturer (Euroimmun AG) of the Euroimmun Analyzer I-2P. Results were evaluated semi quantitatively by calculating a ratio of the extinction value of serum sample to the extinction value of the calibrator 2 which was included into the test. Results were considered as positive (ratio equal to or grater than 1,1), borderline (ratio between 0,8 and 1,1) or negative (ratio less than 0,8). All borderline samples (5) were grouped negative for interpretation of the results.

First attempt to investigate the presence of WNV antibodies in humans was performed in a sample of 27 patients (aged 11 to 68, from 4 municipalities in Vojvodina – Ada, Beocin, Novi Sad, Novo Milosevo) who were hospitalized at Clinic for Infectious Diseases Novi Sad for encephalitis or meningoencephalitis in the period 2001-2005. During September and October 2007, serum samples were collected from 152 randomly chosen healthy persons from 4 municipalities of Vojvodina (Backa Palanka, Beocin, Kisac, Novi Sad). Sera consisted of 101 males and 51 females, aged 18-74 years, were tested for IgG antibodies. In 2009, 182 inhabitants from Novi Sad with no history of neurological diseases (72 children aged from 1 to 18, 34 boys and 38 girls, and 110 adults aged from 19 – 84, 105 females and 77 males) were tested. In the study conducted in 2010, 72 sera samples of randomly chosen healthy person and 18 patients with meningoencephalitis from Novi Sad were tested.

All persons included in the study were interviewed about possible exposure to mosquito bites. Type of housing, proximity to mosquito breeding sites, length of time spent outdoors, presence of window/door mosquito screens, use of mosquito repellents, presence of mosquitoes in the home

and fever/neurological disease history were considered as the risk factors and possible indicators (the last one) of WNV infection.

Mosquitoes were collected from June to October by dry ice-baited NS-2 trap (14) at 66 sampling locations distributed in 29 settlements of Vojvodina province (Ada, Backo Gradiste, Backo Petrovo Selo, Banatsko Novo Selo, Becej, Bela Crkva, Begec, Beocin, Bukovac, Coka, Futog, Glogonj, Hajdukovo, Jabuka, Kanjiža, Kovilj, Kupinovo, Mol, Novi Kneževac, Novi Ledinci, Novi Sad, Novo Milosevo, Palic, Pancevo, Petrovaradin, Rakovac, Sremska Kamenica, Starcevo and Subotica) within the area ranging from 44°42'12" to 46°06'06" North and 19°37'14" to 21°25'01" East. From 2005 to 2009 sampling sites were chosen according to hypothetically higher probability for virus occurrence eg. migrating birds resting places, high abundance of *Culex* spp. mosquitoes. In 2010, traps were placed in "hot places" identified by the previous year serological surveillance in human population. Specimens sampled were anesthetized by dry ice, identified to species (15,2) on dry ice cooled paper, pooled according to date, location, and species, transported on dry ice to the laboratory and stored at -70°C before testing. Pool size for rapid antigen panel assay (VecTest®) did not exceeded 50 mosquito specimens while for virus isolation in Vero cells, and RT-PCR maximum number was 200 specimens per pool.

Rapid antigen panel assay (VecTest®) and virus isolation in Vero cells were performed according to manufacturer instruction and standard procedure (16). After being processed for virus isolation, mosquito cultures were inoculated into Vero cells and examined daily for evidence of viral cytopathic effect (CPE) for 7 days.

From 2005-2009, 61 mosquito pools were tested by rapid antigen panel assay (VecTest®). Additional 730 were screened for cytopathic effect on Vero cell. Positive and suspect samples (115 pools) were further analyzed by generic RT-nested-PCR for detection of flaviviruses (17). In 2010 when WNV was circulating in surrounding areas the scheme was changed, and 50 pools were analyzed by specific RT-PCR in mosquitoes sampled at hot spots only.

For detection of viral RNA in 2010, pools were homogenized in sterile phosphate buffer and centrifuged at 13 000 rpm for 1 minute. The WNV Real – TM (Sacace Biotechnologies S.r.l., Como, Italy) kit was applied according to the instructions provided by the manufacturer. In order to control the process of isolation Internal Control, Negative Control and Pos WNV-RNA-rec control were applied. For control of amplification RNA-buffer and cDNA-WNVc were used. RT PCR was performed using Applied Biosystems 7500 instrument.

Results

ELISA IgG testing of human sera revealed the seroprevalence of WNV in Vojvodina (Serbia) and determined the frequency of persons who were probably in contact with the WNV regardless of the time when the contact occurred. Considering the results of ELISA IgG tests (Table 1), 15 out of 406 healthy persons (3.69%) and 3 out of 45 persons who suffered from viral meningoencephalitis (6.67%) were seropositive for WNW antibodies. Average seroprevalence of WNV in samples taken from 2001-2010 was 3.99% (18 out of 451).

Table 1. Seroprevalence of WNV in persons from Vojvodina (2001-2010)

Status	IgG positive (ratio ≥1,1)	IgG negative* (ratio <0,8)
Healthy (N=406)	15 (3,69%)	391(96,31%)
Meningoencephalitis in the past or acute meningoencephalitis (N=45)	3 (6,67%)	42 (99,33%)

** all bordereline samples (5) with ratio 0,8≥ n <1,1 were treated negative*

IgG ELISA of sera of the 27 patients who were hospitalized for encephalitis or meningoencephalitis in the period 2001-2005 revealed presence of WNV antibodies in two samples (7.41%), a woman aged 36 from Ada and an 11 years old boy from Novo Milosevo. In 2010, IgG antibodies were found in 1 of 18 patients with viral meningoencephalitis (5.55%). During 2007, IgG antibodies to WNV were found in 3/152 serum samples (1,97%) obtained from Backa Palanka, Kisac and Novi Sad and in 2009 in 11 out of 182 healthy persons (6.04%) with no history of neurological diseases. Throughout 2010 IgG antibodies to WNV were found in 2/90 (2,22%) serum samples (1/78

random and 1/18 with viral meningoencephalitis history) samples taken from inhabitants of Novi Sad. Sex and age distribution of seropositive persons was slightly shifted to males and age group above 35 years (Table 2).

A total of 337 persons were exposed to at least one risk factor (Table 3). Among them, 5.04% were seropositive to West Nile virus. Most of the probably infected people did not screen windows and doors in their houses, either alone or combined with one of the other risk factors. In the group of persons who denied exposure to any of risk factors only 0,88% were seropositive to West Nile virus.

Table 2. Distribution of seropositive (ratio ≥ 1.1) persons according to sex and age (2001–2010)

Age group (years)	Males	Females
< 18	4	1
18-25	2	1
26-35	0	0
≥36	5	5
Total	11	7

Table 3. Seroprevalence of WNV in persons from Vojvodina by risk factors (2001-2010)

IgG against WNV	Risk factor/s	
	Present Number (%)	Absent Number (%)
Seropositive	17 (5,04)	1 (0,88)
Seronegative*	320 (94,96)	113 (99,12)
Total	337 (100)	114 (100)

** all bodreline samples (5) with ratio 0,8≥ n <1,1 were treated negative*

During six years of surveillance, a total of 56757 mosquitoes belonging to 15 species (*Anopheles claviger*, *An. hyrcanus*, *An. maculipennis*, *An. plumbeus*, *Aedes cinereus*, *Ae. rossicus*, *Ae. vexans*, *Ochlerotatus annulipes*, *Oc. caspius*, *Oc. dorsalis*, *Oc. sticticus*, *Coquelletidia richiardii*, *Culex modestus*, *Cx. pipiens*, *Culiseta annulata*) were sampled by dry ice baited traps and separated into 841 pools.

None of 176 pools of mosquitoes sampled in 2005 and processed by VecTest® (61 pools) or on Vero cells (115 pools) was WNV positive but positive results after RT-PCR for flavivirus amplification were obtained in 3 pools, sequences being related to cell fusing agent virus (CFAV).

All three pools were sampled at Vojvodina northernmost location, Ludasko lake near Hajdukovo (46°06'06" N and 19°49'53" E) and made of *Ae. vexans* mosquitoes.

No WNV was found in the 615 pools processed on Vero cells between 2006 and 2009. Data obtained from serological surveillance in 2009 had indicated seven "hot spots" of possible WNV transmission in the municipality of Novi Sad (capital of Vojvodina Province). In 2010 mosquito trapping was focused to previously identified "hot spot" streets, city districts and suburbs. Three out of 50 pools of *Cx. pipiens* sampled in one of seven identified "hot spot" areas (within the perimeter 1 km, Detelinara borough of Novi Sad - 45°15'18" N, 19°50'41"E) were positive for WNV RNA.

Both CFAV and WNV detections in mosquitoes are the first records of such type in Serbia.

Discusion

Surveillance of WNV in Serbia is characterized by more then a 30 years long gap, last survey being published by Bordjoski et al. (13). Despite the epidemic in neighboring Romania in 1996 (9) surveillance of WNV has continued to be neglected until this work. Only recently surveillance in birds, mosquitoes and horses had been conducted (18,19).

Serological findings throughout 2005 to 2009 showed that WNV is probably present in Vojvodina. First positive sera were obtained from patients who were hospitalized for viral encephalitis or meningoencephalitis of uncertain etiology during a five-year (2001-2005) period, IgG seroprevalence to WNV being 7.41%. In serum samples taken from 105 patients from Serbia, occupationally exposed to mosquito bites, IgG antibodies to WNV were confirmed in 4,76% by indirect immunofluorescence assay (20) while during this work IgG antibodies were detected in 5.04% of blood samples from people exposed to one or more WNV risk factors (most of them without window/door screens). Average prevalence of IgG antibodies in human sera registered during our survey was 3.99%, with yearly rates varying between 1.97% and 6.04%. In different parts of Italy the incidence of hemagglutinin inhibition antibodies to WNV ranged from 2 to 23%, in Greece from 1 to 27%, in Bulgaria 3%, in Slovakia from 1 to 4% and in

Austria from 1 to 6% (6). In Hungary, 5312 persons were examined and 30 (0,56%) had antibodies against WNV (21). One study carried out in Germany included 14437 blood donors tested by ELISA IgG test for WNV and revealed that 5.9% were initially anti-WNV reactive (22).

The incidence of antibodies in the tropics was higher than that reported for Europe. In Ghana seroprevalence of IgG antibodies to WNV ranges from 4,8% in children to 27,9% in adults. The same study found that 2,4% of children had IgM antibodies against this virus (23). In a similar study, IgG antibodies to WNV ranged from 18,4% in children under 14 years of age to 51,7% in patients aged over 44 (24). In this work, seroprevalence in children was 6.94% (5/72), and 9.09% (10/110) in samples from people over 36 years of age. No considerable bias of the cases concerning gender of individuals tested within older age groups (above 18) was observed, but probable infections were diagnosed in more boys (80%) than girls (20%), the ratio not being biased by the sample size.

During the last decade, human infections with WNV were registered in France, Germany, Denmark and Scandinavia (25,26,27,28), with an increasing trend of number of patients with WNV infections of central nervous system in northeastern Italy and Hungary (21,29). In 2010 major outbreaks of WNV infection in Europe were registered in Greece, Romania and Russia with the case fatality rate of 13.3%, 8.8% and 2.60% respectively (11,12,28,30). Small genetic changes in viral RNA can significantly enhance virulence to humans, possible scenario of the outbreak of WNV lineage 2 in 2010 in Greece. Lineage 2 WNV strains were previously thought to be of low virulence. Nevertheless recent studies in South Africa suggest that lineage 2 WNV strains are a cause of neurological disease in horses and humans (31). "Parental" WNV lineage 2 strain was of much lesser virulence in Hungary and Austria (32,33) and then after registered change by one of (neuro-) virulence and pathogenicity markers (Nowotny personal communication) suddenly induced outbreaks in human population in Greece in 2010. Sequencing of viral RNA found in *Cx. pipiens* mosquitoes in Novi Sad is in progress, and up to now it is proven that it belongs to WNV lineage 2 strain.

Serologic research indicated the likely presence of West Nile virus in Vojvodina. However, former attempts to detect the virus in mosquitoes in Vojvodina and the other parts of Serbia were not successful (18). In this work, RNA of WNV was detected in urban *Culex pipiens* mosquitoes. Many other species of the genus *Culex* are involved in WNV transmission worldwide (34,35,36). The proliferation of the probably main vector, *Culex pipiens*, in certain parts of Europe (12), the increasing virulence of the virus and the recent outbreaks of WNV infections in humans in USA and Greece are timely reminders that WN fever is an important reemerging vector borne disease in Europe.

Detection of a flavivirus sequence similar to CFAV in *Ae. vexans* was the second record of similar viruses in Europe, after Spain from 2001 to 2004. The flavivirus sequences identified in Spain (37,38) were different from all known Flavivirus mosquito viruses, but very close to Kamiti River virus or cell fusing agent virus.

Currently, the routine diagnosis of WNV infection in humans is not carried out in Vojvodina. The incidence of clinically manifested human cases is not known. According to the results of this study the virus is present in Vojvodina and circulates among urban mosquitoes and likely humans. The introduction of diagnostics of WNV infection in regular medical practice is needed in order to establish the system for accurate diagnosis and consequently more efficient treatment of serious clinical manifestation of WNV infection. Due to absence of routine diagnose praxis and limited financial resources in hospitals of Serbia the human cases that should be submitted to RT-PCR tests during the infection are overlooked. In addition, the regular sentinel chickens and mosquito surveillance does not exist. Therefore, system of searching for the virus and its detection in Serbia have been set backwards, based on serological testing of humans in order to optimally utilize available number of test kits (restricted by funding) and minimize the area (and costs of kits) for mosquito surveillance. When grouping of the IgG positive people (likely cases) is detected, "hotspots" for sustainable virus surveillance in mosquitoes were defined in order to detect virus, raise the awareness of public health officials and prevent probable outbreaks and future infections in humans and horses.

Conclusion

Currently, the routine diagnosis or WNV infection is not carried out in Vojvodina. The incidence of clinically manifested human cases is not known. Nowadays, for majority of health professionals in Serbia WNV is the only one in a long line of ARBO viruses transmitted by mosquitoes in remote tropical regions. According to the results of this and previous studies the virus is present in Vojvodina and circulates among urban mosquitoes and humans. The introduction of diagnosis of WNV infection in regular medical practice would contribute to faster establishment of accurate diagnosis and consequently more efficient treatment of serious clinical presentations of WNV infection. Both CFAV and WNV detections in mosquitoes are the first records of such type in Serbia.

Acknowledgment

This paper was realized as a part of the projects TR 6920B (2005-2007), TR 31084 and III 43007 (2011-2014), financed by the Ministry of Education and Science of the Republic of Serbia, The City of Novi Sad, City Administration for Environmental Protection (2009-2010) and 114-451-2142/2011-01 (2011-2014) by Provincial Secretariat for Science and Technological Development, AP Vojvodina. This work received financial support in the form of a grant from the Fondo de Investigación Sanitaria of the Spanish Ministerio de Sanidad y Consumo as part of the "RICET" project

Abbreviations

WNV West Nile Virus
CFAV cell fusing agent virus
ELISA enzyme immuno test
RT PCR real time polymerase chain reaction

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Evaluation of patient satisfaction with family physicians after implementation of family medicine in Turkey

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Abstract

Patient's satisfaction is a desired out-come measure of quality of health care. It is direct feedback measurement and easy method. We aimed to evaluate level of patient's satisfaction with family physician after family medicine implementation at primary care settings in Turkey. This cross-sectional study was conducted in Turkey between January and May 2011 in 45 family medicine offices. The self-administered study survey including EUROPEP tool was delivered with hand-out by researchers who were not family physicians to patients admitted to family health center. They were collected in closed-box. 1500 surveys were distributed. 1112 surveys were returned. Response rate was 74.13%. Of them, 54% (601) was female and 46% (511) was male. Mean age was 36.1±13.9 years-old. Overall rate of patient satisfaction was 70.9%. Rate for high satisfaction level (good-excellent) ranked between 53.5% and 82.4%. Item of" listening to patients" was rated by patients as highest satisfaction (82.4%). Item related with accessibility to physicians on phone was rated as lowest satisfaction (53.5%) by patients. Level of patient satisfaction with clinical behavior was considerably better than health care organization. In conclusion, the study showed that level of patient satisfaction was relatively high, especially with clinical behavior. The patients were less satisfied with health care organization, particularly with accessibility to physicians via phone and waiting time. We observed that patient satisfaction was improved, compared to before implementation of family medicine, and additionally lower than north European countries, but similar with some of the south European ones.

Key words: Patient satisfaction, Family Physician, Primary Care settings

Introduction

In changing Turkey, improving health care services was one of the foremost issues. In this regard, many strategies and policies have been discussed and developed to improve both quality of care and performance of their health services. Over the past few years, Turkey has made several attempts to modernize and improve national health care services. In Turkey, Family medicine implementation at primary care settings after 2005 was one of the most important attempts.[1]

Family medicine implementation in health system is indispensable basement of health services. Prevention and medication are directly provided by health professionals for the community.[2] There are many measurements to evaluate quality of health care. Vaccination coverage, patient's satisfaction and neonatal and maternal mortality ratio are good indicator for measurements of quality in health services. Patient's satisfaction is direct feedback measurement and easy method. Patient's satisfaction is an important and desired out-come measure of quality of health care.[3-5] Patients have a legitimate and important role as evaluators of health care. Obtaining feedback from patients about the quality of primary health care is a powerful way to develop more patient-centered approaches to healthcare delivery. A number of new tools have

been developed to measure patient satisfaction and quality of care in general practice. However, patients from different countries and varied health care systems all report high level of satisfaction with general practice, which makes the results difficult to use in quality improvement.[6-8]

Satisfaction is a complex and difficult concept. Use of exclusively quantitative methods for its measure is inadequate. This especially applies to efforts aimed at determining the components of satisfaction as they actually are, and not those accepted a priori by researchers. Understanding patient satisfaction with the healthcare provided by family doctors is, first, foremost and essential for improving the quality of medical care.[9-11]

Since past 5 years, patient's satisfaction has not been extremely evaluated throughout Turkey. The present study was first study on satisfaction of Turkish patients admitted to family health centers, covering widespread of country. Herein we aimed to evaluate level of patient satisfaction with health care services provided by family physicians at primary care settings in Turkey after family medicine practice has been implemented since 2005.

Methods

Study design

The population-based study was designed as cross-sectional and conducted by Department of Family Medicine, Duzce University School of Medicine between January and May 2011. 15 cities were selected throughout Turkey. The study was carried out in totally 45 family medicine offices was Ethical issue was approved by ethic committee of our institute. Permission for the study conduction was obtained from local directorship of health of ministry.

Recruitment and sample size

The participants were randomly determined from the patients admitted to family medicine offices. The survey including EUROPEP (European Task Force on Patient Evaluations of General Practice) was applied. The survey was delivered with hand-out by researchers, who were not family physicians, to patients admitted to family health center to participants waiting in waiting room before consultation. Questionnaires were collected in the study box. Participation in the study was depending on willingness and all were informed about study. Informed consent was obtained. The cities in which family medicine has been implemented since at least 1 year were selected.

Data collection

The study survey included demographic features (age, gender, education level, occupation and residency) and EUROPEP tool items. By using this tool, patient's opinion about health care provided by their primary care physicians and nurses last 12-month period was asked. There are total 23 items in this tool. Each item has 5 different answers, ranking from poorest (1) to excellent (5), depending on 5-point Likert scale. The participants were asked to mark only one choice for every item.

Statistics

Data was entered into PC and analyzed by using SPSS (Chicago, IL version **15**). Numerical variables were stated as mean+standard deviation. Categorical variables were stated as percentage and number.

Results

Totally 1500 study survey was distributed, but 1172 ones from 45 family medicine offices returned, resulting in a response rate of 78.2%. Level of patient satisfaction was evaluated in two dimensions of clinical behaviors and health care organizations. Rate of high satisfaction level composed of good/excellent, but that of low satisfaction level composed of poor of point mark of Likert scale. The percentage of patient satisfaction level responded by all participitants was stated in figure 1.

Figure 1. The percentage of patient satisfaction level in clinical behavior

Sociodemographic features

The study enrolled 1172 subjects. 60 surveys were excluded because they were not completely filled. Remaining 1112 surveys were analyzed.

54% of them female (601) and 46% was male (511) Mean age of all participitants was 36.1±13.9 (18-85 years-old). Majority were graduated from primary-secondary school (n=450, 40.5%). 50.6% of subjects were employed. Generally, the participitants were married (n=733, 65.9%). In smoking status, percentage of current, the former and never smokers was 28.1% (312), 14.6% (162) and 57.3% (638), respectively (Table 1).

Table 1. Socio-demographic features of participants

	Number, mean	%, ±sd
Age (years-old)	36.1	13.9
Gender		
Male	511	54.0
Female	601	46.0
Education		
Primary-secondary	450	40.5
High school	341	30.6
University	321	28.9
Smoking Status		
Current	312	28.1
The former	162	14.6
Never	638	57.3
Marital Status		
Married	733	65.9
Single (unmarried, divorced)	379	34.1
Occupation Status		
Employed	563	50.6
Unemployed	549	49.4

**sd: standard deviation*

Evaluation of patient satisfaction level in clinical behaviors

Table 2 and Figure 1 showed level of patient satisfaction in clinical behaviors provided by their family physicians. Clinical behaviors included physician-patient communication, giving information and technical experience. Clinical behaviors were evaluated by 14 items of Europep instruments. 74.1 % of subjects ranked Questionnaire item1 (Q1) "making you feel you had time during consultation" as good/excellent response, and mean score was 4.1±1.1. Q2 item "interest in your personal situation" was pointed as good/excellent by 72.8%, and mean score 4.1±1.2. Mean score of Q3 item "making it easy for you tell him/her about your problem" was 4.2±1.1 and responded as good/excellent by 77.1% of participitants. Item "involving you in decision about your medical care" (Q4) was ranked as good/excellent by 73.2% and mean score was 4.1±1.2. Q5 item "listening to you" had the highest mean score (4.4±0.9) and responded by 82.4% of patients. For Q6 item "keeping your records and data confidential", mean score was 4.3±1.2 and 80.6% of participitants responded as good/excellent. The good/excellent response rate of item "quick relief of your symptoms" (Q7) was 72.1% and its mean score was 4.0±1.2. Mean score and good/excellent response rate for Q8 item "helping you to feel well so that you can perform your normal daily activities" was 4.1±1.2 and 73.6%, respectively. Q9 item "thoroughness" was item which was mostly left empty (n=84, 7.55%), but rate of its response as good/excellent and mean score was not low (74.1% and 4.3±1.1, respectively). Q10 item "physical examination of you" was responded by 79% of patients as good/excellent and scored mean 4.2±1.0. Primary and indispensable health task of primary care settings, prevention medicine, was evaluated by Q11 item "offering your services for preventing diseases", and marked as good/excellent with total mean score 4.1±1.2 by 74.4% of patients. Giving information to patients about their symptoms, illness, tests, and treatment modalities was evaluated by item Q12 (explaining the purpose of tests and treatments) and Q13 (telling you what you wanted to know about your symptoms and/or illnesses. They were responded as good/excellent by 72.1% and 75.0%; their mean score 4.1±1.2 and 4.2±1.1, respectively. The percentage of patients responded Q14 item "help in dealing with emotional problems related to your health status" as good/excellent was lowest (66.0%), compared to other items evaluating clinical behaviors, and mean score was 3.9±1.3. 71.8% of patients responded Q15 item "helping you understand the importance of following his or her advice" as good/excellent, its mean score was 4.1±1.2. Item "knowing what s/he had done or told you during previous contacts" (Q16) had the highest percentage of poor responded by 51 patients, resulting 5.1% and scoring mean 3.9±1.3. Lastly, Q17 item "preparing you for what to expect from specialist or hospital care" was also responded by low number of patients as good/excellent (68.9% and 3.9±1.3 of mean score).

Evaluation of patient satisfaction level in health care organization

Level of patient satisfaction with health care organization provided by their family physicians was

Table 2. Status of patient's satisfaction in clinical behaviors

Items	Poorest (%)	Poor (%)	Indecisive (%)	Good (%)	Excellent (%)	Irrelevant/ Not responded (%)
Q1	3.2	4.8	15.7	22.9	51.7	2.2
Q2	2.9	6.5	15.1	23.8	49.0	2.7
Q3	2.3	5.4	13.5	25.6	51.2	1.7
Q4	3.2	5.5	15.1	25.5	47.7	2.9
Q5	1.9	3.5	11.0	23.4	59.0	1.3
Q6	1.7	3.8	10.1	22.6	58.0	3.8
Q7	3.3	6.2	14.7	26.5	45.6	3.7
Q8	2.9	5.4	15.0	26.1	47.5	3.1
Q9	3.0	2.9	11.9	20.5	53.6	8.0
Q10	2.5	4.2	12.9	27.2	51.8	1.3
Q11	4.0	5.0	12.4	26.9	47.5	4.1
Q12	3.1	5.1	16.2	24.5	47.6	3.5
Q13	2.2	4.8	15.1	26.7	48.3	2.9
Q14	3.7	5.6	18.1	21.2	44.8	6.5
Q15	2.5	5.0	16.9	23.7	48.1	3.8
Q16	4.6	6.6	14.2	23.1	47.5	4.1
Q17	2.7	4.9	17.3	24.8	44.1	6.2

Q1: *making you feel you had time during consultation;* **Q2:** *interest in your personal situation;* **Q3:** *Making it easy for you tell him/her about your problem;* **Q4:** *involving you in decision about your medical care;* **Q5:** *listening to you;* **Q6:** *keeping your records and data confidential;* **Q7:** *quick relief of your symptoms;* **Q8:** *helping you to feel well so that you can perform your normal daily activities;* **Q9:** *thoroughness;* **Q10:** *physical examination of you;* **Q11:** *offering your services for preventing diseases;* **Q12:** *explaining the purpose of tests and treatments;* **Q13:** *telling you what you wanted to know about your symptoms and/or illnesses;* **Q14:** *help in dealing with emotional problems related to your health status;* **Q15:** *helping you understand the importance of following his or her advice;* **Q16:** *knowing what s/he had done or told you during previous contacts;* **Q17:** *preparing you for what to expect from specialist or hospital care.*

evaluated by 6 items from Europep instruments, and percentage of responders and mean score was shown in Table 3 and Figure 2. Mean score and response rate of good/excellent for items evaluating health care organization was lower, compared to items of clinical behaviors. Q18 item asked satisfaction with personal staffs other than physicians, and it is highest mean score among other 6 items evaluating health care organization, resulting mean score 4.0±1.3 and 70.8% patients responded as good/excellent. Mean score for Q19 item "getting an appointment to suit your situation" was 3.8±1.5 and responded as good/excellent by 64.9% of them. Item Q20, Q21, and Q22 had lowest mean score. The percentage of patients who left empty and responded as not relevant for those items was high. Q20, item "getting through to the practice on the phone" and Q21 item "being able to speak to the general practitioner on the telephone" evaluated the level of patient satisfaction with access by phone to physician. Q20 had lowest mean score and response rate for good/excellent point among all items of Europep instrument, it was 3.5±1.7 and 53.5%, respectively. Q22 item "waiting time in the waiting room" was also important item evaluating health care organization. Ratio of good/excellent response for Q22 was 58.8% with mean scoring 3.5±1.5. Finally, Q23 item "providing quick services for urgent health problems" was responded as good/excellent by 66.3% patients, resulting in 3.8±1.5 of mean score. The number of patients who rated irrelevant or did not responded was comparably high in items of health care organization.

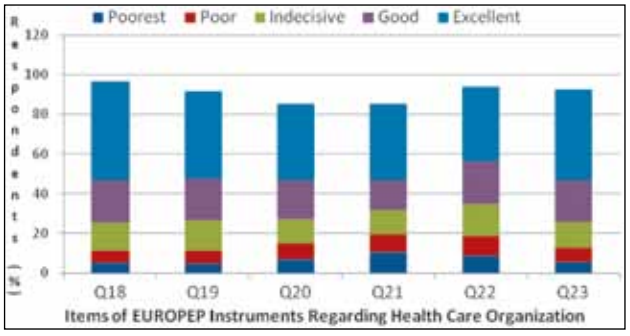

Figure 2. The percentage of patient satisfaction level in health care organization

Table 3. Status of patient's satisfaction in health care organization

Items	Poorest (%)	Poor (%)	Indecisive (%)	Good (%)	Excellent (%)	Irrelevant/ Not responded (%)
Q18	5.0	6.5	14.1	21.6	49.2	3.6
Q19	4.7	6.9	15.0	21.1	43.8	8.4
Q20	7.0	8.1	12.3	19.2	38.8	14.6
Q21	10.5	8.9	12.2	15.6	37.9	14.8
Q22	8.8	9.9	16.3	21.2	37.6	6.9
Q23	5.5	7.1	13.4	21.2	45.1	7.7

Q18: *the helpfulness of the stuff*; **Q19:** *getting an appointment to suit your situation;* **Q20:** *getting through to the practice on the phone;* **Q21:** *being able to speak to the general practitioner on the phone;* **Q22:** *waiting time in the waiting room;* **Q23:** *providing quick services for urgent health problems.*

Discussion

Patient satisfaction is generally considered as the extent to which the patients feel that their expectations and needs are met by the health care services. It is an important issue both for evaluation and improvement of healthcare services.[12,13] The present study, to our knowledge, was the first study with large sample size, evaluating the level of patient satisfaction in primary care settings after family medicine practice was completed throughout Turkey in 2010.

The assessment of patient satisfaction is an important concern in the evaluation of health care system. Measures of satisfaction must be valid and reliable if they are to be used widely. Use of an internationally developed and validated instrument, EUROPEP, for patients' satisfaction with health care provided by their family physicians ensures reliable international comparisons.[14] EUROPEP (European Task Force on Patient Evaluations of General Practice) tool was developed for evaluating patient's opinion and contracts between health authorities and primary care physicians and nurses. Turkish version of EUROPEP tool was validated by Akturk and colleagues.[15] In the present study, Turkish validated type of EUROPEP instrument was used. The study design ensured participation of the patients from 45 practice settings all over Turkey.

We found that patient satisfaction was lower than north European countries, but similar with some of the south European countries. Results of this study showed that the satisfaction with family physician in Turkey can be compared to the satisfaction level in previous study before family medicine practice in primary care settings and also other European countries. Overall level of patient satisfaction was reported as different rate in countries, depending on several factors such as cultural diversity, patient's perspectives and expectations. Fan and college reported that level of patient satisfaction was influenced by method of questionnaire administration and the response rate.[16] Responding rate of our study was relatively high (78.2 %). In the present study, the high response rate and relatively high satisfaction make us ensured that the results are a valid evaluation of patient satisfaction with family medicine practice. Education level and status of economically development might have effect on patients' perspective and expectation. Hence, difference in patient satisfaction between countries can be observed.

We found that rate of response for good/excellent ranked between 53.5% and 82.4%. In the present study, the highest response rate was observed in clinical behavior and lowest rate response was observed in health care organization. According to results of our study, the level of satisfaction with family practice in Turkey was relatively high, a mean of 70.9% of respondents rated items of the questionnaire very good excellent/ after transmission to family medicine in primary care settings. It was less than the findings of Kersnik et al, reporting 88.3%.[17] Our results indicated that it was better than Al-Eisa et al. did. They reported that overall rate of patient satisfaction was 63.9% in their study.[18]

Majority of patients (53.5%) dissatisfied with "not easily to speak to their family physicians on phone". Next item the patients dissatisfied was Q22 item "waiting time in the waiting room". Generally, the patients dissatisfied with waiting in waiting or resting room for long time. The highest response rate of survey (good/excellent) was item

of "listening to you", resulting in 82.4% and mean score of 4.4±0.9. In primary health care provided by family physicians, organization of health care has important effect on patient satisfaction. Determents of health care organization such as "getting an appointment", "through on phone", "being able to speak to physicians on the phone,", waiting time in the waiting room", and " providing quick service for urgent health problem" were lower responded for good/excellent by the patients in the study. Hjortdahl et al. reported that patients more satisfied with general practice if they experienced short waiting times.[19] In European countries, different satisfaction in item of "getting through the practice on phone" was reported. In Denmark (53%) and Norway (56%) were the countries in which lowest patient satisfaction level was detected.[20] Here, we found rate of response (good/excellent) 53.5% (mean: 3.5±1.7). It was comparably lower than reported in those results. Akturk and colleagues conducted a study on patient satisfaction in primary care setting throughout Turkey before family medicine practice. They reported similar results as in our results. They also found that satisfaction with health care organization was comparably low.[21] We observed that patient satisfaction was improved, compared to before implementation of family medicine.

There was one reported study on evaluating patient satisfaction in family medicine practice after 2005 in Turkey, but it was regional. It was conducted by Baltaci and colleagues. in Duzce province, where it was pilot area family medicine practice started to practice in Turkey in 2005. They reported that overall rate of patient satisfaction was 94.5%. They also found that "speaking to their physicians on phone and getting through to the practice on the phone" was relatively low (48.1% and 58.4%), compared to other items. However, they were lower than our results.[22] Low rate of patient satisfaction with some items of health care organization might be due to limited time and overwork load in family health offices, because the number of population attributed to family medicine office is quite large compared to European countries. We also observed that level of patient satisfaction with clinical behavior provided by their family physicians was consistent with their results regarding health care dimension.

It has long been recognized that difficulties in the effective delivery of health care can arise usually from problems in communication between patient and physicians rather than from any failing in the technical aspects of medical care. Improvements in physician-patient communication can have beneficial effects on health outcomes.[23-26]

There are some limitations and priorities in our study. Firstly, the study survey was applied to patients waiting in waiting room. It could be effect on some items and patient perspectives. Secondly, it was applied to patients by their family physicians. Sometimes, physicians might be selective and adherent. Thirdly, the patients over 18 years-old were included. The perspective of patients under 18 years-old was not elicited. The cities in which family medicine has been implemented since one year were selected, so that, difficulties to be faced in transition period were minimized.

Conclusion

Patients' satisfaction from healthcare decides the fate of healthcare providers and healthcare delivery system, and hence needs to be periodically measured to enhance the quality of services. The present study revealed some points for improvement in health care provided by family physicians: physician-patient communication skills should be part of the core curricula faculty development for postgraduate and undergraduate education. For effective communication, family physicians need more time during consultations and better practice management including appointment system and easy accessibility with phone, indicating some health care organizational change should take place on the national level allowing family physicians taking enough time and easy access for their patients. To achieve better primary health care services: the physicians should set suitable time for appointment and allow the patients to easy get through phone.

Acknowledge

We would like to thank nurses working in family health centers for their co-operation. We also appreciate local health directory for their facilitation.

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Influence of Nordic walking on body composition of elderly women

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Abstract

This research included 60 women (N=60) with the residence on the territory of the municipality of Novi Sad. The sample of the examinees consisted of women with the average age 58.5±6.90, mean body weight 70.9 kg±15.32 and mean body height 164.8 m±7.24. The examinees were divided in two groups- one experimental (E) which consisted of 30 members and one control (C) with 30 members. The experimental program lasted 3 months and the activities took place three times a week. The subject of examination was the analysis of the effects which occurred as a consequence of implementing an experimental treatment on body composition of women at the older age. During the period of three months the women from the experimental group walked using Nordic walking poles.

According to the obtained results it was established that during longitudinal processes there were some changes in the body composition of women. The variables Body weight (BW-g)- (E=66,54<C=70,46), Percentage of adipose tissue (FP-%) – (E=37,36<C=39,85) and Percentage of muscles (MP-%) – (E=25,82<C=28,00) showed higher values in the experimental than in the control group in the final measurement. This activity, which is not too imposing on the body of the examined women, but is enough to cause changes, is recommended for elderly women. Under the influence of this program of exercises body weight was reduced, as well as the percentage of adipose tissue and muscles in the experimental group.

Key words: Nordic walking, adipose tissue.

Introduction

The essential and most valuable aim of doing physical exercises is not achieving top sport results, but continuous exposure to recreational, planned physical effort. An innate feature of human body is the necessity to do physical exercises and with its lasting and adequate implementation we can create basic preconditions for good shape, nice physical appearance, higher intellectual abilities and emotional satisfaction, not to mention the reduction or total elimination of the risks of degenerative changes and diseases.

Walking is one of few activities which suits majority of people: it is pleasant, people spend time outside in the fresh air, it is good for socializing, it does not put such an effort on body as other activities, injuries are rare and there are no special preconditions for its implementation. We meet walkers everywhere, in a park, in the street, individually or in groups.

In 1980 clinical studies decided to connect the use of walking poles with the elements of fitness. Later studies demonstrated that this simple, but efficient exercise significantly improves cardio-vascular activity, musculature and vitality of people (Bös, 2004).

Stoughton, Larkin and Karavan (1992) from the University of Oregon were the first to publish the results of the research about Nordic walking. They examined muscular and aerobic capabilities of sedentary women before and after 12 weeks of training with Nordic poles. The experimental group consisted of 86 examinees aged 20-50. Their maximum oxygen intake (VO2 max.) varied between 34-37 ml/kg/min. The control group continued with their previous habits of doing exercises. Both groups, the one which used Nordic walking poles and the one that did not, walked 30 to 45 minutes four times a week. The intensity was set to 70-85 % from the maximum heart frequency. In both experimental groups, maximum oxygen intake and maximum time spent on a treadmill increased significantly.

Nordic walking is convenient for intensifying trainings and what is especially important is the fact

that joints are protected. The use of walking poles unburdens the passive walking apparatus, such as tendons and connective tissue, back and joints (especially knees) at the rate of 15-30 t per hour. That is why Nordic walking is ideal as a rehabilitation sport for the people with orthopedic injuries.

The study conducted by Baatile et al. (2000) on 16 men aged 72 included exercising during 8 weeks, three times a week, 60 minutes per session. The researchers concluded that regular Nordic walking increases the functional independence and quality of life for the people with Parkinson's disease.

The questionnaire made by Schmidt et al. 2004 included 226 adults from Germany (66% were women). They used Nordic walking as a regular exercise during the winter of 2003-2004. The average age was 52. The main motivation for regular walking was health. 12% of them wanted to try something new, while 6% used Nordic walking as an alternative for ski running in summer. We were interested in the effects of using Nordic walking poles on the body composition of women. The changes which occur during the maturity and old age are common for all people, but at the same time there are significant differences in an individual or among individuals which can be a consequence of genetic factors, behavior of an individual (in the sense of eating or some other habits) and health condition or diseases (Heymsfield, Lohman, Wang & Going, 2005).

Methods

Subjects

This research included 60 women (N=60) with the residence on the territory of the municipality of Novi Sad. The sample of the examinees consisted of women with the average age 58.5±6.90, mean body weight 70.9 kg±15.32 and mean body height 164.8 m±7.24. The examinees were divided in two groups of 30 subjects- one experimental (E) and one control (C). All subjects were enrolled in the study on a voluntary basis. Experimental group included elderly women attending fitness program specially designed for this age group at a recreation center, while control group included 30 randomly selected women.

Treatment

The experimental program was performed three times a week for three months. During the experiment examinees took part in no other physical activity. During the period of three months the women from the experimental group walked using Nordic walking poles. Complete program took place in Limanski Park which offers good conditions for this activity. Students from the fourth year of studies at the Faculty of Sport and Physical Education – Department of Recreation controlled the correctness, range and intensity of exercising during the whole treatment. They were acquainted with the treatment and trained for its implementation in advance. Prior to each activity the examinees knew what the necessary heart rate during the training was. The program of walking was conceptualized in such a way that the examinees were always in the aerobic zone of performance. During walking exercise heart rate was monitored by use of pulse meter and used to determine load intensity. The program was divided into three parts different by the volume and intensity.

The program was divided in three parts which differed according to the range and intensity, which can be best represented by the table:

Table 2 shows differences of burdening according to the age. It was concluded that there was no need to include a lot of burdening since elderly people need lower values of heart frequency to allow aerobic exercising. The only thing that is necessary is to follow the given table data and then there is no need for running because aerobic performance can be achieved by walking only, which is much safer and less demanding.

The optimal intensity of burdening is within the limits of 50% to 90% of maximum heart frequency (Adams, 2004). According to the formula of maximum heart frequency which, according to Earl and Beachle (2004), is 220 – A (two hundred and twenty minus the age), the intensity of

Table 1. Exercise plan

WEEKS	1 – 4	5 – 8	9 – 12
RANGE (minutes)	15 – 30	30 – 40	40 – 55
INTENSITY (the percent of maximum pulse)	60 – 65	65 – 70	75 – 80

Table 2. Aerobic burdening

	Maximum pulse	Heart rate on exercises				
Age	100%	80%	75%	70%	65%	60%
40	180	144	135	126	117	108
45	175	140	131	122	114	105
50	170	136	128	119	110	102
55	165	132	124	115	107	99
60	160	128	120	112	104	96

burdening can be easily calculated. The obtained number represents the maximum recommendable heart beats for safe exercising. In relation to the calculated values, we should determine 60 to 80% of the maximum frequency, depending on the burdening we want to achieve.

Measurements

The content of body composition is a subject of many researches, not only in the field of medicine, but also in sports sciences. The focus, owing to the analysis of health status and estimation of possible health risks, is mostly on the content of fat (Ostojić, 2005). Body composition includes the content of a human organism represented by the size of its existing measurable segments and their division into groups (Ugarković, 2001). According to the American Alliance for Health, Physical Education, Recreation and Dance (AAHPERD, 1989), body composition is a ratio of adipose, muscular and bone tissue in the total body weight. Mišigoj-Duraković (2006) claims that a non-adipose tissue consists of muscles, skeleton and internal organs, while an adipose tissue contains so-called 'important' and 'unimportant' fat. Taking the amount of certain components of body composition into account, it can be concluded that women have a significantly higher share of fat in their body composition than men, which is an indicator of sexual dimorphism. The ratio of adipose and non-adipose tissue changes throughout life and it can be modified by external factors, adequate diet which is adjusted to physical and sports activities.

The values of body composition are calculated according to the measured values of skin folds, body weight and height incorporated in the formula given by Matiegka (1921), taken from (Jović, Perunović and Radivojević, 1982), and modified according to (Stojiljković, Mitić, Mandarić and Nešić, 2005). For the estimation of body composition the following measuring instruments are used:

1. Body height (BH-cm)

2. Body mass (BM-g)

- Thickness of skin folds on an upper arm (TSF Uar-cm)
- Thickness of skin folds on a forearm (TSF Far-cm)
- Thickness of skin folds on a thigh (TSF Th-cm)
- Thickness of skin folds on a calf (TSF Ca-cm)
- Thickness of skin folds on an abdomen (TSF Abd-cm)
- Thickness of skin folds on the back (TSF Ba-cm)

3. Percent of adipose tissue (FP-%) – is calculated using the equation of the program according to Matiegka. Percent of adipose tissue in total body mass is determined according to the calculated variables of skin folds and the constant values.

FP – percent of adipose tissue in the total body mass

D – mass of hypodermic adipose tissue in grams

MT – body mass in grams

d – half of the mean value of calculated skin folds in centimeters

K3 – constant value expressed by the number 1.3

TV – body height in centimetres

These values are included in the following formulae:

$FP = (D \times 100) / MT$

$D = d \times TP \times K3$

$d = (AKNNAD + AKNPAD + AKNBU + AKNPOT + AKNLE + AKNTB) / 12$

$TP = 167{,}2 \times \sqrt{\{(MT \times TV) / 1000\}}$

4. Percent of muscles (MP-%) – For the purpose of calculating percent of muscles, the formulae according to Matiegka were used as well. The

percent of the share of muscles in the total body mass was obtained by measuring the variables of circumference, skin folds and constant values.

MP – percent of muscular tissue in the total body mass
M – mass of muscular tissue in grams
MT – body mass in grams
r – mean value of the radius calculated from the circumference of the measured body segments in centimeters minus half of the mean value of skin folds in centimeters on the same segments.
BH – body height in centimetres
K2 – Constant value 6.5

$MP = (M \times 100) / TM$

$M = r^2 \times BH \times K2$

r = (AONAD + AOPOD + AOBUT + AOPOT)/ / 25,12 – (AKNNAD + AKNPAD + + AKNBU + AKNPOT) / 8

Statistical analyses

The SPSS package (SPSS for Windows, version 15.0, SPSS, Chicago, IL, USA) for personal computers was used for the statistical analyses.

The Multivariate Analysis of Covariance (MANCOVA) was used in the process of establishing the effects of applied experimental treatment. The condition under which the multivariate analysis of covariance was implemented was elimination of the differences between the groups during the initial measurement. After the results were equalized, the realistic effects of experimental program for certain groups of examinees were established. The differences among groups on the univariate level with neutralization on the initial measurement were established by using Univariate Analysis of Covariance (ANCOVA), through altered mean values (M).

Results

Tables 4 and 5 contain basic parameters of descriptive statistics. The average value of body mass in the initial measurement showed somewhat lower value than recommended for that age group (in comparison to recommended 72 kg, Čutović and Borkovački, 1994) and sedentary people at the same age (Đurašković, Janković, Jovanović and Ranković, 1985). These skin folds were used for the purpose of establishing the percent of adipose tissue in the total body mass of all examinees in the initial measurement. World Health Organization (WHO, 1995) gives recommendations for the percent of fat in an organism, depending on the age and sex. For women aged 40 to 59 recommended values vary from 23% to 34%. The mean values obtained in the research are slightly higher and for the experimental group it is 41%, while for the control one it is 40%

Using the values of skin folds and body circumference, percent of muscles in the total body mass was calculated. For the experimental group it was 29%, while for the control it was 27%. Mean values for all examinees were a bit lower in comparison to the values recommended for the elderly (Forbes, 1987) and they were 30%.

The subject of research was the analysis of the effects which occurred as a consequence of applying the experimental treatment on body compo-

Table 4. Descriptive statistic of performance of experimental group during initial measurement (n=30)

	M (±SD)		Min	Max	R
Body mass (TM-g)	70,27	10,62	53,50	90,10	36,60
Percent of adipose tissue (FP-%)	41,64	4,93	30,87	50,39	19,53
Percent of muscles (MP-%)	29,01	5,19	19,16	38,69	19,53

TM – Body mass, FP – Percent of adipose tissue, MP – Percent of muscles

Table 5. Descriptive statistic of performance of control group during initial measurement (n=30)

	M (±SD)		Min	Max	R
Body mass (TM-g)	69,51	9,82	52,50	89,00	36,50
Percent of adipose tissue (FP-%)	40,28	7,63	28,18	60,60	32,43
Percent of muscles (MP-%)	27,06	4,23	17,65	35,48	17,84

TM – Body mass, FP – Percent of adipose tissue, MP – Percent of muscles

Table 6. Multivariate analysis of co-variant for variables for estimating body composition between experimental and control group during final measurement.

Wilks	F	df1	df2	p
,26	8,13	16,00	138,00	**,00**

Table 7. Univariate analysis of co-variants in case of variables for estimating body composition between experimental and control group during final measurement

	M		F	p
	E	C		
Body mass (TM-g)	66,54	70,46	58,19	**,00**
Percent of adipose tissue (FP-%)	37,36	39,85	3,97	**,02**
Percent of muscles (MP-%)	25,82	28,00	6,80	**,00**

TM – Body mass, FP – Percent of adipose tissue, MP – Percent of muscles

sition of elderly women. Taking insight into the results of the multivariate analysis of covariance for the applied variables of body composition in both experimental (E) and control (C) group in the final measurement showed in the Table 6, it can be concluded that there is a statistically significant difference in body composition on the multivariate level at the level of significance .01 (p=.000) between the groups.

Univariate analysis of covariance of variables for the evaluation of body composition between the experimental and control group in Table 7 showed a statistically significant difference in all measured variables at the level of significance of .05. Variables: Body mass (BM-g) - (E=66,54 < C=70,46), Percent of adipose tissue (FP-%) - (E=37,36 < C=39,85) and Percent of muscles (MP-%) - (E=25,82<C=28,00) had lower results for the experimental than control group in the final measurement. Owing to the programme of exercising there was a reduction of the value of body mass, percent of adipose tissue and percent of muscles in the experimental group.

Discussion and conclusions

The survey conducted on 17000 Harvard graduates showed that the people who take regular mild exercises have significantly better health conditions. At the time of conducting the survey most of them used walking as a recreation exercise (Tudor-Locke, Lee, Morgan, Beighle, & Pangrazi, 2006).

Another study at Harvard included 11130 people aged between 40 and 60 and it once again confirmed that doing physical exercises, in the first place walking, reduces the risk of stroke. The longer people walk, the longer they live (Tudor-Locke, Lee, Morgan, Beighle and Pangrazi, 2006).

The exercising described in this paper which lasted three months was the first organized and expertly led program of Nordic walking in Novi Sad. The aerobic character of this activity was achieved by monitoring the given burdening on pulse meter.

According to the obtained results it was established that during the longitudinal processes there were some changes in body composition of the examinees. The table 7 shows the results of every individual variable between the experimental and control group. The values of body mass, percent of adipose tissue and percent of muscles showed lower results for the experimental group than the control one in the final measurement. Reduced results in the final measurement are a confirmation of the influence of Nordic walking on the values of body mass, percent of adipose tissue and percent of muscles. Nordic walking is specific because of the use of walking poles which additionally activate the muscles of back and arms. The influence of Nordic walking on lower limbs must not be neglected either since it successfully reduced the value of adipose tissue (Murphy & Hardman 1998; Murphy, Nevill, Neville, Biddle & Hardman 2002). After the implementation of the program, the results of the percent of adipose tissue, even though they are lower in the final measurement, cannot be labeled as recommended on behalf of World Health Organization (WHO, 1995). However, according to the authors Deurenberg, Yap, & Van Staveren (1998) the examinees now belong to slightly overweight women.

Poehlman et al. (1995) take into account the fact that a part of changes happening in body composition of women is due to menopause, or a consequence of reduced secretion of female sex hormones, first of all estrogen. Entering menopause brings about significant changes in body composition.

The calculated percent of muscles showed that the women who used Nordic walking as a regular exercise experienced reduction of muscular tissue. However, it was not logical that the amount of muscles for the group of women who used Nordic walking poles was reduced. The key lies in the fact that Nordic walking reduced the adipose tissue on the relative level, which resulted in the reduction of the participation of muscular tissue on the absolute level. The changes in body composition which are the consequence of ageing, observed from the aspect of biological theories of ageing, especially neuroendocrine theory of ageing, refer to the occurrence of sarcopenia – gradual loss of muscular tissue during the age which is connected to the deterioration of aerobic abilities (Lexell et al., 1988, Coggan et al., 1992). Physically active women experience this in a milder form since their level of muscle maintenance stays on the same level (Sternfield, 2004; Kylie et al. 2006).

Basic form of walking, which has been used as a recreational activity for centuries, has been accompanied by Nordic walking in order to fill in and improve the effects of walking as an exercise. Finnish study conducted by the authors (Anttila, Holopainen, & Jokinen, 1999) compared basic walking with Nordic walking during 12 weeks for 55 office workers. The results of EMG showed that the electrical measurements of the activities of muscles belonging to the higher part of the body (neck, shoulder and upper back) is significantly higher if walking poles are used. Training with poles increased the mobility of the upper part of back as well.

Introducing Nordic walking poles added burdening to the upper part of a body, arm muscles, chests and back, which was not possible by ordinary walking. Engaging a higher percent of muscles produced better effects of exercising. The use of Nordic walking poles unburdens the joints of lower limbs: foot, knee and hip. Owing solely to a greater engagement of the upper part of a body and the increased support it is possible to unburden limbs. The use of walking poles enables the elderly to be more stable since the surface of support is bigger, which reduces the risk of falling and hurting themselves which can cause fractures. Parkkari et al. (2004) estimated the risk of injuries in various sports-recreational activities. 3657 Finns, aged 15-74 were tested. The highest risk in all recreational and competitive activities is present in squash (18.3%), judo (16.3%) and orienteering (13.6%). Nordic walking showed a very low risk (1.7%). Exactly this type of activity which is not too imposing but is sufficient to cause positive changes is recommendable for elderly women. For elderly people in general safety during physical exercising is very important and this reason is sufficient to recommend Nordic walking as an activity adequate for the elderly. Leg movements necessary for running require the strength which is equal to at least triple value of body weight. During walking joints have to involve only a single or double value of the body weight since one foot is always on the ground.

Owing to all previously mentioned facts, Nordic walking belongs to the mildest endurance sports and it is adequate for all age groups and every level of fitness. Exactly this type of activity which is not too imposing but is sufficient to cause positive changes is recommendable for elderly women.

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Developing and implementing flow diagrams for nursing processes in family planning, genital infections and menopausal period

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Abstract

Establishing standards in healthcare safeguards patients and society as a whole, ensures high quality in healthcare services provided and guides the nursing process. A standard terminology and common language facilitates nurses' and midwives' communication with their colleagues. Quality in patient care may only be achieved by establishing, implementing and updating meaningful standards. In view of the importance and efficacy of flow diagrams, this study was designed as a semi-experimental study with a single group to guide development and implementation of flow diagrams for nursing practices in family planning, genital infections and menopausal period. The study was carried out at 6 gynecology hospitals in Istanbul (3 public hospitals, 2 private hospitals and 1 university hospital; 3 hospitals declined to consent) between May 2009 and March 2010. The sample of the research included a total of 90 nurses and midwifes who had been employed at gynecology, family planning and maternal units of these hospitals for at least six months at the time of the study. Consents from relevant authorities and from an ethics committee was received before the start of the research. Data were collected using "Nurse and Midwife Information Form" and "Efficacy of Flow Diagrams Form". In addition, 11 flow diagrams were developed for nursing practices in family planning, genital infections and problems during menopausal period. Participants were asked to provide their opinions regarding these flow diagrams and revisions were made accordingly. They were asked to use the flow diagrams with at least one patient and then to provide further comments on the flow diagrams and the final versions of the flow diagrams were developed following these comments. There was a significant difference between participants' comments on using the processes described in flow diagrams before and after administering them. The nurses and midwifes found our flow diagrams effective and they showed increased confidence in the benefit of flow diagrams ($p=0.000$ $p<0.01$). Routine use of the developed flow diagrams in nursing is recommended.

Key words: Flow Diagram, Nursing and Midwifery, Family Planning, Menopause, Genital Infections

Introduction and objective

The objectives of standardizing nursing care are to facilitate information exchange and good communication among nurses and to achieve the utmost economization of labor force, supplies, time and other sources during provision of healthcare services. The objectives also involve safeguarding nurses' interests as well as those of the patients, and to protect human health and safety by offering high-quality services (Akdemir et al. 1992). In order that these standards constitute good criteria in achieving the targets set, however, they should be established by a relevant authority, should be based on principles, should clarify functions and should be acceptable and interpretable by the users and should allow for quantitative and qualitative review of and provide the opportunity to revise nursing services (Thomas JE., 2004).

With standards of care, it may be possible to plan all phases of nursing standards in advance. These standards allow for rapid provision of services and ensure high quality thereof by each and every nurse. To this end, utilization of brief, directive, visual flowcharts and clinical guidelines can prove effective in meeting the needs of nurses and midwives (Ertem & Sevil, 2007; Kardağ et al. 2004). Standard care or service means "uniformity of care" in terms of both the pattern and extent. Translating standard nursing care processes into visual formats including healthcare maps and flowcharts will both allow for a more clear vision to the stages of the he-

althcare process and ensure that no step is omitted during the provision of healthcare (Akdemir et al. 1992; Akdemir, 1994; Wersorick B., 1990).

A flowchart is a schematic presentation of a process in simple steps that are required to accomplish a certain task. In brief, flowcharts present algorithms in the form of diagrams. The aim is to offer diagrams and to provide graphical presentations of processes that are easy to use and easy to understand by any user. Owing to these properties, flowcharts constitute a good basis for effective insight, analyses and optimization. Nursing flowcharts are applications which cover all the healthcare aspects of patients with respective diseases and are used to ensure high-quality standards in healthcare. Although there are studies on healthcare protocols and case management methods in Turkey, a review of the literature did not yield any studies on the use of flowcharts in nursing.

Based on this need, the present study was performed with a single-group quasi-experimental design to develop flowcharts for nursing practices in family planning, genital infections and the period of menopause, and to implement these flowcharts in actual practice.

Method

Location and Time of the Study

It was planned to include gynecology departments and family planning and menopause clinics of nine hospitals (3 state hospitals, 3 private hospitals and 3 university hospitals) located in both Anatolian and European sides of Istanbul. Due to the issues in obtaining the required approvals for the study from the two university hospitals, and since one private hospital refused to participate due to high workload, three of these nine hospitals were excluded from the study. Thus, the study was performed at three state, one university and two private hospitals, for a total of six hospitals, between May 2009 and March 2010.

Study design

This is a single-group quasi-experimental study.

The Universe and Sample of the Study

The universe of the study included 137 nurses and midwives in total employed at the family planning outpatient clinics and gynecology and obstetrics departments of six hospitals. The inclusion criteria were nurses and midwives who:

- had been employed at the said departments for at least 6 months at the time of the study,
- volunteered to participate in the study.

A total of 90 participants, 41 nurses and 49 midwives, were enrolled in the study. Seventeen nurses and midwives who served at other departments at the time of the study because the obstetrics department was out of service and 8 nurses and midwives who were on leave during the data collection period, 14 nurses and midwives who had been employed at obstetrics, gynecology and outpatient clinics for less than 6 months and 8 nurses and midwives who refused to participate were excluded from the sample.

Data Collecting Tools

Data collection forms developed by the investigator based on a review of the relevant literature were used. The study data were collected by Nurse/Midwife Information Form (Attachment-1) and Forms to Determine Effectiveness of Work Flowcharts (Attachments-2A and 2b).

1. Nurse/Midwife Information Form (Attachment-1)

This tool includes 23 items questioning nurses' or midwives' ages, educational levels, staff status, years of employment as nurses or midwives, years of employment at obstetrics departments, hospitals and clinics they were currently employed, sources of information they utilized for updates on patient care and treatment, type of media they use to obtain information on occupational updates, whether they regularly followed professional journals/books, whether they received in-service training and/or participated workshops etc, their opinions regarding whether a standardized care is necessary in nursing, their opinions regarding whether a standardized care is necessary in all hospitals and their insights as to what work flowcharts are.

2. Forms to Determine Effectiveness of Work Flowcharts (Attachments-2A and 2b)

Forms to Determine Effectiveness of Work Flowcharts were developed by the investigator based on a review of the relevant literature and

aims to determine the benefits of using flowcharts during nursing and midwifery practices and their effectiveness in nursing care. Based on the nursing process and the PDCA cycle, the forms are a questionnaire with 4 subscales (Planning stage, Implementation stage, Evaluation stage and General review stage) and 17 items of 4 choices. Of the subscales of the questionnaire, the planning stage includes 4 items, the practice stage includes 6 items, the evaluation stage includes 5 items and the general review stage includes 2 items. The effectiveness of the flowcharts were evaluated as based on scores from 1 to 4 (4=highly, 3= partly, 2= somewhat, and 1= not at all). High scores indicate high effectiveness of the flowcharts.

The nurses' and midwives perspectives of the flowcharts were first assessed before their implementation by using Attachment-2A. This measurement was performed for the 4 individual stages of the flowcharts, namely the planning stage, implementation stage, evaluation stage and general review stage. Overall rating score was estimated based on this measurement. Following practice, Attachment-2B was administered to evaluate changes in the nurses' and midwives perceptions of flowcharts.

Approval for the Study

After receiving permissions from nursing managements of the six hospitals included in the study and from the Medical Board, a submission was presented to the Ethics Committee of the Marmara University for ethics committee approval. After obtaining the approval, a further submission was made to the Marmara University BAPKO (Scientific Trials Projects Units) for planning of the thesis as a project (project no: SAG-C-DRP-090909-0291). Written consents of the nurses and midwives stating their approval to participate in the study were obtained before administering the questionnaires.

Data Collection Approach

Planning Stage

- A total of 11 flowcharts for nursing practices were developed based on literature review and contribution of the investigators.
- The 23-item Nurse/Midwife Information Form (Attachment-1) and 17-item Forms to Determine Effectiveness of Work Flowcharts (Attachments-2A and 2b) were developed by the investigators to determine nurses' and midwives individual/professional characteristics, and their opinions regarding current sources for practice and utilization of work flowcharts.

Implementation Stage

First implementation
(May 2009-December 2009)

- Training sessions were organized by relevant nurses by the help of hospitals' nursing managers.
- Training sessions involved administration of Nurse/Midwife Information Form (Attachment-1) first to find out individual descriptive characteristics followed by the Forms to Determine Effectiveness of Work Flowcharts (Attachments-2A and 2b) to identify their perspectives of the flowcharts. They were instructed to put a reminder mark in both forms.
- Following completion of questionnaires, a training session was given by the investigator on the importance and necessity of flowcharts in nursing.
- After training, the developed flowcharts were handed to nurses at corresponding units and they were asked to review them, indicating the revisions they felt necessary on the flowcharts.
- Flowcharts with comments on them were collected back 2-3 weeks later and necessary revisions were made.

Second implementation
(February 2010 - Mach 2010)

- The revised flowcharts were delivered to nurses and midwives. They were asked to use each flowchart at least once during their healthcare practices for a period of 2 weeks to 1 month which aimed to have the nurses and midwives review the flowcharts and test their applicability.
- Besides the final implementation, Form to Determine Effectiveness of Work Flowcharts (Attachments-2b) was administered to find

out whether the nurses used the flowcharts effectively.
- Flowcharts after final assessment and Forms to Determine Effectiveness of Work Flowcharts were collected back during a period of 2 weeks to 1 month.
- Final versions of the flowcharts were established based on the feedbacks provided.

Data Analysis

Results of the questionnaires and applicability outcomes of the flowcharts were evaluated.

Percentage calculations by the SPSS software, t-test, variance analysis and Tukey test were used for statistical evaluation of the results.

Results

The youngest of the participants was 20 years old while the oldest was 53 years old, with a mean age of 31.76 ± 7.78 years. The analysis of the participants' educational levels showed that the percentage of participants with bachelor's degree was quite high (79.41%) at private hospitals, while those with two-year degrees were more common in university and state hospitals (55.5 and 48.93%, respectively).

The analysis of the participants' professional characteristics according to hospital types demonstrated that nurse staff was more predominant in university hospitals and private hospitals (100.0 and 55.88%, respectively), while midwives were more common in state hospitals (72.34%). The highest number of staff (58.82%) with 6 months to 5 years of professional experience was at private hospitals, whereas the number of those with more years of service, i.e. 11 years and above, were higher in public and university hospitals (44.44 and 59.58%, respectively).

As a means to access information, the participants employed at university and private hospitals preferred the Internet (55.56 and 44.11%, respectively), while those employed at state hospitals chose consulting physicians as the primary means (40.43%). Nurses and midwives from all types hospitals remained updated of occupational advances by using scientific sources (the Internet, textbooks, etc.) and most of them participated to occupational workshops (91.17, 85.10 and 77.7% from private, public and university hospitals, respectively).

Most of the participants from all three types of hospitals described flowcharts as "Visual and stepwise presentation of healthcare practices" (38.23,

Table 1. Sources of Information the Participants Access by the Types of Hospital Employed (n= 90)

Sources of Information Accessed	University Hospital		State hospital		Private Hospital	
	n=9	%	n=47	%	n=34	%
Sources Used for Patient Care and Treatment						
Internet, TV etc.	5	55.56	5	10.63	15	44.11
Text books, healthcare journals	4	44.44	9	19.14	13	38.23
I consult to a physician	-	-	19	40.43	3	8.82
I consult to nurse colleagues	-	-	8	17.03	-	-
Other (congress, in-service training etc.)	-	-	6	12.77	3	8.82
Sources Used for Accessing Occupational News and Updates						
Internet, TV etc.						
Text books, healthcare journals	4	44.44	16	34.04	21	61.76
I consult to a physician	3	33.33	13	27.65	8	23.52
I learn from nurse colleagues	-	-	11	23.40	3	8.82
Other (congress, in-service training etc.)	2	22.22	7	14.89	2	5.88
Participation to occupational workshops						
Yes	7	77.77	40	85.10	31	91.17
No	2	22.23	7	14.89	3	8.83

(column percentages taken)*

Table 2. Participations' Perceptions of Flowcharts by the Hospitals Employed (n=90)

Perceptions of Flowcharts	University Hospital		State hospital		Private Hospital	
	n=9	%	n=47	%	n=34	%
Flowchart Description						
Care plan	1	11.11	9	19.15	9	26.47
Visual and stepwise presentation of healthcare practices	5	55.56	37	78.72	13	38.23
Has no idea	3	33.33	1	2.13	12	35.30
Previous Usage of Flowcharts						
Yes	-		29	61.70	16	47.05
No	9	100.0	18	38.30	18	52.95
Wishes that flowcharts are in place in the work setting						
Yes	6	66.66	42	89.36	30	88.23
No	3	33.34	5	10.63	4	11.76

*(*column percentages taken)*

Table 3. Participants' Assessment of Flowcharts Before and After Implementation

Forms to Determine the Effectiveness of Flow Charts and Subscales		Median	n	Standard Deviation	Standard Error	Statistical test (p)
Planning	Pre-test	3.497	90	0.450	0.047	t = -6.391
	Final test	3.878	90	0.453	0.051	p=0.000
Implementation	Pre-test	3.333	90	0.481	0.057	t = -6.920
	Final test	3.802	90	0.460	0.054	p=0.000
Evaluation	Pre-test	3.331	90	0.537	0.929	t = -7.803
	Final test	3.349	90	0.464	0.047	p=0.000
Review	Pre-test	3.572	90	0.511	0.051	t = -5.384
	Final test	3.944	90	0.427	0.057	p=0.000
Overall	Pre-test	57.156	90	8.813	0.054	t = -13.704
	Final test	65.444	90	7.396	0.929	p=0.000

(Paired sample t-test=0.000; $p < 0.01$)

78.72 and 55.56% from private, public and university hospitals, respectively). The participants from state hospitals were those who most frequently used flowcharts (61.70%), although they were not specific to nursing, whereas participants from university hospitals in particular never used flowcharts. Regardless of the type of hospitals, all the participants considered flowcharts necessary and wanted them to be implemented in all areas of practice (88.23, 89.36 and 66.66 from private, public and university hospitals, respectively).

The median values column of the above table demonstrates that there is an increase in the scores after implementation, both for the planning, implementation, assessment and review stages and the overall score. The comparison of participants' assessments using paired sample t-test showed that the column of the table listing values of significance differed significantly between the two measurements in planning, implementing, assessment and review stages as well as in the overall outcome ($p = 0.000$; $p<0.01$).

Discussion

Discussion of the Findings Relevant to Flowcharts

Most of the participants from all three types of hospitals described flowcharts as "Visual and stepwise presentation of healthcare practices" (38.23, 78.72 and 55.56% from private, public and university hospitals, respectively). The participants from

state hospitals were those who most frequently used flowcharts (61.70%), although these were not specific to nursing, whereas the participants from university hospitals in particular never used flowcharts. Regardless of the type of hospitals, all the participants considered flowcharts necessary and wanted them to be implemented in all areas of practice (88.23, 89.36 and 66.66 from private, public and university hospitals, respectively). It is interesting that the participants employed at private hospitals regarded standards as necessary but did not use them during clinical practice. Similarly, the participants' descriptions of flowcharts were in accordance with the literature, which showed that they were knowledgeable about these tools, although they made limited use of them in practice, indicating that there are issues in putting these standards to practice.

Flowcharts are schematic presentation of a process in simple steps required to accomplish a certain task. In brief, flowcharts present algorithms in the form of diagrams. The aim is to offer diagrams and to provide graphical presentations of processes that are easy to use and easy to understand by any user. Owing to these properties, flowcharts constitute a good basis for effective insight, analysis and optimization. Steps that should be taken to achieve resolution are presented verbally with no space for any alternative interpretation. They are then described and schematized in detail according to priority (Güven, 2007). These schemes also enable users to know what they may encounter in the next step and helps to see the steps that should be followed. It helps to identify the deficiencies and to make improvements.

In the present study, most of the participants' descriptions of flowcharts were in accordance with the descriptions provided in the relevant literature and again, most of them stated that they were knowledgeable about these tools, although most of them defined that they did not use flowcharts of nursing and midwifery at all in practice, indicating a problem in translating knowledge to practice. According to feedbacks received from the participants, flowcharts were necessary in all settings while occupational flowcharts were lacking or even missing in some areas, and, therefore, they were unable to make use of them, while nurses, in particular, stated that flowcharts developed by peers would offer convenience in every respect of the profession.

In the study by Ergun (1998), the results obtained from the control and experiment groups showed that the patients in the experiment group, who received a standard nursing protocol, had higher levels of quality of life and considered the given nursing care to be very satisfactory (Ergun 1998). In the study by Turan Fedai (2002), it was reported that implementation of standardized flowcharts in treatment planning in patients with cerebral palsy resulted in a significant reduction in the mean period of hospitalization and improvement in the measurable outcomes or treatment (Fedai, 2002). Utilization of standards, with proven validity in the literature, in healthcare ensures that patients receive satisfactory and high-quality nursing care (Erefe, 1993; Kaya, 2004). Standards and treatment protocols also have positive impacts on the quality, cost, patient satisfaction and job satisfaction of the healthcare staff (Daş, 1999). These findings support the findings of the present study. Although there are studies into standards, we found it difficult to identify studies examining nursing care standards in obstetrics. We, therefore, believe that studies which will develop standards and investigate their effectiveness in patient care and treatment in nursing settings are needed.

Discussion of the Findings on the Effectiveness of Flowcharts Before and After Implementation

The comparison of participants' assessments using the paired sample t-test demonstrated that the column of the table listing values of significance differed significantly between the two measurements in the stages of planning, implementing, assessment and review as well as in the overall outcome ($p = 0.000$; $p < 0.01$).

The comparison of the results of the pre-test and final-test performed to measure the effectiveness of flowcharts in the present study showed that the participants considered the flowcharts we developed as effective and demonstrated increased confidence in the benefit and integrity of the charts.

In the study by Corpus and Conforti (1996) on the organization of written clinical standards, the authors described that both the period of hospitalization and treatment costs decreased when such written standards are implemented (Uğur & Işık, 2001; Umur, 2008). Eryılmaz (2001) reported improved care quality and patient satisfaction when

nursing care is standardized (Eryılmaz, 1999). In their study, Ayral et al. (2003) found out a marked improvement in the quality of service offered in a rehabilitation center following implementation of a systematic approach to nursing care (Ayral et al. 2003). Many other studies have also reported similar results (Ergül, 1998; Fedai, 2002; Kaya, 2004). A common point that the findings of the present study indicate is that the expectation from implementing a standardized care plan is a shorter time required for patients to recover and, consequently, reduced cost, as well as improved satisfaction from healthcare given, assurance of invariability in care and increased quality of nursing services (Taşçı, 2005). The similar results between the present and other relevant studies support this point of view.

Discussion of the Participants' Perceptions of Flowchart Development and Assessment

Five flowcharts for nursing practices in family planning practices were developed. Participants from family planning centers and outpatient clinics from six hospitals were asked to review and provide their opinions on these flowcharts. During this review, the participants expressed that the steps facilitated tracking of the process and that the flowcharts provided guidance in identifying the overlooked tasks and deficiencies. Participants from family planning units of state hospitals provided minor additions to the points to consider in barrier methods and in hormonal methods and to the issue of obtaining written consent prior to surgical sterilization.

Two flowcharts for genital infections and sexually transmitting diseases were developed. Participants from family planning centers, outpatient clinics and obstetrics departments from the hospitals were asked to review and provide their opinions on these flowcharts. The feedbacks received from the participants indicated that providing nursing services for problems associated with genital infections in a holistic approach resulted in convenience in practice settings, that overlooked or confused tasks could be observed in this holistic picture and that no points were left deficient in practice, with no difficulties experienced in services, as a result of implementing the flowcharts.

Four further flowcharts for menopausal complaints and their treatment were developed. The participants expressed that the highly detailed and informative characteristics of flowcharts for the menopausal process would prove very effective in fulfilling the unmet needs in this area and that they will also provide guidance in the long term. Participants from all types of hospitals provided minor additions to the flowcharts regarding the steps to be taken in eliminating the complaints.

Conclusion and suggestions

The mean age of the participants was 31.76±7.78 years. The number of participants with bachelor's degree was higher at private hospitals (79.41%), while those with two-year degrees were more common in university and state hospitals (55.5 and 48.93%, respectively). As a means to access information, participants employed at university and private hospitals preferred the Internet (55.56 and 44.11%, respectively), while those employed at state hospitals chose consulting physicians (40.43%) as the primary means. Most of the participants from all three types of hospitals described flowcharts as "Visual and stepwise presentation of healthcare practices" (38.23, 78.72 and 55.56% from private, public and university hospitals, respectively). The participants from state hospitals were those who most frequently used flowcharts (61.70%), although these were not specific to nursing, whereas the participants from university hospitals in particular never used flowcharts. The comparison of the participants' assessments using paired sample t-test showed that the column of the table listing values of significance differed significantly between the two measurements in planning, implementing, assessment and review stages as well as in the overall outcome ($p = 0.000$; $p < 0.01$).

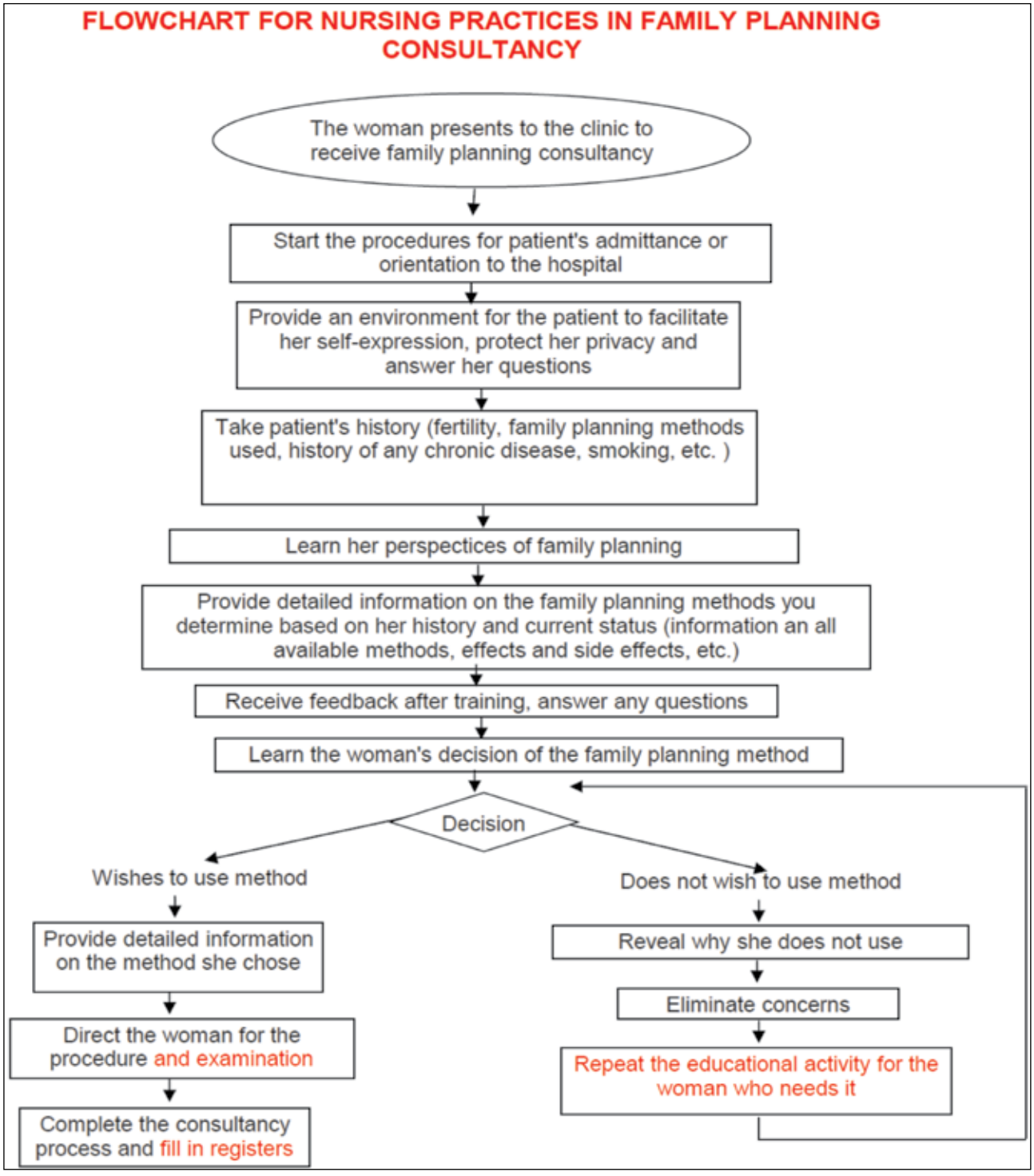
Suggestions

- The standardized flowcharts developed as part of the present study should be used by all nurses and midwives in all areas of practice and their effectiveness should be measured,
- All nurses and midwives should be encouraged to use the flowcharts developed,
- It should be ensured that the student nurses and midwives understand the importance of using standardized healthcare procedures during their training,

- Standardized flowcharts should be developed not only in this are but also in others, through teamwork with involvement and cooperation of academician and clinician nurses and midwives,
- The effectiveness of the developed standards should be reviewed periodically,
- The training of all newly-employed staff at hospitals should include flowcharts.

Acknowledgments

This research was presented and approved as a doctoral thesis in 2011 at the Obstetrics and Gynecology Nursing Program of the Institute of Health Sciences at Marmara University. The research was funded by the Marmara University BAPKO (Scientific Trials Projects Units) for planning of the thesis as a project (project no: SAG-C-DRP-090909-0291).



Example Flowchart 1

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Bioethical Aspects Across Medicine: The Care of Cardiovascular Diseases in Brazil

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Abstract

Bioethics applied to medicine extrapolates the traditional medical concepts of non-maleficence (primum non nocere) and beneficence (bonum facere) and regards to justice, autonomy, equity, protection, compassion and humanization, not considering people just like patients, but understanding the complex existence of each single person. Worldwide, the morbidity and mortality indices regarding to diseases of heart and blood vessels became progressively grater. For countries in development, like Brazil, these numbers are even more expressive and this increase trend seems to be caused by wider exposition of population to some risk factors. This article broaches an intersection between bioethics and medicine, focusing the reality of cardiovascular diseases in Brazil and the necessity of doctors to base their behavior in bioethical paradigms.

Key words: Bioethics; Medicine; Disease; Morbidity; Mortality; Cardiovascular.

Introduction

Bioethics has, since last decade, gained attention in media, for healthcare professionals and for academic circles, especially due to debates about euthanasia, abortion, cloning, genetic engineering, contraception methods. This term was first proposed by Van Rensselaer Potter and André Hellegers in the seventies of the twentieth century[1] and is composed of two Greek roots: "bios", related to life, and "ethics", that refers to custom, character, and human behaviour. Its etymological derivation, however, neither indicates what bioethics represents today nor the activities of those who claim to practice it[2]. Bioethics has become an important knowledge for conflict identification, decision making and policy formulation.

Both proposals, from Potter and Hellegers, aimed to create bridges between the hard sciences, with their rationality and purpose, and those traditional concerns of philosophers and thinkers loosely included under the umbrella term "humanities". This is particularly clear in Potter's designation for this new field as "bridge to the future"[1]. For him, it was a "science of survival" and his point of view consisted in raising awareness about environmental preservation and the interrelatedness of all living beings. A Belgian gynaecologist called André Hellegers, then at the Kennedy Institute of Ethics established at Georgetown University, proposed a link between humanistic thinking and the practice of medicine[2].

For medicine practice, the range of aspects involved in bioethics are wider and brighter than those from traditional medical ethics study, inspired on Hypocrates oath's ideas of nonmaleficence (*primum non nocere*) and beneficence (*bonum facere*)[3]. It transcends the mere concept of prescribing the best tratment options for the patients in professional activities. Bioethics includes in its theorical-practical approach other references like justice, autonomy, equity, protection and compassion, for giving a few examples, to get near from human beings and humanization, not considering people just like patients, but understanding the complex existence of each single person[4]. This is a significant aspect for current health care process.

For those physicians who work in some areas of medicine like cardiology, cardiovascular surgery, neurology, neurosurgery and intensive therapy, to give examples, the nearness of disease and death make even more important the comprehension of bioethical concepts.

Increasing morbidity and mortality rates related to cardiovascular conditions are making more common for these professionals to face situations of providing critic and emergency care or continuous assistance for these patients, needful of attention and respect. Supply these individuals on these needs, obviously beside the technically correct medical treatment, represents practicing bioethics.

Methods

Epidemiological information on this article was given by DATASUS, the national public health databank of Brazil, which is composed by various specific and independent databases. For tracking the number of deaths due to cardiovascular pathologies all over the country, we surveyed the national mortality records system, called SINAM (Sistema Informatizado Nacional de Mortalidade). Quantitative mortality data are classified according to the categories from tenth version of International Classification of Diseases (ICD-10), published by World Health Organization (WHO). Our object of study, cardiovascular pathologies, refers to ICD chapter X, codes I01 to I99, represented as "diseases of the circulatory system".

Results and Discussion

Morbidity and mortality profiles related to cardiovascular motives in Brazil have followed a trend observed in most of world's countries[7]. Risk of developing this sort of diseases increases exponentially according to exposition of people to a certain joint of factors, some of them correctly evaluated and disclosed by well-known clinical trials like Framingham study[8].

In Brazil's specific case, a country in development located on South America with a population near 190 million of inhabitants[9], society's urbanization process, condensing population around major metropolitan areas, beside of the recent increase of population's medium income, has amplified the portion of Brazilian citizens exposed to some of related agents, notably the nourishment and situations of mental and physical stress[8].

Alimentary habits have changed. The consumption of industrialized, high fat foods and excess of carbohydrates raised obesity rates along the years. Modern life obliges people to spend most or their time at work or stressing activities, what takes human body to extreme conditions, secreting excessive quantities of cathecolamines and cortisol. Most of people have a sedentary lifestyle. All of these factors contribute to predispose organism to develop chronic patologies like systemic arterial hypertension and diabetes mellitus, just to cite two of them[8].

According to SIM (Sistema de Informações de Mortalidade), an informatized system that unites national records of mortality numbers and its reasons, and DATASUS, national databank of public health services, 956.337 Brazilian citizens died between 2007 and 2009 due to diseases of the circulatory system. It represents an average mortality index of 1,84 per thousand/year, considered each year population[10]. If this amount is stratified per year, we disclose a significant increase of mortality number in this period.

In 2007, the quantity of deaths in Brazil because of cardiovascular diseases was 308.466. This number became progressively greater along the posterior two years. Was 317.707 in 2008 and ended 2009 at 330.074 [10]. We can infer a well-founded average increase index of 3,4% per year along the analyzed three years, what makes Brazilian rates coherent to another countries' data.

Most of these deaths, about 45 % of total, accounting to 135.438 in 2009, due to specific and fatal events: acute myocardial infarction (55%) and ischemic or hemorrhagic cerebrovascular accidents (45%) [11]. In these cases, doctor's attitude facing bioethical parameters overdraws the relationship between doctor and patient. Beside of managing last critical care and posterior consequences of patient's death, notably if it occurs under doctor's care, the professional faces patient's relatives, needful of attention and sympathy.

But some of patients facing cardiac conditions don't die in acute events. They support for years or decades some clinical conditions that made them unable to do their normal activities. Sociedade Brasileira de Cardiologia (SBC), Brazilian national association of cardiologists says that 3,8 million people, what represents 2,5% of general population, have some type of chronic cardiac disease in Brazil[12].

Nowadays, even after an important technologic and scientific development, the mortality rates of

heart failure can overpass 50% in five years after diagnosis[13]. After 65 years, the incidence of this condition achieve 10 cases per 1.000 individuals[14]. Worldwide, nearly 400.000 patients die each year due to cardiac insufficiency[15].

Information of DATASUS shows that 414.000 patients were admitted by Brazilian hospitals because of heart failure and its complications in 2009 and 28.000 of them died[11]. About 33% of patients at cardiologic units from Brazilian public health system have some stage of heart failure[12].

This patients with heart failure, for example, depending on their disease classification, can't even run up stairways without help[15]. Limitations like this make the diagnosis of cardiac diseases surrounded by stigmas, making patients even more needful of assistance. Caring of these chronic conditions is impossible to be done without compromise with bioethics.

Doctor's behavior based on bioethics, over and above technically correct measures, is disclosed by giving these people (patients itself or its relatives) the necessary, correct and understandable information, being solicitous, accessible and kind[5]. This expedient, although seems effortless, is seldom consummate by healthcare professionals, notably physicians and surgeons.

Physicians and surgeons behavior facing patients and relatives is often a mix of autocracy, superiority and inaccessibility[4]. This type of comportment is completely opposed to those attitudes in which bioethical conduct consists. Bioethical should be the basic paradigm of medical practice.

Conclusions

Concepts about bioethics became more popular recently. Medical schools and academic circles made the study of bioethics a part of its curricula. But most of health professionals seem to not put the bioethical ideas in practice on their daily professional activities, notably on assistance to patients.

Epidemiologic data discloses that doctors will face even more patients with cardiovascular illnesses in their services. These patients will be suffering extreme conditions, several times taking them to death. It is imperative these professionals to have their behavior based on bioethical parameters.

Generally, these individuals waiting or under medical care and treatment are needful of care and attention. Patients with cardiovascular conditions or their relatives, when patient dies, are particularly vulnerable, because of stigmas of this kind of pathologies.

Because of modern life obligations and medium population lifestyle, especially about alimentary habits and sedentary habits, morbidity and mortality due to cardiovascular diseases has increased in Brazil at high rates in last years. Healthcare professionals, principally physicians that develop its professional activities in areas that involve care of patients with these kind of pathologies, need to be able to put in daily practice all of bioethical concepts.

Cardiovascular pathologies carry patients to death through fast or slow ways. In both cases, the health professional will be required to deal with arduous situations. Patients and families don't require only technical measures and prescriptions. They need to be heard, respected, understood and comprehended. All of these abstract notions make part of most particular instances of bioethics.

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Using Conjoint Analysis to Elicit Patients' Preferences for Public Primary Care Service in Serbia

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Abstract

Due to the constant reforms in the field of health care services in Serbia going on in the recent years and the attempt to raise them up to the standards of the developed European countries, a need emerged that the attitudes and the priorities of those for whom these services are designed be heard. Hence the aim of this paper was to identify the preferences of the primary care service consumers in Serbia, having in mind predominantly the non-economic factors and the physician-patient relationship, in order to increase the level of patients' satisfaction without investing large material resources. For that purpose, conjoint analysis was applied. Survey was fielded in Belgrade, Serbia. Out of the 60 patients that were approached, 54 agreed to complete the survey (90% response rate). We found that the cost of appointment is the most important attribute on the aggregate level. Of slightly smaller importance is the option of making appointment by phone, while the treatment selection method is the least important attribute. We also found that the results obtained for a priori defined segments slightly differ from those on aggregate level. On the other hand, using conjoint data we isolated two groups of patients, whose preferences significantly differ from the averaged. The most important attribute for the larger segment is related to physician-patient relationship, followed by the option of making appointment by phone, while the cost of appointment is the last but one by the importance criterion. Accordingly, we have shown that conjoint analysis can provide information to policy-makers to help them more effectively provide an appropriate level of primary health care service.

Key words. Conjoint analysis, primary care, patients' preferences, segmentation.

1. Introduction

Understanding and responding to the patients' preferences is a critical aspect of the current health policy worldwide. This is especially relevant to primary care, which has traditionally been viewed as health care, which is oriented to the needs of patients rather than focused on technology [1].

Generally, preferences are viewed as ideas about what steps should be taken, which can be identified from patients' satisfaction, and is the assessment of care that has been provided [2]. It has been hypothesized that preferences will drive assessments of satisfaction, because meeting the needs of patients will generally lead to higher levels of satisfaction [3, 4].

Two key dimensions of the quality of primary care are: technical care (i.e. the quality of clinical care) and interpersonal care (i.e. the quality of communication between physician and patient). The aspects of the interactions between patients and physicians that are important for patient satisfaction are relatively well known [1, 5, 6]. However, designing services that are sensitive to patients' preferences in the context of limited resources may require patients to choose among attributes of service.

The paper deals with the services of primary health care in Serbia. Namely, due to the constant reforms in the field of health care services in Serbia going on in the recent years and the attempt to raise them up to the standards of the developed European countries, a need emerged that the attitudes and the priorities of those for whom these services are designed be heard, that is, that the preferences of these consumers be determined. Besides, the media in Serbia keep reporting on numerous problems in treating people, often with a fatal end, even after some routine health care services, the consequence of which was a negative attitude of the nation towards the entire health care system in Serbia.

Every reform requires resources, and given that Serbia is a transition country, and that the whole world is affected by the economic crisis, our attention in this paper is focused upon identifying the factors affecting the patients' satisfaction with the primary care service, predominantly the non-economic factors. The identification of the patients' preferences, according to these factors, can highlight the importance of the human factor in increasing the patients' satisfaction, especially when it does not require additional large material resources.

Following the initiative of the Ministry of Health of the Republic of Serbia, and starting from 2004, research has been conducted in Serbia on the patients' satisfaction with varied aspects of health care services, among other things, the satisfaction with the physician-patient relationship [7]. That surveys are based on simple questionnaires in which the respondents are requested to mark the level of current satisfaction on a 1 to 5 scale, however, no deeper analysis is performed to identify the factors or agents that determine the satisfaction level and the measure to which their impact is really felt.

Hence our goal in this paper was to identify the preferences of the primary care service consumers in Serbia, having in mind predominantly the non-economic factors and the physician-patient relationship, in order to increase the level of patients' satisfaction and to change the negative attitude towards the country's health care system, without investing large material resources. For that purpose, in this study conjoint analysis, one of the most widely used preference-based techniques, was applied.

The conjoint analysis is a consumer research technique developed to provide a method for determining the relative contribution of multiple factors to consumer satisfaction. It has been widely used in several fields of economics as well as in marketing research [8, 9, 10, 11]. In the field of health care, it has been used by non-economists to elicit preferences for health care [12, 13, 14]; to identify which factors are important to establish consumer preferences for dental services [15], health insurance [16] and mental health service [17].

During the 1990s this technique was increasingly implemented by health economists. It has been used in a wide variety of contexts, including: analysing optimal service provision [18]; estimating WTP indirectly [19]; looking at patient preferences in the doctor-patient relationship [6]; looking at patient preferences for various attributes of asthma [20, 21] and smoking cessation treatments [22]; discussing prioritizing across clinical service developments [23].

We chose conjoint analysis in this study for several reasons. First, conjoint analysis has the advantage over conventional patient satisfaction models, in that it can be used to identify and, more importantly, quantify the trade-offs that service users are prepared to make between conflicting attributes. The approaches used in the conventional survey of patients' preferences which are usually carried out by applying the simple scaled questions are found to be less effective in health care applications. These approaches often produce low discrimination on attribute importance or sometimes even unreasonable results. Respondents often insist that all attributes are highly important to them. They usually appear to want the highest quality medical treatments and best-trained physicians at the least cost with no waiting time and probably available just around the block from their home. With limited resources it is impossible to fulfill all the wishes of the patients. Second, conjoint analysis allows for the utility estimation for any combination of attributes, including combinations that represent goods or services that may not currently be available. Third, conjoint analysis allows for the preference based segmentation. Namely, conjoint analysis is a micro-based measurement technique, i.e. preferences are measured at the individual level. Hence, if preference heterogeneity is present, the researcher can find it. Another reason we chose conjoint analysis in this study is its cost-effectiveness. Mainly, conjoint analysis does not require a large sample size [24]. The reason is that analysis results in a set of utilities for each individual.

2. Method

2.1. Conjoint analysis: Conceptual Framework

Conjoint measurement has psychometric origins as a theory, to decompose an ordinal scale of holistic judgment into interval scales for each component attribute. Originally developed by psychologist Luce and statistician Tukey [25], conjoint analysis has attracted considerable attention

in the field of mathematical psychology since the mid 70's, especially in marketing research, as a method that portrays consumers' decisions.

Conjoint analysis, sometimes called 'trade-off analysis', reveals how people make complex judgments. The technique is based on the assumption that complex decisions are made not based on a single factor or criterion, but on several factors CONsidered JOINTly, hence the term conjoint. Conjoint analysis enables the investigator to better understand the interrelationship of multiple factors as they contribute to preferences. This approach has been broadly defined as "any decomposition method that estimates the structure of a consumer's preferences given his or her overall evaluations of a set of alternatives that are pre-specified in terms of levels of different attributes" [26]. The basic assumption that underlies the decomposition approach is that customers evaluate the total utility of a product or service by combining the separate utilities to assess the attribute levels of that product/service.

2.2. Survey Procedure

There are five main steps in the design and analysis of conjoint analysis studies [13, 27]. The initial step is to identify the attributes of importance to the study question. We identified six key attributes based on literature review [1], previous work and the pilot survey. In stage two, attribute levels have to be defined. The identified attributes and levels assigned to them are shown in Table 1.

The "Appointment" is the attribute which was not available in the public health care services in Serbia at the time when survey was conducted, and refers to the possibility of making an appointment by phone. However, in the key attribute identification process, primarily in direct interviews with the service consumers, this attribute constantly emerges as desirable, therefore it is included into the research in order to determine the extent to which the patients' satisfaction would be higher if this option were included into the service. The second quoted attribute is the "Cost", for which the following levels are defined "40RSD fee", "Fee up to 750RSD" and "Fee higher than 750RSD". The "40RSD fee" level is included into the analysis because the majority of Serbian population uses the services of the public health care system of Serbia, paying 40RSD for each examination. In case an individual has no health insurance, he/she pays up to 750RSD for each examination service in the public health care centres, or higher than 750RSD in private clinics. The next three attributes, "Manner", "Attitude" and "Knowledge", are related to the interpersonal care i.e. quality of communication between physician and patient. The last defined attribute is the "Treatment" which refers to the patient's involvement in the process of choosing between two or more options of treating certain diseases.

Certain attributes, such as the physician's expertise, the distance to the health care centre, etc., are not included in to the research for a number of reasons. The pre-research has shown that the patients find certain factors less important or simply taken

Table 1. Attributes and levels used in the study.

Attributes	Description	Levels
Appointment	Option of making an appointment by phone	Yes, with the choice of appointment time Yes, with no choice of appointment time No
Cost	Cost of appointment to patient	40 RSD fee Fee up to 750 RSD Fee higher than 750 RSD
Manner	Physician's interpersonal manner	Warm and friendly Formal and businesslike
Attitude	Physician's patience and thoroughness	Listens patiently, does not hasten the patient Performs examination by routine and fast
Knowledge	Physician's knowledge of the patient	Physician knows patient's medical record Physician does not know patient's medical record
Treatment	The treatment selection method	The patient is involved in decision-making as regards treatment The patient is not involved in making decisions as regards treatment

for granted, e.g., the physician's expertise. Besides, the spread of public health care centres is rather satisfactory on the territory of Belgrade, and the construction of new centres would require ample resources. The idea in this study is to identify those factors that do not demand large investments.

The next stage in conjoint analysis study is to decide which scenarios to present to individuals, i.e. to generate experimental design [28]. The attribute and levels in Table 1 gave rise to 144 possible scenarios (3^2x2^4). Since respondents could not realistically be expected to consider such a large number of different scenarios, a component of the statistical package SPSS (Orthoplan) was used to reduce the possible number of profiles to a manageable level, while still allowing the preferences to be inferred for all of the combinations of levels and attributes. The use of Orthoplan results in an orthogonal main effects design, thus ensuring the absence of multicollinearity between attributes. Through the use of this design, the 144 possible profiles were reduced to 16.

Having established the experimental design, the next stage is to elicit preferences for the scenarios. In this study the rating approach was used. Individuals were presented with each of the 18 profile scenarios, 16 from the experimental design and 2 holdout tasks. Individuals were asked to state their level of preference for each scenario on a Likert's scale of 1 to 9, where 1 indicated "dislike very much", and 9 indicated "like very much". Holdout cases are judged by the individuals but are not used to estimate utilities. They are used as a check on the validity of the estimated utilities.

Having collected the information on individual preference, the responses need to be analyzed. To determine the relative importance of different attributes to respondents, the trade-offs that individuals make between these attributes, as well as the overall benefit taking into account these trade-offs, a relationship must be specified between the attributes' utility and the rated responses. The simplest and most commonly used model is the linear additive model. This model assumes that the overall utility derived from any combination of attributes of a given good or service is obtained from the sum of the separate part-worths of the attributes. Thus, respondent *i*'s predicted conjoint utility for profile *j* can be specified as follows:

$$U_{ij} = \sum_{k=1}^{K} \sum_{l=1}^{L_k} \beta_{ikl} x_{jkl} + \varepsilon_{ij}\ ,\ i = 1,...,I,\ \ j = 1,...,J\ , \quad \text{..... (1)}$$

where I is the number of respondents; J is the number of scenarios; K is the number of attributes ($k = 1,...,K$); L_k is the number of levels of attribute k ($l = 1,...,L_k$). β_{ikl} is respondent i's utility with respect to level l of attribute k. x_{jkl} is such a (0,1) variable that it equals 1 if profile j has attribute k at level l, otherwise it equals 0. ε_{ij} is a error term.

To estimate the parameters of the model (part-worths), the statistical package SPSS 16.0 (Conjoint procedure) was used. The parameters were estimated for each respondent in the sample individually (individual preferences), as well as for the total sample (averaged preferences).

2.3. Validity issue

Conjoint analysis tasks are cognitively challenging, and even the most attentive subjects with well-behaved preferences may report some inconsistent responses. Thus, the challenge is to evaluate whether the consistency failures are serious enough to invalidate the welfare-theoretic validity of a subject's responses. In this study, two approaches to measuring validity were used: (1) internal consistency of preferences, and (2) consistency with theoretical predictions.

Internal consistency was measured in three ways. Firstly, in order to provide measures of the correlation between the observed and estimated preferences, Pearson's R and Kendall's tau statistics were used. Secondly, to check the validity of the utilities for just the holdout profiles, Kendall's tau were used. Holdout profiles were rated by the subjects, but were not used by the conjoint procedure for estimating the utilities. Instead, the conjoint procedure calculates correlations between the observed and predicted rank orders for these profiles, as a check of the validity of the utilities. Thirdly, a test of monotonicity was used which postulates that a subject should prefer more, rather than less, of any given good. For example, it can be assumed that, all other things being equal, that respondents prefer warm and friendly approach

and lower prices. Theoretical validity was explored by examining the sign of the parameters.

3. Results

Survey was fielded in Belgrade, Serbia. The only criterion for eligibility was that respondents were 18 or older. Individuals who agreed to participate completed a form that is a small part on demographics. Of 60 individuals who were asked to participate in the survey, 54 completed the questionnaire, giving a response rate of 90%. There were 50 valid questionnaires (giving a total of 900 observations). Slightly more than half of respondents (56%) were women. The average age of participants was 39.04 years (range 18-65 years) with the following age distribution: 18-23 years (34%) 24-50 years (34%) 51 years + (32%). 40% of respondents were students, 20% unemployed, 20% employees and 20% retired.

3.1. Aggregate level preferences

The averaged results from the regression analysis are shown in Table 2. The size of the coefficients suggests that the cost of appointment is the most important attribute. Of slightly smaller importance is the option of making appointment by phone, while the treatment selection method is the least important attribute. The signs of the regression coefficients were all as expected, including the negative coefficient for the lowest levels of attributes. The statistics for the estimated models are presented in Table 2. A high value of the Pearson coefficient, 0.982, confirms the high level of significance of the obtained results. Similarly, a high value of the Kendall correlation coefficient, 0.833, indicates a high level of correlation between the input and the estimated preferences. The Kendall coefficient for two holdout profiles has a value of 1.000, which is an additional indicator of the high quality of the obtained data.

Table 2. Aggregate level analysis (averaged results)

Variable	Attributes' importance	Part-worth utilities	Std. Error
Appointment	**21.831%**		
Yes, with the choice of appointment time		0.513	0.128
Yes, with no choice of appointment time		-0.022	0.151
No		-0.492	0.151
Cost	**22.641%**		
40 RSD fee		0.690	0.128
Fee up to 750 RSD		-0.242	0.151
Fee higher than 750 RSD		-0.448	0.151
Manner	**12.799%**		
Warm and friendly		0.485	0.096
Formal and businesslike		-0.485	0.096
Attitude	**14.596%**		
Listens patiently, does not hasten the patient		0.632	0.096
Performs examination by routine and fast		-0.632	0.096
Knowledge	**18.763 %**		
Physician knows patient's medical record		0.768	0.096
Physician does not know patient's medical record		-0.768	0.096
Treatment	**9.370%**		
The patient is involved in decision-making as regards treatment		0.325	0.096
The patient is not involved in making decisions as regards treatment		-0.325	0.096
Constant		4.209	0.107
Correlations between observed and estimated preferences			
	Value	**Sig.**	
Pearson's R	0.982	0.000	
Kendall's tau	0.833	0.000	
Kendall's tau for Holdouts	1.000		

The most preferred scenario on aggregate level is the following: that it is possible to make an appointment by phone, with an opportunity to choose the time, that the fee for the appointment should be no higher than 40RSD, that the physician is polite and friendly, listens to the patient patiently, already having an insight into the patient's files and being familiar with his medical record, and also to enable the patient to participate in decision-making in the treatment selection. The total utility of such a scenario is 7.62. Since the option of making an appointment by phone was not available in Serbia at the time when survey was conducted, and provided that all the other attributes are at their highest levels, the total utility of the scenario is 6.617. With the introduction of the option of making appointments by phone, the preferences would rise by approximately 15%. On the other hand, in case the physician did not know the patient's case history, the preferences would fall by as much as 23%; if the physician examined the patient in a routine way and hastily, the preferences would fall by approximately 20%, while the rise in the cost of examination up to 750RSD would reduce the patients' preferences by 14%. Such an analysis is possible to be conducted for all the potential attribute combinations.

3.2. Preferences of a priori defined segments

In order to identify whether there are differences among the preferences of certain consumer groups, an analysis was conducted for the a priori defined segments as well. The a priori segmentation was carried out on the basis of the status and gender criteria.

The preferences of the patients segmented on the basis of the status are presented in Table 3. They are perceived to be rather in accord in the groups of students, the employed and the unemployed, while a certain difference is observed in retiree group. The basic difference is the relative importance of the "Cost" attribute, to which retired persons assign a surprising third important position, whereas in the three other groups this attribute tops the list. Similarly, the "Knowledge" attribute has a priority of the first order with the retirees, which can be explained by their need to be socially accepted to a higher degree, which is characteristic of that age. The "Appointment" attribute is second important according to all the segments, whereas the "Treatment" occupies the very bottom of the list.

The research has shown that the preferences of the respondents segmented on the basis of gender differ only slightly; however, these nuances could still serve as an indicator for a slightly different approach to women in comparison to that used for men. Namely, the differences refer to the priorities of the two major attributes that distinguished themselves in the course of the research. The female respondents put the cost of appointment to the top position, while the males find the option of making appointments by phone to be most important. The difference in preferences in these two attributes is not, however, so express, since men place the cost to the second position, meaning that this attribute is important for this segment of population too, while the option of making appointments by phone is assigned the third position by the women. Similarly, the knowledge of patient is a second priority with women, while the men consider it third important. The relative importances of other attributes are similar for both genders.

3.3. Preference based segmentation

Regardless of the fact that no significant differences in the preferences of the demographic se-

Table 3. Preferences of a priori defined segments

	Status				Gender	
	Students	**Unemployed**	**Employed**	**Retired**	**Female**	**Male**
Number of respondents	20 (40%)	10 (20%)	10 (20%)	10 (20%)	28 (56%)	22 (44%)
Appointment	20.00%	19.00%	24.00%	22.00%	19.00%	24.00%
Cost	21.00%	23.00%	25.00%	19.00%	22.00%	21.00%
Manner	15.00%	12.00%	14.00%	8.00%	14.00%	12.00%
Attitude	19.00%	12.00%	13.00%	13.00%	15.00%	16.00%
Knowledge	15.00%	23.00%	20.00%	26.00%	21.00%	18.00%
Treatment	9.00%	12.00%	4.00%	13.00%	10.00%	9.00%

gments have been identified, we used the data on the individual level to conduct the preference based segmentation. For that purpose, *K-means* analysis was applied. The analysis yielded two segments whose preferences are presented in Figure 1.

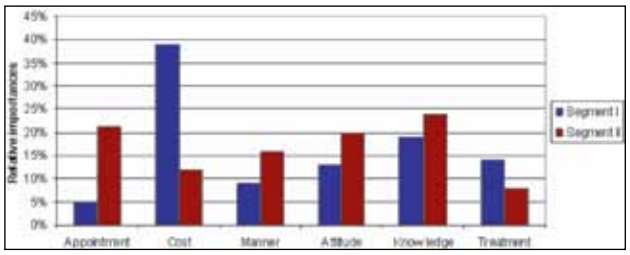

Figure 1. Preferences of post hoc identified segments

The first segment includes 17 respondents (34% of the entire sample), and these are the basic health care beneficiaries, who consider the "Cost" to be absolutely most important attribute (39%). In addition to the cost attribute, these respondents find "Knowledge" to be highly important too (19%), however its importance is twice as low compared to the "Cost" attribute. The very bottom of the list, according to the importance criterion, is occupied by the "Appointment" attribute (5%). This segment can be viewed as a conservative segment who considers it most important to pay as little as possible for the service and that there is a chance that the physician knows them, the other factors remaining relatively negligible. It is possible, however, that such findings result from the level of the respondents' earnings, which are rather low, therefore they cannot but value the cost as the most important attribute of all.

The second segment includes 33 respondents (66% of the entire sample). These are the basic health care beneficiaries, who rate the "Knowledge" as most important attribute (24%), which means that they consider it most important that their physician is well acquainted with their case history, while the "Appointment" and "Attitude" attributes share the second and the third positions on the scale. It is evident that this segment primarily values the so-called non-economic attributes, those concerning the interpersonal relationships on the physician-patient relation, while cost of appointment occupies the last but one position on the importance scale (12%). On the basis of such ratings of the key attributes of the primary care services, a conclusion can be drawn that the patients in Serbia are simply fed up with impoliteness, the physician's formal approach, etc., and that they are willing to pay a higher price, but to get an adequate service in return.

4. Discussion and conclusions

To our knowledge, this is the first study to use conjoint analysis, a theoretically based and statistically rigorous approach, to determine a relative contribution of multiple factors to patients' satisfaction with public primary care service in Serbia.

The findings of the research on an aggregate level show that the "Cost" is the most important attribute, however, not the absolutely dominant, since it is closely followed by the "Appointment", with less than one percentage point down. That the "Cost" attribute will be one of the dominant could be expected for several reasons. Firstly, the price as the criterion in decision making is generally one of the rather important factors taken into consideration when selecting any product or service. This is one of the elementary preconditions of a customers' rational market behaviour. Besides, since the public system of primary care, where the costs for such services are symbolic and are expressed by the amount of 40RSD paid for the service is still dominant in Serbia, it is obvious why the respondents stressed the importance of this attribute: why should they pay 1,000RSD for the service in private clinics, when they can get this same service in public institutions, at far more favourable conditions. Similarly, the economic crisis during which this research was conducted, and when the need for savings was stressed at all times, certainly had a fair impact upon the respondents to assign so much importance to price.

A high level of importance of the "Appointment" attribute although indeed intriguing, is not surprising. There are several reasons that the respondent rated this attribute so highly, although it was not part of the present system of primary care services. One reason could be that people do not like to wait and that they realise that time is money. The reason for this relatively high importance could also be that people in Serbia are always waiting for something – they are always standing in one queue or another.

A rather high level of importance of the "Knowledge" attribute shows that the service users indeed care that the physician knows their case history, as they probably consider it a precondition

of good quality treatment. We can see that this is the first in rank attribute, reflecting interpersonal relations between the physician and the patient, and that it is positioned at the top of the priority list, which shows to the primary care services providers that they can significantly improve the patients' satisfaction by improving this factor. This is also important because these attributes are generally no-cost attributes – no financial investments are necessary to get a higher quality of the service delivered at the shortest possible time.

The reason that the treatment selection method is considered to be the least important attribute may be due to the fact that the attributes deemed highly important by the primary care consumers still remain not satisfied in practice, therefore the priorities of the patients are that it is these attributes that should be satisfied first. It is only then that this attribute can gain in importance, as is the case worldwide. Another reason that the importance of this attribute is so small can be a specific state of mind of the people in Serbia, who have for years lived in a social system in which a passive attitude was systematically encouraged, hence people are still unaware that they can have their say when it comes to the decisions concerning their own well being.

As regards the findings obtained for the a priori defined segments, they differ only slightly from the results obtained on the level of the entire sample. The preference based segmentation of respondents, however, distinguished two groups of respondents. It is in this way that rather intriguing data are obtained. Hence, the findings for the segment including a much larger number of respondents are totally different from those obtained on the aggregate level. The basic reason is that the aggregate data are calculated on the basis of average values; therefore, in case of heterogeneous market they can be misleading.

Namely, the respondents in a larger segment rated the "Knowledge" attribute highest, followed by the "Appointment", "Attitude", and the "Manner" attributes, whereas the "Cost" attribute is the last but one by the importance criterion, which is absolutely contrary to the results obtained on the aggregate level. The possible reason for such results is that the people in Serbia are sick with the so-called "free treatment", characterized by the general indifference of the physician towards the health care service consumers' wishes and priorities, where the physician's approach to the patient is usually formal and businesslike and where examinations are routine. Therefore they are willing to pay a higher price for the service, on condition the service comes in a package together with warmth and kindness, as well as with patience and the knowledge of the patient. These findings may have significant implications in deciding whether to focus upon the cost as a key element of satisfaction, or turn attention to other, non-economic attributes deemed to be more significant by a majority of population.

Since the goal of the research was to show the applicability of conjoint analysis in determining the patients' preferences for primary care service in Serbia, with a special emphasis upon the non-economic factors and their impact upon the patients' satisfaction, the findings obtained and presented above confirm that our task is successfully accomplished.

The findings of the study are significant on both a theoretical and applied levels. On a theoretical level, they add to our knowledge of the relative importance of non-economic factors influencing patients' satisfaction. On the applied level, the results provide information to policy makers to help them more effectively provide appropriate service levels in the area of primary care. In fact, the results can be used to promote the public health system in Serbia, which would result in an increased overall patient satisfaction as well as in changing the public attitude as regards primary care in general.

Acknowledgement

This research was partially supported by the Ministry of Science and Technological Development, Republic of Serbia, Project numbers: III44007 and TR33044.

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Effects of Individual Training of Primary Schoolchildren on Tooth Brushing Skills

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Abstract

Purpose: Regular tooth brushing habits are not satisfactory among children in Turkey. It is important to encourage children to adopt proper health behaviors, such as proper tooth brushing, during their school years.

Methods: The current controlled, quasi-experimental study was conducted by nurses in order to evaluate the effects of individual training on tooth brushing skills of primary schoolchildren. A total of 267 children from primary schools participated in this study. The brushing skills of the children were evaluated by using a tool for measuring brushing skills prepared by the investigators in accordance with the literature. Pre- and post-training-tests were performed to evaluate the brushing skills of the children before and after the training.

Results: Significant differences were noted between the study and control groups in terms of post-training test scores. In the study group, a significant increase was noted in the test scores after the training as compared with their pre-training test scores.

Conclusion: The current study demonstrated that the individual training conducted by nurses was effective in improving the tooth brushing skills of primary schoolchildren.

Key words: tooth brushing, individual training, nursing, children, dental health, Turkey

Introduction

Oral and dental diseases lead to significant work loss. Furthermore, physiologic and social implications of oral and dental diseases have adverse effects on daily life (1) and quality of life (2,3). It has been reported that dental caries and gingival diseases are common in children (4,5)) and have an increasing prevalence (3) in developing countries. The high prevalence of oral and dental diseases in children in Turkey makes this a leading public health problem (6,7,8).

It was emphasized in the World Health Assembly in 2007 that standard hygiene practices could prevent many chronic oral diseases (3). The literature also points out that dental health problems can mostly be overcome by personal oral hygiene (9,10,11). One of the indispensable criteria for providing oral hygiene is tooth-brushing (5,9,12). Tooth brushing, when performed at regular intervals for an adequate time and in the proper manner, is effective in the control of dental plaque formation (9,13) and in preventing dental caries (14).

It is known that regular tooth brushing habits are not satisfactory in developing countries (5,7,15). Studies in Turkey have also revealed inadequate tooth brushing habits in children (7,15). It has been recommended to encourage children to adopt proper health behaviors, such as tooth brushing, during their school years (10,11).

School health services in Turkey are carried out within the framework of basic healthcare services of the Ministry of Health. Healthcare workers (physicians, midwives, and nurses) conduct screening and vaccination programs. However, healthcare workers do not provide a systematic education on adoption of proper oral health behaviors and prevention of oral-dental diseases to schoolchildren. It has been suggested that nurses can participate educational programs on oral health, and that school nurses can play an effective role in preventing dental diseases (16,17,18).

The aim of the current study, in the context of the above-mentioned approach, was to evaluate the effect of an individual training program implemented by nurses on the tooth brushing skills of primary schoolchildren.

Methods

The current study was conducted in a quasi-experimental model. The data for the study were collected between April 2007 and June 2007. The study was carried out at three primary schools in Aydin, a city in Western Turkey. Although there were 28 primary schools in Aydin at the time of the study, the schools included were chosen from areas where children of low- and middle-income families generally attended, considering the fact that such children require more education to improve their health behaviors. Based on the fact that children between the ages of nine and 10 years have the highest risk for dental caries (8), third grade children (aged 9-10 years) were enrolled in the study.

The classes in the schools that would be assigned either the study or control group were randomly selected. Among 339 students who were attending the third grade at the time of the study, 281 (145 in the study group, and 136 in the control group) were enrolled in the study; however, a total of 14 children were excluded due to absence from school during the data collection, follow-up, and evaluation periods. Consequently, the study was completed with a total of 267 children (133 in the study group, and 134 in the control group).

Data collection tools

A questionnaire and a tool for measuring brushing skills were used for data collection. The questionnaire consisted of 8 items concerning the school and the class attended, age, gender and dental health habits of the children. In order to evaluate tooth brushing skills, a tool was prepared by the investigators by reviewing the literature (9,19,20). The tool consisted of 34 items. Each "yes" scored 1 point and each "no" scored 0 points, yielding a minimum of 0 and a maximum of 34. The total score obtained was used in evaluating the level of tooth-brushing skills, with higher scores indicating better tooth brushing skills.

Data collection

Prior to the data collection procedure, the school administrations and teachers were informed about the study. Furthermore, an appointment was made with each school administration for a specific time to collect data. The children were asked to bring their toothbrushes to school on the appointment day.

Prior to the training program, characteristics of the children were recorded by means of personal interviews. The children who did not have their own toothbrushes were provided with toothbrushes. A pre-training test was performed simultaneously in the study and control groups, to evaluate the tooth brushing skills of the children prior to the training program.

Training sessions

After performing the pre-training test, the children in the study group participated in a training session on tooth-brushing skills, conducted by the investigators. The session was held in school libraries. The students were taken into the library in groups of 4-5 and were given a lecture on the significance of tooth brushing, which was followed by a demonstration on how to brush teeth.

It has been suggested that training programs conducted to teach skills should be divided into seven basic elements to facilitate the learning of psychomotor behaviors. It has been recommended that the trainers demonstrate the entire skill during the first step, then focus on the fundamentals in the second step, and repeat the entire skill in the third step. In the fourth step, students should be asked to perform a simple part of the skill on their own, and trainers are recommended to help students to perform the entire skill in the fifth step. The entire skill should be performed again by students under trainer supervision in the sixth step. Finally, in the seventh step, students should perform the skill on their own and repeat it until the skills are improved (21).

Consequently, the steps of the training program on tooth brushing were based on those seven basic factors of teaching skills. The tooth brushing skills training program was then performed by following those steps. First, the investigators demonstrated and repeated the entire procedure used in tooth brushing step-by-step to the students, including how a toothbrush should be held. At this stage, the students observed the investigators and then repeated the process in front of a mirror. It was ensured that the students brushed properly, first the outside surfaces of the upper and lower teeth, and then the inside surfaces of the upper and lower teeth, followed by the inside surfaces of the upper and lower front teeth, and finished by the cutting edges of the teeth. The parts of the procedure that had not been we-

ll-understood were performed by the trainers once again. The children were asked to repeat the procedure until they performed the skill correctly. Each child was allocated about 5-7 minutes of training time. The students were asked to perform the tooth brushing procedure at home exactly as they had been taught. In order to evaluate the impact of the training program, the tooth brushing skills of the children were evaluated by a post-training test 15 days after the training program. Although the children in the control group did not undergo a training program, a post-training test was performed simultaneously with the study group.

Ethical considerations

Written approval of the relevant provincial directorate for national education was obtained for the study. The approval process also included ethical consideration. The children and their teachers were informed about the purpose of the study, and verbal consent was obtained as well. After the post-training test, the children in the control group also underwent a training program on tooth brushing skills.

Statistical analysis

Statistical analysis was conducted using SPSS for Windows (Version 11.5; SPSS Inc., Chicago, IL, USA). Descriptive statistics were used for the children's demographic characteristics and habits regarding dental health. Age, gender, and dental health habits of the children in the study and control groups were compared by using a chi-square test. The Kolmogorov-Smirnov test was used to test normality of data and it was noted that data regarding the pre- and post-training test scores were non-normally distributed. As a result, the Mann-Whitney U test was used to assess the differences between the groups and the Wilcoxon signed-rank test was used to compare the differences between the pre- and post-training test scores of the study group. A p value <0.05 was considered statistically significant.

Results

It was noted that over one-half of the children in the study and control groups were nine years of age; no significant difference was noted between the groups with respect to age (χ^2=0.002; p>0.05). Over one-half of the children in both groups were boys and no difference was noted between the groups with respect to gender (χ^2=0.100; p>0.05; Table 1).

Table 1. Comparison of the groups in terms of age and gender

	Study Group, n (%)	Control Group, n (%)	p
Age (years)			
9	92 (69.2)	93 (69.4)	0.967
10	41 (30.8)	41 (30.6)	
Gender			
Female	57 (42.9)	60 (44.8)	0.752
Male	76 (57.1)	74 (55.2)	

Df=1

Table 2 shows the dental health habits of the children enrolled in the study. Three-fourths and two-thirds of the children in the study and control groups reported daily brushing, respectively. The majority of the children in the study and control groups reported having their own toothbrushes, but few children reported using dental floss. Chi-square analysis revealed no significant differences between the study and control groups in terms of daily tooth brushing habit (χ^2=2.525; df=1; p>0.05), owning a toothbrush (χ^2=0.431; p>0.05), flossing (χ^2=0.603; p>0.05), and consumption of sweet foods between meals (χ^2=0.013; p>0.05; Table 2).

Table 2. Comparison of the study and control groups in terms of dental health habits

Habits	Study Group (n=133), n (%)	Control Group (n=134), n (%)	p
Tooth brushing daily			
Yes	101 (75.9)	90 (67.2)	0.112
No	32 (24.1)	44 (32.8)	
Owning a toothbrush			
Yes	127 (95.5)	130 (97.0)	0.511
No	6 (4.5)	4 (3.0)	
Flossing			
Yes	18 (13.5)	14 (10.4)	0.438
No	115 (86.5)	120 (89.6)	
Consumption of sweet foods between meals			
Yes	29 (21.8)	30 (22.4)	0.909
No	104 (78.2)	104 (77.6)	

Df=1

The comparison of the pre-training test scores of the children in the study and control groups is

presented in Table 3. No significant differences were noted between the two groups regarding pre-training test scores ($p>0.05$). The post-training test scores of the children in the study group were found to be significantly higher than control group ($p<0.001$; Table 4). Analysis revealed a significant difference between the pre- and post-training test scores of the children in the study group ($p<0.001$). The post-training test scores of the children in the study group were significantly higher as compared to their pre-training test scores (Table 5).

*Table 3. Comparison of the pre-training test scores of the children in the study and control groups**

	Mean rank	Sum of rank	**p**
Study group (n=133)	131.62	17505.50	0.606
Control group (n=134)	136.36	18272.50	

**Mann-Whitney U test was used (U=8594.500)*

*Table 4. Comparison of the post-training test scores of the children in the study and control groups**

	Mean rank	Sum of rank	**p**
Study group (n=133)	194.82	25911.00	0.000
Control group (n=134)	73.63	9867.00	

**Mann-Whitney U test was used (U=822.000)*

*Table 5. Comparison of the pre- and post-training test scores of the children in the study group**

Scores for Tooth brushing Skills	**Mean rank**	**Sum of rank**	**p**
Negative rank, (n=2)	1.50	3.00	0.000
Positive rank, (n=129)	67.00	8643.00	
Equal, (n=2)	-	-	

**Wilcoxon signed-rank test was used (Z=9.926)*

Discussion

It is known that practical training significantly improves the tooth brushing skills of children (12). We noted a significant increase in the post-training test scores of children in the study group as compared with their pre-training test scores. It is known that psychomotor skills are largely acquired by modeling, and reinforcing proper behaviors. A step-by-step demonstration of the psychomotor skill that is to be taught is also essential. The entire skill has to be broken down into rational parts and each part has to be taught only after the previous part has been satisfactorily learned. This approach is known to make learning easier, and it helps to commit the skill into long-term memory (21,22). Therefore, the approach utilized by the nurses was observed to have a positive impact on the brushing skills of the children because they had broken down the skill into parts, as brushing the upper and lower front teeth, the upper and lower back teeth, the inside surfaces of the upper and lower front teeth, and the cutting edges of the teeth. Considering the fact that children acquire five motor skills by the age of 8 years (17), the significant increase noted in test scores after the training of the children who had completed their motor development, can be attributed to the fact that they understood and practiced the tooth brushing skill well.

Swain, Allard and Holborn (23) emphasized the necessity of providing training on dental health to schoolchildren. They also reported that home control of proper oral hygiene was not always successful, and thus schools were optimal environments to ensure oral hygiene. In that particular study, it was emphasized that schools provided a positive environment to teach oral hygiene as well. Petersen and colleagues (24) reported that systematic health education of schoolchildren might further improve oral health. It has been emphasized that carrying out training programs for small groups has also a significant impact on improving oral hygiene (25). In a study investigating the effects of school-based oral health education, significant improvements in oral health behaviors and gingival bleeding scores of children were noted (24). In light of the existing data, it can be suggested that training programs carried out at schools for small groups of children are effective in improving their tooth brushing skills, and repeating the programs can have a significant impact on reinforcing the skill.

The results of this study are limited to the data obtained from the children participating in the study and cannot be generalized to the children from other schools. The study did not establish whether the tooth brushing skills acquired by the children became a habit.

Conclusions

In 2007, the World Health Assembly reported that the aim of the programs for improving oral health in schools was to develop individual care practices and healthy lifestyle behaviors in children (3). One of the most important roles of nurses is health education. The school nurse provides health education by providing health information to individual students and groups of students through health education, science, and other classes. Health education topics may include oral health, nutrition, exercise, smoking prevention and cessation, and others (18). Nurses can actively participate in the training programs on oral health and school nurses may play an effective role in the prevention of dental diseases in schoolchildren. In this study, the importance of the role of nurses, as educators in programs aimed to improve the tooth brushing skills of schoolchildren, was emphasized. Moreover, children can acquire regular and proper tooth brushing habit if the tooth brushing skills program is carried out over a longer period of time and is repeated.

Acknowledgements

The authors would like to thank all of the children and families who participated in this study, as well as the administrators and teachers in the schools where the study was conducted, for their valuable contributions. No external or intramural funding was received.

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Evaluation of biochemical markers in hepatocyte like cells differentiated from adipose derived stem cells

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Abstract

Recent evidence suggested that the cells with the properties of mesenchymal stem cells (MSCs) can be derived from adult peripheral tissues, including muscle, dermis and adipose tissue. Adipose tissue is ubiquitous and easily obtainable in large quantities.

We isolated Adipose derived stem cells (ADSCs) from the portion of subcutaneous adipose tissue of rat. These cells could be readily differentiated into cells such as chondrocyte, osteocyte, hepatocyte and adipocyte lineage demonstrating their multipotency. To characterize the ADSCs surface expression of the typical markers used flow cytometric analysis and finally these cells differentiated into hepatocyte like cell. Immunocytochemical analysis showed that ADSCs expressed Albumin and α-fetoprotein during differentiation. Also we determined biochemical markers of hepatocyte like cells such as SGPT, SGOT, urea Production and glycogen synthesis. Finally we isolated total RNA from hepatocyte like cells at indicated days after differentiation, and performed RT-PCR by using the specific primers (albumin, α Fetoprotein and Alpha 1-Antitrypsin).

Key words: Differentiation, Mesenchymal stem cell, Hepatocyte-like cell, Adipose derived stem cell and Biochemical markers.

Introduction

Mesenchymal stem cells (MSCs) display multipotency and under perfect conditions they had the ability to differentiate into lineages of mesenchymal tissues including muscle, bone, cartilage and fat.[1] MSCs were first characterized in bone marrow, but a wealth of studies have demonstrated the presence of uncommitted MSCs progenitor cells in the connective tissue of several organs including muscle, adipose tissue and trabecular bone.[2] The role of these cells was not entirely clear, but they are generally believed to constitute a reserve cellular fraction for tissue maintenance and repair. Although several authors have shown that adipose tissue MSC can differentiate into morphologically clearly discernable cell types[3], few studies have demonstrated that the functional properties of MSCs differentiated in vitro are equivalent to those of primary cell cultures from the same organ.

Adipose tissue is ubiquitous and easily obtainable in large quantities, it may provide an alternative source of stem cells for mesenchymal tissue regeneration and engineering. Adipose derived stem cells (ADSCs) have similar characteristics with Bone marrow-derived mesenchymal stem cells (BMSCs) in vitro and in vivo. [4] This property renders them interesting as a model system for studies of differentiation pathways and potentially useful for cell and gene therapy.

On this manuscript we focused on the isolation of ADSCs from rat adipose tissue and Evaluated of biochemical markers in hepatocyte like cells differentiated of them.

Experimental

Reagents

Antibodies for the flow cytometric assay, mouse anti-human monoclonal antibodies for albumin and αFP and the goat anti-mouse FITC-conjugated immunoglobulin G (IgG) were obtained from DAKO (Denmark) and Oxford Biomedical Research, Inc (UK).Dulbecco's Modified Eagles Media (DMEM), fetal bovine serum (FBS), peni-

cillin- streptomycin, phosphate-buffered saline (PBS), 0.25% EDTA solution was obtained from Gibco Bio Cult (Paisley, Scotland, UK). Stem Span media were purchased from Stem Cell Technology (USA). L-glutamine, Hepatocyte growth factor, dexamethasone, oncostatin M, Alizarin red staining and, Oil red staining kits and other reagents were purchased from Sigma Aldrich Co (USA). 1% periodic acid (Sigma), Periodic Acid Schiff's (PAS) reagent (Sigma), Mayer's hematoxylin (Sigma). The RNA extraction, cDNA synthesis, hot start PCR master mix and plasmid extraction kits were purchased from Qiagene (USA).

Isolation, Culture and expansion of ADSCs

Adipose tissue is washed extensively with sterile phosphate-buffered saline to remove blood cells, and debris. The extracellular matrix is digested with 0.075% collagenase I at 37°C for 30 minutes to release the cellular fraction. Collagenase I is inactivated with an equal volume of DMEM containing 10% FBS. The infranatant is centrifuged at 250 g for 10 minutes to obtain a high density cell pellet. The pellet is resuspended in proliferation medium consisting of low glucose DMEM supplemented with 10% FBS, 2 mM L-glutamine, 100 U/ml of penicillin, and 100 µg of streptomycin, at 37°C, 5% CO_2, and humidified atmosphere containing 95% air. After 3 days, the nonadherent cell fraction was removed by washing with PBS. These cells are maintained in control medium, until they reached 70%–90% confluence. Cells were passage 4 times prior to further analysis so as to ensure removal of contaminating hematopoietic cells.[5]

Immunophenotyping of ADSCs by flow cytometry

To characterize the ADSCs surface expression of the typical marker proteins, approximately 20×10^5 cells were incubated with fluorescent isothiocyanate(FITC) and phycoerythrin labeled antibodies for CD_{90},CD_{29} and CD_{45},CD_{31},CD_{11} and CD_{34}. Isotype-matched irrelevant monoclonal antibodies were used as negative controls. For the purpose of cell-surface staining, cells were incubated in the dark for 30 minutes at 4°C in PBS supplemented with 1% BSA. After washing, cells were resuspended in PBS and measured using a Coulter Epics-XL flow cytometer, and the results were analyzed with the Win MDI 2.8 software.

Adipogenic differentiation of ADSCs

3×10^3 ADSCs cells were plated in 24-well culture plates. The cultured cells were incubated in the adipogenic medium for 14 days. Fat droplets within differentiated adipocytes derived from hMSCs were observed using the oil red O- staining method. Cell monolayers were fixed in 10% (v/v) formaldehyde solution in aqueous phosphate buffer, washed in 60% isoporpanol and stained with a 0.6% (w/v) oil red O- solution for 10 min at room temperature. This was followed by extensive washing with distilled water prior to destainig in 100% (v/v) isoporpanol for 15 min. The stained material was examined with phase constrast microscopy.[6]

Osteogenic differentiation of ADSCs

The potential of ADSCs to differentiate into osteogenic lineages was examined. To induce osteogenesis, hMSCs were incubated at 3×10^3 cells/cm^2 in an osteogenic medium for 2 weeks, with a medium change every third day.[7] To assess osteogenic differentiation, the cells were fixed with 90% methanol for 10 min at room temperature and identified by specific histochemical staining for calcium, using the Alizarin red staining kit. The stained material was examined with phase constrast microscopy.

Hepatic differentiation protocol

When ADSCs treated with Hepatocyte growth factor, oncostatin M, and dexamethasone, it has been shown that they have the potential to differentiate in to hepatocyte-like phenotype by expressing albumin. Hepatic differentiation was performed using a two-steps protocol. Briefly in the first step which lasted for seven days, the cells were cultured in medium consisting of low glucose DMEM supplemented with 15% FBS, 20ng/ml of dexamethasone and 10^{-7} mol/L of dexamethasone, followed by 20 ng/mL of oncostatin M for 2 weeks.[8]

Immunocytochemical staining

After 3 weeks of cell culture under hepatocyte condition medium, cells were fixed with 4% paraformaldehyde in PBS for 30 minutes at room temperature, washed with PBS and then perme-

able with 0.4 % (v/v) Triton X-100 for 20 min. Corresponding primary antibodies including the mouse anti-human albumin (1:1000) and mouse anti-human αFP (1:500) were then added to the cells and incubated overnight at 4°C. The cell were subsequently washed three times with PBS and incubated with a second fluorescence-labeled antibody and FITC-labeled goat anti-mouse IgG at 37°C, for 3 h in the dark. After washing with PBS the cells were incubated with 4, 6-diamidino-2-phenylindole (DAPI) (1:1000) for the purpose of nuclear staining. The cells were then visualized using a fluorescence microscope.

Determination of SGPT and SGOT

Cultured cells were harvested with 0.25% trypsin-EDTA solution on day 21 of differentiation. After centrifugation of cells at 1000 rpm for 5 min, the pellet was resuspended in 300 µM lysis buffer containing 50 mM Tris-HCl, 1% Triton X-100, 150 mM NaCl, 1 mM phenylmethylsulfonylfluoride (PMSF) (pH ~7.5) on ice. The cell lysate was then homogenized with a short sonication and centrifugation at 12 000 rpm for 10 min. SGOT and SGPT levels in supernatant media derived from 10^5 cells per well were measured with quantitative kits. The methods were based on kinetics UV-test.[9]

Urea production

Differentiated cells were incubated with medium containing 5 mM NH_4Cl for 24 h in 5% CO_2 at 37°C on day 21 of differentiation. Following incubation, obtained supernatant from 1×10^5 cells per well was collected and urea concentration was measured by a colorimetric assay kit. This assay is based on reduction of ammonia produced via urea hydrolysis. Undifferentiated ADSCs were used as negative control.[9]

Periodic acid-Schiff (PAS) staining for glycogen

Glycogen storage was evaluated using paraffin-embedded tissues. Briefly, sections were oxidized in 1% periodic acid for 5 min and rinsed three times in deionized water. Slides were then treated with PAS reagent for 15 min, rinsed in deionized water for 5–10 min, stained with Mayer's hematoxylin for 1 min and finally rinsed in deionized water.[10]

Reverse transcription-polymerase chain reaction (RT-PCR)

RT-PCR was used to determine the expression of albumin, αFP(α Fetoprotein), β-actin (as an internal control) and AAT(Alpha 1-Antitrypsin) .Total RNA was isolated from ADSCs and HepG2 cells using the RNase Mini Kit protocol (Qiagene). Single-stranded cDNA was performed using the reverse transcription-PCR protocol of the First Strand cDNA Synthesis Kit. PCR parameters for amplification of the hepatocyte marker genes were as follows: reverse transcribed into first strand cDNA using oligo(dT) primer, and amplified by 35 cycles (94 °C, 1 min; 55 °C, 1 min; and 72 °C, 1 min) of PCR using 10 pmole of specific primers.[11] The primers sequence used is shown in Table 1.

Results

ADSCs isolation

1 g of adipose tissue yielded approximately 5×10^3 stem cell, which was 500-fold greater than the number of MSCs in 1 g of bone marrow.

Table 1. Primers used for Reverse transcription polymerase chain reaction (RT-PCR) of liver specific gene expression

Primer	Sequence	Product size (bp)
Albumin	F 5' CTT TGG CAC AAT GAA GTG GGT AAC 3' R 5'GCA GTC AGC CAT TTC ACC ATA GG 3'	351
α- Fetoprotein	F 5' CCT GTA ACC TGT GAG ACT GG 3' R 5'ATT CAA GCA CCG AAA TCT GTA G 3'	252
AAT	F 5'AGA CCC TTT GAA GTC AAG GAC ACC 3' R 5'CCA TTG CTG AAG ACC TTA GTG ATG 3'	400
β-actin	F 5' CTG GAA CGG TGA AGG TGA CA 3' R 5' AAG GGA CTT CCT GTA ACA ATG CA3'	185

AAT (Alpha-1 antitrypsin)

Characterization of ADSCs

Flow cytometry analysis revealed that the ADSCs cells were positive for CD_{90}, CD_{29}, and negative for CD_{34}, CD_{45}, CD_{11} and CD_{31} (figure 1).

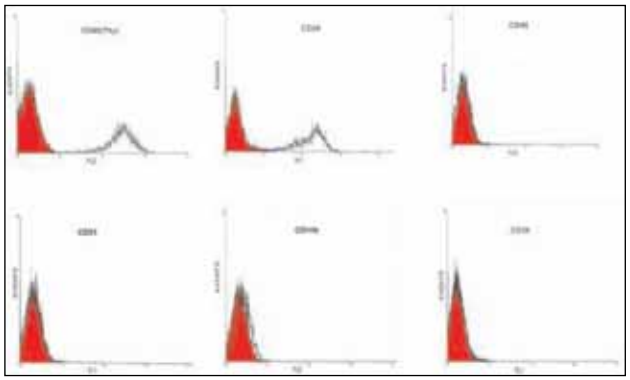
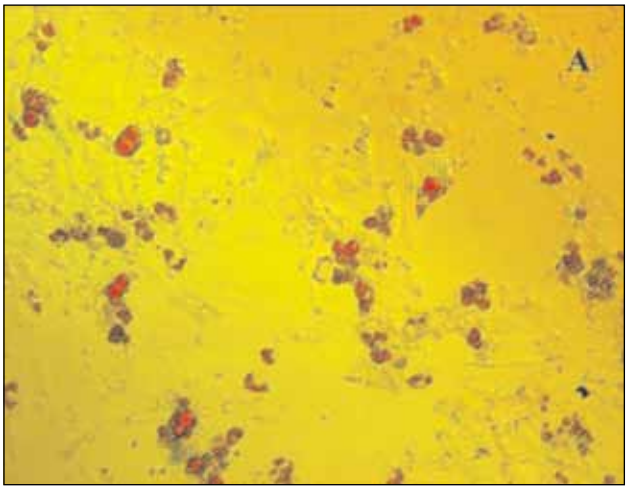


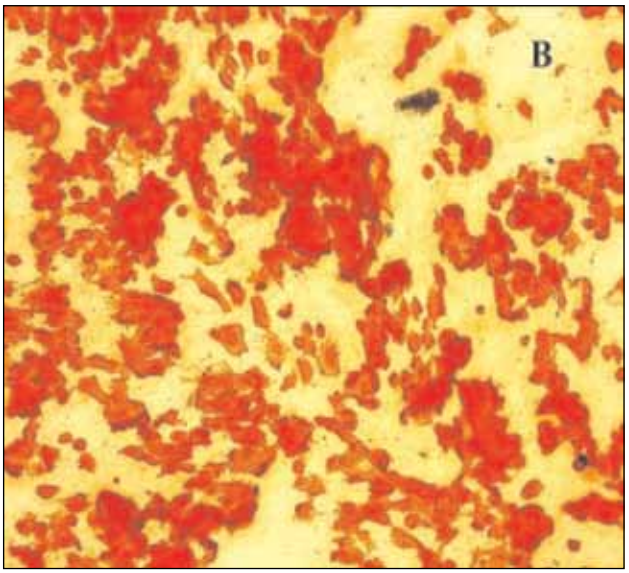
Figure 1. Flow cytometric analysis of the ADSCs. Flow cytometric analysis was performed for the specific markers of hematopoitic markers. ADSCs were positive for CD_{90}, CD_{29}, and negative for CD_{34}, CD_{45}, CD_{11} and CD_{31}.

Adipogenic and Osteogenic differentiation of ADSCs

To examine whether ADSCs have potential to differentiate into adipocytes, oil red O-staining was performed after 12 days of culturing in adipogenic medium. Fat droplets were seen in red on the surface of cells (figure 2A). We examined further whether ADSCs can differentiate into osteoblasts by Alizarin red staining assay. Presence of calcium deposits, characteristic of osteogenic cells, in differentiated cells after 12 days of culturing in osteogenic medium were observed (figure 2B). ADSCs cultured in normal medium served as a control and were negative for both staining.



A



B

Figure 2. Differentiation of ADSCs into adipocytes (A) and osteoblasts (B)

Hepatic differentiation of ADSCs

During the initiation step of hepatic differentiation, the cells showed a remarkable transition from bipolar fibroblast-like morphology to a round epithelial-like shape.[12]

Immunocytochemical staining

To determine in vitro hepatic differentiation of ADSCs, the expressions of albumin (liver specific protein) and αFP (a protein indicative of hepatocyte morphology) were examined. The differentiated cells were positively stained for albumin and αFP on day 21. The percentage of albumin and αFP positive cells were 79.23±2.95 and 69.37±3.79 in the differentiated cells. After washing with PBS, cells were incubated with DAPI (4, 6-diamidino-2-phenylindole; 1:1000) for nuclear staining. The cells were visualized and photomicrographed by using a fluorescence microscope.

Functional assay

Typical functional hepatic features such as synthesis and/or secretion of SGPT, SGOT, and urea were measured in hepatocyte-like cells derived from ADSCs.The levels of urea excreted in culture media from differentiated cells were 15.2 ± 4 (mg/dL) on day 21 ($P < 0.04$). Moreover, differentiated cells produced higher levels of SGPT (5.2 ± 2 mg/dL) and SGOT (7.3 ± 1 mg/dL) as

compared to undifferentiated ADSCs ($P < 0.01$). The levels of urea, and SGPT in culture media or cell lysate derived from undifferentiated ADSCs were negligible.

Glycogen synthesis

Glycogen storage was determined by Periodic Acid Schiff's (PAS) staining in hepatic like cells cultured at day 21. Positively stained glycogen granules were detected in the cytoplasm of cells. Spontaneously differentiated cells were negative for PAS staining.

Functional characterization of differentiated ADSCs into hepatocyte-like cells

To determine whether the cells have functional properties of hepatocytes, total RNA was isolated at 0, 7, 14 and 21 days after differentiation of the ADSCs into hepatocyte like cell and the expression of several hepatic proteins were examined by RT-PCR. On day 0, ADSCs did not express. Undifferentiated ADSCs did not express albumin. During differentiation expression of AFP increased and expression of albumin induced in ADSC-derived hepatocytes cultured with HGF, OSM, and Dexamethazone at 7_{th} day after differentiation. All genes expression was demonstrated by hepG2 (positive control). The relative amount of albumin in differentiated ADSCs in to HepG2 cells was 24.7% and 27.7% at 30 and 35 PCR cycles, respectively (Figure 3). These results indicated which the hepatocyte-like cells have characteristics that closely resembled those of adult hepatocytes.

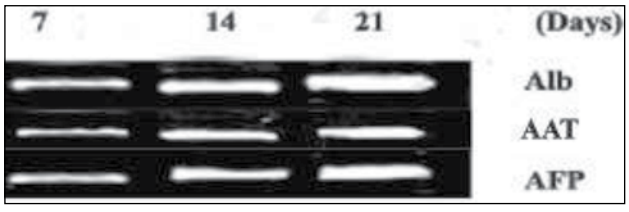


Figure 3. Determination of hepatic lineage genes by RT-PCR. We isolated total RNA from hepatocyte-like cells at indicated days after differentiation, and performed RT-PCR by using the specific primers

Discussion

MSC could differentiate into cells of all mesodermal origin, including adipocytes, osteocytes, chondrocytes, myocytes, and endothelial cells. [5, 13] Besides these, MSC were also capable of "transdifferentiation" into ectodermal cells, such as neural cells. [14, 15] Recently, BMSC have been shown to differentiate into hepatocyte-like cells. [16] In this study, we showed that ADSCs can be differentiated into functional hepatocyte like cells by differentiation Media. HGF originally identified and cloned as a potent mitogen for hepatocytes, showed mitogenic, motogenic, and morphogenic activities for a wide variety of cells that expressed the HGF receptor c-Met, a transmembrane protein possessing an intracellular tyrosine kinase domain. Moreover, HGF plays an essential role in development and regeneration of the liver. [17] It has been reported that treatment of the cultures with OSM, a member of the interleukin-6 cytokine family, increased the cell size of hepatocytes and enhanced cell differentiation and formation of bile canaliculi.[8]

In this study, we established the method for differentiation of ADSCs into hepatocytes in vitro. When ADSCs were cultured in the media containing HGF and OSM, and Dexamethasone, cells showed morphology of mature hepatocytes and expressed albumin. The most important problem is immunorejection in liver cell transplantation. However, autologous stromal cells isolated from adipose tissue are immunocompatible and easily isolated. Different studies have shown that transplanted hepatocytes can repopulate liver disease in several mouse models, including a mouse model of metabolic liver disease termed hereditary tyrosinemia. Transplantation of amniotic epithelium-derived hepatocyte-like cells into recipient mice also resulted in detectable levels of donor cell derived AT in the circulation. [18, 19] Recent animal studies have been shown that infusion of MSCs through the tail vein can protect against experimental rat liver fibrosis.Therefore, we expected that ADSCs would become very useful source to hepatocyte regeneration or liver cell transplantation. This property makes them interesting as a model system for studies of differentiation pathways and potentially useful for cell and gene therapy.

Acknwoledgement

Financial support of the National Institute for Genetic Engineering, Biotechnology and Tarbiat Modares University is acknowledged.

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Student-centred medical education for the future physicians in the community: An experience from Serbia

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Abstract

Introduction: The aim of this paper is to present an experience from the School of Medicine (University of Belgrade, Serbia) in restructuring of study programs to be compliant with Bologna standards and European best practices in public and community health. We devised the new module with the idea to implement both the concept of patient-centered medicine and community-based learning activity, in the pre-clinical phase of medical education. It emphasis importance of social determinants of health, community activities on health promotion, early contacts with patients, developing communication skills and introduction of primary health care settings

Method: All participants of overall four generations of this curriculum (students, patients and physicians) were asked to evaluate their participation in the module, using three types of self-administered questionnaires**.**

Results: Overall 1529 students (response rate 74.1%), 1011 patients (response rate 57.2%) and 118 physicians (response rate 69.5%) had participated in this evaluation. One-way analysis of variance (ANOVA) demonstrated significantly higher satisfaction of patient with the participation in the new module (4.48), than the students (3.90) and physicians (4.08) ($F=69.882$, $p = 0.000$).

Conclusion: The satisfaction of patients enrolled in this module encourages us to continue. Broadening of medical training to primary health care and other "non-medical" community settings enabled students to get insight into the different social determinants of health. However, the instructors need to improve the student's understanding and devotion for this conception.

Key words: patient-centred medicine, community, education, curriculum

Introduction

Medical schools are dominated by disease-centred medicine. The main goal is to teach students several hundred nosologic entities enabling them to assign patients to these entities according to the relevant signs and symptoms. This kind of education is based on the professional model rooted in medical paternalism, a legacy of the 19th century.[1]

Life, within its dominant aspects, has taken another course. Instead of submission to the expert authority, there is a clear movement towards centrality of an individual's autonomy and increasing multivocality. In medicine, that movement has given rise to the concept of patient-centred medicine.[2] Patient-centred medicine, at present, does not mean that a professional's task is to ensure that patients are empowered to adopt an autonomous stance.[3] This would require a radical change of medical technology. Currently, patient-centred medicine is giving rise to another development; it is increasing the complexity of medical care. Namely, the shift from disease-centredness to patient-centredness means the following: 1) in addition to signs and symptoms (typical for the disease), other factors (typical for the patient) should be considered as well, 2) in addition to the presribed protocols, other therapeutic/preventive options should be introduced as well, and 3) in additon to medical institutions and medical personel, other institutions and professionals should participate in alleviating patient suffering and complaints as well.

That development corresponds to the postmodern trends in developed societies and it should be supported. According to some opinions, even if medicine does not respond to the ideas of postmodernism, it may become increasingly irrelevant to the needs of a changing society.[4] In regard of that, the practice is generally lagging behind the theory. Physicians tend to show limited ability to

achieve patient-centered outcomes.[5] Thus, the logical conclusion is that something has to be done within medical education. Among the initiatives that could move medical education towards postmodern trends, and through that move medical practitioners toward patient-centred medicine, several of them should be emphasized.

The first initiative is the deconstruction of the medical meta-narrative characterized by a search for a definitive discourse that makes the medical knowledge coherent, meaningful, and masterful. The complementary move emphasizes the small narrative, a post-modernist model of unique, lived experience, based on lived lives of patients/clients, on the diverse, on the complex, and the unique.[6] That orientation goes with education, which is putting less emphasis on the "hard science," and more emphasis on the context. That means the broadening of medical training to include "non-medical" settings so that students could get insight into the problems and contexts of patients' lives.[7]

The initiative which is next in line is putting the emphasis on social responsiveness. Social responsiveness is raised to the status of the fourth obligation of medicine (in addition to medical teaching, research, and service) and it consists of responsiveness and moral accountability to the local community and society at large.[8] Connected with social responsiveness are two demands. The first demand is criticizing today's culture of medicine as hostile to altruism, compassion, fidelity, and self-effecing. It is arguing for overcoming of tension between self-interest and altruism by "engaging the heart".[9] The second demand is paying attention to socio-cultural and behavioural determinants of disease, requiring students to understand the social nature of ill-health processes and to have skills in the area of prevention and health promotion.[10]

Deconstructive educational initiatives are, of course, coupled with deconstructive pedagogical initiatives. Among them, the most outstanding is the initiative deriving from "critical pedagogy"[11], which is introducing "student-centred" medical education.[12] Student-centred education overcomes the clasical model of medical apprenticeship. Instead, it enables and encourages students to discover as well as generate the knowledge relevant to their needs without explicit guidance and direction from their teachers.

Following democratic changes in the Serbia in 2001, there was a pronounced tendency to ponder existing medical curricula, and restructure both organization and the content of study programs so that they are compliant with Bologna standards and European best practices in public and community health. Having these trends in mind, staff at the Institute of Social Medicine of the Medical Faculty of Belgrade devised the module "Physician in the Community." The aim of this paper is to present the process of development and subsequently, the evaluation of the module, after the four years of implementation.

Methods

Description of the module

The module, "Physician in the Community," was derived as a result of collaboration of the Institute of Social Medicine (as a part of the School of Medicine) and 11 Primary Healthcare Centers in Belgrade. The development of the module included three phases: the planning, implementation, and evaluation. In the first phase (2004/2005), planning focused on the content of the Module, on organizational aspects (recruitment of physicians and patients willing to participate), and on the types of the evaluation. Teaching staff from the Institute of Social Medicine were responsible for that part. Their key partners were managers of 11 primary health care institutions from the Belgrade community. They were involved in assessing the acceptability and feasibility of the module. The positive assessment was followed by signing of the contract, and by incorporation of the module, "Physician in the community," in the core curriculum of the School of Medicine as a part of the "Basics of the Clinical Practice II" in the second year of medical studies. Participation in the module was on a voluntary basis for physicians and patients. Informed consent was obtained from all patients involved in the module.

The second phase, implementation, started from the 2005/2006 school year. One teaching cycle for this module lasted 10 weeks, overall 20 hours. The organization of the learning format of each week consisted of the introductory lesson at the Institute of Social Medicine, followed by a stu-

dent visit to the patient. Every visit was debriefed on the next lesson with the trainer. At the first lesson, the students were introduced and trained to use a specially designed method for this module: "My patient."

Description of the method "My patient"

Each student received "his/her patient" in a Primary Healthcare Center. In cooperation with a physician, each student followed one patient in his/hers contact with health care services and other health or social institutions, as well in the family settings. Each visit to the patient was followed by the interview that lasted at least 45 minutes. The students were prepared for the interview with the patient at the theoretical lessons, during which they have discussed with their teachers the topic of the each interview. The detailed content of the theoretical lessons and visit to the patient is provided in Table 1.

This module didn't include end-of-module examination. The obligation of the students was to prepare short group presentations of their experience with the patients, following the topics of the interview. Each group defined the special aims of their work based on a program. The emphasis was on the community role in improving the health and well-being of citizens. Based on written records of their patient's interview, theoretical knowledge, national and international documents, each group presented the synthesis of their findings in the last week of this module. The presentations of the students' work had different forms: Power Point, video, oral and poster presentations, as well as role–play. The concluding session of each teaching year of the module is the public presentation of the student's works in the City Hall in front of the representatives of the community (Health Secretary Board of the City), patients and their families, key partners from the primary health care institutions, members of the media, students, and teaching staff of the School of Medicine of the University of Belgrade.

Evaluation

Evaluation of the module was performed at the end of each cycle. Three types of short questionnaires (questionnaires for students, physicians, and patients) were designed to explore satisfaction with the participation in the module, contributions of the new curriculum, and possibilities for improvement. To insure content validity, we performed pilot testing and each questionnaire was first peer reviewed by 5 students, physicians or patients, depending on the type of the questionnaire. The offered categories of the answers were on the five-point Likert scale, from 1 (minimum agreement) to 5 (maximum agreement). The questionnaire for students included eight questions (Cronbach's α coefficient=0,835), which referred to knowledge and experience regarding factors that influence health of patients, communication with patients and teaching staff, as well as overall satisfaction with the module. The questionnaire for patients included seven questions (Cronbach's α coefficient=0,723) regarding the relationship with students, possible adverse effects of students' visits on their lives, and the overall satisfaction with the module. The questionnaire for physician trainers consisted of seven questions (Cronbach's α coefficient=0,701). Trainers were asked to estimate the significance of the new training module for primary health care, patients' perceptions of the module, and their own communication with students, as well as overall satisfaction with the module. In all questionnaires an open-ended question was added at the end, offering the respondents to give additional comments and suggestions. Partially completed questionnaires were excluded from the analysis.

The participation in the evaluation was voluntary and anonymous. Electronic database and statistical analysis were done in the STATISTICA using descriptive and analytical methods.

Results

During four teaching years, 1529 students were included in the curriculum and asked to complete the questionnaire. The response rate was 77.7%. Of the 1011 patients included in the module, we received 630 completed questionnaires (a response rate of 62.3%). The total number of valid questionnaires from physicians in the primary health care centers was 78 (a response rate of 66.1%).

Table 1. Content of the theoretical lessons and visit to the patient

Week of the Module	Content
I	▪ At the first lesson with the teacher, students were acquainted with the practical guidelines about the module and their first contact with the patient. ▪ The teachers discussed the baseline principles of communication skills. ▪ The students got instructions how to collect basic date about the patient (age, level of education, occupation, family status, place of residence) and about health status (health problem) of the patient
II	▪ Second lesson and following visit to the patient addressed socio-economic environment. Students talked with the patients in order to perceive their economic position and employment status as well the economic status of their family. Students assessed how much these factors allow or limit the life activities of the family's members relevant to the health (nutrition, leisure time, risk factors)
III	▪ The next topic refered to social support and marginalization with the aim to summarize the social status of the patients (relation with friends and relatives, who were the persons they relied on when they needed a support, did they ever felt marginalized, social networks and other issues). Students assessed the social-cultural context for development of the social capital and also, the potentials, which patients used and other, which could be more used.
IV	▪ The students learned to systematize and define the mechanisms of the social organizations with the aim to save and improve health in the society (at work, in the family, in the town). Set of questions addressed patient's opinions and satisfaction with health care services.
V	▪ The students were prepared to assess the following issues concerning family life of their patients: type of family (for example, patriarchal family, civil family, open family, enclosed family...), family relations and the patient's needs of help from their family (who provided that help, what the prospects and the real possibilities were, and how the disease affected family life). Furthermore, students estimated in which way the specific family routine influenced the health of family members.
VI	▪ The students were prepared to ask appropriate questions in order to assess the patient's attitudes to violence in family and its influence on family relations (especially when women, children and personal experience were in question). They looked for possible patient's experiences or experiences from patient's social network, especially, what the reactions on violence were and what the effects on health were. Students discussed with the patients the possible solutions and role of community in preventing the problem of violence in family.
VII	▪ Through the conversation with the patients students perceived to which extent specific cultural patterns and social influences had formed the attitudes towards health and risk behavior. Students assessed lifestyles of their patients, asking questions about healthy habits (adequate nutrition, physical activity and others) and preventive health behaviors, including adherence to screenings, advices and regular check-ups with their chosen doctors (GP or gynecologist from the community health care institutions). Also, students asked patients about presence of certain types of risk behaviors (alcohol consumption, tobacco, sexual risk behavior, inadequate diet.)
VIII	▪ The last interview with patient addressed the principles of the contemporary cities development. Students intended to perceive the impact of urban environment on health and well-being of the citizens (quality of air and water, green areas, perceived noise, communication with the destinations of great importance, stimulation of walking, safeness and others).
IX	▪ During the ninth week, students amended the notes taken during the interviews with the patients in order to prepare the final presentation. With the teacher's assistance, students were organized in small groups (5-7) and started to prepare the final presentation.
X	▪ Based on written records of their patient's interview, theoretical knowledge, and international documents, each group presented the synthesis of their findings.

Students' responses

After the module was completed, 39.3% students reported to be fully aware of factors that influence their patients' health and quality of life, and 52.0% were able to quote at least three groups of social factors that influence patients' health. Questions related to improvement in student's awareness and insight in factors influencing patients' health and quality of life showed that mean score was 3.94 for the awareness of overall factors that influence health and quality of life of patients, and the score was significantly higher for the knowledge of social factors (4.29;Wilcoxon, Z=-4.169, p=0.000). We noted a high mean score for insight in activities of the local community for improving health (4.05). Students gave the highest scores for the easiness of communication with patients (4.45). The majority of students (85.7%) rated teachers' presentations with a 4 or 5 (on a five-point scale) with a mean score of 4.38. However, the overall satisfaction with participation in the module was not scored so highly; the mean was under 4 (3.90), and students perceived that their expectations were moderately fulfilled (mean=3.45; Table 2).

Patients' responses

Almost all patients (91.0%) were satisfied with their participation in the module. Compared to students and physicians, patients were significantly more satisfied with their participation in the module, with a mean score of 4.48 (F=69.882, p = 0.000). Scores for questions addressing the relationship between patients and students as perceived by patients were high. The patients had a high level of confidence in the students (mean = 4.67), understood importance of student-patient cooperation (mean = 4.29) and were satisfied with the student's approach to them (mean=4.94). Almost all patients agreed that students did not ask too many questions regarding their personal life (89.6%). Patients perceived participation as a positive experience as they felt better at the end of the module (mean = 3.79). Participation in the module didn't cause much difficulty in the patients' everyday activities (Table 3).

Physicians' responses

The overall satisfaction with the participation in the module of physicians' form the primary health care institutions was 4.08. The physicians'

Table 2. Mean responses of students to questionnaire items

Question	Mean level agreement* (n=1134)	SD
Overall satisfaction with participation in the module	3.90	1.09
Awareness of factors that influence patients' health and quality of life	3.94	1.12
Knowledge of social factors which could improve health	4.29	0.88
Insight in activities of local community for improving health	4.05	1.06
Easiness of communication with patients	4.45	0.75
Satisfaction with teachers presentations	4.38	0.89
Fulfilled expectations	3.45	1.08

** (5=maximum agreement, 1=minimum agreement)*

Table 3. Mean responses of patients to questionnaire items

Question	Mean level agreement* (n=579)	SD
Overall satisfaction with participation in the module	4.48	0.72
Importance (meaning) of cooperation with student	4.29	0.94
Satisfaction with student's approach to patient	4.94	0.24
Level of confidence in student	4.67	0.60
Did the participation in module caused difficulties for everyday activities	1.98	1.36
Feeling better at the end of the module	3.79	0.87

**(5=maximum agreement, 1=minimum agreement)*

Table 4. Mean responses of Primary Health Center physicians trainers to questionnaire items

Question	Mean level agreement* (n=82)	SD
Overall satisfaction with participation in the module	4.08	0.87
Importance of the module for work in PHC	3.92	1.06
Estimation of patients' reactions	4.22	0.72
Satisfaction with communication with students	4.56	0.69
Need for closer communication with students	1.53	0.50

** (5=maximum agreement, 1=minimum agreement)*

opinion about the importance of the knowledge and experiences acquired in the module for the future work in primary health care had a mean score of 3.92. The physicians noticed positive reactions of their patients to the module (mean = 4.22), perceived communication with the students as very good (mean = 4.56) and stressed no need for additional time for deeper communication with the students (Table 4).

Discussion

The module "Physician in the Community" is created and implemented using experiences of similar modules and projects at various universities since the 1990s, with the aim to include the concept of patient-centered medicine and community–based learning activity at the very beginning of the medical education.[12-15] Mutually to these modules, it emphasis importance of health determinants, community activities on health promotion, early contacts with patients, developing communication skills and introduction of primary health care settings. Furthermore, the example from China revealed that many dental schools integrated community-based education into their curricula as well. These programs are designed to integrate behavioral and population-based sciences and expose students to social, environmental, and cultural influences that affect health and diseases.[16] The study about learning experiences of students from seven dental schools from USA (SWOT analysis of the educational program) inform future curriculum development with the notion that one of the most important opportunities of the dental schools are to develop strategies of early contact with the patients.[17]

Although the students at the School of Medicine are engaged in the work of primary health care at the later years of study, this is the first time they get insight with practice of general physicians. Experience has showed that medical students' visits to pre-clinical primary health care center may influence their attitudes towards primary health care work and the physician-patient relationship.[18, 19]

After finishing the module, we evaluated the opinion and satisfaction with it among the students, their patients, and the physician-trainers in the primary health care centers. The students were especially impressed by the ease of communication with the patients. They were also satisfied with the way the teachers presented topics for the conversation with patients. The possible reasons for this may be the way of doing it, working in small groups and discussions, which resulted in a positive approach to learning.[20] Even though 86% of our students assessed their satisfaction with teacher performance as very high and high, it was lower when compared with students of the University of Hong Kong, whose satisfaction with tutors' performance was 95% in 1999 and 98% in 2000.[21] Slightly more than 50% of the students were able to enumerate at least three groups of social factors, which when enhanced, improved peoples' health and the quality of life. Compared with the knowledge, students' awareness about the existence of different factors which influence peoples' health was lower. Also, the possibility to look into community activities which would contribute health promotion was assessed as high. Two-thirds of students were "satisfied" and "very satisfied" with their participation in the module. At the University of Hong Kong, two-thirds to three-quarters of students perceived that the core objectives had been satisfactorily attained as communication skills and determinants of health.

On the other hand, patients assessed as very high the way students treated them, the attained

confidence level, their satisfaction with the participation in the module and the importance of cooperation with students. Their satisfaction with the participation in the module may be seen through the collaboration with students which had a little influence on their daily activities as well as in the fact that more than one-half of the patients were feeling better after finishing the module. Different studies addressed the satisfaction of the patients included in work with students. Some of them showed a high level of satisfaction of the patients, some showed the patients' anxiety because of the potential pressure which the work with students could bring and because of the possible violation of privacy and confidentiality.[22,23] In our study, physicians and students have thoroughly informed patients about the content and the objectives of the module as well as with the fact that their privacy would not be violated. Being well-informed is recognized as one of the factors which contribute to the involvement of patients in work with students and their good communication.[12] Furthermore, patients in this kind of programmes recognize their active role and they represent partners who contribute the students' education.[24]

Physicians who were involved in the module were satisfied with the communication with students. They assessed patients' satisfaction with the module as very high. Patients "found" people who were ready to listen to them, not only about their disease, but about their daily and life problems as well. For some of them meetings with students represented a little "rituals." Other similar programmes also showed that the benefits for patients were multiple.[25] Physicians believe that this kind of module is important for their work in primary health care and they have evaluated it as "very good" (the mark was 3.92).

However, there were some limitations to this study. The comparison with the experiences of others is limited due to peculiarity of the educational model and the instruments for the evaluation. As a positive aspect of this study, we point out the number of participants which has been involved, since the evaluation included all students, patients and tutors during passed four teaching years, from the time of commencing with the new module. The evaluation done in this way has been carried out at the School of Medicine for the first time and it could be good example for other courses. Students were motivated by the fact that the best work and their recommendations will be presented in the City of Belgrade Hall and that they will be able to present their suggestions to the local government and health authorities. Presented evaluation opens the options for further research which would be helpful for improving our work with students.

This paper represents our intention to share a good experience in the development and implementation of a new curriculum. We strongly believe that presented educational approach empowers our students to develop future relationship with the patient beyond the classical medical trainings, organization aspects of services, hi-tech medicine devises and cost-containment strategies. It is too early to judge the relation of outcomes of present developments and quality of patient treatment. In future study and with a further evaluation of the progress in the module we will stem to explore this issue. From our point of view, basic altruistic values, communicational skills and holistic approach are *condition sine qua non* for the future physicians in the community and during the medical education it is our obligation and mission to cherish and develop these values.

Lessons for Practice

- Classical model of medical education don't enable and encourage students to practice patient-centered medicine.
- Broadening of medical training to primary health care and other "non-medical" community settings enable students to get insight into the different social determinants of health;
- An orientation of medical education, which is putting less emphasis on the "hard science," and more emphasis on the context and patient empowerment resulted with patient satisfaction.
- However, next steps are to improve the student's satisfaction, understanding and devotion for this conception.

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Discharge After Surgical Treatment: What do patients in Turkey want to know?

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Abstract

The shortened length of stay in hospitals and the increased emphasis on health prevention and promotion makes patient education very important before discharge. The purpose of this study is determining information requirements and priorities of patients who underwent a surgical intervention regarding their home care prior to their discharge. It was found that post-discharge learning needs of the patients, when prioritized, were "medications", "treatment and complications", "quality of life", "skin care", "activities of living", "feelings related to condition", and "community and follow-up" and that information requirements varied depending on personal characteristics. In order for an efficient discharge training to be planned, it is necessary to take individual characteristics of patients into account and to determine their requirements in a proper way.

Key words: discharge, discharge planning, information requirements,

Introduction

The primary task of nursing is to help healthy or sick individuals. This help includes those activities that can enable the healthy and sick to acquire the necessary information, desire, and power for maintaining their healthy condition and recovery respectively. While carrying out these activities, nurses might frequently need to educate individuals (Bayat, 2005).

The admission process consists of a series of activities that are intended to facilitate an individuals entry to the clinical care environment and to prepare them for their progress through the health system with minimal anxiety. From the day of admission, planning the process for patient discharge is often commenced. The patients should be discharged with the necessary knowledge and skills to meet their own care requirements (Coşkun & Akbayrak, 2001).

Today, planning the discharge process has become even more important with the increased cost of health services, sophisticated technology, and the increasingly reduced duration of hospitalization as a result of the desire to prevent such complications as hospital infection.

Patients need a proper discharge plan in order to continue their treatments at home, to support their own care, to conduct self-care, to protect themselves against complications, to determine potential complications at an early stage, and to cope with disrupted body functions (Bowles, et al.,2003; Paul, 2008; Şenyuva & Taşocak, 2007;Yıdırım, 2007).

The literature mentions that a well-planned and efficient discharge plan shortens the period of hospitalization, has a positive effect on the recovery process, and prevents unnecessary hospitalization and complications that might develop following an operation Rhudy, et al., 2010; Aydoğan, 2005).

According to the modern understanding of health services, an individual has to play an active role in protecting, improving and promoting a healthy status. Through the instructor role carried out independently, nurses can enable individuals to develop those skills and behaviors that will protect their health through well-planned instruction, based on particular requirements. Patient instruction is an indispensable part of nursing and an important part of patient-nurse interaction (Şenyuva & Taşocak, 2007; Güler&Taşkın, 2001; Javorski, 2005).

As the key person during the process of discharge planning, nurses have significant responsibilities for planning and implementing the instruction with which the patient/family of the patient are provided with, and for ensuring team cooperation (Bowles, et al., 2003). However, the literature includes evidence that nurses cannot fulfill their roles in discharge planning in an efficient manner because of many nurses disbelief in discharge planning, their inability to carry out the process of

discharge planning in a proper way, their heavy workload, their lack of knowledge regarding the process of discharge planning, insufficient team communication, and lack of time. Moreover the physical environment required for discharge planning lead to difficulties in developing and implementing the discharge plan (Yıldırım, 2007). In a study carried out on nurses in Turkey, it was found that a majority of the nurses did not set a place or a time for the necessary instruction of patient aftercare (Avşar & Kaşikçi, 2011).

In order to satisfy patients' expectations, nurses need to know what these expectations are. The literature indicates that patients benefit from the care provided and get accustomed to post-discharge therapy at a higher level when their perceptions and goals are consistent with those of the nurses (Algıer et al., 2005).

Therefore, the purpose of this study is to determine the information requirements and the priorities of patients' homecare treatment after undergoing surgery prior to their discharge.

Method

The study was conducted at the Department of General Surgery, in the University School of Medicine in Istanbul/Turkey, between December 1, 2008 and January 30, 2009.

The population of the study comprised of patients who were hospitalized, underwent surgery and were in the process of being prepared for discharge. The sample of the study included patients who were above 18, literate, able to communicate, did not suffer from any psychiatric disorder or pain and agreed to participate in the study. The sample size was determined by power analysis with 216 patients producing the sample. A total of 220 patients were contacted between the dates specified. The data was collected through face-to-face interviews with the patients included in the sample group. The patients undergoing surgery were exposed to the scale 24-48 hours before their discharge.

The data was collected through a "Personal Information Form" and "The Patient Learning Needs Scale" (PLNS), a Personal Information Form, designed by the researcher through a review of the literature, which comprised of a total of 22 questions regarding the socio-demographic characteristics of the patients, together the discharge instructions of the clinic. The PLNS was originally developed by Bubela, Galloway, McCay, McKibbon, Nagle, Pringle, Ross & Shamian in 1990 in an attempt to determine information requirements of patients during discharge, and was adapted into Turkish and tested for its validity and reliability by Catal&Dicle (2008). Comprising of a total of 50 items and 7 sub-scales, the scale is a 5-point Likert scale with scores (possible range 50-250) varying from "not important" to "extremely important". The sub-scales of the scale are "Medications", "Activities of Living", "Community and Follow-up", "Feelings related to condition", "Treatment and Complications", "Quality of Life", and "Skin Care". In this study the internal consistency co efficiency of the scale was $\alpha = .95$.

The data was statistically analyzed via SPSS (Statistical Package for Social Sciences) for Windows 10.0 under the supervision of a specialist in statistics. The data was analyzed using percentages, means and median, the Student's T-test and Pearson Correlation Method.

The necessary permission was received from the "Research Ethics Committee" from the hospital in which the study would be conducted.

Results

It was found that 60% of the patients within the sample were women; 45% of them were primary-secondary school graduates; 45% of them were housewives; 73.2% of them were married; and 98.6% of them had social security health insurance. Furthermore, it was concluded that 47.7% of the patients were treated for a period of 0 to 7 days; that 26.4% of them underwent surgery for gallbladder stones, ıncisional hernias, appendicitis and ınguinal hernias.

The learning requirement of the patients included within the study according to The PLNS was found to be 199.19±26.62 points. When this figure is divided by the number of questions, the mean score is 3.98 in total.

Table 2 presented that, according to the patients, the five most important ones out of the learning needs specified were "how to care for their operational wounds" (4.58), "what they should

Table 1. The Patient Learning Needs Scale and sub-scales average points (N:220)

	Patient Learning Needs			
	Min	**Max**	**Mean**	**Std. Deviation**
Medications (8-40)	16	40	34.16	5.00
Activites of living (9-45)	21	45	35.25	5.10
Community and follow up (6-30)	8	30	21.26	4.55
Feelings related to condition (5-25)	6	25	17.58	4.68
Treatment and complications (9-45)	22	45	38.25	4.58
Quality of life (8-40)	14	40	32.60	5.54
Skin care (5-25)	9	25	20.08	3.02
Total (50-250)	123	250	199.19	26.62

Table 2. The mean scores of the items regarding the patients who needed the most information (N: 220)

	Learning Needs			
	Min	**Max**	$\bar{x}$	*SD*
How should I care for a post operational wound? (Item 25)	1.00	5.00	4.58	0.66
What should not I do regarding heavy lifting? (Item 30)	1.00	5.00	4.49	0.71
What are the problems that might arise in relation to my illness? (Item 10)	1.00	5.00	4.47	0.76
How can I identify any problem that could arise at home? (Item 4)	1.00	5.00	4.46	0.86
How long should I use any particular medication? (Item 16)	2.00	5.00	4.45	0.67

Table 3. Learning needs of patients according to gender and comparison of mean subscale score (N:220)

	Gender					
	Female (n:132)		Male: (n:88)			
	$\bar{x}$	SD	$\bar{x}$	SD	t	p
Medications	34.65	4.53	33.43	5.57	1.783	0.076
Activities of Living	34.97	4.76	35.68	5.56	-1.016	0.311
Community and Follow-up	21.64	4.29	20.70	4.87	1.493	0.137
Feelings related to condition	**18.11**	**4.27**	16.78	5.16	**2.069**	**0.040***
Treatment and Complications	38.53	4.17	37.82	5.14	1.130	0.260
Quality of Life	**33.21**	**5.02**	31.68	6.15	**2.021**	**0.044***
Skin Care	20.27	2.48	19.81	3.68	1.103	0.271
Total Points	201.37	23.11	195.91	31.01	1.495	0.136

not do" (4.49), "what the problems that might arise in relation with their diseases are" (4.47), "how to notice any problem that could arise at home" (4.46), and "how long they should use any particular medication" (4.45).

As seen in the Table 3; the discrepancy between the point averages of the learning requirements depending on gender, suggested that the total score of the female patients regarding "feelings related to condition" and "quality of life" were statistically significantly higher than those of the male patients ($p<0.05$).

In table 4; there is a comparison between the groups depending on educational status, indicating that the scores of the literate patients regarding "medications", "treatment and complications" and "skin care" were significantly high ($p<0.05$).

Discussion

The findings in the study indicated that patients had a variety of discharge information needs as determined by The Patient Learning Need Scale (PLNS). Patients found it important to be infor-

Table 4. Learning needs of patients according to the education and comparison of mean subscale score (N:220)

	Education									
	Literacy (n:36)		Primary Education (n:101)		High School (n:45)		University (n:38)			
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	F	p
Medications	**36.33**	**4.10**	**34.52**	**5.25**	33.04	4.27	32.47	5.12	**4.889**	**0.003****
Activities of Living	36.53	4.61	35.09	5.03	34.80	4.78	35.03	6.01	0.929	0.428
Community and Follow-up	22.22	4.73	21.25	5.16	21.22	3.75	20.45	3.32	0.942	0.421
Feelings related to condition	18.06	4.86	18.16	4.84	16.73	4.02	16.58	4.64	1.727	0.162
Treatment and Complications	**39.61**	**3.28**	37.70	5.20	37.49	4.27	**39.29**	**3.85**	**2.666**	**0.049***
Quality of Life	34.36	5.18	32.44	5.57	31.27	5.87	32.95	5.11	2.197	0.089
Skin Care	**21.33**	**2.19**	20.22	2.67	19.16	3.52	19.63	3.55	**3.975**	**0.009****
Total Point	208.4	21.63	199.3	28.08	193.7	26.07	196.3	26.15	2.266	0.082

med of the issues specified and had all the information requirements (Çatal &Dicle, 2008; Carew, 1999; Jacobs, 2000; Merkley, 1997; Rich-vander-Bij, 1999).[13- 17]

Studies conducted both in Turkey and other countries, report that a great majority of patients needed information during discharge and the level of information provided were not satisfactory in regard to the meeting of those the requirements (Aydoğan, 2005; Jacobs, 2000; Rich-vanderBij, 1999; Henderson&Chien, 2004; Jickling&Graydon, 1997; Maloney&Weiss, 2008; Suhonen et al., 2005).

When prioritized in the sub-scale of PLNS, information regarding "medications", "treatment and complications", "quality of life", and "skin care" respectively were found by patients to be highly significant whereas knowledge regarding "activities of living", "feelings related to condition" and "community and follow-up" were considered by them as neither unimportant nor very important (Table 1). A study found that patients with an experience of repetitive hospitalization had a history of irregular use of medications (Tel& Akdemir 1998). This raises suspicion that patients are discharged without the necessary knowledge of their medication. Similarly, in another study conducted by Henderson and Chien (2004) on Chinese patients in Hong Kong, the participants reported that their greatest need following surgery was regarding the medication and complications during the post-operative period.

Many studies concerning the information requirements during discharge found, as the present study did, that the leading priorities of patients regarding their information needs are treatment and complications (Carew,1999; Rich-vanderBij, 1999; Jickling& Graydon,1997; Suhonen, et al., 2005; Johansson, et al., 2005). In their study concerning the information requirements of patients who underwent a hip replacement, Johansson et al.(2005) found that community and follow-up were the least significant needs. The findings of that study are parallel with those of the present study. In their study, where patients assessed the instruction provided by nurses, Oerman et al. (2001) reported that patients found it important to be informed by the nurses regarding about diseases, medication, and treatment.

In the light of these findings, patients are more concerned about medical information, but they are not aware of the kind of information that necessary for them to carry out their own aftercare program following discharge, or the importance of medical follow-ups to aid in their recovery.

Similarly, several study results showed that information about "how to notice any problem that could arise at home" and "what the problems that might arise in relation with their diseases are" were among the first five items in order of impor-

tance (Carew,1999; Merkley, 1997; Rich-vander-Bij, 1999; Jickling& Graydon,1997) .

Information requirement was highest among women. This might be attributed to the fact that women, when compared to men, are more emotional, vulnerable, and sensitive and are able to express their worries in an easier manner than men. Suhonen et al.(2005) stated that patients who undergo surgery differ individually in their learning requirements , Rich-vanderbij's (1999) study results shows that the discharge information needs were high and with women it was significantly higher.

Similarly, the study conducted by Johansson et al.(2005) found that patients with a lower educational status needed more information. These findings suggest that those with a lower educational status need more information during the process of discharge and such patients are more aware of their lack of knowledge compared to other patients.

The study also concluded that the scores of those patients informed of home care, illnesses , and aftercare visits following their discharge regarding "community and follow-up" were higher than those who were not provided with such information ($p<0.05$). This finding might be attributed to the fact that the patients who are informed are more conscious of the importance of social support and medical follow-up for themselves and caregivers.

Moreover, according to the results of the study it was found that the patients had high information requirements. It was discovered that the issues regarding what patients needed to be informed about following the discharge were, in order of priorities, "medications", "treatment and complications", "quality of life", "skin care", "activities of living", "feelings related to condition", and "community and follow-up". It was observed that information requirements were high among women, patients with a lower educational status, those patients hospitalized for a longer period of time, and those patients who are provided with information. As a result, educating patients is a key component of nursing care. It has an effect on patient and family satisfaction, an increase in patient autonomy, an improvement in quality of life, and a decrease in overall medical costs.

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Occupational skin diseases in Autonomous Province of Vojvodina

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Abstract

Background: Occupational skin diseases (OSD) are skin lesions that occur due to exposure to various occupational hazards at the workplace.

Objective: to examine the characteristics of OSD in Vojvodina by industry, and to determine the types of occupational hazards that lead to OSD.

Methods: The study was based on records of OSD from the Register of Occupational diseases in Vojvodina during the 12-year-period (1997-2008).

Results: From 1997 to 2008 a total of 70 persons were affected by OSD. The number of affected female workers in comparison to affected male workers was significantly higher ($p<0.001$). The diagnosis of occupational contact dermatitis was established in 90%, while the rest of the workers (10%) were diagnosed as occupational contact urticaria. The highest number of cases was in healthcare with 18.5 % affected, followed by employees in the leather industry with 14.3 % cases, and workers in the textile industry with 12.8 % cases. The total incidence was 1.6, with the highest incidence recorded in hairdressers with 81.6 cases per 100.000 workers per year. The five most common detected allergens were, as follows: cobalt chloride, nickel sulphate, potassium dichromate, para-phenylenediamine and penicillin. In health care the most frequent allergen was penicillin, while in leather industry the most frequent were nickel suphate and cobalt chloride.

Conclusions: Identification of occupational hazards plays the key role in evaluation of worker's health risks at workplace. Implementation of work safety measures and health surveillance by occupational health services are the most important preventive measures in reducing occupational skin diseases.

Key words: Occupational Disease, Skin Disease, Workplace

Introduction

Occupational skin diseases (OSD) are skin lesions that occur due to exposure to various occupational hazards at the workplace. OSD are among the most frequent work related diseases in industrialized countries [1, 2].

Autonomous Province of Vojvodina is the most developed economic region of Republic of Serbia with a population of roughly 2 million.

The purpose of the study was to examine the characteristics of OSD in Vojvodina by industry, and to determine the types of occupational hazards that lead to OSD. To the best of our knowledge, there are no published studies of OSD in Vojvodina to date.

Materials and methods

The study was based on records of OSD from the Register of Occupational diseases in Autonomous Province of Vojvodina at the Institute of Occupational Health Novi Sad, during the 12-year-period (1997-2008). The Register is established on the legislation concerning occupational diseases [3, 4].

Information available from these sources included, as follows: sex and age of workers; type of industry; work experience and related exposition to occupational hazards; induction period for the occurrence of skin disease; the final diagnosis of skin disease; the use of personal protection; and the assessment of work ability.

Occupational disease rates per 100000 employees by occupational groups were calculated whenever possible. Employment data used for in-

cidence rate calculations were based on the number of workers by industry in Vojvodina [5, 6]. The statistical significance of the results was analyzed by Student's t-test and chi-square ($\chi 2$) test, and $p<0.05$ was considered significant.

Results

In the observed 12-year-period, from 1997 to 2008, a total of 70 persons were affected by OSD. The number of cases was the highest in 1998 when 14 cases were registered (Figure 1).

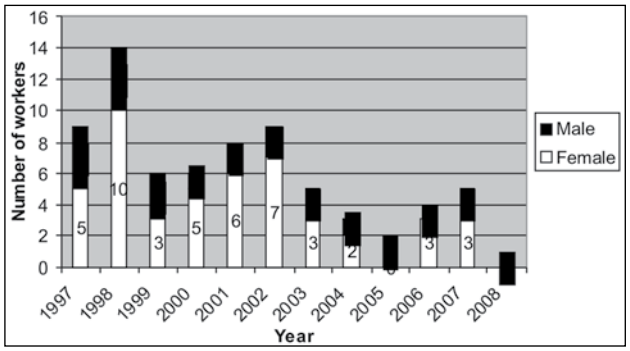

Figure 1. Number of workers with occupational skin diseases by gender from1997 to 2008

The number of female workers was 47 while the number of male workers was 23, resulting a ratio of 2.04:1, which was statistically significant ($\chi 2$, $p<0.001$).

On average, age of affected workers was 44 (SD 8.2) years, their work experience was 20 (SD 7.5) years and induction period for the occurrence of skin disease was 17 (SD 8.9) years. Although there was statistically significant difference between male and female workers at average age (t-test, $p<0.05$), the same was not recorded considering their average induction periods (t-test, $p>0.05$).

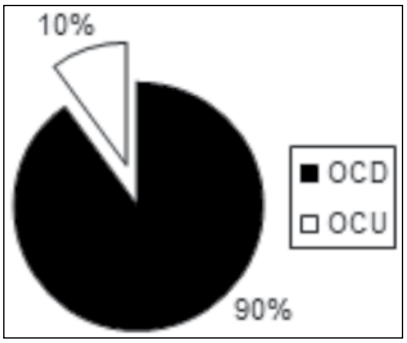

Figure 2. Number of workers with occupational contact dermatitis (OCD) and occupational contact urticaria (OCU)

The diagnosis of occupational contact dermatitis (OCD) was established in 63 (90%) cases, while the rest of the workers 7 (10%) were diagnosed as occupational contact urticaria (OCU) (Figure 2).

In total of 63 cases of OCD, 60 cases (about 95%) were allergic, 2 were irritant, and 1 case was phototoxic type.

Cases of occupational skin diseases were recorded in 17 different occupational groups (Table 1).

Table 1. Number of occupational skin diseases cases in occupational groups (1997-2008)

Occupational group	Total number	
	N	%
Healthcare	13	18.5
Leather industry	10	14.3
Textile industry	9	12.8
Food industry	8	11.4
Agriculture	6	8.5
Metal products industry	5	7.1
Printing industry	3	4.2
Mineral industry	3	4.2
Hairdressing	2	2.8
Furniture industry	2	2.8
Chemical industry	2	2.8
Construction	2	2.8
Paper industry	1	1.4
Retail trade	1	1.4
Catering	1	1.4
Oil industry	1	1.4
Wood processing industry	1	1.4
TOTAL	70	100

Regarding the total number of cases per occupational group, for period 1997-2008, the highest number of cases was in healthcare with 13 (18.5 %) affected, followed by employees in the leather industry with 10 (14.3 %) cases, and workers in the textile industry with 9 (12.8 %) cases (Table 1).

In all occupational groups, out of 94 identified occupational allergens, the five most common in relation to the number of allergic reactions were: cobalt chloride with 20 positive reactions, nickel sulphate with 18, potassium dichromate with 14, para-phenylenediamine with 7 and penicillin with 6 positive reactions. In health care the most frequent allergen was penicillin, while in leather industry the most frequent were nickel suphate and cobalt chloride (Table 2).

Table 2. The five most common allergens and the number of positive reactions in occupational groups with the highest number of occupational skin diseases cases: healthcare, leather and textile industry, as well as in all occupational groups

Occupational group	The five most prevalent allergens	Number of positive reaction
Healthcare	Penicillin	5
	Nickel sulphate	3
	Hydroquinone	3
	Thiuram mix	2
	Epoxy resins	2
Leather industry	Nickel sulphate	7
	Cobalt chloride	7
	Leather	4
	Lining	3
	Para-phenylenediamine	2
Textile industry	Potassium dichromate	2
	Balsm of peru	2
	Wool	2
	Synthetics	2
	Grass pollen	2
All occupational groups	Cobalt chloride	20
	Nickel sulphate	18
	Potassium dichromate	14
	Para-phenylenediamine	7
	Penicillin	6

Table 3. Incidence rate of occupational skin diseases in occupational groups (2002-2008)

Occupational group	Incidence rate per 100.000 employees
Healthcare	2.5
Leather industry	15.7
Textile industry	7.9
Food industry	1.2
Agriculture	1.4
Metal products industry	8.4
Printing industry	4.6
Mineral industry	1.9
Hairdressing	81.6
Furniture industry	8.8
Chemical industry	-
Construction	-
Paper industry	-
Retail trade	-
Catering	-
Oil industry	-
Wood processing industry	-
TOTAL	1.6

Unfortunately, due to lack of employment data on occupational groups from 1997-2001, we could have calculated incidences only for 7-year-period (2002-2008). The incidence was 1.6 cases per 100.000 workers per year, with the highest incidence recorded in hairdressers with 81.6 cases per 100.000 workers per year, followed by workers in the leather and the furniture industry with 15.7 and 8.8 cases per 100.000 workers per year, respectively (Table 3).

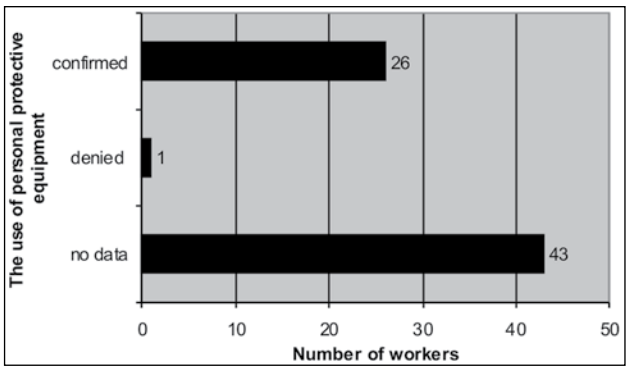

Figure 3. The use of personal protective equipment according to statements of affected workers

Concerning the use of personal protection, 26 (about 37 %) out of 70 affected workers confirmed the use of gloves and other personal protective equipment during work, one worker denied using, while for the rest of 43 (about 62 %) affected workers no data were available (Figure 3).

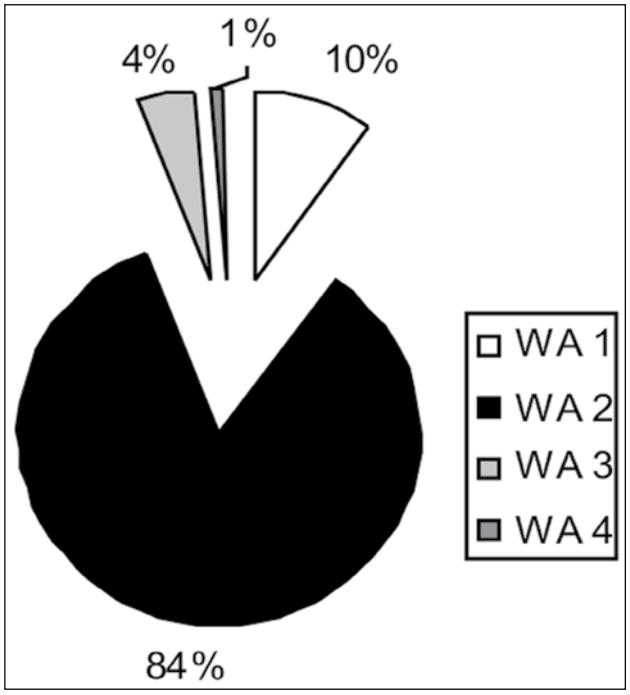

Figure 4. The assessment of work ability (WA): WA 1 - able to work at the same work place with certain restrictions; WA 2 - work at the other work places with certain restrictions; WA 3 - the loss of work ability; WA 4 - undetermined due to cardiovascular comorbidity.

According to the assessment of work ability, 7 (10 %) workers were able to work at the same work place with certain restrictions, 59 (84 %) workers were able to work at the other work places with certain restrictions, and in 3 (4 %) workers the loss of work ability was determined. However, in one patient the work ability was not determined because a cardiologist's opinion was needed due to cardiovascular comorbidity (Figure 4).

Discussion

In the USA, as well as in the most European countries, contact dermatitis (both allergic and irritant) account for between 90 and 95 % of cases of OSD, in our research it was 90 % [1, 7]. Generally, it is believed that 80 % of people suffering contact dermatitis have an irritant type of contact dermatitis. However, from occupational point of view, the situation is slightly different. In Singapore, in period between 2003 and 2004, occupational irritant contact dermatitis (OICD) accounted for 62.4 % cases of OCD, which was similar to research done in Northern Italy [8, 9]. In Western Australia 1999 occupational allergic contact dermatitis (OACD) was diagnosed in 38 %, while in New South Wales was diagnosed in 35 % of cases [10, 11]. In Vojvodina, OACD had by far the largest proportion in OCD which could have been explained with very stringent criteria for the recognition of skin disease as occupational related, and allergic reactions to occupational hazards in the working environment were the most objective evidence of occupational disease etiology.

The statistically significant higher number of affected female workers comparing to male workers in our study could be explained by their more frequent occupational exposure to contact alleregens. It is believed that female workers are more prone to delayed-type hypersensitvity [12]. Subtle, but important, endogenous gender differences in this type of immune reactions demand further defining [13, 14].

It is estimated that the annual incidence of work-related skin diseases in the world is 0.9-1.5 cases per 1000 workers [7]. Nonetheless, it is believed that the real incidence in the USA is 10 to 50 times higher than published, because the mild cases of skin diseases usually are not reported [15]. Based on the literature review, it could be concluded that most of the studies lack appropriate standardization [16]. Notification of OSD is compulsory in Germany, Finland and Denmark. In Germany, prospective standardized registries in the region of the Saarland and Northern Bavaria are established. According to these registries the estimated annual incidence in the entire working population is 70 cases per 100000 employees [17]. The simmilar indicence rates were recorded in Finland and Denmark, as well [18, 19]. The difference in incidences between Vojvodina and abovementioned countries not only could be interpreted with obvious economic development of these European countries, but intensifying previously rigorous criteria set out in the Regulations on occupational diseases in Republic of Serbia [4].

Health and Safety Executive (HSE) in Great Britain in 2005 declared hairdressers as an occupational group who were most at risk for suffering from OSD with the annual incidence of 98 cases per 100000 workers [20]. The similar incidence was recorded in our study (81.6 cases per 100000 workers). The process of hair shampooing carries risks for the development of skin irritation. Although wearing of gloves during this process is legally regulated in the European countries in 1992, the great majority of the hairdressers fail to comply with this regulation [20].

Neither our nor the other studies neglected the importance of antibiotics as potential allergens in the occupational setting. Contact allergy to penicillin and it's derivatives (ampicillin, amoxicillin, cloxacillin) can be assocciated with early-type hypersensitivity such as anaphylaxis, therefore general preventive measures against anaphylactic shock must be applied even in patients with contact dermatitis to penicillin. Since most of the patients tested on natural and semisynthetic penicillins already had contact with them before, it is difficult to assess whether hypersenstitivity is the result of multiple sensitisation or crossed reaction [21].

The role of nickel as an occupational allergen is still unclear and up to 12% of the total estimated cases of occupational contact dermatitis are thought to be due, at least partially, to nickel [22]. Nickel, as well as cobalt sensitization is highly associated with leather work. However, cobalt rarely is a unique sensitizer; more frequently this allergen is found to be associated with either nickel or chromate sensitization. It is believed that personal and familiar atopy is not associated with nickel and cobalt sensitization [23].

In our study in 62% of workers suffering from OSD no data could be obtained on use of personal protective equipment (PPE) in the work process. Lack of data can be interpreted as a worker's fear of loss of financial compensation or attitude of the employer if they admit that PPE were not even used. PPE is primarily related to the use of gloves during work. For example, small molecular acrylates can permeate thin protective disposable gloves [24]. Using adequate PPE is considered the most important protective measure [25]. Diepgen emphasized that although clinical studies suggested that many PPE had the expected effect, there was insufficient epidemiologic evidence for or against the use of PPE. For example, gloves are often recommended in the work process, but in fact may contribute to increased risk of developing contact dermatitis, as microenvironment inside the glove is drastically changed, and the wearing of unsuitable gloves are often worse than not wearing them [26].

Assessment of work ability of workers suffering from OSD in our study in the highest percentage (84%) was that they were able to work at another workplace with restrictions. Hogan described that the change of workplace was mostly reserved for those workers with severe hypersensitivity to certain allergens, which in the work process in any way could not be eliminated or suppressed (which was usually the case in our study), as well as for those workers with atopic eczema of hands whose workplace demanded repeated skin exposure to irritants such as water, soap and solvents [27].

In the first study on the impact of OSD on the quality of life in 2005 year, Hutchings et al. concluded that OSD had notable impact on quality of life which was reflected primarily through the symptoms and feelings and at least through the daily activities [28]. Quality of life in patients with OSD compared with patients with atopic dermatitis appears to be better. In patients suffering OSD hands are the most common place of affection as atopic dermatitis may affect the face and other regions which can cause more inconvenience and thus in large proportions affect daily activities, leisure, business and social relationships. Most of the studies that estimated the role of workplace changes in patients with OSD did not prove the improvement in the prognosis of OSD after changing workplace [28].

Identification of occupational hazards plays the key role in evaluation of worker's health risks at workplace. Implementation of work safety measures and health surveillance by occupational health services are the most important preventive measures in reducing occupational skin diseases.

Acknowledgement

This research was supported by Ministry of Science of the Republic of Serbia, Project number 41012.

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Physical education and sport students' awareness and attitudes regarding HIV and AIDS in Turkey

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Abstract

Background: Research regarding Turkish university students' knowledge, attitudes/beliefs, and perceptions of risk about HIV/ AIDS is fairly limited, and studies mainly result in information that is far from defining the real situation.

Objective: The aim of this study is to examine Turkish physical education and sport students' awareness and attitudes regarding HIV and AIDS.

Methods: A structured questionnaire was designed primarily to obtain information on HIV- and AIDS-related knowledge and attitudes of the Physical education and sports students. The software generated descriptive statistics relating to demographics and Physical education and sports students knowledge, and data were expressed as both means ± standard deviations and percentages. Analysis of variance (ANOVA) and Student's t-test analyses were used to assess statistical significance. In all analyses, the usual significance level was 5%.

Results: A sample of 545 physical education and sport students in Turkey were included in this study. The respondents exhibited a moderate level of knowledge about HIV and AIDS. However, the survey revealed some common misconceptions, indicating that physical education and sport students require additional training in the mechanisms of HIV transmission. We found some differences in the level of HIV-related knowledge between different demographic groups. Female students have a high level of awareness in terms of how HIV/ AIDS is spread by contagion, the ways of guarding against HIV/AIDS, and the effects of HIV/AIDS. We also observed that knowledge levels show significant differences with both age and class. Our findings show that the major source of knowledge about AIDS was mass media, including television, the Internet and newspapers. These results are discussed within the framework of critical studies on HIV-related knowledge. Implications for the development of curricula and in-service training programs for physical education and sport students, organizations and institutions are discussed.

Conclusion: It was concluded that Female students have a high level of awareness in terms of how HIV/AIDS is spread by contagion, the ways of guarding against HIV/AIDS, and the effects of HIV/AIDS. Further, knowledge levels show significant differences with both age and class whereas the major source of knowledge about AIDS was mass media, including television, the Internet and newspapers.

Key words: HIV/AIDS; knowledge; attitudes; sexual behavior; Physical Education and Sport Students; Turkey

Introduction

Acquired Immune Deficiency Syndrome (AIDS) is an important social health problem which is threatening the whole world, and is still incurable. According to the reports of WHO released in December 2008, of about 33.4 million HIV-infected people, there are 31.3 million adults, and 2.1 million children and the adolescences younger than 15. It was reported that 33.9 million people have died of AIDS since the disease was identified (1,2) . HIV/AIDS has no definite cure, may only be protected by taking necessary precautions, and is a costly disease for its expensive and long treatment process. On the other hand, health education and precautions are the main priorities to be protected from AIDS, as it is not curable yet. Thus, having sufficient information about AIDS is vital for the control of the infection. Moreover, raising information level decreases risk behaviors. Although adolescences and adults are expected to be more informed to avoid high-risk behaviors, but the expected fact cannot be always achieved (3).

Many older adolescents and young adults were infected with AIDS as teenagers[4]. According to the United Nations Joint Programme on HIV/AIDS, young people between the ages of 15 and 24 account for 45% of all new HIV infections (5,6). Particularly, in many parts of the world, young adult individuals are in sexual relations at early ages without having inadequate knowledge and with misconceptions about sexually transmitted diseases which increases their chance of contacts with HIV/ AIDS and STDs(7,8,9). There are several factors raising the risk of HIV infection among young people, such as first sexual experiences, drug addiction at this ages and high rates of sexually infected diseases(10). Nevertheless, there is a chance to diagnose protective health behaviors in young people.

Students are characterized as having minimum illness and death rates and healthy individuals of the young population. Moreover, students can also be considered as the vulnerable risk population. According to the results of the 1995 National College Health Risk Behavior Survey by The Centers for Disease Control(11). There are high levels of a variety of behaviors that put college and university students' health at risk(12). Sexually transmitted diseases and HIV/AIDS resulting from risky sexual behaviors cause a big burden over the students. Of all the HIV transmission, 50% is observed in young population(13,14). Thus, it is important to provide and support young generations who will shape the future with healthy sexual behaviors and attitudes. University students have a special and important place among young people since they constitute a mixed culture with different value judgments and feel the socio-economic changes intensively. Students risky sexual behaviors generally result from their inadequate knowledge levels, lack of education about STDs and HIV/ AIDS. 75% of the students answered the question of common information source as doctors, while the answer to the same question in a similar survey in the other countries is peers and friends(15).

In contrast to other studies conducted in non-Islamic countries such as Nepal(16) , sexual education and discussion is minimal in Islamic countries such as Turkey[17].Younger people prefer to share and talk about their sexual problems and needs to their peers rather than their families or parents(18,19). They may have a chance to get wrong information or misconceptions from their peers as they have no definite information sources and levels(19). Therefore, it is vital to inform younger people about HIV/ AIDS.

The ironical common belief among young adolescents is that HIV cannot affect them but affects others(20,21). For example, in a national survey conducted in Zambia, it was found that 32% of the adolescent /young females and 30% of the adolescent males of all the participants reported that AIDS could not be avoided. Likewise, 25% of the adolescent females and 21% of the adolescent males reported that they had no knowledge that AIDS could infect a healthy person(22).

Turkey, there is relatively an increase on diagnosed HIV/AIDS cases as a result of the awareness and test facilities on HIV/AIDS. However, Turkey is still considered as having rare HIV/ AIDS cases. From the period 1985 to June 2010, 4177 HIV (+) cases were reported to the Ministry of Health. 70% of these cases are males. People between the ages 25 and 39 are the most affected age group from the infection. The most common ways of the transmission of the infection are the sexual intercourse (65%) and the injections (3%) (23). Moreover, HIV/AIDS education is poorly developed in the Turkish school system. According to teacher and counselor trainers, HIV/AIDS information is not included in the curricula of secondary and high school system. Furthermore, teachers and counselors do not receive HIV/AIDS information in their training programs(24). Sexuality is a taboo in Turkish society and with a 97% of Muslim society; girls are not allowed to have sexual relation before marriage(25,18).

Although Turkey is one of the rare countries that has a low of epidemic level, exposing to HIV and AIDS seems a considerable health issue. For instance, the first AIDS case was reported in 1985, whereas the total number of the cases reported officially was 2711 (638 AIDS and 2073 HIV+). The data reported officially indicate that heterosexual relations, (75%), man to man sex (16%) and drug injection (7) were the most common transmission among HIV cases (Joint United Nations Programme on HIV/AIDS (5). On the other hand, it cannot be stated that the official data were true due to the low level of consultation of people with STDs at hospitals and inadequate scientific data(26).

People's attitudes towards HIV/AIDS and infected people are affected by the various aspects of culture. Cultural and social values some of which Islamic based are still widespread in Turkish culture. Turkish social norms play an important role on individual's sexual normality and his/her perception of sexual norms(27). Nevertheless, as a result of the social and cultural changes in Turkey, people have much more sexual freedom than the previous generations in some parts of the country. Thus, people are under the risk of infections including HIV(28). As Turkish culture is made up different value systems, Turkish society is very complex from the aspects of sexual behaviors and sexually transmitted diseases(29).

University students have been targeted in many countries for HIV/AIDS prevention programs. This group's greatest risk stems from relatively high levels of sexual activity and a tendency to have multiple sex partners(30). Information regarding Turkish university students' knowledge, attitudes/beliefs, and perceptions of risk about HIV/ AIDS is limited, not in published form, or available solely in Turkish publications where translation to English is necessary(31,32,33,34,35). Limitations of these studies, mainly due to small samples, result in information that is far from defining the real situation. The studies primarily focus on factual knowledge about HIV/ AIDS, and do not include extensive information about university student's attitudes toward people with HIV/AIDS, university student sexual behaviors, personal perceptions of risk, nor university student sexual communication behaviors. For instance, Pehlivan (1994) showed that when Turkish students from different universities were asked to list STDs, the majority of them (93%) mentioned AIDS(32). Eylen (1996) reported at the Fourth Turkish Counseling and Guidance Conference that the majority of Turkish teacher university students in her 100 person sample had both accurate information about AIDS and limited knowledge about specific AIDS facts. It was found that the main information source of these university students was the media. The researcher suggested the need for further HIV/AIDS education of Turkish teachers-in-training(34).

Below is a review of the results of prior research regarding the attitudes towards HIV/AIDS. To begin with, According to the students of Nis University, mass media is the most important tool to get information about HIV/AIDS. 36.8% of the students who participated to the survey pointed out the 'media'. The same results have also been pointed out in the other countries that television and printed media is the most outstanding way for getting information about HIV/AIDS and STDs(36,37). The common view of all the young people in all over the world is that they seldom state their parents as the source of information about HIV/AIDS(38,39). In a survey conducted in Bosnia- Herzogovina, only 10 % of the students stated that they got information from doctors(40). Statistically, there are no differences between males and females about the answers to the question of the most common source of information about HIV and AIDS. However, there are differences in the distribution of answers to the same question between medical and non-medical students. As medical students are in a more close contact with doctors during their education, 81.7 % of them indicated doctors as people from whom they generally received information, while the answer to the same question among non-medical students is 71.3%(36). In addition, Nwokocha and Nwakoby (2002) stated that Nigerian kigh school students did not have enough information on basic facts about HIV/AIDS, including the reasons, the ways of transmission and the prevention of the disease(41) Tavoosi et al. (2004) propounded that Iranian students had misconceptions about the transmission ways of the disease(42). For example, mosquito bites (33%); public swimming pools (21%) and public toilets (20%) were stated incorrectly as the ways of transmission. It is likely that, in more liberal countries where HIV/AIDS cases were in high levels, the attitudes and the knowledge of young people were relatively much more than the conservative countries where the cases were in low levels. For example, Marcelin et al., (2006) conducted a survey having 300 items in the USA, Florida, and Miami-Dade Country to Haitian adolescents about their knowledge and beliefs on HIV and AIDS (43). They discovered that participants had high levels of knowledge on the ways of transmission such as sharing needles and having unprotected sexual relations. Moreover, they found that condom was used as a prevention method. Moreover, in a survey conducted by Polat,Yüzer and Başer (2011) it was found

that 42.4 % of the students used a condom during sexual intercourse (44). In another study conducted on Greek students of a technical high school, it was found that 72% of the questions related to HIV were answered correctly and the 45% of the participants stated that they believed to have enough knowledge about HIV and AIDS (45). Similarly, Dias et al. (2006) found that Portuguese adolescents had high levels of knowledge about HIV transmission with positive opinions and tolerance towards infected people (46). Nevertheless, Dias et al. discovered that the adolescents had also high level of misconceptions.

As a result, research regarding Turkish university students' knowledge, attitudes/beliefs, and perceptions of risk about HIV/ AIDS is fairly limited, and studies mainly result in information that is far from defining the real situation.

Thus, the current study aims to investigate Turkish physical education and sports students' level of knowledge, sources of information and attitudes towards HIV and AIDS and educational needs concerning the disease. With these concerns in mind, the research asks three research questions:

1. What are the level of physical education and sports students' level of knowledge about HIV and AIDS?
2. What are their sources of information about HIV and AIDS?
3. What are their attitudes towards HIV and AIDS?

Methods

Samle and Settings

Physical education and sports students were the target population of this study. The sample group in the study consisted of 545 Physical education and sports students from universities in Balikesir, Istanbul, Bursa, Sakarya, Antalya and Burdur in Turkey. Socio-demographic characteristics of respondents of Physical education and sports students came from a wide range of backgrounds, as presented in Table 1. Of our respondents, 63.5% were male and 36.5% female, 36.5% were between the ages of 20 and 24 years and 50.1% The population of the study consisted of 545 freshmen, sophomores, juniors, and seniors at the Departments of Physical Education and Sports, Coaching Education and Sports Management in Turkey. Of the 545 students, 48.3% (263) were in the Physical Education and Sport Department; 20.9% (114) of the students were in the Coaching Education Department, and 30.8% (137) of the students were in the Sport Management Department. With respect to class, 217 (39.8%) were freshmen, 101 (18.5%) of the students were sophomores, 112 (20.6%) were juniors, and 115 (21.1%) were seniors. Nearly half of the respondents (93.8%) were single, (45.9%) emphasized that they had little knowledge about HIV and AIDS.

Table 1. Socio-demographic characteristics of respondents (N = 545)

Variable		n	%
Gender	Male	346	63.5
	Female	199	36.5
Age	≤18	191	35.0
	20-24	273	50.1
	25-30	81	14.9
Departments	Sport Management	167	30.8
	Physical education and sport	263	48.3
	Coaching Education	114	20.9
Marital status	Single	511	93.8
	Married	12	2.2
	Widowed	11	2.0
	Living together	11	2.0
Class	Freshmen	217	39.8
	Sophomores	101	18.5
	Juniors	112	20.6
	Seniors	115	21.1
Sport area	Football	188	34.5
	Basketball	101	18.5
	Volleyball	117	21.5
	Wrestling	20	3.7
	Judo	9	1.7
	Swimming	28	5.1
	Athletics	44	8.1
	Taekwondo	16	2.9
	Karate	15	2.8
	Gymnastics	7	1.3
Level of HIV/AIDS knowledge	None	35	6.4
	A little	250	45.9
	Some	214	39.3
	A lot	46	8.4

A structured questionnaire was designed primarily to obtain information on HIV- and AIDS-related knowledge and attitudes of the Physical

education and sports students. The questions were selected based on a review of previous studies(47,41,16,48,49,50,51). The questionnaire was initially tested with 125 Physical Education and Sport Students who were not included in the main study. Based on the results of the pilot study, some survey items were changed. The final questionnaire was composed of the following four parts: (1) demographic items, including gender, age, educational level, marital status, departments, class, sport area and level of HIV- and AIDS-related knowledge (6 items); questions regarding HIV- and AIDS-related knowledge covering three main topics of general information (7 items); (2) questions regarding modes of HIV transmission (15 items) and methods of prevention and treatment (7 items); (3) questions regarding respondents' attitudes towards HIV and AIDS (12 items); and (4) finally questions regarding the source of respondents' information on HIV and AIDS (13 items). The response categories for each section were as follows: for their- and AIDS-related knowledge section, questions were in a 'yes', 'no' and 'undecided' format; items on attitudes relating to HIV/AIDS were in a 3-point scale format ('agree', 'neither agree nor disagree' and 'disagree') and finally, 'yes' and 'no' responses were used for questions relating to sources of information. The information used in the main study was collected from 545 Physical education and sports students in face-to face interviews. The questionnaire was administered during the last week of Spring semester of 2010-2011 academic year.

The outcomes of this survey were statistically analyzed using SPSS software for Windows 11.5. The software generated descriptive statistics relating to demographics and Physical education and sports students knowledge, and data were expressed as both means ± standard deviations and percentages. Analysis of variance (ANOVA) and Student's t-test analyses were used to assess statistical significance. In all analyses, the usual significance level was 5%.

Results

HIV/AIDS knowledge of respondents

Table 2 shows the physical education and sports students responses to the questions regarding general HIV- and AIDS related knowledge, the mode of transmission and methods of treatment and protection. For the most part, the physical education and sports students had a good level of knowledge about HIV and AIDS. Most of the respondents knew that AIDS is caused by a virus (89.0%), and that HIV can be detected through blood test (83.7%). However, 26.1% of the respondents were undecided about whether mosquitoes can transmit HIV and 44.6% of the respondents agreed with the misconception that mosquitoes can transmit HIV.

Regarding knowledge of the mode of transmission, most of the Physical education and sports students surveyed knew that HIV is transmitted through the having unprotected sexual intercourse with an infected person (82.2%), using a hypodermic needle contaminated with HIV/AID (80.9%) and Receiving transfusions of infected blood (80.4%). The majority of the physical education and sports students knew that HIV is not transmitted by shaking hands or touching someone with AIDS (74.3%) or by kissing or hugging an infected person (62.4%). However, only 47.9% of the respondents responded to the statement 'HIV is transmitted from the breast milk of an infected woman to her baby' correctly. Their responses to the statements 'HIV is transmitted by sharing personal items with an infected person such as a drinking cup, towel or clothing' and 'HIV is transmitted by sharing a toilet with an infected person' also revealed some misconceptions.

Regarding methods of treatment and protection, the Physical education and sports students knew that AIDS cannot be avoided by exercising regularly (64.0%), and that the best single way to prevent HIV is through education (71.6%). Condom use can help protect against HIV infection (72.3%). However, the respondents held some misconceptions about the items 'there is a new effective vaccine against the disease' and 'avoiding people with AIDS' as a method of protection.

Variables associated with socio-demographic characteristics of respondents and relating to general knowledge, knowledge of the mode of transmission and knowledge of methods of treatment and protection in the univariate analysis were analyzed using analysis of variance (ANOVA) and Student's t-test analyses. No statistically significant differences in the Physical education and sports students' knowledge were found with respect to educational status and gender class.

Table 2. HIV- and AIDS-related knowledge of the respondents (N = 545)

General knowledge	Yes		Non		Undecided	
	n	%	n	%	n	%
AIDS is caused by a virus.	485	89.0√	23	4.2	37	6.8
HIV can be detected through blood test.	456	83.7√	19	3.5	70	12.8
HIV is a virus that weakens the immune system.	436	80.0√	26	4.8	83	15.2
People with HIV can look healthy.	340	62.4√	168	30.8	37	6.8
It can take a long time for an infected person to show any symptoms of the disease.	335	61.5√	82	15.0	128	23.5
AIDS is a disease that is only transmitted sexually.	266	48.4	224	41.1√	57	10.5
Mosquitoes can transmit HIV.	243	44.6	160	29.4√	142	26.1
Mode of transmission						
Having unprotected sexual intercourse with an infected person.	448	82.2√	42	7.7	55	10.1
Using a hypodermic needle contaminated with HIV/AID.	441	80.9√	47	8.6	57	10.5
Receiving transfusions of infected blood.	438	80.4√	52	9.5	55	10.1
Having a tattoo done with a device that was used on an infected person.	409	75.0√	50	9.2	86	15.8
From an infected pregnant woman to her unborn baby.	340	62.4√	69	12.7	136	25.0
Sharing a razor blade with an infected person.	314	57.6√	163	29.9	68	12.5
From the breast milk of an infected woman to her baby.	261	47.9√	132	24.2	152	27.9
Kissing or hugging an infected person.	119	21.8	340	62.4√	86	15.8
Coughing and sneezing of an infected person.	113	20.7	317	58.2√	115	21.1
From sharing personal items with an infected person, such as a drinking cup, a towel or clothing.	111	20.4	329	60.4√	105	19.3
A massage given to or received from an infected person.	106	19.4	327	60.0√	112	20.6
Sharing a toilet with an infected person.	101	18.5	354	65.0√	90	16.5
Sharing a swimming pool with an infected person.	91	16.7	328	60.2√	126	23.1
Shaking hands or touching someone with AIDS.	80	14.7	405	74.3√	60	11.0
Eating from the same plate as an infected person.	80	14.7	370	67.9√	95	17.4
Methods of treatment and protection						
AIDS can be avoided by exercising regularly.	71	13.0	349	64.0√	125	22.9
The best single way to prevent AIDS/HIV is through education	390	71.6√	79	14.5	76	13.9
Condom use can help protect against HIV infection.	394	72.3√	52	9.5	99	18.2
Having only one partner/spouse	421	77.2√	59	10.8	65	11.9
AIDS has a cure/vaccination	202	37.1	233	42.8√	110	20.2
There is a new, effective vaccine against the disease.	112	20.6	236	43.3√	197	36.1
Avoiding people with AIDS	278	51.0	182	33.4√	85	15.6

Note: √ correct answer

The respondents' attitudes towards HIV and infected persons are shown in Table 3. The vast majority of the respondents agreed with the following statements: 86.8 % education on how to avoid HIV should be given to all pupils at school; 83.3% HIV- and AIDS-related education, including information on protection against the disease, should be provided to Physical education and sports students in service training seminars; 81.8% AIDS-related education, including information on protection against the disease, should be provided to Physical education and sports students in service training seminars and associations should publish books, brochures or posters about AIDS and methods of protection against the disease for Physical education and sports students; 82.9% infected persons should be supported, treated and assisted; and AIDS is a serious public health problem and infected persons should inform other people about their disease. However, 44.0 %

Table 3. Respondents' attitudes towards HIV and infected persons (N = 545)

Statements	Agree		Disagree		Neither agree nor disagree	
	n	%	n	%	N	%
AIDS is not a serious public health problem.	110	20.2	58	10.2	377	69.2
HIV/AIDS-infected persons should be restricted from work.	196	36.0	123	22.6	226	41.5
HIV/AIDS-infected students should not be allowed to go to school with uninfected children.	173	31.7	154	28.3	218	40.0
Social contact with infected persons is dangerous.	188	34.5	115	21.1	242	44.4
Infected persons should inform other people about their disease.	410	75.2	93	17.1	42	7.7
Infected persons should be supported, treated and assisted.	452	82.9	52	9.5	41	7.5
Education on how to avoid HIV/AIDS should be provided to all pupils at school.	473	86.8	39	7.2	33	6.1
AIDS-related education, including information on protection against the disease, should be provided to Physical education and sports students in service training seminars.	454	83.3	49	9.0	42	7.7
Education-related organizations and associations should publish books, brochures or posters about AIDS and methods of protection against the disease for Physical education and sports students.	446	81.8	61	12.2	38	7.0
I would not like to share a home with an infected person.	240	44.0	169	31.0	136	25.0
I would not like to serve an infected person in the workplace.	169	31.0	175	32.1	201	36.9
I would not feel any compassion towards an infected person.	126	23.1	148	27.2	271	49.7

of Physical education and sports students stated that they would not like to share a home with an infected person. This can be considered a negative or discriminatory attitude towards people living with HIV. This attitude was not significantly related to levels of HIV and AIDS awareness and demographic factors.

Table 4. Respondents' reported sources of information (N = 545)

Source of information	Yes		No	
	n	%	n	%
Television	424	77.8	121	22.2
Internet	379	69.5	166	30.5
Newspapers	330	60.6	215	39.4
School	222	40.3	323	59.7
Health staff	208	38.2	337	61.8
Magazines	207	38.0	338	62.0
Books	172	31.6	373	68.4
Conferences/ seminars	141	25.9	404	74.1
Radio	139	25.5	406	74.5
Brochures	108	19.8	437	80.2
Family	100	18.3	445	81.7
Tourists	39	7.2	506	92.8
Posters	36	6.6	509	93.4

Table 4 shows that the major source of knowledge about AIDS was mass media, including television, the Internet, newspapers, and Health staff Television was reported as the single most important source of knowledge by 77.8% of the respondents. Other important sources of information were the Internet (69.5%) newspapers (60.6%).

Below is the presentation of correlational data. To begin with, T-test results indicate that gender is a significant variable that affects HIV/AIDS Awareness and attitudes. That is, female students have a high level of awareness in terms of how HIV/AIDS is spread by contagion, the ways of guarding against HIV/AIDS, and the effects of HIV/AIDS. (p=0.00). Interestingly enough, the ANOVA results show that younger students are more aware than older students in terms of the spread of HIV/AIDS, preservation from it and the ways how to cope with it. (p=0.00) In a similar way, grade is also a significant variable. (p=0.00) That is the ANOVA results demonstrate that lower grade students seem more aware than higher ones regarding the knowledge of HIV/AIDS, spread of HIV/AIDS, preservation from it and the ways how to cope with it. (p=0.00) Moreover, the findings in-

dicate that the level of knowledge on HIV/AIDS constitutes a significant factor. (p=0.00) On other words, the more the students know about it, the higher they have awareness of spread of HIV/AIDS, preservation from it and the ways how to cope with it. Last of all, the ANOVA results show that the students in the department of physical education teaching have a higher level of awareness of knowledge on HIV/AIDS, spread of HIV/AIDS, preservation from it and the ways how to cope with it. (p=0.00)

Discussion

In this study, most of the Physical education and sport students were male and relatively young. Almost everybody was single. More than half of the respondents emphasized that they had A little level of knowledge about HIV and AIDS; the respondents identified the concept of HIV correctly (virus, infection) in 89% of the responses. The results were similar to the previous studies concerning the knowledge of the Finnish adolescents(52,53), nursing staff and German nursing students(54,55,39,51). However, their survey responses suggested that they were not knowledgeable enough about the mode of transmission or methods of treatment and protection. We also observed that Physical education and sport students were undecided about some statements and were sometimes unable to give exact responses to questions.

The results of the study show similar results to the findings suggested by Montazeri (2005) (48). This may be explained that there is a a low level of public awareness about HIV and AIDS in some countries such as Iran and Turkey. In a broader sense, a considerable portion of turkish people believes that having a sexually transmitted disease is a source of shame, and that it is impossible to discuss HIV and AIDS with anyone. Moreover, in contrast to some other studies carried out in non-Islamic countries such as Nepal(16), sexual education and the discussion of sexuality is at a minimum level in Muslim countries such as Turkey(17). In this sense, in a study examined Turkish medical university students' sexual attitudes and behaviors, Ozan et al. (2005) reported that physical pleasure, curiosity, and desire for experience were the main reasons for the first sexual intercourse for men, while love was a leading reason for women (56). That is, both men and women had more negative attitudes towards the premarital sexual experience of women than of men, as found by Aras et al., (2003) who researched the attitudes of Turkish university students (57). Shapurian and Hojjat (1985) also noted similarities and differences regarding the attitudes of younger Iranian men and women. , and found that Iranian men and women differed considerably in their attitudes towards premarital sex for men, and that a higher percentage of women agreed on premarital sex for their male peers than for their female peers(58). Gender-based differences were observed in the knowledge levels of Physical education and sport students. The results of the study reveal that the male respondents have a higher knowledge level regarding HIV and AIDS when they compared to females. Moreover, female participants were most often undecided about the statements in the survey. The result in supports the findings noted by(59,60,61),while it conflicts with some other studies(62,41,27,48,51). Finally, the findings can be explained by the opinion that educational programs aiming to establish an awareness of HIV and AIDS mainly focus on men. That is, societal and social values in Turkey grant men more privileges and freedom, and, thus, they may more be exposed to sexually transmitted diseases in contrast to the Islamic rules that dictate the sexuality of the woman is limited to only her husband(49). It as also observed that there is a significant difference in the levels of knowledge and attitudes between Physical education and sport students between different age groups. The results shoed that younger students are more aware than the older ones regarding the knowledge and attitudes of AIDS/HIV. The data also suggested that respondents' knowledge of HIV and AIDS does not originate from school while it is derived from their personal experiences.

Physical education and sport students' statements explaining their social and cultural behavior were evaluated to determine their attitudes about HIV and infected persons. In a general sense, Physical education and sport students showed a positive or neutral attitude towards AIDS and infected persons, with the exception of one item stating 'I would not like to share a home with an infected person' and 'I would not like to serve an

infected person in the workplace' Among the overall population, there is a fear of establishing social contact with ones living with HIV. That is, even surgeons who have the adequate knowledge of HIV and AIDSfeel worried about possible social contact with HIV-positive people as reported in a study conducted in Turkey (63,28,51). Similar results have also been found in studies conducted in Iran (41), Tunisia (64) and Central Asian countries (65). This can be explained by the similarity between Iranian, Nigeria, Tunisian, Central Asian and Turkish people's attitudes towards HIV that are associated with the taboo of extramarital sexual relations. Physical education and sport students' major source of knowledge about AIDS was mass media such as television, the Internet and newspapers. Television was the most important source for AIDS awareness in this study. Knowledge sources of knowledge of HIV and AIDS seemed similar to the results found in previous studies(17,66,36,27,48,49,67,68,69). To add, Maswanya et al. (2000) stated that 97.5% of the Japanese high school students perceive that television is the main source in addition to written and visual press (70).

However, only a few Physical education and sport students stated that they had acquired knowledge about AIDS from school. First aid and health are taught to the students studying in the departments of Physical education and sport in Turkey, On other words, qualified education at schools can also play an important role in student's good performance. In this sense, students can be encouraged to use condom, and trained about some significant issues such as self-confidence and a healthy sexual life. In addition, a well-known fact that should not be ignored is that today's students waste time in an interactional and communicational environments such as the Internet and social media in which they can easily access to the HIV information when compared to the knowledge sources decades ago. However, there are still opportunities of improvement. In other words, students should be informed about the mode of transmission and prevention as as 44.6% students still believed mosquitoes. It is also obvious that students do not have sufficient and accurate information about HIV/AIDS, and as have not been given sufficient training while AIDS is an important public health problem with its complex, emotional, behavioral, and psychosocial complications that accompany the physical illness. The only way to cope with this disease is prevention. The best way to prevent the disease is through education as implicated by Altun, (2004) and Islam et al.(2002) As a final note, it was observed that there was a significant difference in the levels of knowledge and attitudes between Physical education and sport students between different age groups(71,72).

Based on the findings of this research, some recommendations can be made. As the mass media has an important role in the distribution and dissemination of information about HIV and AIDS to both students and teachers, television usage to inform people about the disease must continue. As Ahmed, Hassali, Bukhari and Sulaiman (2011) stated that success in AIDS education program is best assured when communication and education are carried out continuously based on long-range strategies (73). Moreover, education in terms of all aspects of HIV and IDS should be placed in school curricula, as well as education on how to avoid HIV should be given to university students. For this purpose, education and training related to sexually transmitted infections and their prevention should also be provided to students through the in-service training programs developed by the Ministry of National Education. That is, information is vital to train students about accurate understanding of the modes of transmission and prevention strategies of HIV/AIDS. In this sense, teachers play a major role in the provision of information to increase awareness leading to behavioral change among their students as their knowledge and perception about the disease can have positive influence their on their students. It is also important for the Turkish government to start plan for the implementation of a systematic HIV/AIDS education programme for university students. The starting point should be the acknowledgement that there is a large sexually active university population in Turkey. In addition, the efforts of Turkish HIV/AIDS organizations currently working at grassroots level should be encouraged.

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A successful rescue of a pregnant woman complicated with Influenza A H1N1, severe pneumonia and respiratory failure

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Abstract

The pandemic influenza A (H1N1) virus emerged since 2009, and has reached worldwide. Pulmonary complications of H1N1 influenza A severely endanger survival of pregnant women. we successfully rescued a pregnant woman complicated with influenza A/H1N1 and severe pneumonia. A 32-year-old pregnant woman 36w+ was presented with fever, productive cough and dyspnea, and was diagnosed with influenza A subtype H1N1 with severe pneumonia and respiratory failure. She received assisted ventilation and other therapies and fetus was successfully delivered by cesarian section. After 39 days, the patient was clinically supported and discharged. In this report, we have discussed the greater risks to pregnant women infected with the H1N1 virus, and emphasize the importance of cesarean section to improve ventilation.

Key words: influenza A (H1N1); pregnancy; pneumonia; respiratory failure.

Introduction

The pandemic influenza A (subtype H1N1) virus emerged in the United States and Mexico since 2009, and has rapidly spread worldwide [1,2]. Pregnant women are at especially high risk for the development of complications due to the H1N1 virus [3-5]. Treatments are made more difficult by other concomitant disorders. We report here a case of a pregnant woman with influenza A (H1N1), severe pneumonia and respiratory failure.

Case Report

A 32-year-old pregnant woman 36w+ was admitted to our hospital due to fever, productive cough and dyspnea. The patient had been otherwise well until 6 days prior to admission, when she presented with nonproductive cough and fever which appeared to be flu-like, with a temperature ~37.8 °C. Three days before admission, she complained of thin yellow sputum and dyspnea, and her respiratory distress worsened.

The patient had no history of other diseases or exposure. The vital signs included a temperature of 36.9 °C, pulse 104 beats per min, blood pressure 117/66 mmHg, respiratory rate 50 breaths per min and oxygen saturation 55-60%. The physical examination revealed an acute suffering expression, consciousness and orthopnea. She displayed anoxic manifestations of cyanosis and enhanced respiratory movements. The respiratory rhythm was irregular, accompanied by coarse breathing sounds, and moist rales were heard in both lungs without presentation of rash, lymphadenopathy, sore throat, swollen tonsil or edema in the lower extremities. Laboratory tests revealed a sign of acidosis and severe hypoxemia, abnormal liver and renal function. A chest radiograph showed extensive exudative process in both lungs, which is a sign of white lung (Fig. 1A).

Immediately on admission, the patient received non-invasive assisted ventilation in ICU. The presumptive diagnosis was severe pneumonia, respiratory failure, and suspected influenza A H1N1. She underwent a cesarean section and a live female fetus was delivered 3 hours after admission. However, when the mother returned to ICU, she became unconscious and presented respiratory distress. She received endotracheal intubations and was assisted by mechanical ventilator with pressure-controlled synchronized intermittent mandatory ventilation (SIMV-P), fractional in-

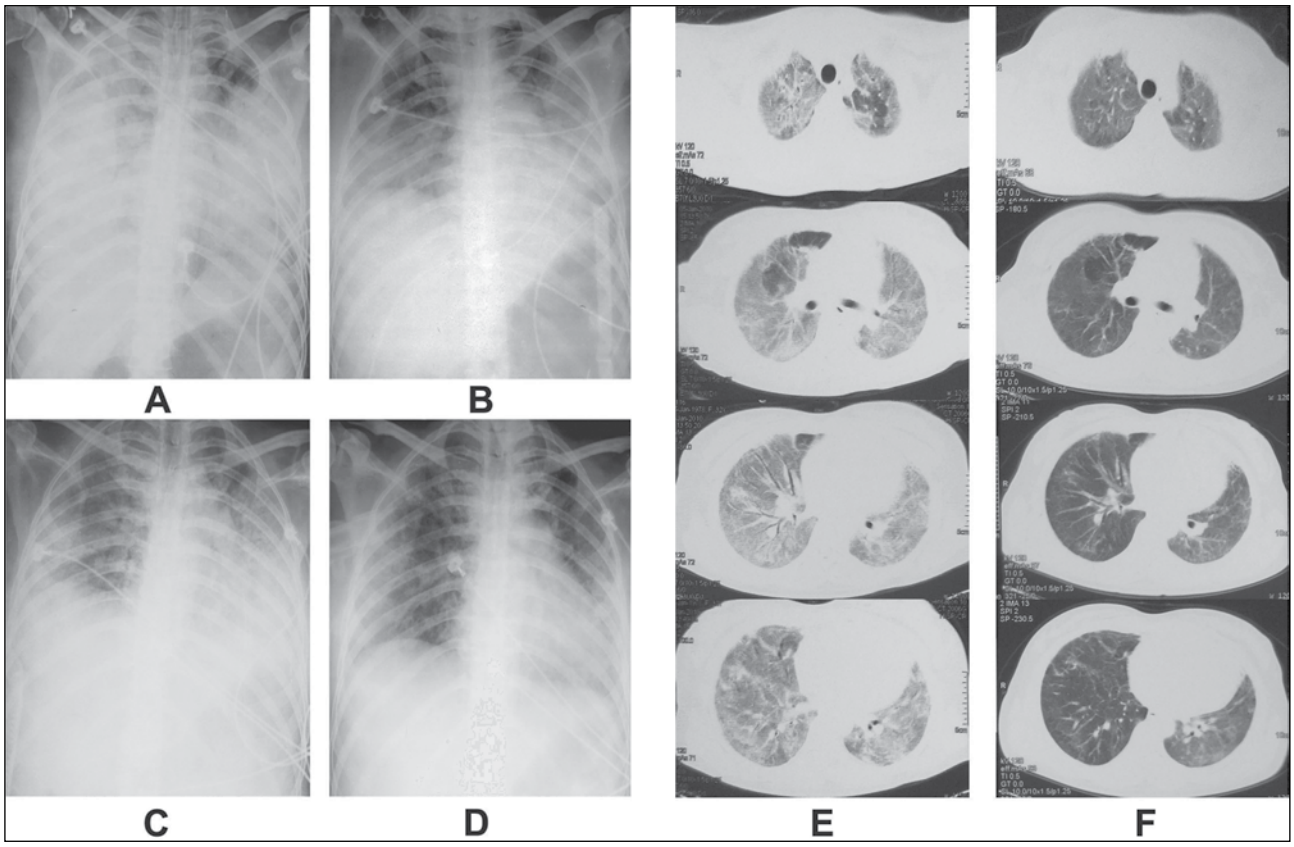

Figure 1. Chest Imaging Presentation
A: Chest radiograph on day 1 of admission; B: Chest radiograph on day 5 after admission; C: Chest radiograph on day 11 after admission; D: Chest radiograph on day 20 after admission; E: Chest CT scan on day 26 after admission: F: Chest CT scan on day 38 after admission.

spired oxygen (FIO_2) of 100%, and positive end-expiratory pressure (PEEP). A specimen from a pharynx swab tested positive for the 2009 H1N1 virus (confirmed by the provincial Center for Disease Control). Multiple bacterial cultures of sputum and blood specimens since admission have been negative. A combination of anti-viral, anti-bacterial and additional symptomatic supportive measures (shown in Figure 2) was administered. Screen of chest imaging was seen in Figure 1. A chest CT (Fig. 1F) on day 38 suggested a few bilateral pulmonary inflammations and bulla in both upper lungs. Because all vital signs and other tests had returned to normal, the patient was discharged.

Discussion

During pregnancy, healthy women have a 4- to 5-fold increased rate of serious illness and hospitalization with influenza[6-7] . According to the Immunization Strategic Advisory Group, pregnant women with H1N1 have a 10 times higher likelihood of requiring admission to an ICU as compared with the general population, and 7-10% of all hospitalized cases are women in their second or third trimester of pregnancy[8] .

Several risk factors are responsible for threatening pregnant patients complicated with influenza A H1N1 with more severe conditions and rapid progression. These include a decreased number of T helper cells and depressed activity of natural killer (NK) cells[9,10]. These changes lower the ability of pregnant patients to remove secretions in the respiratory tract and reduce functional residual capacity, while increasing oxygen consumption and water content in the lungs[11] . Pulmonary infection, hypoxemia and increased oxygen consumption finally cause respiratory failure. Misdiagnosis during the early stage of a pregnancy complicated with pneumonia and different levels of dyspnea during the late stage of pregnancy might result in medical neglect. X-ray examination of pregnant women is safe if the abdomen is appropriately protected [12]. For the case reported here, the patient had not been ino-

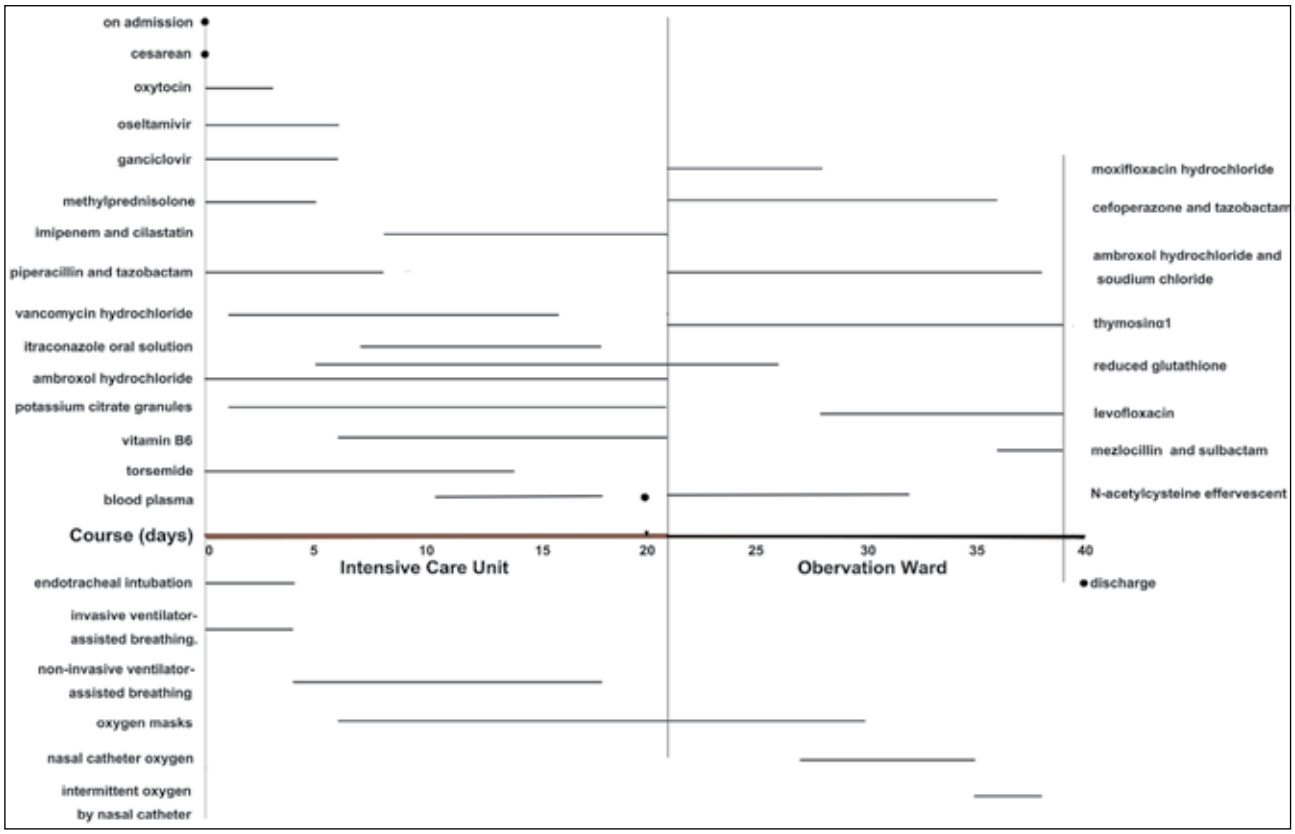

Figure 2. Schematic Diagram of Treatments
The summary of treatments

culated with influenza A H1N1 vaccine, nor did she receive a chest x-ray, and these factors eventually lead to a severe condition.

The patient's ventilation improved and respiratory failure was relieved after she received a combination of cesarean section, assisted ventilation, and methylprednisolone, antiviral and empirical antibiotic treatments. Thus, we think that pregnant women above 36 weeks complicated with influenza A H1N1 pneumonia should have the pregnancy delivered and receive appropriate treatments for the following reasons: 1. Pregnancy promotes progression of disease, as the enlarged abdominal cavity reduces pulmonary function and the extra blood supply to the placenta results in elevated oxygen consumption. 2. Fetal distress might occur because of the mother's fever, hypoxemia and disturbance of electrolytes caused by disease evolution. 3. Damage to the liver and kidneys of pregnant patients complicated with influenza A H1N1 is more severe compared to that of non-pregnant patients. Therefore, continuous monitoring of liver and kidney function and support treatments are desirable after termination of the pregnancy. Pregnant women should receive the inactivated H1N1 vaccine during the influenza season and those with suspected or documented H1N1 infection should be treated with oseltamivir[13]. Because pregnant women are prone to severe complications from H1N1 virus infection, the benefit of treatment with this antiviral medication outweighs its theoretical risk[14]. Also, assisted ventilation support can correct metabolic disorders. Nevertheless, having pregnancy delivered would be more beneficial for the treatment of mother [15]. In our case, application of broad-spectrum antibiotics and appropriate adjustment of medicine were critical for her improvement. We believe that an empirical antibiotic treatment is necessary for viral pneumonia although this is still controversial[16].

Conclusion

For the reasons mentioned above, it is extremely important to strengthen measures for preventing infection and treating pregnant woman complicated with H1N1. Here we successfully rescued a pregnant woman infected with H1N1

influenza and severe pneumonia. This patient's condition and a review of her therapeutic management might provide a reference for clinicians. In this report, we have discussed the greater risks to pregnant women infected with the H1N1 virus, and emphasize the importance of cesarean section to improve ventilation.

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Preoperative and postoperative histological findings in patients with hysterectomy after endometrial hyperplasia or endometrial cancer

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Abstract

Objective: Comparison of preoperative and postoperative histology findings and extent of surgical treatment, as well as presence of disease recurrence and postoperative survival, during the five-year follow-up period in patients included in this study.

Patients and methods: A total of 11 patients with endometrial hyperplasia and 42 patients with endometrial cancer, surgically treated with total abdominal hysterectomy/bilateral salpingo-oophorectomy during one calendar year, were included in our study. Patients' clinical status was followed for five years after the surgery.

Results: Preoperative histological finding of atypical endometrial hyperplasia was confirmed in 63.6% patients after the surgery, while in 36.4% of all lesions were without atypia postoperatively. Dominant histological type of endometrial cancer was endometrioid adenocarcinoma. Cervical involvement was verified in only one patient (2.4%) before the surgery, compared to six cases (14.3%) postoperatively. In three patients out of six (50%) with cervical involvement, distant recurrent disease was registered during the follow-up period. The 5-year disease-free rate for patients with endometrial cancer was 83.33% (35/42), and the overall survival rate was 92.86% (39/42). Disease recurrence occurred in seven patients (16.67%); the median time to recurrence was 24 months (range 2 to 54 months).

Conclusion: We recommend that surgical treatment of patients with endometrial cancer and cervical involvement include para-aortic lymph node dissection, in order to increase disease-free interval and diminish the risk of recurrent disease.

Key words: endometrial cancer, endometrial hyperplasia, total abdominal hysterectomy/bilateral salpingo-oophorectomy

Introduction

Endometrial hyperplasia and endometrial cancers are frequent oncological conditions which affect mainly peri- and postmenopausal women, mostly diagnosed at an early stage with an excellent prognosis. Their usual clinical presentation includes abnormal vaginal bleeding, followed by endometrial biopsy or curettage, and surgical treatment (1, 2, 3).

Endometrial hyperplasia is a heterogeneous histopathological diagnosis, within a wide range from subtle and spontaneously reversible proliferative lesions, to incipient carcinoma (1, 3, 4). The currently accepted WHO terminology, based on tissue architectural features, separates endometrial proliferations into simple or complex hyperplasia, whereas classification based on cytological features (originally proposed by Kurman et al. in 1985) defines typical and atypical hyperplasia (5). This terminology was adopted by the WHO, because of increased progression risk of lesions classified as *complex hyperplasia with atypia* to cancer was reported, in contrast to lesions diagnosed as *hyperplasia without atypia* (23% of cases of atypical hyperplasia progressed while only 2% of those without atypia progressed, within a mean follow-up of 13.4 years) (5,6).

Endometrial cancer comprises about 4% of all malignant tumors in women globally, and occurs predominantly after the menopause (7**).** Cross-sectional incidence rates of endometrial cancer have been increasing among postmenopausal women in most European populations, whereas mortality rates have been declining, with few exceptions. In premenopausal women, endometrial cancer is relatively rare, and where data on incidence are available, observed trends are mainly decreasing. Family history of endometrial cancer is associated with an

increase in risk, while high parity and late age at last birth is considered to give long-lasting protection (8). The role of obesity as a risk factor in both premenopausal and postmenopausal women is also firmly established (9), whereas there is limited evidence that physical activity has a protective effect (9). Use of combined oral contraceptives (COC) confers a long-lasting protection against endometrial cancer, particularly among long-term users (10). Hormone therapy use for treatment of menopausal symptoms (hormone replacement therapy, HRT) is an important risk factor in countries where their prescription has been common practice in recent decades (8). Risk increases markedly with use of estrogen only and sequential estrogen-progestin HRT, although it may be mitigated by the continuous addition of progestin's (11, 12). There is also substantive evidence that smoking is protective (13). Ewertz and Jensen reported increasing rates between 1943 and 1980 in Denmark, suggesting an increase in estrogen use was responsible among postmenopausal women (7).

In comparison to other types of cancer, endometrial cancer has a relatively low mortality, due to its clinical presentation and early diagnosis in FIGO grade I. Surgical management of endometrial cancer is a challenging task. A complete surgical staging, including lymphadenectomy is the gold standard to evaluate lymph node involvement, the most common site of extra uterine spread of endometrial cancer (14). It is also important to balance risks and benefits of each surgical option, avoiding both over- and undertreatment. The crucial change from clinical to surgical staging was performed by the Gynaecologic Oncology Group (GOG) trial, published in 1987. According to this study, pelvic and para-aortic lymphadenectomy have been introduced in the oncological practice of endometrial cancer, bases on the International Federation of Gynaecologists and Obstetricians (FIGO) criteria (9). The new FIGO classification addressed new information about prognostic predictors (9-15).

Many factors (tumor extension, histological grade, depth of myometrial invasion, cervical involvement and lymph-vascular space involvement) influence the clinical course and outcome of endometrial cancer, and have to be discriminating in therapeutic strategy (16, 17). The management of stages I and II has been recently amended by ESMO (European Society for Medical Oncology), who proposed surveillance, brachytherapy, and radiation therapy followed by brachytherapy for respectively low, intermediate and high risk groups (16). These recommendations are controversial and must be confirmed. Locally advanced stages represent a heterogeneous population in which surgery should be proposed if it is feasible, then followed by radiotherapy and/or chemotherapy. The essential surgical treatment for endometrial hyperplasia and endometrial cancer is total abdominal hysterectomy-bilateral salpingo-oophorectomy (TAH/BSO) (16, 17).

The objectives of this study were to compare the accuracy of endometrial biopsy in reference to the final hysterectomy pathohistology, to compare preoperative histology findings to the extent of surgery, as well as the postoperative histology and extent of surgical treatment. Finally, the aim was to establish the presence of complications, recurrence of the disease, and postoperative survival, during the five-year follow-up period in patients included in this study.

Materials and methods

Patients

This investigation included 53 patients (11 with endometrial hyperplasia and 42 patients with endometrial carcinoma) surgically treated with total abdominal hysterectomy/bilateral salpingo-oophorectomy (TAH/BSO) at the Clinics for Gynaecology and Obstetrics in Clinical Centre Nis, Serbia, in 2005. Patients' clinical status was followed for five years after the surgery, until December 2010, at the Clinics of Oncology in Clinical Centre, Nis. Pathohistology was performed at the Institute of Pathology in the Clinical Centre in Nis. This investigation was performed in accordance with the regulations of local Research Ethical Committee at the Medical Faculty in Nis, University in Nis.

Methods

Patients included in this study were prepared for the surgery according to the protocols used at the Surgical Department of Clinics for Gynaecology and Obstetrics at the Clinical Centre in Nis. All patients underwent appropriate diagnostic procedures prior to surgical treatment. Pathohistologi-

cal verification of the specimens was performed at the Institute of Pathology in the Clinical Centre in Nis in order to obtain the preoperative histological findings. All patients were followed by means of physical and/or imaging studies every three to four months during the first two years and every six months thereafter at the same institution.

From available medical documentation the following data were used: age of patients, anamnestical data considering risk factors and reproductive anamnestic data, preoperative histological findings and decision about the extent and type of surgery, and postoperative histological findings, as well as data about further course and outcome of disease.

Statistical analysis

Results were analyzed with descriptive statistics and processed with Windows Office Excel 2000. Parameters calculated were mean and standard deviation, index of structure, interval of variation, Fisher's exact test and Wilcoxon's test. The last two were performed with SPSS software package (ver 12.0) for Windows.

Results

Between January 2005 and December 2005, a total of 53 patients were considered eligible for this study, 42 with endometrial cancer, and 11 with endometrial hyperplasia. Basic clinical characteristics of patients with AEH and endometrial cancer are given in Table 1.

All patients underwent surgical treatment with TAH/BSO. Pathohistological findings of patients with endometrial hyperplasia were compared prior to and after the surgery (Table 2). Preoperative pathohistology was confirmed in 63.6%, while the others were diagnosed with hyperplasia without atypia, and benign endometrial alterations (27.3% and 9.1%, respectively).

Table 1. Clinical features of patients with endometrial hyperplasia and endometrial cancer

Characteristic	Endometrial hyperplasia Cases (n=11) N (%)	Endometrial cancer Cases (n=42) N (%)
Age, mean ± SD (years)	52 ± 6,07	61.88 ± 8.09
Range (min-max)	43-63	46-78
Median age of menarche (yrs)	12	13
Number of pregnancies		
0	1 (9,1)	3 (7,1)
1	1 (9,1)	11 (26,2)
2	8 (72,7)	23 (54,8)
3	1 (9,1)	4 (9,5)
>3	/	1 (2,4)
Number of miscarriages		
0	2 (18,2)	12 (28,55)
1	3 (27,2)	10 (23,8)
2	2 (18,2)	6 (14,3)
3	2 (18,2)	2 (4,8)
>3	2 (18,2)	12 (28,55)
Risk factors - yes	4 (36,4)	28 (66,7)
Risk factors – no	7 (63,6)	14 (33,3)

Table 2. Preoperative and postoperative findings in clinical group with endometrial hyperplasia

Histological variant	Preoperative histological findings		Postoperative histological findings	
	N	%	N	%
Atypical hyperplasia	11	100.0%	7	**63.6%**
Non-atypical hyperplasia	0	0.0%	3	**27,3%**
Other benign alterations	0	0.0%	1	**9.1%**
TOTAL	**11**	**100.0%**	**11**	**100.0%**

Patohistological types of endometrial cancer found in our clinical group are shown in Table 3. Dominant histological type was endometrioid adenocarcinoma (92.8% pre-, and 90.4% postoperatively). Histological grade is given in Table 4, with majority of patients with grade II (G2) (prior to surgery 52.4%, after the surgery 61.9%). Number of patients in which cervical involvement was histologically verified is given in Table 5.

Patients with endometrial cancer received adjuvant therapy after the surgical treatment (4 of them received chemoradiation, 38 received radiotherapy). The 5-year disease-free rate for patients with endometrial cancer was 83.33% (35/42), and the overall survival rate was 92.86% (39/42). Disease recurrence occurred in seven patients (16.67%). (Table 6); the median time to recurrence was 24 months (range 2 to 54 months).

Discussion

Atypical hyperplasia is a reliable high risk predictor in women with subsequent or concurrent endometrial carcinoma. Diagnosis of atypical hyperplasia usually leads to a recommendation of hysterectomy (6, 18, 19).

Average age of our patients with atypical hyperplasia was 52 years (range: 43-63), similar to findings of other authors (16). Risk factors were present in 36.4% patients of the clinical group. After the surgery, preoperative histological findings were confirmed in 63.6% patients, while in remaining 36.4% lesions without atypia (27.3 %) or other benign alterations (9.1%) were verified. No statistically significant difference was found in preoperative and postoperative findings in clinical group with atypical hyperplasia and clinical group without atypia and other benign lesions. Discrepancy between

Table 3. Histological findings in patients with endometrial cancer before and after the surgery

Histological variant	Preoperative histological findings		Postoperative histological findings	
	N	%	N	%
EA*	37	88.0%	35	**83.3%**
EA* with squamous differentiation	2	4.8%	3	**7.1%**
Mucinous adenocarcinoma	1	2.4%	1	**2.4%**
Serous adenocarcinoma	1	2.4%	2	**4.8%**
Mixed cell carcinoma	1	2.4%	0	**0.0%**
Carcinosarcoma	0	0.0%	1	**2.4%**
TOTAL	**42**	**100.0%**	**42**	**100.0%**

**endometrioid adenocarcinoma*

Table 4. Histological grade of endometrial cancer in preoperative and postoperative pathohistological findings

Histological grade	Preoperative pathohistological findings		Postoperative pathohistological findings	
	N	%	N	%
G 1	14	33.3%	12	**28.6%**
G 2	22	52.4%	26	**61.9%**
G 3	6	14.3%	4	**9.5%**
TOTAL	**42**	**100.0%**	**42**	**100.0%**

Table 5. Pathohistological verification of cervical involvement on curettage material and endometrial tissue obtained through hysterectomy

Cervical involvement	Preoperative histological findings		Postoperative histological findings	
	N	%	N	%
Positive	1	2.4%	6	**14,3%**
Absent	41	97.6%	36	**85,7%**
TOTAL	**42**	**100%**	**42**	**100%**

Table 6. Clinical characteristics of seven recurrent patients

Patient number	Age	Grade	Myometrial invasion	Cervical involvement	Adjuvant therapy	Recurrent site	Time to recurrence (month)
1	51	G3	>1/2	-	Chemoradiation	Para-aortic lymphadenopathy. Malignant ascites.	12
2	71	G2	>1/2	+	Chemoradiation	Para-aortic lymphadenopathy.	2
3	61	G2	>1/2	+	Chemoradiation	Colon,liver, stump, peritoneal carcinosis.	25
4	58	G2	>1/2	+	Radiation	Liver, lungs	54
5	56	G2	<1/2	-	Radiation	Vertebra	34
6	69	G3	>1/2	-	Radiation	Colon	22
7	69	G1	<1/2	-	Chemotherapy	Liver	24

the preoperative and postoperative pathohistology can be explained by biological characteristics of atypical endometrial hyperplasia (progression, persistence, regression) (20), interval between curettage and hysterectomy (21), and criteria used for diagnosis of endometrial hyperplasia (4, 5, 6).

At the Clinics for Gynaecology and Obstetrics of the Clinical Centre in Nis, all patients with atypical hyperplasia were treated with total abdominal hysterectomy/bilateral salpingooophorectomy (TAH/BSO). The extent of the surgery was defined by preoperative pathohistology, and was re-evaluated after the postoperative pathohistology. The result of this comparison was also without statistical significance.

The most common histological subtype of endometrial cancer is endometrioid adenocarcinoma, accounting for about 80-85% of endometrial cancers. Less common histologies include serous adenocarcinoma (1-10%) and mucinous adenocarcinoma (1-9%) (17). Total of 90.4% of our patients were diagnosed with endometrioid cancer, while all other types counted for the remaining 9.6%, with serous carcinoma at the second place (4.8%).

It has been suggested that 19-30% of all endometrioid cancers classified as grade 1 after curettage or endometrial biopsy, show higher histological grade (G2 or G3) on operatively extirpated uterus. These findings are easy to understand, since during the curettage superficial, more differentiated part of endometial cancer is removed, and only deeper, more invasive part remains (22, 23, 24, 25, 26). No significant differences between preoperative and postoperative patohohistology were found in our study considering tumor histological grade.

Involvement of endocervical glands and cervical stroma is an important prognostic parameter in endometrial cancer (12, 22). Cervical involvement is a risk factor for extrauterine tumor expansion, lymphonodal metastasis and recurrent disease (16, 17). In our study, cervical involvement was verified in only one patient (2.4%) before the surgery, compared to six cases (14.3%) postoperatively (without statistical significance). In three patients out of six (50%) with cervical involvement, distant recurrent disease was registered during the follow-up period, similar to 44% found by other authors (27). 14.28% (1/7) of our patients with recurrent disease in para-aortic lymph nodes had confirmed cervical involvement, which is similar to data reported by other authors (27).

Because of the typical early clinical presentation, most cases of endometrial cancer are endometrioid adenocarcinoma that is well-differentiated and stage I disease. Overall 5-year survival rates for all grades and histological subtypes are approximately 85-95%, 75%, 50%, and 20% for surgical stage I, II, III, and IV disease, respectively (24). In our study, during the follow-up period of 5 years, 7 (16.67%) patients had recurrence and 3 (7, 14%) died from their disease. The 5-year disease-free and overall survival was 83.33% and 92.86%, respectively.

In five of seven patients included in this study, who suffered from recurrent disease, deeper myometrial invasion was present. Deeper myometrial

invasion is associated with pelvic and para-aortic lymph node metastases. Recurrence rates up to 46% are observed with deep myometrial invasion (greater than one-half) as compared to 8-13% with superficial or no invasion (24). In our study, 2 out of 5 (40%) patients with deeper myometrial invasion had para-aortic lymph node metastases.

The most common extrapelvic sites for recurrences include the lungs, abdomen, para-aortic lymph nodes, brain, bones, and liver. In our patients, the most common sites were liver (three patients), colon (two patients) and para-aortic lymph nodes (two patients).

The literature has consistently shown that the most recurrences of endometrial cancer are diagnosed within 2 years (28). Our findings confirm such findings, since the median time of recurrence in our clinical group with endometrial cancer was 24 months.

Based on results of our study, as well as on available data published by other authors, we recommend that surgical treatment of patients with endometrial cancer with cervical involvement includes para-aortic lymph node dissection, in order to increase disease-free interval and diminish the risk of recurrent disease.

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Decision making and problem-solving skills of midwifery students in Turkey

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Abstract

Objective: This descriptive study was undertaken to assess the change in decision-making and problem-solving skills of a group of Turkish midwifery students across a 4-year educational course and according to age groups.

Methods: The study sample consisted of 296 students. Subjects were given DMQ I-II (Melbourne Decision Making Scale I and II) and PSI (Problem Solving Inventory) questionnaires to examine the effect of age and education on decision-making and problem solving skills.

Results: DMQ I-II total and PSI subscale scores were in the intermediate range, with no significant difference between age groups in terms of self-esteem, DMQ-II and PSI scores. However, significantly higher self-esteem scores were observed for 4th grade students as compared to 1st and 3rd grade students, and 4th grade students had better PSI scores. In addition, higher self-esteem and vigilance type decision making was associated with better PSI scores.

Conclusion: Our results suggest a room for improvement in self-esteem, decision-making style, and problem-solving skills of this group of midwifery students.

Key words: Decision making skills, Problem-solving skills, Midwifery students

Introduction

Individuals use decision-making and problem-solving skills to resolve life's daily problems. Problem-solving skills are a natural element of the practice of health professionals forming the basis for the development of professional skills and high-quality patient care [1,5]. Midwives, accountable for what they do or refrain from doing, have helped to widen the traditional boundaries of their professional accountability in order to improve and maintain the quality of care and services provided to mothers and babies [6,7]. This resulted in a dynamic and evolving role for midwives, with an associated increase in the number of required decisions. Midwives are no longer only responsible for providing care, but are also responsible for the effective use of resources.

Midwives who are confident and assertive decision-makers are considered ideal role models for students and newly qualified midwives. When students encounter midwifery practice in hospitals, such as midwife-led units and birthing centres where policies and procedures are entirely woman-centred and formulated by midwives, they are more inclined to appreciate the full scope of a midwife's role and her ability to make autonomous decisions without the interference of others [6,8]. Therefore, the type of clinical environment to which student midwives have been primarily exposed during their training is of significant importance for the development of confident and autonomous decision makers [9,10].

There has been considerable research on the effect of decision-making style on decision-making itself [11,13]. For instance Mann et al. tested the competence of students' course planning and satisfaction using domains of vigilance, hypervigilance, and defensive avoidance [14] and found a significant positive relationship between decision and vigilance, course planning, and satisfaction.

Decision-making is required at every step of problem solving, and a number of decision-making/problem-solving models have been developed [15, 16]. As shown below, a five-step model has proven effective in emergency situations such as those encountered by midwives. Within the context of five-step model, each step may be complet-

ed promptly provided that a thorough consideration is given prior to it. In this model, documentation for each step is not necessary. The first step is to identify the problem, then explore alternatives, select an alternative, implement the solution, and finally evaluate the solution [3, 17, 21].

A student's attitudes and actions may be influenced by the stage of growth and development, age, self-image, value system, level of knowledge, interpersonal skills, performance ability, and the capacity to assist clients [22, 25]. It has also been reported that the ability to solve problems can be improved through skill training [5], simulations, and verbal protocols [26, 27]. Therefore, in our educational courses, special emphasis is given to improving the professional skills of the students of midwifery who will always require problem-solving and decision-making skills for the care of mother and newborn throughout their career.

As a matter of fact, the published literature in health sciences have generally focused on "clinical" decision making skills rather than the general decision making skills. However, in the present study the latter has been the subject of primary interest in a group of university students of midwifery before they have reached the "clinical" decision making stage. As described in Materials and Methods, our study had two phases: In the first phase self-respect as well as decision-making and problem solving skills were assessed according to age and grade of the participants. In the second phase, the change or improvement in above named domains during a 4-year university course were followed. This approach for data analysis was expected to provide useful information for more effective educational activities.

Materials and methods

This descriptive study was designed to assess the association between age, grade and problem solving and decision making skills of midwifery students throughout a 4-year academic course.

Participants

The target sample consisted of all students (n=317) from the Department of Midwifery, Ege University School of Health, with an actual participation rate of 93.4% resulting in 296 students actively taking part in the study. The mean age was 21.38 ± 2.23 years (range: 18-32); 26% were in their 4th grade and 96.6% were single.

Of the total sample, 58.8% and 37.2% were between 18-21 and 22-25 years of age, respectively, and the remaining 4.1% were 26 years or older; 41.6% resided in university dormitory, 30.7% shared a house with friends, and 27.8% were living with their parents. A minority of the students (8.4%) reported working at paying jobs.

Age and grade were the only demographic data used for study analyses. Other demographic data were used in the 2nd phase of the study.

Assessments

The data was collected using a questionnaire, which was developed from previous studies. The first part of the questionnaire contained 10 questions identifying the students' demographic characteristics. The second part comprised of Melbourne Decision Making Questionnaire (DMQ I-II) and the Problem Solving Inventory (PSI). DMQ I-II was developed by Mann et al. [14] in a cross-cultural study involving six countries and aims to compare self-esteem and decision-making styles of university students. The reliability and validity studies for Turkish students were conducted by Deniz [15].

Decision-making questionnaire I (DMQ I) (Self-esteem) measures self-esteem as a factor in decision-making. It consists of six items [sample item: 'True for me' (score 2); 'Sometimes true' (score 1); 'Not true for me' (score 0)]. The maximum score is 12 and higher scores represent higher self-esteem in decision-making [14,15]. The Cronbach alpha level was 0.74 in the study by Mann et al. [14] while it was 0.66 in ours.

Decision-making questionnaire II (DMQ II) consists of 22 items and measures decision-making styles. This scale has the same choice of responses and is graded in the same way as DMQ I. The scale contains four subscales: vigilance (6 items), buck passing (6 items), procrastination (5 items), and hypervigilance (5 items) as decision-making styles [14,15]. The reliability coefficients of the subscales were calculated as follows: for vigilance, 0.80; buck passing, 0.87; procrastination, 0.81, and hypervigi-

lance, 0.74 [14]. The Cronbach alpha coefficients of DMQ I and DMQ II varied between alpha values of 0.65 and 0.80 [15]. In our study, the corresponding figure was 0.70.

The Problem Solving Inventory (PSI) assesses the self-confidence and feeling of self-control of the individual in problem solving, as well as the way in which an individual approaches problem solving. The inventory can be used to determine how an individual approaches or copes with a problem and consists of 35 items, developed by Heppner and Petersen [28]. Adaptation for Turkish respondents was performed by Sahin et al. [29]. Scores between 1 and 6 are given on a Likert scale by the participants, from '1' denoting 'totally agree' to '6' denoting 'totally disagree'. Low scores indicate effectiveness as well as having the behaviour and attitudes for successful problem solving. High scores indicate an inability to reach a successful solution when faced with a problem. The score range of PSI is 32-192. The PSI has six subscales: hasty approach, thinking approach, buck-passing approach, assessing approach, self-confident approach, and planned approach [29,30]. The alpha level of PSI was 0.90.

Statistical analysis

The SPSS 15.0 programme evaluated and analysed the data with descriptive and inferential statistics such as percentage, means, and analysis of variance (ANOVA). Dependent Variables were scores of DMQ I-II and PSI whereas independent variables were age and grade (1st, 2nd, 3rd, 4th year) of the participants. The correlation between PSI mean score of the decision-making style and decision self-esteem was analysed using Pearson correlation coefficients. Further analyses were performed by taking the number and percentage distributions of the data obtained.

Ethics

The study protocol was approved by the Scientific Ethics Commitee of Ege University School of Health and by the Administration of Ege University School of Health. The students were given information regarding the purpose and content of the study; they were told that their participation was voluntary, and their verbal consent was obtained. Among the departments of Ege University School of Health, only students of midwifery were included.

Results

Means, standard deviations and ranges for DMQ I-II and PSI scores are shown in Table 1. The mean self-esteem, total DMQ II, and total PSI scores were 8.82 ± 2.14, 21.87 ± 5.43 and 86.93 ± 21.02, respectively.

A distribution of self-esteem, DMQ II and PSI scores with respect to age and grade can be found

Table 1. The DMQ I-II and PSI scores of midwifery student

DMQ I-II	Mean-SD	Min-Max	Scale Score Range
Decision self-esteem (DMQ I)	8.82±2.14	2.00–12.00	.00-12.00
DMQ II			
Vigilance	9.37±2.12	3.00–12.00	.00-12.00
Buck-passing	4.18±2.49	.00–11.00	.00-12.00
Procrastination	3.64±2.24	.00–10.00	.00-10.00
Hyper vigilance	4.66±2.27	.00–10.00	.00-10.00
DMQ-II Total Score	**21.87±5.43**	**10.00–40.00**	**.00-56.00**
PSI Subscales			
Hasty approach	28.05±6.69	9.00–47.00	9.00-54.00
Thinking approach	12.17±4.60	5.00–25.00	5.00-30.00
Buck-passing approach	10.31±4.02	4.00–23.00	4.00-24.00
Assessing approach	7.29±2.90	3.00–18.00	3.00-18.00
Self confident approach	16.05±5.40	6.00–33.00	6.00-36.00
Planned approach	9.96±3.53	4.00–21.00	4.00-24.00
PSI Total Score	**86.93±21.02**	**44.00–138.00**	**32.00–192.00**

in Table 2. There were no significant differences between age groups in self-esteem, DMQ II and the PSI scores ($p > 0.05$). However, significantly higher self-esteem scores were observed for 4th grade students as compared to 1st and 3rd grade students. Similarly, 3rd grade students had significantly higher PSI scores as compared to other grade groups. No significant differences were observed between the grades in DMQ II scores ($p > 0.05$).

Table 3 shows the correlations between self-esteem, DMQ II and PSI scores. There were significant correlations between DMQ I-II and PSI scores. Self-esteem and vigilance were negatively correlated with PSI subscale scores; thus higher self-esteem and vigilance being associated with lower (i.e better) PSI scores. On the other hand, buck-passing, procrastination, and hypervigilance type decision making were positively correlated with PSI subscale scores; thus, these decision-making styles were associated with worse PSI scores.

Discussion

In this study the relationship between self-esteem, decision-making skills and problem-solving skills of midwifery students were examined as well as the impact of basic variables of the study on decision-making skills. In addition, the association between decision-making behaviours and demographic variables was explored. Proper clinical decisions based on skilful cognitive performance in midwifery has important implications with regard to health and safety of the mother and newborn [6]. Hence, acquisition of these skills should

Table 2. Comparisons of different age groups and classes with regard to DMQ I-II and PSI scores

Age Groups		Self- esteem	DMQ II Total scores	PSI Total Scores
		Mean-SD	Mean-SD	Mean-SD
18-21 age		8.7± 2.1	21.6±5.2	87.0±20.6
22-25 age		8.8±2.1	22.0±5.5	87.1±21.2
26 and upper		8.7±2.3	23.9±7.1	83.5±25.3
	F	.047	1.032	.166
	df	(2;293)	(2;293)	(2;292)
	p	.954	.358	.847
Class				
First Class		8.43±2.26	22.2±5.2	84.2±19.1
Second Class		9.02±1.89	21.6±4.6	86.5±18.8
Third Class		8.39±2.58	22.1±5.8	96.6±22.8
Last Class (Fourth)		9.37±1.63	21.5±5.9	81.0±20.3
	F	**3.762**	.325	**7.991**
	df	(3;292)	(3;292)	(3;291)
	p	**.011***	.808	**.000***

**p<0.05*

Table 3. Correlations between DMQ I-II scores and PSI scores

		PSI						
		Hasty approach	**Thinking approach**	**Buck-passing approach**	**Assessing approach**	**Self confident approach**	**Planned approach**	**Total Score**
Self esteem	**r**	-.337*	-.334*	-.323*	-.267*	-.421*	-.409*	-.470*
DMQ II								
Vigilance	**r**	-.474*	-.493*	-.489*	-.439*	-.440*	-.450*	-.608*
Buck-passing	**r**	.461*	.330*	.458*	.282*	.434*	.423*	.547*
Procrastination	**r**	.368*	.202*	.379*	.210*	.308*	.325*	.412*
Hyper vigilance	**r**	.382*	.339*	.393*	.271*	.440*	.390*	.501*
Total Score	**r**	.338*	.184*	.340*	.158*	.339*	.316*	.393*

**p<0.05 level*

be regarded as a priority in training and educating midwifery students [8].

In this group of midwifery students, self-esteem and vigilant decision-making style scores were above the intermediate range, while buck-passing, procrastination, and hypervigilance decision-making style subscale scores were low. Higher self-esteem and vigilance decision-making style scores suggest a higher skill level for decision-making. On the other hand, other styles, i.e. buck passing, procrastination and hypervigilance, were associated with lower decision-making skills. Similarly, Sari showed a negative correlation between conflict resolution [21]. According to Deniz, the most frequently used decision-making style usually has the highest score recorded in the decision-making style subscale [15].

PSI total mean scores in this group of midwifery students were at the intermediate range, with a mean score of 32-192. In this assessment tool, higher total scores indicate that the individual perceives himself/herself to be incompetent in problem-solving skills [29, 30]. With regard to PSI subscale scores, midwifery students appear to implement a considerate, buck-passing, assessing, and planned approach to problem solving. However, PSI scores suggest the absence of a self-confident attitude and adoption of a hasty approach. The lower than intermediate scores for thinking approach, buck-passing, assessing, and planned approach indicate that the problem-skills of these group of midwifery students were above average. On the other hand, intermediate scores for self-confidence and hasty approach are supportive of a lower problem-solving skill level.

Previous studies have reported similar findings. For example, Yurttas and Yetkin found an intermediate level of problem-solving skill and absence of a self-image as a "competent problem solver" [31]. In that study, there were no significant differences in mean problem-solving scores between university departments, with highest and lowest scores for problem-solving skills recorded in midwifery/nursing and dental hygiene departments, respectively [19]. Another study found positive correlation between the grade and problem-solving skills of nursing school students in Taiwan [13]. Can et al. observed higher problem-solving scores among health school students (nursing and midwifery) compared to technical school students, and environmental factors were proposed as a possible explanation for the observed difference [32].

In this study, self-esteem, DMQ II and PSI total scores were similar across the age groups. However, several previous studies have found improved problem solving with increasing age, i.e. individuals learn how to solve problems and benefit from the experiences of others as they live [32]. On the other hand, Yurttas and Yetkin examined the empathy and problem-solving skills of healthy college students and found no effect of age on problem solving [31]. This might be due to unrealistic self-assessment of problem solving skills among younger students due to overestimation of their capabilities.

Significantly higher mean self-esteem scores were found in forth grade students compared to the 1st and 3rd grades. In addition, a low mean PSI total score was found in fourth grade students. A lower problem-solving score indicates a higher skill level for problem solving. Both can be an indication of the efficacy of university education.

Despite some discrepancies, our findings are generally in agreement with previous studies. For instance, Can et al. observed a lower skill level for problem-solving in 1st year students compared to 4th year students [32], while some other studies reported the opposite, i.e. lower problem solving skills in 4th grade students than in 1st grade students [22]. Baxter and Boblin expressed their view that communication with patients is an effective way of improving decision-making skills in nursing students [11]; they also emphasized that proper decisions would be based on the level of self-development, evidence based practices, and the schedule followed. As the students progress through their educational course, decision making and problem solving skills would be expected to improve due to increased knowledge and experience level along with the effect of observing other health personnel and spending more time in actual practice.

Personality traits are also important determinants of decision-making skills. For example higher levels of ambivalence, anxiety, frustration, and lower personal identity, self-esteem and external locus of control were observed in individuals who tended to be more indecisive [33]. According to Mann et al., hypervigilance is associated with severe emotional stress [17].

Many factors such as a mismatch between age and type of the problem, prior level of information, personal abilities, attitudes, potential benefits of the solution, and personal traits influence the problem solving process. Also cognitive state, values, emotions, self-image, level of intelligence, motivation, and readiness for a problem can be included in this list [25]. From a cultural perspective, Mann et al. found higher self esteem scores in decision making in a group of Western students compared to students from East Asia [14]. It is well established that social roles, personal rights and responsibilities are also important in decision-making processes.

Higher self-esteem and vigilance decision-making style were associated with better PSI subscale and total scores. However, buck-passing, procrastinating and hypervigilance decision-making styles were associated with worse PSI outcomes. subscales and the total score. Similar to our findings, Deniz reported a significant relationship between high self-esteem levels in decision making and better PSI subscale and the total scores [15]. As stated by Abaan and Altıntoprak [1], nurses who perceive themselves as efficient problem solvers are inner- and control-focused people with self-confidence in decision making who can respond suitably to interpersonal and environmental demands. Deniz showed a positive relationship between problem solving and self-esteem and the vigilance decision-making style, and found a negative relationship between problem solving and the buck-passing, procrastinating, and hypervigilance decision-making style subscales [12].

Decision-making is considered as a phase of the problem-solving process. This conclusion is consistent with the findings of Philips et. al. [34], who examined the relationships between decision-making behaviours and problem-solving skills. Their findings indicate that individuals who use reasonable decision-making strategies do not avoid problems, but rather approach them. Use of a compatible and reasonable decision-making style is associated with better problem-solving competence. Similarly, self-esteem in decision making increases with a higher capacity of problem-solving [16, 34].

While problem-solving skills develop in a time course with positive reinforcement from educational activities [8, 27], decision-making is instantaneous. After graduation, students of midwifery will encounter situations where they would be required to define and resolve problems. Thus, incorporating case-based problem solving training in educational activities will provide an invaluable contribution to problem-solving skills of the students. In the same line of thinking, scenario-based case studies may provide similar improvements in problem solving skills [9]. For instance, Stacey et al. recommended the use of different decision making models and underscored the importance of novel practice methods and need for new research on this topic.

It would appear that many of the efforts to provide support for new students in higher education have been aimed at helping students 'fit the system' of the university, rather than encourage independent decision making and problem solving. There is considerable evidence that 'add-on' academic skills courses, which aim to improve the academic abilities of first-year students, have remained a popular way of dealing with the perceived problems of at-risk students [23].

Conclusion

In this group of midwifery students, an improvement in self-esteem, decision-making skills and problem-solving skills was observed from the 1st grade to the 4th, as evidenced by DMQ I-II and PSI scores. In this respect, the differences observed between grades may point out to the need for incorporating related instructional methods among those currently used. The university education environment should prepare students for the real world, emphasizing problem-based instructional methods, with realistic educational and clinical circumstances for midwifery students. Lecturers should be advised to facilitate the development of problem-solving and decision-making skills in midwifery students and to encourage their progress. In addition, compulsory case-based problem solving training can be included in the current educational courses.

Acknowledgment

We thank the midwifery students participating in this study.

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Non-functionant urinary bladder paraganglioma

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Abstract

Introduction: Paragangliomas are rare tumors origined on chromaffin-cells of neural crest and can be located from skull base to pelvis, on sympathetic or parasympathetic paraganglia. They account on less than 0,06% of all urinary bladder tumors, with only few hundreds of cases reported in literature since the first record by Zimmermmann in 1953.

Case Report: A 63 year-old woman referring irritative urinary symptoms was submitted to an ultrassonography that disclosed an irregular-shaped nodulation on her bladder. CT confirmed the existence of a nodulation on bladder's anterior wall. Patient had normal levels of urinary catecholamins and Vanilmandelic acid. Tumor was excised and posterior immunohistochemical study revealed it was a paraganglioma. Nowadays, ten months after surgery, patient stills healthy and disease-free.

Discussion: Paragangliomas can be classified as functionant or non-functionant, according to its production of cathecolamins, which can cause the same symptom complex of pheocromocytomas. About 10-15% of bladder paragangliomas are malignant, and potential metastasis are more common to lymph nodes, lungs and bones. 131-MIBG iodine cyntilography is the most sensitive method for diagnosis and surgery (transurethral resection or cystectomy) is the best choice for treatment.

Key words: Paraganglioma, Urinary Bladder, Neuroendocrine Tumors.

Introduction

Paragangliomas are neuroendocrine neoplasms that arise from chromaffin cells on sympathetic or parasympathetic autonomic nervous system paraganglia. They can develop from skull base to pelvis, but 80% of them are located on abdomen(1), along the paraortic and paravertebral axes. Paragangliomas located at urinary bladder are rare. This kind of tumor accounts on less than 0,06% of all neoplasms of the urinary bladder.

Marchand, in 1891, reported the first case of paraganglioma: a nodulation from carotid body. Zimmermmann et. al. described the first case of urinary bladder paraganglioma in 1953 (2) and subsequently, only a few hundreds of cases are registered in medical literature. Because of its uncommonness and wide spectrum of clinical presentations, specially about the cathecolamine production, the bladder paragangliomas are difficult to recognize, specially for the general doctor, and its prognostic is not well stablished.

The reported case is from a 55-year-old female patient with a nodulation in her bladder's anterior wall, further excised and confirmed as an non-functionant urinary bladder paraganglioma by the immunohystochemical study.

Case report

M. S. P., a 63-year-old female caucasian patient was admitted on Hospital Sao Francisco, a public hospital located in the city of Crato, on northeast Brazil. She was complaining of irritative urinary symptoms, like dysuria, pollakiuria and micturition urgency, and related a single episode of macroscopic haematuria. The existence of previous systemic or infectious patologies, like hypertension and diabetes mellitus was denied. She also had negative familiar history for neoplasms and hypertension. At the moment of her admission, the blood pressure was 130x90 mmHg and the heart rate was in the normal range. General physical examination showed no significant evidences.

A routine abdominal ultrassonography revealed an unique lesion in the anterior wall of the urinary bladder, with a irregular shape and solid aspect. Further, an helicoidal Computed Tomo-

graphy (CT) of the pelvis confirmed the existence of an intravesical nodulation, measuring 3,5 per 2,5 centimeters in the largest axis, without cystic caracteristics and presenting some enhancement after endovenous iodine contrast infusion. The nodule was adhered to bladder's anterior wall and had no extra-vesical involvment.

Patient underwent a transurethral resection of urinary bladder that made surgical removal of the tumor. Excised material went to anatomopathological study, that made clear it was an atypical mesenchymal proliferation. Biopsied specimen had 3 kilograms of weight and was dark-brown colored. Later, immunohystochemical study confirmed it was an urinary bladder paraganglioma. After surgical excision an immunohystochemical diagnosis, Vanilmandelic acid and urinary cathecolamins have been evaluate and were in normal levels.

Nowadays, ten months past after surgery, patient still disease-free, undergoing a complete review each three months. This follow-up will remain until five years after the tumor resection. If the patient still disease-free after this period, is considered cured. The definitive diagnosis is non-functionant urinary bladder paraganglioma.

Discussion

Neoplasms that arise from cells of endocrine and nervous systems are called neuroendocrine tumors, which can be benign or malign and have the similar characteristic of producing biogenic amines and polypeptide hormones. The annual incidence of neuroendocrine neoplasms with clinical significance varies around 2,5-5 per 100.000, involving many differentiation levels and prognostic assessments[1].

The World Health Organization (WHO) produced a classification scheme that separates neuroendocrine neoplasms into three main categories, further subdivided, according to its differentiation grade, histological features and hormone production[6]. Paragangliomas are classified as 'originated from a neural cell line'.

Chromaffin cells are originated from the neural crest and are part of sympathetic and parasympathetic autonomic nervous systems. Two kind of tumors have its origin on these cells: the pheocromocytomas and the paragangliomas. Pheocromocytomas are the most frequent cathecolamin-secreting tumors in general population [2] and are located in the adrenal medula. Paragangliomas are extra-adrenal tumors with the same embriologic origin, but in extra-adrenal locations, along the paravertebral and paraortic axes.

Although more than four fifths of paragangliomas develop in the abdomen, the involvement of the urinary bladder is rare and this hystologic class of tumors accounts on a very low rate of all bladder's neoplasms, about 0,06%[3]. The frequence of paragangliomas is higher in young female patients, but it may occur over a large range of ages and the difference of prevalence between genders is minimal[4].

Most of paragangliomas are assymptomatic. All of them have neurosecretory granules, but only 1-3% produces enough catecholamins to cause organic symptoms, which are the same of the functinal pheocromocytomas and result from an adrenergic or noradrenergic overstimulation. Cephalgia, tachycardia and diaphoresis form the classis triad of this tumor's systemic symptoms, beside of hypertension, palpitations, anxiety, pallor and weight loss, for example and can occur as paroxystic symptoms[5]. This combination of signals is very sensitive and specific for presumptive diagnosis of cathecolamine-secretion tumors. According to its capacity of producing these systemic symptoms, paragangliomas can be classified as functionant or non-functionant tumors. Urinary tract related symptoms are also associated with almost all bladder neoplasms, like in the reported case. Dysuria and painless haematuria are the most frequent[6].

Imaging and biochemical tests are necessary methods to diagnose a bladder paraganglioma. These tumors are usually small and unique submucosal nodulations with or without ulcerated surface. Cystoscopy can show almost 80% of intravesical paragangliomas. Computed Tomography and Magnetic Nuclear Resonance in suspicious patients that demonstrate solid, homogeneous mass with a ring of calcification are very suggestive of paraganglioma[5].

The diagnosis confirmation is based in biochemical evaluation of urinary cathecolamins and Vanilmandelic acid. Literature shows that the most sensitive and specific method is the 131-metaiodobenzilguanidine iodine cyntilography, the con-

trast with major tumor penetration[7]. According to some authors, the best choice for treatment of urinary bladder paragangliomas is surgical resection. The excision can be done as transurethral resection, like was done for this patient, partial or total cystectomy [5]. If there are proven metastasis, pelvic lymph node dissection is necessary.

Due to its very low prevalence, literature have no consistent studies about bladder paragangliomas prognostic. Is extremely recommended the patient follow-up for five years, like is done for the oncologic patients in general. After this period, the patient achieve discharge.

Conclusion

Urinary bladder paraganglioma is a rare pathology and its various clinical presentations can make it misdiagnosed, specially the non-functionant kind of tumors, because of its absence of systemic symptoms.

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Burnout syndrome in medical students during clinical training

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Abstract

Introduction: Burnout syndrome denotes undesirable stress reactions in one's professional sphere which reduce their work output and create dissatisfaction with their career. The last decade was characterized by intense research on this problem occurring in medical profession, highlighting the fact that the previous formative period of studying and specialization gives a significant contribution to burnout.

Objective: This study was aimed at assessing the influence of stress during medical studies on the development of burnout syndrome in medical students by the end of clinical training.

Methods: This cross-sectional study included all the fifth-year students from the Medical Faculty in Novi Sad and Belgrade in 2011, of whom 178 and 375, from the respective faculties, volunteered to fill in an anonymous questionnaire containing questions on selected socio-demographic data, length and accomplishments of previous courses, stress, various activities (including examinations, contact with patients, relationship with the teaching staff and other staff working at clinics). The 22- item *Maslach Burnout Inventory* (MBI) was applied to assess the risk of developing burnout syndrome.

Results: At the assessment of burnout syndrome the values of depersonalization were very high on the sub-scale MBI-DP in 73.1% of all respondents, whereas 52.7% of medical students from Novi Sad and 31.9% from Belgrade had high values for emotional exhaustion (MBI-EE). Examinations, as defined by half of all students, were the most stressful activity in both groups of students. Among the predictors of high risks of developing burnout syndrome, stressful effects of contact with patients were prominent for high MBI-EE: OR=1.59 (95%CI:1.15-2.21), and for high MBI-DP: OR=1.49 (95%CI: 1.00 –2.24) as well as stressfulness of examinations which significantly affects high MBI-EE: OR=1.33 (95%CI: 1.04 –1.70

Conclusion: The high prevalence of burnout among the medical students by the end of their clinical training underlines the necessity of modifying the existing curriculum so that it would prepare future doctors much better to overcome stress associated with the direct contact with patients. In addition, material to be covered for examinations should be reduced since examinations, besides contact with patients, represent the most stressful individual effect.

Key words: Burnout syndrome, stress, medical students, clinical training

Introduction

For several decades burnout syndrome has been recognized as a measure of the extent of adverse stress reactions which leads towards detachment from the tasks performed by the employees in professions giving support to other people [1]. The frequency of burnout syndrome, which is known as a problem of mental health among health professionals worldwide, including Serbia, is twice as high as in other professions on average, ranging from 25%-76% depending on the medical specialty [2-6], with the highest percentage observed in younger doctors [7]. Burnout is nowadays seen as a phenomenon of distress in educational process as well, and it was recently recorded in as many as 50% of medical students in the USA, with the increasing frequency throughout the studying [8].

Studying medicine is often associated with prolonged state of having to cope with multiple stressors: such as learning workload, lack of free time and mastering complicated medical procedures along with the simultaneous work with patients. Such a widespread presence of distress

among medical students has a harmful effect on the accomplishment and professional development and may cause reduced professional interest, degradation of humanitarian attitudes and decline of empathy [9-12].Several studies have shown that medical students have specific mental health problems, which may even deteriorate during medical school [13-16]. Symptoms of anxiety and depression are much more frequent in medical student population than in aged-matched group of general population or students of other faculties [17,18]. One of the previous studies on mental health condition of medical students from Belgrade University showed that the total mental disorder prevalence was 16.1% during the first month after the enrollment, but two years later it increased to 17.5%, the estimated incidence rate of psychiatric diseases being 5.3% per year [19].

Objective

This study was aimed at assessing the presence of burnout syndrome as a specific problem of mental health among students at clinical training, and the influence of stress on burnout development while studying medicine at two medical faculties in Serbia.

Methods

This cross-sectional study included all the fifth-year students from the Medical Faculty in Novi Sad and Belgrade in 2011. Of 529 students from Belgrade and 178 from Novi Sad, 375 (70.9%) and 138 (77.5%), respectively, volunteered to fill in an anonymous questionnaire. The questionnaire contained the selected socio-demographic data with the questions on their academic accomplishment such as: the length of studying, number of examinations passed and the mean mark. The questions on various aspects of activities during the studying referred to examinations, contact with patients, relationships with the teaching staff and other staff working at clinics. The students applied the proposed four-point Likert scale for stress self-assessment.

Maslach Burnout Inventory (MBI), which is widely used as an instrument for measuring burnout with respect to the professional activity, is a questionnaire with 22 questions categorized into seven possibilities [20]. For the purposes of this survey, MBI had been modified to include specific questions referring to studying-related activities. There were three sub-scales to measure emotional exhaustion (MBI-EE) (the feeling of emotional drain caused by work-load), depersonalization (MBI-DP) (the feeling of being detached from colleagues) and personal accomplishment (MBI-PA) (the feeling of low personal competence and achievement). The total score for each scale was defined as "moderate" or "high" according to the determined average norms from the sample of American health professionals, which had been tested on the sample of health professionals from Serbia [20,3,4].

Student t-test was applied to compare the mean values of the parameter data for the two groups, and for the non-parameter statistics we used *Mann-Whitney* U test and $\chi 2$ test for continuous variables and categorical variables, respectively. The logistic regression was used for determining independent risk factors for burnout. The obtained data were analyzed by SPSS version 17. The study had been approved by the Ethical Committee of Medical Faculty in both Novi Sad and Belgrade.

Results

According to the results presented in Table 1 there were more female students than males in both groups, their number making more than two thirds of the total number of students. The average age of the students ranged from 24 to 27.5 years, being a year higher on average for the students from Novi Sad. The same statistically significant difference could be observed in the length of studying, and the number of passed examinations was significantly higher in the group from Belgrade at the moment of survey (31.6 ± 4.4 versus 26.5 ± 5.2), whereas the mean marks did not vary significantly between the two groups, most frequently ranging from 8 to 9.

Examinations were defined as markedly stressful activity by slightly less than a half of all the students, and more often by those from Novi Sad than from Belgrade (63.9% versus 41.9%, $p < 0.001$). Contacts with patients were defined as non-stressful activity by 57.2% of the students. Contact with staff working at clinics (nurses, doctors and other per-

Table 1. Demographic characteristics and studying success

	Students from University of Belgrade (n=375)	Students from University of Novi Sad (n=138)	Together (n=513)	Significance
males	135 (36%)	38 (27.5%)	173 (33.7%)	p=0.072*
females	240 (64%)	100 (72.5%)	340 (66.3%)	
age	24.4 ± 1.8	25.5 ± 2.7	24.7 ± 2.2	p<0.001*
length of studying	5.3 ± 1.4	6.5 ± 2.6	5.6 ± 1.9	p<0.001**
passed exams	31.6 ± 4.4	26.5 ± 5.2	30.2 ± 5.2	p<0.001**
mean mark (6-10)	8.43 ± 0.72	8.51 ± 0.69	8.45 ± 0.71	p=0.259*

** χ2 significance; **t test significance*

Table 2. Perceived stressful effect of the studying activities

(n) %	Students from University of Belgrade	Students from University of Novi Sad	Together	Significance
Stressful influence of exams	370	133	503	
no	53 (14.3%)	2 (1.5%)	55 (10.9)	p<0.001
mild	37 (10%)	9 (6.8%)	46 (9.1%)	
moderate	125 (33.8%)	37 (27.8%)	162 (32.2%)	
high	155 (41.9%)	85 (63.9%)	240 (47.7%)	
Stressful influence of contact with patients	368	134	502	
no	217 (59%)	70 (52.2%)	287 (57.2%)	p=0.528
mild	119 (32.3%)	48 (35.8%)	167 (33.3%)	
moderate	28 (7.6%)	14 (10.4%)	42 (8.40%)	
high	4 (1.1%)	2 (1.5%)	6 (1.2)	
Stressful influence of contact with staff at clinic	371	134	505	
no	232 (62.5%)	66 (49.3%)	298 (59%)	p=0.024
mild	88 (23.7%)	43 (32.1%)	131 (25.9%)	
moderate	42 (11.3%)	17 (12.7%)	59 (11.7%)	
high	9 (2.4%)	8 (6%)	17 (3.4%)	
Stressful influence of contacts with teaching staff	370	134	504	
no	179 (48.4%)	50 (37.3%)	229 (45.4%)	p=0.043
mild	126 (34.1%)	54 (40.3%)	180 (35.7%)	
moderate	50 (13.5%)	18 (13.4%)	68 (13.5%)	
high	15 (4.1%)	12 (9.0%)	27 (5.4%)	

** χ2 significance*

sonnel) was described as a non-stressful activity by more than a half of the students; however, the students from Novi Sad found this kind of communication as a very stressful one (6% versus 2.4%). Although stress associated with the contact with the teaching staff was assessed as slight or mild, the students from Novi Sad described this contact as markedly stressful (9% versus 4.1%).

The scores on the MBI-EE sub-scale ranged from 22.35±11.02, with significantly higher values among the students from Novi Sad (25.61 ± 10.82 versus 21.17 ± 10.87), and they were frequently positioned among high values in this group of students (52.7% versus 31.9%)

The DP sub-scale values were 13.47±5.63, again in significantly higher in the students from Novi Sad (15.02 ± 6.09 versus 12.90 ± 5.35) and usually in the range of high values in almost three quarters of all the students. The average values of PA sub-scale scores were 30.39±16.36%, with lower levels in more than 50% of the students and markedly higher standard deviation in the students from Belgrade. When being assessed for the presence of burnout syndrome, 73.1% of all the respondents were found to have high values on the MBI-DP sub-scale, whereas high values on the MBI-EE sub-scale were observed in 52.7% of the medical students from Novi Sad and 31.9% from Belgrade. Table 4 gives an overview of all important predictors of high burnout questionnaire scores. The following variables were important for high EE subscale scores: high-valued stressful effect of contact with patients OR=1.59 (95%CI: 1.15 –2.21), as well as of examinations OR=1.33 (95%CI: 1.04 –1.70). The predictor important for high depersonalization was only the stressful effect of contact with patients OR=1.49 (95%CI: 1.00 –2.24), the stressful effect of contacts with staff working at

Table 3. Mean values and distribution of high MBI subscale scores

	Students from University of Belgrade	Students from University of Novi Sad	Together	Significance
Emotional exhaustion				
mean±SD	21.17 ± 10.87	25.61 ± 10.82	22.35 ± 11.02	p<0.001**
n (%) high ≥ 27				
	114 (31.9%)	68 (52.7%)	182 (37.4%)	p<0.001*
Depersonalization				
mean±SD	12.90 ± 5.35	15.02 ± 6.09	13.47 ± 5.63	p=0.001**
n (%) high ≥ 10				p=0.095*
	258 (71.1%)	103 (78.6%)	361 (73.1%)	
Personal accomplishment				
mean±SD	30.47 ±18.53	30.18 ± 8.11	30.39 ± 16.36	p=0.464**
n (%) low < 33				p=0.297*
	214 (61.8%)	73 (56.6%)	387 (60.4%)	

***Mann Whitney U test significance; * χ2 significance*

Table 4. Risk factors for critical scores of MBI sub-scales

	EE high score		DP high score		PA low score	
Variable	OR (95%CI)	p*	OR (95%CI)	p*	OR (95%CI)	p*
Stressful influence of exams	1.33 (1.04 –1.70)	0.02	1.05 (0.81 – 1.36)	0.710	0.84 (0.65 – 1.07)	0.163
Stressful influence of contact with patients	1.59 (1.15 –2.21)	0.005	1.49 (1.00 – 2.24)	0.049	1.12 (0.79 – 1.57)	0.530
Stressful influence of contact with staff on clinic	1.15 (0.83 –1.61)	0.406	1.30 (0.88 – 1.92)	0.184	2.14 (1.47 – 3.12)	0.000

** Significance of predictor in binary logistic regression*

clinics was a highly significant predictor for low PA OR=2.14 (95%CI: 1.47 –3.12).

Discussion

The 5th year medical students have taken the majority of examinations and pre-clinical activities, thus having the clinical training and the subsequent examination as predominant activity [21]. This study has reviewed the influence of stress of clinical training on the development of burnout syndrome among the 5th year medical students who had courses organized by the Bologna curriculum from the beginning. Their achievements resulting from this way of studying have been rather satisfactory. The most stressful academic activities observed in our research are examinations, the statistical significance being much higher among the students from Novi Sad than from Belgrade (63.9% versus 41.9%, $p<0.001$) and at the moment of this survey, the duration of their studying was longer and the number of examinations they had passed was significantly smaller as well. Examinations as instruments for assessing knowledge have already been determined as significant stress triggers during studying [22]. In addition, the students from Novi Sad often described the contact with teaching and non-teaching staff as very stressful. The phenomenon of abuse of students, which is reflected in non-verbal and verbal humiliation expressed by administrative, non-medical and medical staff at clinics, have already been observed and described in literature [23]. By recognizing the relationship with the teaching staff as an important stressor in our study, we have highlighted the importance of implementing programmes for training teachers to improve their pedagogical skills, who should teach their students not only medicine but the informal curriculum of humanitarian attitude [24]. The unfortunate fact is that mental health problems, including burnout syndrome, are observed among teaching staff as well and they can influence students spontaneously by process of identification. [25].

When checking the internal consistency of the sub-scales of the MBI we found the values of Cronbach alpha to be over 0.70, which is considered sufficient enough to validate this kind of questionnaire [26,27]. By assessing the presence of burnout syndrome in our respondents by the widely used MBI questionnaire we found that the values were higher in our students than in medical students from the USA, particularly on the depersonalization sub-scale which is the most relevant for the seniors at clinical training (high values on the MBI-DP sub-scale were present in 73.1% of all our respondents) [28]. In the comparison of MBI scores of the orthopedic surgeons from the University Clinical Centre and the general practitioners from the Health Centres in Belgrade it was revealed that 70% of them had high scores on the emotional exhaustion sub-scale; whereas our future doctors had significantly lower values (high in 52.7% of the students from Novi Sad and 31/9% from Belgrade, but more than twice higher values on the depersonalizations sub-scale) [3,4]. In a multi-center study performed among students at five medical schools in the USA, the multivariate analysis pointed to the following factors independently associated with student burnout: personality characteristics, personal life stressful events and common learning environment as the particular key factors [29]. This finding underlines the necessity of further assessment of conditions of studying and clinical training at medical faculties. According to some authors, certain personality characteristics of students, which were not covered by this study, should also be assessed at the very beginning of studying medicine in order to detect mental health problems in due time [30]. Among predictors for high risk of burnout syndrome in our medical students the following are outstanding: stressful effect of contact with patients for high MBI-EE: OR=1.59 (95%CI: 1.15 –2.21), and for MBI-DP: OR=1.49 (95%CI: 1.00 –2.24) as stressfulness of examinations (only for high MBI-EE: OR=1.33 (95%CI: 1.04 –1.70). Such findings suggest the possibility of aggregation of students' personality characteristics which determine interpersonal sensitivity and tendency to get emotionally exhausted in specific activities, as well as the possibility of improving mechanisms for coping with stress in students by introducing appropriate changes in the curriculum (more practical skills, modified methods of testing the knowledge) and by elective extra-curricular courses.

Conclusion

The high prevalence of burnout syndrome in medical students by the end of clinical training draws attention to the necessity for modifying the curriculum (reduction of the material to be covered for examinations, which are defined to have individually the most stressful effect) and more intense education of future doctors to be able to overcome stress caused by the contact with patients.

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The effect of baby massage on mental-motor development of healthy full term baby

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Abstract

Objective: The purpose of the present study is to define the effects of baby massage on mental-motor development of healthy full term babies.

Design: The research was designed as experimental.

Setting: Istanbul University Istanbul Medical Faculty Gynecology and Obstetry Clinic.

Subjects: The group of the research includes totally 104 healthy and full term babies (of which 52 is in control and 52 is in experimental group) who born in Istanbul University Istanbul Medical Faculty Gynecology and Obstetry Clinic between June 2001 and October 2002, and taking pediatric polyclinic service for healthy children in same hospital and their mothers who comply with criterions of case select. Mothers, who were educated on performing baby massage following birth, were asked to apply the massage 15 minutes once a day for during six months. Mental-motor developments of the babies in the experiment and control groups were evaluated in the third and sixth months after birth using Ankara Developmental Screening Inventory (ADSI). Percentage, average, standard deviation, student's t and chi-square tests were used in statistical evaluations.

Main outcome measures: Demographics of healthy full term baby including; weight, length and cranial circumference at birth, sex, birth modality, apgar score, solely breastfeeding duration and mental-motor development scores.

Results: Experimental and control groups were similar each other in terms of sex, birth modalities, apgar scores, weight, height, and cranial circumference at birth, solely breastfeeding durations. ($p>0.05$). When mental-motor development of two groups were compared with ADSI It seen that lingual-cognitive, fine motor, gross motor, social skill-self-care subscale and overall development of the experiment group were significantly higher than the control group ($p<0.05$).

Conclusions: It was concluded that mental-motor development can be improved with baby massage in healthy full term babies.

Key words: baby massage, newborn, mental-motor development, health promotion.

Introduction

Physical growth and mental-motor development is very rapid during the first years of life. Visual, auditory and tactile stimulants make significant contributions to the mental-motor development of babies during this period. Visual, auditory and tactile stimulants are used systematically and simultaneously during baby massage (Field 2002; Rosalie 2002).

Many benefits of massage on babies and children have been reported in the literature. It improves communication between the mother and the baby (Drummond 1998; Onozawa et al. 2001), increases seratonin levels while decreasing stress hormone levels of babies (Feary 2002; Field et al. 1996_a; $Field_b$ et al.1996), reinforces the immune system (Highlands 1998; Rosalie 2002), regulates the digestive system, reducing the incidences of flatulence and colic (Kılıç 2000; Uzuner 1998), organizes sleep (Agarwal et al., 2000; Field and Hernandez, 1998, Scafidi et al. 1986), supports weight gain (Field et al. 1987; Field et al., 1986; Mathai et al. 2001; Karabudak and Öztürk 2008, Bal Yılmaz H and Conk Z 2009) and motor development and coordination (Ferber et al. 2002; Mathai et al. 2001; Karabudak and Öztürk 2008; Scafidi et al. 1990). Studies, however, were carried out with preterm baby. There aren't any researches about effects of baby massage on mental-motor development of healthy full term baby.

Positive results reported for preterms babies suggest that similar results can also be obtained in

healthy full term baby with baby massage. However, comprehensive studies are needed to verify this presumption.

The present study aims to investigate effects of infant massage on mental-motor development of healthy full term babies. It is a firs study has been conducted in our country, A review of the international literature revealed that no study had been performed to investigate the effects of infant massage on mental-motor development in healthy full term babies. Therefore, the present study will contribute literature on this topic. Although baby massage is effective method to improve baby's health, It is not known enough by nurses in Turkey. We believe that this study will contribute use of baby massage by nurses during mother's training programs in Turkey.

Hypotheses of the Research

Hypothesis 1: Infant massage supports lingual-cognitive development

Hypothesis 2: Infant massage supports fine motor development

Hypothesis 3: Infant massage supports gross motor development

Hypothesis 4: Infant massage supports social skill-self care development

Hypothesis 5: Infant massage supports overall development

Methods

Participants

Study was conducted between June 2001 and October 2003 with 104 healthy full term newborns (52 in the control and 52 in the experimental groups), and their mothers who complied with subject selection criteria. Experimental and control groups were created randomly. The babies were born between June 2001 and October 2003 in Istanbul University Istanbul Medical Faculty Gynecology and Obstetrics Clinic, and received pediatric polyclinic service for healthy children (general infant care, immunization, growth and development follow-up, general health check, etc.) in the same hospital. Statistical evaluations and the study reports were completed in July 2003.

Design

The following criteria were determined in order to similar experimental and control groups:

Inclusion criteria:

***Babies*:**

- Delivered at 38-40 weeks,
- Defined as healthy by a physician during postpartum physical examination,
- Birth weight between 10 – 90th percentiles,
- Apgar score above 7 in the first and fifth minutes,
- Received pediatric polyclinic service for healthy children in the same hospital.

Mothers:

- At least primary school graduates,
- 18-35 years of age,
- No infectious skin diseases
- No previous information or education about baby massage

Families:

- Have social insures
- Have maximum two children

In the first phase of the study permission were obtained from the institutions as well as mothers who participated in the study. In the second phase of the study information was collected from the mothers with "Interview and Observation Form". A total of 152 mothers were asked to participate of the study and 145 mothers were accepted the study. Experimental and control groups were determined randomly. In this phase of the study 76 babies and their mothers in the experiment group and 69 babies and their mothers in the control group.

In the third phase of the study, education was given to mothers in the experimental group; training was carried out one day after the delivery. Educational were completed in 4 sessions, all of which were carried out during hospitalization by same reseacher. The first three sessions of the education were performed in hospital's education room and the last was done in mothers' rooms. In the first session of the education, mothers watched "Baby Massage Videotape". In the second session of the education, baby massage was demonstrated each mother on a baby manikin. Then, in the third session of the education, mothers applied the baby massage on a manikin. Their mistakes were corrected by researcher. Approximately an hour was allocated for the first three sessions. In the fo-

urth session of the education mothers applied the baby massage on their babies under the supervision of the researcher. The fourth session of the education lasted about 45 minutes. Mothers started to apply the baby massage one day after the discharge. Each mothers also used classical music during practicing of baby massage. Baby massage was performed every day for about 15 minutes during six months by mothers, Each mother was given "baby massage brochure", as well as a "Massage Application Record Chart" for recording the days they have performed the baby massage.

In the fourth phase of the study, mothers who experimental group were visited at home on the 15th day of birth. In this phase of the study researcher evaluated mother's baby massage application skill. The assessment of the mother's baby massage application skill was evaluated by "Baby Massage Efficiency Evaluation Form". In this phase of the study researcher excluded 5 babies and their mothers from the experiment group due to inadequate skill.

Fifth phase of the study was carried out in the 3rd month after birth. Mental-motor development of babies in both the experiment and control groups was assessed by using ADSI in this phase.

Sixth phase of the study was conducted in the 6th month after birth. In this phase researcher was evaluated mental-motor development of babies in the experiment and control groups by ADSI. In addition, researcher assessed mothers either applied baby massage regularly or not via "Massage Application Record Chart". Babies who were not applied baby massage regularly (10% of the 6-month period or for a week) were excluded from the experimental group. In the beginning of the study there were 76 infants in the experimental group and 69 in the control group. Numbers of babies dropped out of the study in this phase were six. Six babies excluded because of could not be made home visit (6 infants) and seven babies excluded because of not brought follow-up appointments (7 infants). Thus a total of 24 infants had been excluded from the experimental group.

Reasons for exclusions in the control group were as follows: 11 infants were not brought to follow-up appointments, and 6 mothers could not be contacted due to changing their addresses. Thus This study was conducted 104 healthy term babies who 52 in the experiment, 52 in the control group.

Infant Massage Technique

Infant massage involves gentle strokes on the face, arms, chest, abdomen, back, legs and feet of the babies by his/her mother using softly formulated baby oil. The baby massage was accompanied by classical music. Duration of baby massage is 15 minutes daily. Each baby massage' steps is repeated three times (Johnson & Johnson 1999; Uzuner 1998; Yeğen and Egemen 2000).

Materials

1) Interview and Observation Form: This form developed by the researchers and includes 18 questions on descriptive characteristics of families and children. The form is consisting of three open-ended and 15 close-ended questions.

2) Ankara Development Screening Inventory (ADSI): It is a screening test specific to Turkish children developed by Savaşır et al. (1998) , Inventory is used to identify mental-motor development of children 0-6 years of age. Validity and reliability studies of the inventory for the Turkish population had been performed and its reliability was analyzed by internal consistency and test-retest methods. Cronbach Alpha coefficient of the inventory and its subscales was .91-.99 for infants (0-12 months of age). Test-retest reliability of the inventory was evaluated by Pearson moment multiplication and was r = .99 for months 0-12. Agreement percentage of ADSI with Denver Developmental Screening was .92. ADSI includes 154 items, each scored1 point. The score obtained from the 154 items reflects overall development. Total score (T Score) is obtained by converting the overall development score to a standard score. This score enables a comparison of the child's current development status with normal dispersion. The inventory has 4 subscales defined in the developmental in literature and development tests, which are: Lingual-Cognitive (LC), Fine Motor (FM), Gross Motor (GM), and Social Skill-Self-Care (SS-SC). Ankara Developmental Screening Inventory can be applied in any period between 0-6 years of age and evaluates children's Mental-Motor Development.

3) Baby Massage Brochure: It was developed by Johnson & Johnson and provides detailed information about baby massage.

4) Baby Massage Videotape: It was developed by Johnson & Johnson and provides detailed information about baby massage.

5) Massage Application Record Chart: It was developed by the researchers. Mother marked this chart the days of massage applied.

6) Baby Massage Efficiency Evaluation Form: It was developed by the researchers to measure the efficiency of the massage performed by mothers. The form includes the stages of the massage as a checklist.

7) Baby manikin: It has a normal newborn size and made of plastic.

Analysis

Data obtained during the study were evaluated on SPSS 12.0 by statisticians. Percentage, average, standard deviation, Student's t and chi-square tests were used in statistical evaluations.

Findings

Demographic information

Babies in the experimental group were similar with the control group in sex, birth modalities, apgar scores, weight, height, and cranial circumference at birth and solely breastfeeding durations. There were no statistically significant differences between two groups (p>0.05) (Table 1).

When we compared the mental-motor development of babies in experimental with control group in all groups We found that lingual-cognitive, fine motor, gross motor, social skill-self-care subscale mean scores and overall development score and T score of the experiment group were significantly higher in the experimental group. (Table 2)

Because of solely breastfeeding duration can be effect mental-motor development of babies, mental-motor developments of the babies who solely breastfeeding group evaluated separately. Also It was seen that mental-motor development of experimental groups higher than the control group in solely breastfeeding group (Table 3).

Table 1. Comparison of groups according to some variables that can be effecting mental-motor development (N=104)

	Experimental Group (n=52)		**Control Group (n=52)**		c^2
	n	**%**	**n**	**%**	**p**
Sex					
Female	22	42.3	25	48.1	0.349
Male	30	57.7	27	51,9	0.554
Birth Modality					
Normal, vaginal	36	69.2	33	63.5	0.388
Cesarean	16	30.8	19	36.5	0.534
	Experimental Group (n=52) Mean± SS		**Control Group (n=52) Mean ± SS**		**t p**
Average of apgar score					
1st minute	8.78 ± 0.41		8.81 ± 0.39		-0.240 0.809
5th minute	9.76 ± 0.42		9.80 ± 0.39		-0.476 0.635
Physical Measurements					
Wight at birth (g)	3360.5 ± 293.8		3363.4 ± 331.9		-0.047 0.963
Height at birth (cm)	50.23 ± 0.80		50.19 ± 1.14		0.199 0.843
Cranial circumference at birth (cm)	34.70 ± 0.63		34.64 ± 0.94		0.366 0.715
Solely breastfeeding duration	5.21±1.30		4.71±1.57		1.763 0.081

Table 2. Comparison of mental-motor developmental scores in all groups

Mental-Motor Developmental Domains	Months	Mean ADSI Scores in the Whole Group N=(104)		
		Experimental Group (n=52) Mean ±SS	Control Group (n=52) Mean ±SS	t p
Lingual-Cognitive	3rd	8.50±1.55	6.23±1.57	7.387 0.0001
	6th	13.19±1.37	11.75±0.98	6.151 0.0001
Fine Motor	3rd	6.88±2.19	2.76±1.33	11.553 0.0001
	6th	12.19±1.42	10.53±1.79	5.193 0.0001
Gross Motor	3rd	5.09±1.19	2.96±0.62	11.433 0.0001
	6th	9.48±1.69	7.21±2.34	5.651 0.0001
Social Skill- Self Care	3rd	7.92±1.54	5.17±1.04	10.641 0.0001
	6th	12.57±1.28	9.53±1.78	9.944 0,0001
OVERALL DEVELOPMENT	3rd	28.32±3.70	16.19±2.74	18.967 0.0001
	6th	47.46±3.53	38.96±5.99	8.813 0.0001
TOTAL SCORE (T SCORE)	3rd	74.53±7.59	50.57±5.80	18.081 0.0001
	6th	60.76±4.42	50.88±6.81	8.77 0.0001

Discussion

As seen in Table 1, the babies in the experiment and control group were similar in weight, height and apgar score at birth, solely breastfed and in other factors that can be effect mental-motor development. (Table 1)

As shown in Table 2, baby massage has positive effects on both mental and motor developments of healthy full term babies. Breastfeeding duration is known to be an important factor influential on mental-motor development (Mc Kinney et al. 2000; Neyzi 2002). Therefore mental-motor development in solely breastfeeding group was evaluated separately. Total score (T Score) average in ADSI is acknowledged as 50. T scores less than 35 indicate *developmental deficiency*. The babies in our study, both in the experiment and control groups, had T Scores higher than 35. Mean T scores in the third and sixth months were higher in the experiment group (3rd-month=74,50±8,10, 6thmonth=60,85±4,32) compared to the control group (3rdmonth=49,88±5,47, 6thmonth=50,52±7,32) (Table 4). These results indicate that T scores of the babies in the experimental group have improved with massage. While T scores of experimental group higher than the average score, T score in the control group around average score.

In the literature, some researchers investigated effects of baby massage on both mental and motor development, while others investigated either mental or motor development. Among these, Field et al. (1987) and Rice (1979) have reported a better mental development in massage group of premature infants compared the control group using

Table 3. Comparison of mental-motor developmental scores in solely breastfeeding group

Mental-Motor Developmental Domains	Months	Mean ADSI Scores of Infants Solely Breastfed for 6 Months N=(59)		
		Experimental Group (n=34) Mean ±SS	Control Group n=25) Mean ±SS	t p
Lingual-Cognitive	3rd	8.64±1.82	5.92±1.65	5.897 0.0001
	6th	13.50±1.44	11.48±0.87	6.215 0.0001
Fine Motor	3rd	6.76±2.18	2.72±1.48	7.976 0.0001
	6th	11.94±1.27	10.48±1.75	3.699 0.0001
Gross Motor	3rd	4.94±1.15	3.20±0.50	7.065 0,0001
	6th	9.47±1.59	7.12±2.68	4.201 0.0001
Social Skill- Self Care	3rd	5,04±0,93	5.04±0.93	7.324 0.0001
	6th	9.60±1.93	9.60±1.93	6.986 0.0001
OVERALL DEVELOPMENT	3rd	28.23±3.89	15.92±2.56	13.758 0.0001
	6th	47.52±3.42	38.64±6.46	6.837 0.0001
TOTAL SCORE (T SCORE)	3rd	74.50±8.10	49.88±5.47	13.126 0.0001
	6th	60.85±4.32	50.52±7.32	6.785 0.0001

Bayley Scale. In a study from Turkey, Karabudak and Öztürk (2008) have reported higher mental development scores in massage group of premature infants compared the control group measured with ADSI.

Investigating the effects of massaging on motor development, Field et al. (1986) measured motor development of preterm infants using Bayley Scale and Mathai et al. (2001) measured motor development of preterm infants using Brazleton Newborn Behavior Scale and indicated better motor development in the massage group. In another study $Field_b$ et al. (1996) found that motor development of massage group better than the control group in intrauterine cocaine exposure babies. Scafidi et al. (1996) found that motor development of massage group better than the control group. Scafidi and Field (1997) found that motor development of massage group better than the control group in infants born to HIV positive mothers. Evaluating the effects of massage on motor development using ADSI, Karabudak and Öztürk (2008) has defined a statistically non-significant difference in the massage group. Authors thought that this result may be related to the assessment made in the early period of life (2nd month).

The results of the study are similar with other studies except of Karabudak and Öztürk (2008). The dissimilarity between our findings and study of Karabudak and Öztürk (2008) may be related to the time of evaluation.

Conclusion

In conclusion infant massage supports mental motor development of healty full term babies.

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How do employees respect general precaution principles?: The case of emergency rooms of selected hospitals of Mazandaran university of medical sciences-2010

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Abstract

Introduction: Clinical employees all over the world are at risk of contamination by several microorganisms. In order to avoid this, following general precaution developed by center for disease control and prevention is recommended.

Objective: we aimed to study degree and quality of following the general precaution principles against patient blood and secretion among employees of emergency departments in to provide practical recommendation to improve employees' safety.

Methods: The present descriptive-analytical study was carried out cross sectional. Research population comprised of all employees working at emergency rooms of selected hospitals(n=220). Data were collected using a checklist. They were analyzed using frequency tables, mean, standard deviation) and Chi-square test through SPSS16.

Results: Employees compliance with General Precaution was assessed weak (35.1%) regarding hand washing, however, they had better performance in wearing gloves, using patient care equipment and doing safe injection, with scores 60.5%, 78.1%, 47.7% respectively. The relationship between gender, education, job title, age, participation in training sessions and overall compliance rate was significant (P.V<0.05).

Conclusion: The overall compliance rate with General precautions principles among employees was not satisfactory. This needs urgent attention to improve the situation since the risk of hepatitis B and hiv virus transmission through blood and secretion is very high . Implementing infection control standards, taking actions to improve employees compliance with personal protection issues, using engineering controls, implementing cognitive approaches, behavior modification strategies and combining theoretical bases with educational experiences to promote skills in practicing standard principles of general precaution is necessary.

Key words: General precaution, emergency room, hospital, Mazandaran, Iran

Introduction

Infection in a medical center may transmit from patient to employees, patient to another patient, staff to the patient or it can be spread from infected substances such as sputum, blood, urine and other biological products contained pathogenic elements. Health care workers contact with blood transmitted pathogens is regarded as an important occupational risk for these personnel. (1-3). Hepatitis B (HBV) transmission risk following accidental needle injury is about 20 to 40 percent, this number is 1-4 per thousand for risk of HIV transmission and 1.2-10 percent for risk of hepatitis C (HCV) transmission. In addition, prospective studies have estimated the average risk of HIV transmission after percutaneous exposure to HIV infected blood around 0.3 percent (95% CI: 0.2-0.5) and 0.09 (95% CI: 0.006-0.5) after exposure to mucous membranes. However, there are considerable shortcomings in following general precautions among employees (4-5). According to a study in tabriz city of Iran, 51.7% of employees had weak performance in observing general precaution principles regarding contact with blood, skin and mucous membranes (6-7). In a study in

Shiraz of Iran, knowledge and attitudes of 63.3 percent of medical students in observing cautionary actions were good, however, it did not comply with their practice pattern (8).

Among all health workers who are at risk of infection with blood and secretion borne pathogens, emergency department workers are at a higher risk of contamination with mentioned factors (1).

The Center for Disease Control and Prevention (CDC) developed universal precautions to minimize the chance of contamination of health care workers with pathogens transmitted by blood in 1994. These precautions are designed for preventing employees contact with patients' blood, secretions, all body fluids, fecal material, injured skin and mucous membranes. Studies suggest that health workers contact with blood and secretion decreases as they observe general precautions and following these precautions is the best way for health workers protection against occupational HIV and other blood and secretion transmitted infections (1-4).

Therefore, this study was carried out to determine degree of following general precaution against diseases transmitted via patient blood, body fluids, secretions and fecal at emergency rooms of selected hospitals of Mazandaran university of medical sciences.

Methods

This descriptive – analytical study was carried out cross-sectional. Data collection tool was a checklist containing four parts. First part of the checklist studied demographic characteristics, the second part contained general questions and the third part investigated the equipment necessary for deployment of universal precaution and the fourth part covered questions regarding standards observation. Checklist validity is confirmed in previous study (6). Checklist Reliability was confirmed by test- retest method (79% for general questions and 71% for all questions). We applied single-blind method for data collection. To ensure this process, at first emergency room employees rooster was prepared and a unique number was allocated to each of the employees, which was brought on every page of the checklists. In order to avoid observation bias, at first questions on following standard precautions were completed by observation, then section of demographic and general and question on the existence of essential equipment were completed through interviews. To prevent observers registration error, only four observers were recruited for data collection. Harmony of the data collection was guaranteed by preparing manual and providing theoretical and practical education to the observers. Study population comprised of employees of Emergency rooms of Emam, Booali, Fatemezahra, Razi and Emamebehshhar, who were all included in the study.

To assess the compliance with universal precaution, each of the indices were provided with three options (Yes, No and makes no sense) in the checklist. The "makes no sense" answers in each section were removed and only yes and no answers were considered. The sum of yes answers to the sum of yes and no answers were calculated and interpreted as :0-24.9% very poor, 25-49.9% poor, 50-64.9% moderate, 65-74.9% good, and 75-100% very good, according to previous study (6). Descriptive statistics (frequency tables, mean, standard deviation) and inferential tests (Chi-square test) were used for data analysis, all analysis were performed in SPSS 16.

Results

220 participated in the study (54.4 percent female). Mean age of the participants was 35.11±7. The majority of the participants (59.5 percent) were in the nursing group. Most of them (39.5 percent) were in service less than five years. 63.6 percent of employees participated in the study had training courses while 69.1 percent stated that they have read the educational materials, instructions or books about the universal precautions.

Finding on the Availability of supplies for applying universal precautions in the emergency clinic showed that: 96.4 percent had access to Gloves, 37.3 percent to gown, 82.7 percent to Glasses, 75.5 percent to masks and protector to cover the face, 37.7 percent to water with electronic or foot pedal, 100 percent to cotton and alcohol, soap and to safety box.

Although 35.1Percent of employees wash their hands after contact with blood, body fluids, secretions, body waste, and contaminated instruments, and in the intervals between contacts with

Table 1. Percentage of universal precaution compliance in emergency rooms of selected hospitals of Mazandaran University of Medical Sciences

General compliance of universal precautions	Safe injection	Patient care equipment	wearing gloves	Hand washing	Variable
51.3	47.7	78.1	60.5	35.1	Percentage

patients, 60.5 percent of studied employees used gloves prior to contact with blood, secretions and contaminated waste and equipment, before contact with mucous membranes and injured skin and before diagnostic and therapeutic measures. 78.1 percent of the staff disinfected or wiped out patient care equipment in a proper way.

47.7 percent of workers respected safe injection i.e. avoiding needles and sharp instruments manipulation, not bending needles, not recapping needles and putting needle and sharp instruments in the safety box.

Discussion and conclusion

This study indicated that, employees of emergency rooms compliance with general precaution were at intermediate level (see table 1), This a sign which needs immediate attention by administrators since it has been shown in previous studies (14-21) the incompliance with principles of prevention against blood borne diseases, body fluids, secretions and patient wastes increases the risk of hepatitis B virus and HIV transmission.

Besides the observed difference according gender, profession, working experience and attending training courses were significant (PV<0.05; see table 2). In spite of intermediate overall compliance of employees with general precautions, nursing assistants had better performance in this regard (51.3% vs. 67.7%).

The study conducted by Nazari (2006), applying the principles of universal precautions by midwives working in medical universities were at intermediate level, also Chi-square test did not show any significant difference between universities (9). In a study in three hospitals carried out by Department of Environmental Health, Johns Hopkins University, the compliance of health workers with standard precautions in women were more than men and mean score was highest for nurses and technicians and least for physicians (12,13).

Overall compliance of comprehensive precautions was poor (34.5%) among physicians of Texas Medical School of America (10).

In another study, the mean score of knowledge and attitude of Iranian physicians was acceptable (70%) about precautions during contact with blood and secretion But the average score of their performance was reported poor (26%)(11).

Although, prevention of exposure with microorganisms is one of the most important factors in reducing the incidence of infectious diseases, our study indicated that universal precaution compliance was poor (35.1%) in respect to hand washing and most of the staff (65.4%) in the emergency room did not wash their hands after each contact with patients and 74.4% of them did not wash their hands while examining several areas of patients body to prevent transmission of microorganisms from one area to other parts of the body. Chi-square test results showed that compliance with this precaution (washing hands) in women (43.1%) was more than men (32.6%) more in nurses (40.6%) than physicians and housekeeping employees and more in employees who participated training sessions (see table 2).

In a study in the West Algeria 95 percent of nurses washed their hands after removing gloves and 69 percent of them washed their hands between examinations and serving patients (13). Previous studies on knowledge and practice of health workers and medical students reported, employees' perception of preventive measures weak, nurses adoption in observing personal precautions inappropriate. Unavailability of soap, solutions and hand washing liquids, in addition to low knowledge and attitudes of personnel and time shortage have been reported as the main reasons not washing hands. Besides, confidence to their own skills and a sense of safety against disease have been reported as the most common barriers toward observing standard precautions. (22-27).

Universal Precautions compliance was at intermediate level (60.5%) regarding to wearing glo-

Table 2. Compliance with different aspects of general precaution among studied employees

variable yes		washing hands			wearing gloves			patient care equipment			Safe injection			General precautions overall		
		No	p	yes	no	p	yes	N0	p	yes	no	p	yes	no	p	
Gender	Male	32.6	67.4	0.0002	64.7	35.3	0.2	66.6	33.4	0.0001	43	57	0.0001	48.1	51.9	0.0001
	female	43.1	56.9		57.6	42.4		87.8	12.2		56.1	43.9		58.6	41.4	
Age group	<15	42	58	0.4	45.8	54.2	0.0001	76.5	23.5	0.0001	50.9	49.1	0.0001	52.1	47.9	0.0001
	25-34	35.7	64.3		69.2	30.8		79.6	20.4		47.6	42.4		53.9	46.1	
	35-44	38.3	61.7		51.2	48.8		71.4	28.6		48	52		49.8	50.2	
	45≤	50	50		100	0		100	0		87.5	12.5		86.2	13.8	
Job title	physician	31	69	0.0001	57.9	42.1	0.0001	57.9	42.1	0.0001	44.3	55.7	0.0001	45.3	54.7	0.0001
	Nursing	40.6	59.4		62.7	37.3		84	16		53.6	46.4		57.1	42.9	
	Nursing assistant	41.9	58.1		73.9	26.1		94	6		69.5	30.5		67.7	32.3	
	Housekeeping employees	38.3	61.7		30.8	69.2		71.8	28.2		7.7	92.3		31.9	58.1	
working experience	<5	39.4	60.6	0.09	50.6	49.4	0.0001	75	25	0.0001	47.4	52.6	0.0001	50.7	49.3	0.0001
	5-9	32.5	67.5		63	37		71.3	28.7		48.2	51.8		50.7	49.3	
	10-19	41.2	58.8		75.7	25		82.2	17.8		49.3	50.7		57.7	42.3	
	20-24	37.1	62.9		56	44		90.9	9.1		58	42		57.1	42.9	
	25≤	50	50		100	0		100	0		85.7	14.3		84.6	15.4	
participation in training sessions	Yes	46.3	53.7	0.0001	66.1	33.9	0.0001	80.4	19.6	0.1	51.4	48.6	0.0001	58	42	0.0001
	No	24.4	75.6		50.6	49.4		73.7	26.3		46.8	53.2		45.7	54.3	
availability of equipment	Yes	39.5	60.5	0.0001	63.1	36.9	0.0001	80.8	19.2	0.0001	50	50	0.0001	55	45	0.0001
	No	5	95		0	100		8.3	91.7		50	50		20.6	79.4	

ves. Wearing gloves in male personnel were more than women (64.7% versus 57.6%), in nurses (62.7%) were more than physicians (57.9%) and housekeeping employees (30.8%), in personnel with 10-19 years working experience were more than employees with less than 10 years experience and more than 20 years and in Personnel aged over 45 years (100%) and 25-34 years (69.2%) were more than employees in other age groups. Also wearing gloves in individuals who had participated in training sessions were more than who had not participated training sessions. Chi-square test did not show any significant statistical difference between sex and wearing gloves (P>0.05).

Differences observed in wearing gloves in terms of work experience, age and participation in training sessions was statistically significant (P<0.05). A study on observing preventing measures of viral hepatitis reported that 53% of staff do not wear gloves while working and 97% of who wore gloves do not change the gloves. Similarly a study at Johns Hopkins University regarding general precautions showed that 97% of employees did not use gloves (12).

Additionally in a study of observing standard precautions among nurses of university hospital in the west of Algeria, the use of gloves in male nurses were more than female nurses (13) that is similar to the results of present study. Universal precaution compliance of dealing with patient care equipment in hospitals (proper transportation, disinfection or removal of disposables contaminated with blood, body fluids and secretions) were Very well (78.1%) in studied nurses.

Chi-square test results indicated that compliance with universal precautions standards of dealing with patient care equipment in female employees were more than male employees (87.7% versus 66.7%), in nursing assistants (94%) more than nurses, housekeeping personnel (71.8%) and physicians (9.57%) , in workers with more than 20 years working experience more than employees with less than 20 years experience, in age group above 45 years (100%) more than other age groups and in employees who participated in training sessions (80.4%) more than those who have not participate in training sessions (73.7%).

Observed differences regarding dealing with patient care equipment was significant in relation to gender, working experience and age (P<0.05) however, differences were not significant with participating in training sessions (P>0.05). Respect to safe injection was at a poor level (47.7%). Chi-square test showed that safe injection compliance in women were more than men (56.1% versus 43%), in nurses (53.6%) more than physicians(44.3%) and housekeeping personnel(7.7%), in workers with more than 20 years working experience more than employees with less than 20 years working experience, in age group above 45 years (87.5%) more than other age groups and the employees who participated in training sessions (51.4%) were more than those who have not participated in training sessions (46.8%). Observed difference in all of mentioned measures were significant (P<0.05). A study conducted in Nigeria indicated that 48% of medical students were damaged with the needle tip (28). Another study in US showed the most of whom injured with sharp objects were in nursing category (29). Also Rafiee(17) showed that in most studied centers needles and blood contaminated wastes and fluids were collected in a single bin with other home wastes and equipment disinfection is not conducted properly. This is a matter of concern since previous studies reported that the risk of hepatitis B following needle injury is 33% (18-21).

This study indicated that factors such as participating in training sessions, availability of necessary equipment for observing general precautions, gender, education, occupational category and age were effective on compliance with general precaution principles. Similarly, previous studies have reported following factors efficient on observing general precaution principles: understanding organizational commitment to safety, perceived conflict of interest between the need to protect themselves and the need to provide medical care to patients, occupational category, gender, individual perception of risk and knowledge of disease transmission, on the other hand, factors such as multiplicity of staff duties, work stress, lack of time and skills to ensure against the blood and secretions transmitted diseases has been the main barriers of respecting general precaution principles (10,12,30-33).

Implementing infection control standards, measures to improve staff compliance with personal protection issues, engineering control, cognitive

approaches, behavior modification strategies and combining theoretical education with experience to enhance skills are necessary prerequisites for applying standard principles of universal precautions.

Annual training of personnel and repeating this training on universal precautions, providing all supplies and equipment that reduces the risk of infectious disease, should be considered as a priority by authorities.

Suggestions

According to the results of this study there were some weaknesses in applying various aspects of universal precautions in emergency clinics of hospitals, therefore, it is essential to give special attention to the employees on- Job training programs, employees' Initial training and training of medical and nursing students. According to results of this study, a proper plan for providing personal protection equipments and improving employees' compliance with general precaution standards should be developed.

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Prevailing effect of psychiatric comorbidity in a patient with hot water epilepsy and celiac disease

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Abstract

Comorbidity is common in epilepsy, and particularly mental disorders have a complex and intriguing relationship with epilepsy. Anxiety is one of the most frequently occurring interictal psychopathological symptoms that may affect the patient's quality of life more severely than the epileptic seizures themselves. We report a case of a 31-year-old man with complex comorbidity of celiac disorder, spontaneous and unusual hot-water reflex epileptic seizures, and anxiety disorder.

Key words: epilepsy; reflex epilepsy; comorbidity; anxiety; celiac disease

Introduction

As accepted in modern practice, the term comorbidity refers to the statistical association of two distinct diseases in the same individual at a rate higher than expected by chance [1]. It is crucial to highlight clinical complexity since comorbidity is reported in 79% of all ill people [2]. Comorbidity has serious implications for both the diagnosis and treatment of a primary disease of interest.

Comorbidity is common in epilepsy and an increased prevalence of comorbid conditions in people with epilepsy as compared with the general population has been found in several population-based surveys [3, 4, 5, 6]. Comorbidity in epilepsy is important to address because epilepsy patients and their families often find the effects of comorbidities more troubling than the seizures themselves.

In this paper we describe a patient with complex comorbidity of celiac disease, epilepsy with spontaneous and reflex seizures and the consequent anxiety disorder, which all led to considerable impairment of the patient's quality of life.

Case presentation

A 31-year-old man, who had been previously diagnosed with epilepsy and treated in a health care center in his home town, presented to our tertiary outpatient epilepsy clinic because of increased frequency of seizures during bathing.

Medical records showed that the diagnosis of epilepsy was established in early childhood when he was 3 years old, after repeated episodes of generalized tonic-clonic seizures (GTCSs) during sleep. An interictal EEG done at that time revealed focal epileptogenic abnormalities**.** During childhood and adolescence spontaneous GTCSs were incompletely controlled with carbamazepine and/or phenobarbital. He had no family history of epilepsy or febrile convulsions. However, he had one episode of febrile convulsions during infancy.

In addition, at the same time, when he was 3 years old, he was diagnosed with celiac disease, confirmed by small bowel biopsy, following recurrent diarrhea and inability to gain weight that started at the age of 7 months. Since the age of 3 years, he had been on a strict gluten-free diet.

At the age of 6, he had a seizure while bathing in hot water, characterized by oral automatism and unresponsiveness that lasted for 1-2 minutes, after which he slept for a couple of hours. He had only five such episodes until the age of 11. Thereafter, he had only spontaneous GTCSs (1-2 per year) for the following 17 years that were incompletely controlled with the previously mentioned AEDs (carbamazepine and/or phenobarbital). At the age of 28, the patient was switched to valproate, soon also topiramate was added, and was free of GTCSs under this bitherapy thereafter.

At the age of 29, episodes of unconsciousness during bathing in hot (or warm) water recurred.

Immediately before losing consciousness he would experience unpleasant sensations in the form of peresthesia starting in the legs and spreading towards the head, accompanied by fear and anxiety. At first the seizures occurred once a month, but then increased in frequency so that the patient was avoiding bathing or showered rarely (approximately once a month). Before bathing, he would perform ritualistic behavior, such as preparing himself and the bathroom for several hours, and often eventually give up bathing. Upon his parents' insistence a psychiatrist was consulted, who suspected a phobic disorder and ordered him alprazolam in combination with an antidepressive (sertraline) and a benzodiazepine (lorazepam), which he took for a few months with no satisfactory effect. Hence the patient was referred to our outpatient clinic.

Neurological exam and interictal EEG were normal and MRI revealed left-side hippocampal gliosis with no atrophy (Figure 1). He had been free of GTCSs under bitherapy with valproate (1250mg/day) and topiramate (100 mg/day) for the previous three years. Attempts to provoke seizures by exposing the patient to hot water (40°C) during EEG recording failed.

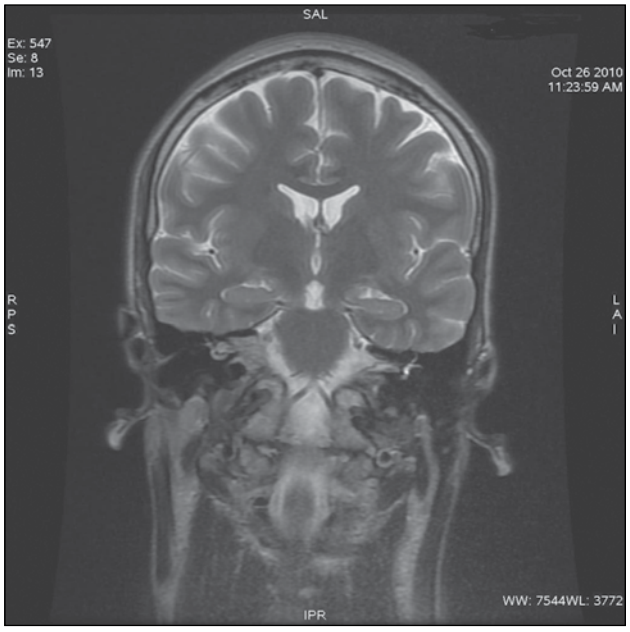

Figure 1. MRI: left-side hippocampal gliosis with no atrophy

The patient was referred for further evaluation and hospital treatment to the psychiatry clinic. Clinical examination and psychological testing revealed anxiety, non-psychotic paranoid ideas, signs of moderate cognitive deterioration and modest intellectual abilities as well as the presence of hypersensitivity, accompanied by hostility, impulsivity, irritability and emotional instability. No seizures or epileptiform abnormalities were registered during hospitalization. However, the patient demonstrated severe anticipatory anxiety, which was alleviated by low-dose antidepressants (escitalopram) in combination with low-dose antipsychotic (risperidone) and benzodiazepine with clonazepam. Nevertheless, after discharge from hospital, the patient reportedly continued avoiding regular bathing.

Discussion

This case is interesting because it represents an unusual complex comorbidity of reflex and spontaneous seizures, celiac disease and consequent anxiety.

Hot water epilepsy (HWE) is an unusual form of reflex epilepsy in which seizures are provoked by immersion in hot water. The exact pathogenesis of HWE is unknown, but complex partial seizures are common in this type of epilepsy. It was suggested that a lesion in the temporal lobe may be responsible for HWE [7]. However, only a few case reports of HWE with structural lesions demonstrated by computed tomograpraphy (CT) or magnetic resonance imaging (MRI) have been reported [8, 9, 10, 11]. Involvement of the temporal lobe in HWE has also been postulated in animal models. Experimentally repeated exposure of the heads of adult rats to hot water increased susceptibility to convulsions [12, 13]. It was also suggested that the seizures in HWE are a type of hyperthermic seizures similar to febrile convulsions [13]. HWE probably has a genetic basis and may have kindling effect on the hippocampus as do febrile convulsions [14]. Our patient had one episode of febrile convulsions and the structural change seen on MRI may have developed as a result of it. On the other hand, the hippocampal pathology may have resulted from the kindling effect of many HWE seizures. Investigations with new detailed neuroimaging techniques in addition to experimental and clinical studies might be helpful in understanding the mechanism of this reflex epilepsy.

Our case is unusual since spontaneous seizures preceded reflex seizures, whereas literature data su-

ggest that the reverse is usually the case [15, 16, 11]. However, a case of spontaneous non-reflex seizures preceding HWE has also been reported [17].

Another interesting point is that our patient had several HWE seizures between the ages of six and eleven years followed by a period of over 15 years when he was free of HWE and had only spontaneous seizures. HWE is generally known to be benign and self-limited if precautions are taken against precipitating factors, but it was not the case in our patient.

Celiac disease, or gluten-sensitive enteropathy, has recently been described in association with epilepsy and other neurological disorders. Over the last decade coexistence of epilepsy and celiac disease has been widely reported, but the cause of neurological complications observed in celiac disease is still unclear [18, 19]. Although published data suggest that gluten-free diet can improve seizure control in patients with epilepsy and comorbid celiac disease [20], we failed to achieve complete seizure control in our patient despite his gluten-free dietary compliance.

Symptoms of mental disorders are common in patients with epilepsy. Around 60%-70% of patients with refractory seizures and 10%-20% of patients with well-controlled epilepsy have a psychiatric comorbidity, most often depression, anxiety and cognitive disturbances. Despite the manifest association between epilepsy and mental disorders, especially anxiety, the underlying pathogenetic mechanisms is still unclear. Anxiety can manifest itself in ritualistic compulsive behavior which can lead to somatoform dissociative disorder, as well as trigger the occurrence of psychogenic non epileptic seizures (PNES) [21]. Anxiety can worsen the patient's seizure state with PNES or with occasional seizures caused by psychic decompensation through biological mechanisms [22, 23]. Our patient probably had both epileptic and psychogenic non epileptic seizures. In our opinion, anticipatory anxiety in our patient developed as a result of reflex epileptic seizures.

Our case adds proof to the observation that anxiety accompanying epilepsy affects the patient's quality of life more severely than the epileptic seizures themselves [24, 25, 22]. Comorbid anxiety in epilepsy is very likely to trap the patient in a vicious circle. An adequate intervention therefore does not involve modifying antiepileptic treatment by all means, but rather improving the seizure state and anxiety by anxiolytic treatment. Only dual treatment of epilepsy and anxiety has been found effective, and it is recommended that anxiety should be treated first.

In conclusion, the overall health status and quality of life of patients with epilepsy is greatly compromised by comorbidity. Although seizure freedom should continue to be a primary clinical goal, the optimal care should also include identification and management of comorbidities.

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First day in clinical practice: evaluating stress of nursing students and their ways to cope with it

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Abstract

Background: Initial clinical experience mostly is highly stressful for nursing student.

Objectives: The study was conducted to evaluate the stress experienced by nursing students, who are about to carry out their first clinical practices, and also, their ways to cope with it.

Methods: The study was experimentally conducted with students studying at 1st grade of nursing department of a university in Turkey in the order including final test control group.

Sampling of the study consisted of a test group and a control group (n=26) accounting for 52 students in total. Data were obtained by "the clinical stress questionnaire", "the inventory of the styles for coping with stress" and Student Questionnaire Form. Frequency, arithmetical mean, Mann-Whitney U, Kruskal Wallis, Spearman's Correlation Analysis and Cronbach alpha test were used in analyzing the data.

Results: It was found that mean score of the test group relating to stress level (31.88 ± 8.60) is lower than that of the control group (32.30 ± 11.57); however, the difference between them is not statistically significant (p>0.05). When it was assessed from the point of view of coping with stress, it was seen that mean score of sub-dimension of optimistic approach of the students in the control group (8,26 ± 2,34) is higher than that of the students in the test group (6,34 ± 1,89) in a statistically significant way (U= -2.80 ; p=0,005).

Conclusions: It was concluded that students experience stress on their very first day that they will begin clinical experience with no respect to circumstances despite training and orientation works to be conducted, more concentration should be paid to laboratorial education in the first grade, simulation practices should be developed, clinical environment should be made suitable for the student and moreover, effective methods in coping with stress should be taught and practiced.

Key words: clinical practice, coping style, nursing students, stress.

Introduction

Theoretical information and clinical practice are integrated parts supplementing each other in nursing education like education programs of all other disciplines based on practice (1). Learning occurs, in real manner, during clinical practices for nursing students. Clinical training provides undergraduate nursing students with opportunities to observe, develop, and practice the intellectual and psychomotor competencies indispensable to nursing. They involve developing critical thinking, analyzing, psychomotor communication and management skills. Clinical experiences also encourage the exploration of personal feelings, the transfer of knowledge from theory to practice. (2,3).

Matching up clinical practices with what is known by a student, as well as diversity and frequency of practices are very important while students convert any information learnt by them in theoretical and practical ways into a behavior in clinical practice fields (1).

Stress, which emerges as response of the body to a situation threatening physically and psychologically or non-conforming conditions, is considered an ordinary part of daily life. On the other hand, coping with it is described as an individual's resi-

stance to the events or factors, which causes stress for him, and also, all of his cognitive, emotional and behavioral responses showed by him for bearing stress (4,5). Some of the methods for coping with stress help as a protective function regulating negative feelings in relation with stress to produce alternative solutions while they may make effects of stress worse and may cause problems in adaptation (6). Using effective methods for coping with stress or practices, which may mitigate the stress, may help an individual in ensuring his adaptation when he encounters a stressful event (7,8). Stress, which is experienced at lower level by a student, motivate the student while stress at high level makes learning and thinking harder and also, may cause physical and mental damages (9,10,11).

Nursing students have the same academic stressors as other collage students such as midterm and final examinations, research papers and other assignments. In addition nursing students experience a clinical component, which is highly stressful (12). Nursing students encounter clinical experience, which will continue during the four-year baccalaureate term, in the lessons on fundamentals of nursing for the first time. Several studies demonstrate that the clinical experience for nursing students having no prior healthcare experience, particularly the first rotation, frequently produces high levels of stress and anxiety, which can adversely affect the learning process (13,14,15,16).

It was determined that the stress experienced by students was caused by the reasons like strange and complex nature of clinical environment, seeing people, who suffer, being emotional, failing in remembering those learnt by them, consideration about having lower scores, and insufficiency in communicating with patients and the team (1,7,16,17,18,19,20,21).

Highly stressful experience in the first clinical practice may have significant effect on professional socialization process of a student and his decision about the profession (17,22). Furthermore, experiencing stress at high level and failing in coping with it may cause a decline in academic performance, depression, having a feeling of loneliness, nervousness, insomnia, anxiety, exhaustion and serious medical problems. Moreover, this may result in dropping out of school for some students (7,19,23,24).

Stressors perceived by students and stress level experienced by them may decrease gradually after the first clinical practice during the entire nursing education period. In other words, clinical stress level occurs at a lower degree and students can cope with it more effectively during their other practices after the first one because they have more experience, skills and knowledge (3,18,20). As a result, students suffer from stress at the highest level and need effective methods to cope with it more during their first clinical practices. Therefore, it is reported that investigating students' opinions about clinical practice and which tasks they have difficulty in will be helpful in developing most effective clinical training strategies in nursing education (21). Thus, the study was conducted to determine the stress felt by nursing students, who would have their first clinical experience, when they were prepared for clinical practices through different methods, and to determine their styles in coping with stress to recommend the most effective ways for solution.

Method

Research Design

The study was experimentally conducted with students studying at 1st grade of nursing department of a university in Turkey in the order including final test control group.

Setting and participants

The sample was comprised of students enrolled in the "Fundamentals of Nursing" course, in a vocational nursing school in Turkey. Test and control groups were established by using simple random sampling method among 106 students, who were studying at first grade of nursing school and also, agreed to participate in the study. 52 students in total were included in the study in two groups, test (n=26) and control groups (n=26).

Data collection

Data were obtained by the clinical stress questionnaire (CSQ), the inventory of the styles for coping with stress (WCQ) and Student Questionnaire Form including socio-demographic features of students.

Student questionnaire form: This form was designed by the researchers and includes questi-

ons probing factors, which are believed that they have an effect students' stress level and their styles for coping with it, like age, sex, which school he graduated from, birth place, student's current residence, as well as why he chose nursing, whether he likes school or not, whether any other family member work for medical sector or not and whether he has been in a hospital before or not.

Clinical Stress Questionnaire (CSQ): The clinical stress questionnaire (CSQ) is a self report Likert- type instrument that was developed by Pagana in 1989 for the purpose of determining the beginning stress value that is threatening or challenging to nursing students in their first clinical experience. CSQ, whose Turkish validity and reliability was established by Sendir and Acaroğlu (2007), may be applied in all internal and operational clinics after the "student's first clinical practice day". The questionnaire has 4 sub-dimensions like threat, fight, damage and benefit emotions. Each item is assessed at 5 degrees and requires one the options of 0-"none", 1- "a little bit", 2- "moderate", 3-"much", 4-"too much" is marked. The score ranges between the least "0" and the most "80" based on points given for each item. Lower scores indicate lower stress level while high scores indicate higher stress levels.

The inventory of the styles for coping with stress (WCQ): The inventory of the styles for coping with stress, which was adapted from Folkman and Lazarus's 'Ways of Coping Inventory' scale to Turkish, was established by Hisli and Durak (1995) from the point of view of validity and reliability. It consists of 5 sub-scales like 'self-confident approach', 'optimistic approach', 'desperate approach', 'obedience approach', and 'social support seeking approach'. Each sub-scale of this four-point Likert type scale including 30 items in total is computed separately to be assessed. The scores to be obtained from the scales range between 0 and 21 for 'self-confident approach', 0 and 24 'desperate approach', 0 and 18 'obedience approach', 0 and 15 'optimistic approach' and 0 and 12 for 'social support seeking approach'. High points are evaluated that the individual uses the relevant way in coping with stress more (25).

Students practice the relevant skill at the end of each lesson in practice laboratory in the existing training program. Students of the control group were required to begin hospital practice for clinical practice after general laboratorial practice of the lesson on Fundamentals of Nursing according to the existing training program. On the other hand, unlike those in the control group, students in the test group were required to repeat all skills, which would be applied in clinical practice, in laboratory for five days after they had completed the existing program before they were sent to hospital for clinical practice. The clinical stress questionnaire (CSQ), the inventory of the styles for coping with stress (WCQ) and Student Questionnaire Form were applied to students of both of the groups at the end of their first day in clinic.

Ethical considerations

The required permission was obtained from the directors' board of the school. Then, students were informed about the objective of the study, content of being a participant and procedures. The students, who had agreed to participate in the study, were informed about the participant's right to withdraw from the study at any time if he feels that his personal rights are violated or damaged during the study.

Data analysis

The obtained data were analyzed by using frequency ,arithmetical mean, standard deviation, Mann-Whitney U, Kruskal Wallis, Spearman's rho Correlation, and Cronbach alpha coefficient tests in SPSS 13.0 package program.

Results

Most of the participants were girls (76.9%), whose ages' mean was 19±30. It was determined that students taking place in control and test groups were socio-demographically similar.

Clinical stress point mean of students in the test group was 31.88 ± 8.60 while it was 32.30 ± 11.57 for students in the control group. Any statistically significant difference was not found between the groups ($p>0.05$) (Table 1). WCQ point means of students of the test and control groups were very close to each other and there was no significant difference between them. Only point mean of the control group's students for optimistic approach sub-dimension (8.26 ± 2.34) was significantly higher than that of the test group's students (6.34 ± 1.89) (U= -2.80; p=0,005; Table 1).

Table 1. Clinical Stres And Coping Strategies Of Nursing Students

The Clinical Stress Questionnaire	Study Group		Control Group			
	$\bar{x}$	*Sd*	$\bar{x}$	*Sd*	*U*	*p*
Threat	9,61	4,64	9,69	4,54	-,220	,826
Challenge	13,73	4,20	14,69	7,49	-,018	,935
Benefit	3,88	1,47	3,84	1,61	-,335	,737
Harm	4,65	3,17	4,07	3,03	-,737	,461
Total	31,88	8,60	32,30	11,57	-,238	,812
Ways of Coping Inventory						
Self-Confident Approach	13,38	2,91	13,00	2,97	-,813	,416
Optimistic Approach	6,34	1,89	8,26	2,34	-2,800	**,005**
Helpless Approach	9,96	3,71	9,34	3,23	-,931	,352
Submissive Approach	4,92	2,11	4,34	2,59	-,637	,524
Seeking Social Support	8,11	1,27	8,19	1,93	-,338	,735

Table 2. The Relation Between Clinical Stress' Level And Coping Strategies Of Nurses Students

CSQ \ WCQ			Self-Confident Approach	Optimistic Approach	Helpless Approach	Submissive Approach	Seeking Social Support
Study Group (n=26)	Threat	r	-,040	-,401*	,597**	-,043	,070
		p	,846	**,042**	**,001**	,836	,735
	Challenge	r	,223	,477*	-,095	,089	,178
		p	,273	**,014**	,644	,665	,384
	Harm	r	-,322	-,492*	,369	,088	-,136
		p	,109	**,011**	,063	,668	,508
	Benefit	r	,506**	,599**	-,192	-,170	,057
		p	**,008**	**,001**	,348	,408	,781
	Total	r	,027	-,125	,372	-,044	-,015
		p	,896	,544	,061	,830	,943
Control Group (n=26)	Threat	r	-,092	,023	,626**	,344	-,252
		p	,655	,912	**,001**	,085	,215
	Challenge	r	,326	,235	,335	-,152	,117
		p	,105	,247	,094	,459	,568
	Harm	r	-,010	-,078	,401*	,357	-,247
		p	,961	,704	**,042**	,074	,223
	Benefit	r	,281	,499**	,166	-,142	-,019
		p	,164	**,010**	,417	,488	,927
	Total	r	,240	,199	,582**	,060	-,105
		p	,237	,329	**,002**	,771	,611

** p<.05 ** p<.01*

Considering correlation between point means obtained from the two scales, for test group's students, there is a positive significant correlation between sub-dimension of self-confident approach and benefit emotion (r = ,506; p<0,01), a negative significant correlation between sub-dimension of optimistic approach and threat (r =-,401, p<0,05) as well as damage emotions (r=-,492; p<0,05), and a positive significant correlation between fight (r=,477, p<0,05) and benefit emotions (r=,599; p<0,01). Furthermore, a positive significant correlation was seen between sub-dimension of desperate approach and threat emotion (r =,597; p<0,01) (Table 2). In case of students of the control group, only three significant correlations take place. These significant correlations exist between sub-dimensions of optimistic approach and benefit and between sub-dimension of desperate approach and threat and damage emotions (p<0.01)

Discussion

The essential objective of clinical training in nursing education is to ensure that students gain competence in using values, attitudes, knowledge and skills existing in nursing profession and to ensure they pass to professionalism from studentship (26). Eşer et al., (2008) Nursing students may face many factors causing stress during their educational process. If students experience stress in one of these factors, in clinical practice, and they cannot manage it properly, this may affect their academic performance and their perceptions about the profession. Moreover, a clinical practice, which is completed successfully by taking advantage at a desired level, lays the ground for the clinical practices to be performed in the future and has an effect in perception of professional qualifications (26). Eşer et al., (2008) and Gorostidi et al., (2007) reported in their study that clinical stress experienced by nursing students decreases gradually in the course of time as they become more experienced in the future.

In the present study, it is seen that clinical stresses of students taking place in test and control groups are moderate and although there is no statistically significant difference between these two groups, students of control group experienced more stress compared with those in test group (Table 1). Sheu et al., (2002) reported that nursing students experience stress at a moderate level in their first clinical practice and this supports our findings. Gül and Bozkurt (2003) reported that midwifery students experience anxiety at moderate level during invasive operations in clinic and there is no statistically significant difference between state anxiety levels of students before and after invasive operation. Bayar et al., (2009) compared state-continues anxiety point means of students before and after clinical practice and found that anxiety level before clinical practice is significantly higher. In another study conducted for the same purpose, any statistically significant difference was not found between anxiety point means (3). Lo (2002), in his longitudinal study, evidenced that 2nd grade students experienced temporary stress at higher level than those at 1st grade did because they had more clinical practices. According to the finding of our study, although laboratorial exercises decrease stress, it is inevitable that students experience stress in hospital.

Students' ways in coping with stress in both of control and test groups are similar (Table 1). However, it is seen that students in the control group need more to use the optimistic approach compared with those in the test group ($p<0.05$). This finding suggests that students in the test group were able to cope with stress more easily because they had an opportunity to make more exercises in laboratory. Other studies in the literature show that nursing students use optimistic approach mostly in coping with stress (8,9,27).

It was determined that potentials in benefit and fight emotions relating to clinical stress increase as students in the test group use optimistic approach for coping with stress while emotions relating to threat and damage decrease significantly. Also, they use self-confident approaches significantly more for coping with stress as their stress in benefit dimensions including being relax and self-confident increases ($r= 0.506$, $p<0.05$). Any similar correlation could not be found for students in the control group. They use desperate approach significantly more when they see the potential for being damaged. Unlike those in the test group, they do not use optimistic approach. They use optimistic approach only when benefit emotion causes stress. Şahin (1995) reported in his study on validity and reliability of WCQ that self-confidence and optimism are useful methods for coping with stress (28).

In addition, both of the groups showed a significant desperate approach when they face threat relating to stress. This is an inevitable result because being desperate causes failing in problem solving and more stress. However, if significant correlations relating to coping with stress in case of clinical stress in the test group are evaluated as a whole, repeating practices may have an effect on this result.

Conclusion

Nursing students studying at 1st grade experience stress on their first day in clinical practice. Even if they repeat the practices several times to develop their psychomotor skills, first they stress never decreases. If positive problem-focused ways are employed for coping with stress (self-confidence and opportunism approaches), positive emotions (fight, benefit) mitigating stress will increase. Stu-

dents in the test group had an opportunity to repeat the practices several times before clinical practice and as a result, they more used positive methods for coping with clinical stress while this did not occur in case of students taking place in the control group. It may be believed that increasing number of laboratorial practices may cause students use positive methods for coping with stress more.

Further Research and Recommendations

Encouraging students to use positive methods for coping with stress may decrease their clinical stress. To prevent their first clinical practices from making negative effects on them that they will have their first impressions about the profession:

* More time should be given for laboratorial practices for 1st grade nursing students and they should be met with a simulative patient instead of real cases, and models should be employed for developing skills,
* Physically and mentally assuring and informative adaptation programs should be arranged for students under consultancy of lecturers before clinical practice lessons,
* Suitable environment and facilities should be provided for students to ensure that students are more self-confident and to develop problem solving skills of students,
* Comparative studies should be conducted with research groups in higher number

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Technical Efficiency of Iranian Medical-services Insurance Organization Using Data Envelopment Analysis Approach

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Abstract

Background: Performance evaluation is one of the most important topics in management literature. There is a variety of criteria for such an evaluation including efficiency, effectiveness, and quality assessment. Meanwhile, efficiency is a current criterion with a different method of measurement.

Objectives: Iran's Medical-services Insurance Organization (IRI- MSIO) is one of the major financial health institutions nationwide which, in this paper, has been studied to model its technical efficiency.

Methods and material: This study was descriptive-practical. Technical efficiency of 31 provincial offices of MSIO was determined by using Data Envelopment Analysis (DEA). The information (2010 data) was constructed through the forms designed by researchers from Information center of IRI-MSIO. The efficiency of provincial offices was surveyed by MSIO-experts and classified the provincial offices. Differences between groups of technical efficiency were evaluated with ANOVA and Kruskall-Wallis Tests.

Results: Total average technical efficiency of all provincial offices of MSIO was 0.513. The estimation of province-efficiency was surveyed by MSIO-experts who, with regard to environmental factors influencing the efficiency of MSIO, classified the provincial offices into four groups (Kruskall-Wallis Tests: ($P<0.001$).

Conclusion: Input-oriented BCC model to be the optimal efficiency measurement for IRI-MSIO. Based on the impact of efficiency environmental factors (access to services and overhead refers indices), the MSIO provincial offices can be classified into four main groups. Therefore, in order to increase the efficiency of MSIO in national level it is suggested to consider this classification as an on-factor in the referral system to delivery of medical services.

Key words: Medical Services Insurance Organization, Iran, Technical efficiency, Managerial efficiency, Efficiency of scale, Data envelopment analysis (DEA)

Introduction

The Medical-services Insurance Organization (MSIO) is one of the major financial health sectors in I. R. Iran offering, in 2009, a wide range of financial security products with a mission to protect nearly 39,965,000 Iranians in medical services[1]. This organization was founded with a governmental nature in 1995 on Iranian Health Insurance Coverage law; its workflow basically consists of four main processes: investing, insuring target population, activating contracts with healthcare providers, and purchasing the medical services needed. The MSIO holds local offices in all 31 Iranian provinces with a wide network of agencies from the private sector nationwide.

Limitations in resource and facilities have ever since adversely affected the socio-economic conditions of all communities[2]; enhancing the productivity level and improving the efficiency variables are increasingly critical points of concern for any nation planning to effectively manage such challenges[3]. This topic is even more important in health sector, being the core of sustainable development and a fundamental segment of infrastructure for all other sectors. In addition to moral ethical aspects of the above mentioned issue, health promotion has also a social-econo-

mic aspect which must be taken into consideration by policy-makers as a part of holistic sustainable development plans[4].

Act no. 88 in the 4th Development Plan of Islamic Republic of Iran (2007-2010)[5] has emphasized the enhancement of productivity and efficiency in health sector. Social policies of Iran's 5th Development Plan(2009) has, too, focused on implementing an integrated system of policy-making, planning, evaluating and allocating public resources for the sake of human health; to achieve higher levels of health insurance both in quantity and quality are the targets of these Plans, out of packet must be decreased by 30 percentages[6].

One can consequently observe the attempts for increasing productivity level in health sector, particularly in health insurance systems, in recent Plans of IRI. Major organizations have been so far established to measure productivity and efficiency; therefore the objective of the present paper is to measure the technical and scale efficiency of Iranian Medical-services Insurance Organization; a Data Envelopment Analysis (DEA) method was applied with an eye to these reasons.

There are two general approaches to measuring technical efficiency, namely parametric and nonparametric. Data Envelopment Analysis (DEA) is the nonparametric method, and can easily handle multiple input/output cases.

Moreover, in DEA application, inputs and outputs can have significantly different units of measurement without requiring any a priori tradeoffs nor any input- output prices. Given these highly desirable features of the nonparametric methods, it is not surprising that they have recently become very popular among researchers[7].

The main DEA models have been described in details by several authors[8-10] thus a detailed description is not provided here. The first DEA model was developed by Charnes et al in 1978. This model, known as the CCR model, assumes constant returns to scale (CRS)[9]. The efficiency score (θ) in the presence of multiple- input and -output factor is defined as[9]:

$$\text{Technical efficiency} = \frac{\text{weighted sum of outputs}}{\text{weighted sum of Inputs}} \quad \text{..... (1a)}$$

Or mathematically as[9]:

$$\theta = \frac{u_1 y_{1j} + u_2 y_{2j} + \cdots + u_s y_{sj}}{v_1 x_{1j} + v_2 x_{2j} + \cdots + v_m x_{mj}} = \frac{\sum_{r=1}^{s} u_r y_{sj}}{\sum_{i=1}^{m} v_i x_{ij}} \quad \text{..... (1b)}$$

Let the DMU_j to be evaluated on any trial be designated as DMU_o where o ranges over 1, 2, ..., n. To measure the relative efficiency of a DMU_o based on a series of n DMUs, the model is structured as a fractional programming problem as follows[10]:

$$\max_{u,v}: \theta = \frac{\sum_{r=1}^{s} u_r y_{ro}}{\sum_{i=1}^{m} v_i x_{io}} \quad \text{..... (2)}$$

$$\text{s.t:}$$

$$\frac{\sum_{r=1}^{s} u_r y_{rj}}{\sum_{i=1}^{m} v_i x_{ij}} \leq 1 \quad j = 1, 2, 3, \ldots, n$$

$$u_r, v_i \geq 0$$

where n is the number of DMUs in the comparison, s the number of outputs, m the number of inputs, u_r (r = 1, 2, ..., s) the weighting of output y_r in the comparison, v_i (i = 1, 2, …, m) the weighting of input x_i, and y_{rj} and x_{ij} represent the values of the outputs and inputs y_j and x_i for DMU_j, Eq.(2) respectively can equivalently be written as a linear programming (LP) problem as follows[10]:

$$\max_{u,v}: \theta = \sum_{r=1}^{s} u_r y_{ro} \quad \text{..... (3)}$$

$$\text{s.t:}$$

$$\sum_{i=1}^{m} v_i x_{io} = 1$$

$$\sum_{r=1}^{s} u_r y_{rj} \leq \sum_{i=1}^{m} v_i x_{ij} \quad = j = 1, 2, \ldots, n$$

$$u_r, v_i \geq 0$$

In reality, the dual (envelopment) form of the DEA-LP problem is simpler to solve than (3) due to fewer constraints. Mathematically, the dual li-

near problem is written in vector–matrix notation as follows:

$$\min_{\lambda,\theta} \theta \quad \text{..... (4)}$$

$$\text{s.t:}$$

$$Y\lambda \geq y_0$$

$$X\lambda - \theta x_0 \leq 0$$

$$\lambda \geq 0$$

Where y_o is the s × 1 vector of the value of original outputs produced and x_o is the m ×1 vector of the value of original inputs used by the oth greenhouse. Y is the s × n matrix of outputs and X is the m × n matrix of inputs of all n units included in the sample. λ is a n × 1 vector of weights and θ is a scalar with boundaries of one and zero that determines the efficiency score of each DMU, i.e., $\theta = 1$ shows a technically efficient DMU; $\theta <$ 1 shows a technically inefficient DMU [10].

For each inefficient DMU, target input and output levels have to be prescribed. These targets are the results of respective slack values added to outputs[11]. To calculate the target values for inputs ($\hat{x}_{io}$), the input value (x_{io}) is multiplied with an optimal efficiency score (θ), and then slack amounts (s-) are subtracted from this amount,[11]:

$$\hat{x}_{io} = \theta * x_{io} + s_i^- \quad i = 1,2,\ldots,m \quad \text{..... (5a)}$$

Similarly, in an input-oriented model, efficient output targets are calculated as [11]:

$$\hat{y}_{ro} = y_{ro} + s_r^+ \quad r = 1,2,\ldots,s \quad \text{..... (5b)}$$

If there is no restriction on λ, Eq.(4) represents constant returns to scale[12, 13]. CRS implies that a given increase in inputs would result in a proportionate increase in outputs and the feasible region of the envelopment problem becomes a conical hull. A restriction on $\lambda(\lambda = 1)$ leads to no condition on the allowable returns to scale, also called variable returns to scale (VRS). Under this condition, the performance frontier line is not then restricted to pass through the origin, and an increase in inputs may not result in a proportionate increase in outputs in this case [10]. Due to convexity, the efficient DMUs form a convex hull on which all inefficient points are projected. Because the VRS is more flexible and envelops the data in a tighter way than the CRS, the score or pure TE (θVRS) is equal to or greater than the CRS or overall TE score (θCRS). The relationship can be used to measure scale efficiency (SE) of the oth greenhouse as:

$$SE = \frac{\theta^*_{CRS}}{\theta^*_{VRS}} \quad \text{..... (6)}$$

Where SE = 1 implies scale efficiency (or CRS) and SE < 1 indicates scale inefficiency [10].

In Iran, likewise, researchers have used DEA for efficiency measurement of different organizations[14, 15]; it has, in some cases, been carried out in insurance companies[16-18].However, few studies have so far involved health sector and even among this minority, most were carried out in hospitals concluding that data envelopment analysis should be applied rather than parametric methods [19-21].In a research on efficiency of health care among selected Islamic countries, Hosseini-nasab and Baskha (2010) evaluated the performance of 24 countries regarding their application of financial resources for improving health through data envelopment analysis method[22].In health-insurance sectors only one article was found with a topic of "Performance Evaluation in Tehran province branch of social security by merge of fuzzy data envelopment and balance score card (FDEA & BSC)" the indicators of which being designed by BSC and the performance of their organizations evaluated through FDEA[23].

In world level, there were several studies regarding performance of hospitals[24-26]in health insurance, however, only one study was found in which Wu et al. (2007) simulated the production and investment functions in Canadian health and life insurance companies by Data Envelopment Analysis; they presented a new model of data envelopment analysis which simultaneously measures the production and investment performances of Canadian health insurance companies[27].

Methods

This study was descriptive-practical in nature and cross-sectional regarding the method of conduction. In the first step, DEA-model of MSIO was designed through holding several interviews with experts prior to doing system analysis; the information (2010 data) was then constructed through the forms designed by researchers from Information center of IRI-MSIO. In the next step, DEA-model was run in $Deap_2$ software and the estimation of province-efficiency was surveyed by MSIO-experts who, with regard to environmental factors influencing the efficiency of MSIO, classified the provinces into four groups. Finally, differences between groups of technical efficiency were evaluated by SPSS software (ver. 17) through parametric ANOVA and nonparametric Kruskall-Wallis Tests.

To give a brief description of the DEA-model of MSIO, a non-parametric Data Envelopment Analysis (DEA) method is employed with variable return-to-scale (VRS) assumption to measure input-oriented technical efficiency of MSIO.

MSIO intrinsically bears four main processes: investing, insuring target population, activating contracts with health care providers, and purchasing the medical services. As local offices of MSIO in Iranian provinces have no investing activity, this factor is eliminated in our model. Therefore, three variables were selected as input of BCC-model (the number of manpower, overhead costs, building and land area) as well as another three as output (namely, proportion of insured population from the total target population, proportion of contracted institutions from the total activity institutions, proportion of once-referring from the medical expenses)(figure 1).

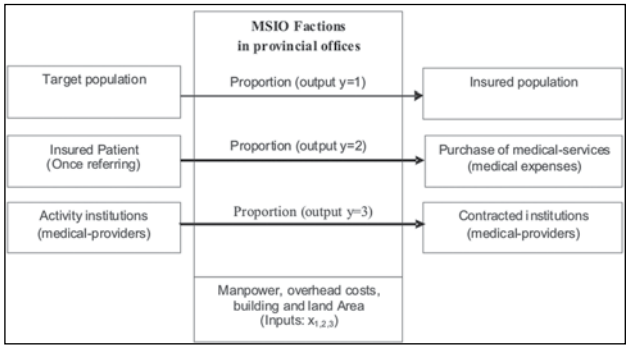

Figure 1. MSIO system analysis and inputs-output definitions for BCC model

Results

Total average technical efficiency of all provincial offices of MSIO was 0.513;the mean of managerial efficiency was 0.610; the scale efficiency was 0.840. Relying on the results of Data Envelopment Analysis model with variable return to scale (VRS) assumption, there was % 16 capacities to promote efficiency in all provincial offices of MSIO, using the same amount of inputs without any increase in fees.

4 of the provincial offices (13%) were fully efficient. Efficiency of 12 of the offices (39%) were between 1 and 0.5 while 15 of them (8%) were less than 0.5; therefore, 87 percent (27) of province office of MSIO working inefficiently.

Results also showed that 4 of the MSIO local (provincial) offices (13%) have constant returns to scale (CRS), 13 (42%) have descending returns to scale (DRS) and 14 of them (45%) have increasing returns to scale(IRS).In 2008, the lowest rate of technical efficiency was reported to be that of Tehran provinceoffice(0.048) which has increasing returns to scale (IRS) (Table 1).

Environmental factors are the variables which were not normal in delivery of service nor were under the control of management. In this study, following the interviews conducted with the MSIO experts, provincial nature indicated with access to services and the amount of overhead (i.e., the number of patients with insurance coverage who were referred to other provinces to receive services) was considered as environmental factor before expert classifying the provincial offices into four main groups (according to the experts' views);according to Table 1, there was an increase in efficiency in provinces with: smaller size, less population, limited access to levels of public service accommodations, and smaller amounts of overhead, while, on the other hand, provinces with: larger size, more population, more access to public service accommodations, and larger amounts of overhead have lower efficiency.

To examine the difference between these groups of offices, we performed a series of parametric (ANOVA and Scheffe) tests and non-parametric (Kruskal-Wallis and Mann-Whitney) ones. The results of ANOVA test supported the findings that the difference between the mentioned groups was statistically significant with a 95% confidence

Table 1. Efficiency measurement & Ranking of MSIO provincial offices by DEA-VRS model

Rank	Name of province holding an MSIO office	Efficiency			Type of returns to scale
		Technical	Managerial	Scale	
1	ILAM	1	1	1	crs
2	S. KHORASSAN	1	1	1	crs
3	SIMNAN	1	1	1	crs
4	N. KHORASSAN	1	1	1	crs
5	BUSHEHR	0.9375	1	0.9375	drs
6	KOHKILUYE & BOYERAHMAD	0.8842	0.9225	0.958482	drs
7	QOM	0.7426	0.7644	0.971481	irs
8	HORMOZGAN	0.6658	1	0.6658	drs
9	CHAHAR MAHAL& BAKHTIARI	0.6503	1	0.6503	drs
10	ZANJAN	0.6047	0.6229	0.970782	irs
11	QAZVIN	0.6002	0.8938	0.671515	irs
12	YAZD	0.5988	0.639	0.937089	drs
13	MARKAZI	0.5982	0.6988	0.856039	irs
14	ARDABIL	0.5641	0.7753	0.727589	drs
15	KARAJ	0.5565	0.5934	0.937816	irs
16	GOLISTAN	0.5415	1	0.5415	drs
17	KORDISTAN	0.4904	0.5192	0.94453	irs
18	LORISTAN	0.4655	0.4856	0.958608	drs
19	HAMADAN	0.3832	0.3928	0.97556	irs
20	KERMANSHAH	0.3199	0.3493	0.915832	irs
21	SISTAN & BALOOCHISTAN	0.3155	0.5378	0.586649	drs
22	KERMAN	0.2899	0.3136	0.924426	irs
23	W. AZARBAYJAN	0.2787	0.7408	0.376215	drs
24	GUILAN	0.2531	0.266	0.951504	irs
25	RAZAVI KHORASSAN	0.2176	0.2827	0.769721	drs
26	MAZANDARAN	0.2006	0.2174	0.922723	drs
27	KHUZISTAN	0.197	0.2851	0.690986	drs
28	ISFAHAN	0.1807	0.1866	0.968382	irs
29	E. AZARBAYJAN	0.1715	0.1809	0.948038	irs
30	FARS	0.1524	0.163	0.934969	irs
31	TEHRAN	0.0483	0.0928	0.520474	irs
	Total mean	**0.513184**	**0.610442**	**0.840676**	

level($P<0.001$) and the results of Kruskal-Wallis test showed significant difference between the groups ($P<0.001$). So it can be concluded that the efficiency of provincial offices classified by the level of their access to services as well as the amount of overhead refers as environmental-factors was corrected. Result of Mann-Whitney test between pair groups is demonstrated in Table 2.

Conclusion

To adopt an appropriate model for measuring the efficiency of MSIO, we firstly measured the efficiency of provincial offices by running both output and input-oriented CCR and BCC models. Then, comparison of the findings indicated different results from CCR and BCC models, which means the assumption of constant return to scale (CRS) to measure efficiency of MSIO isn't applied. Since the inputs of provincial offices of MSIO are more controllable than their outputs, we concluded that the input-oriented BCC model to be the optimal efficiency measurement for IRI- MSIO.

Also, based on the results of DEA input-oriented model, the total efficiency of MSIO was relatively low; to justify this, one reason could be that

Table 2. Result of Mann-Whitney Test to calcify provinces

Groups of provinces office	Name of provinces	Mean of efficiency group	Standard Deviation	Result of Mann-Whitney Test			
				Group 1	Group 2	Group 3	Group 4
Group 1	ILAM, S. KHORASSAN, N. KHORASSAN, KOHKILUYE & BOYERAHMAD	0.97105	0.0579				
Group 2	SIMNAN, MARKAZI, QAZVIN, BUSHEHR, KARAJ, ARDABIL, LORISTAN, HORMOZGAN, CHAHAR MAHAL& BAKHTIARI, ZANJAN, KORDISTAN, SISTAN & BALOOCHISTAN	0.620725	0.074846	0.012			
Group 3	GOLISTAN, HAMADAN, W. AZARBAYJAN, QOM, KHUZISTAN, KERMAN, MAZANDARAN, GUILAN, KERMANSHAH, YAZD	0.38053	0.205769	0.004	0.010		
Group 4	E. AZARBAYJAN, RAZAVI KHORASSAN, ISFAHAN, FARS, TEHRAN	0.1541	0.063716	0.013	0.002	0.005	

short-term function was not positioned properly in relation with the long-term one, and the results of short-term function were not in appropriate situation. Structure and information systems of MSIO ought to be reformed in order to obtain an economically relevant production function moving to services associated with a small amount spent on resources. Therefore, it can be concluded that the low level of efficiency is due to a defect in managerial structure or a systemic failure. In this regard, structural reorganization and organizational re-engineering seem to be strategic changes necessary.

As the provincial offices of MSIO all bear a surplus-capacity and in order to decrease this capacity, a comprehensive plan ought to be developed as a change-package with a capability to conserve all required aspects. For instance, more than 50% of the total costs of MSIO is related to manpower costs which is by itself a large fixed cost. Therefore, based on results of Data Envelopment Analysis, adjusting the surplus of manpower performs a pivotal role in reducing the costs of this organization; needless to say, attention to all other aspects of the organization should be devoted.

Based on the impact of efficiency environmental factors (access to services and overhead refers indices), the MSIO provincial offices can be classified into four main groups. Therefore, in order to increase the efficiency of MSIO in national level it is suggested to consider the above-mentioned classification as an on-factor in the referral system to delivery of medical services.

Acknowledgments

The authors gratefully acknowledge the participation of Administrators and personnel of the Iran's Medical-services Insurance Organization (MSIO) and the support provided by Science & Research branch of Islamic Azad University of Tehran.

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The specificity of clinical presentation and prognosis in adolescent Acute Lymphoblastic Leukemia

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Abstract

Introduction: The outcome and prognosis of childhood acute lymphoblastic leukemia (ALL) has been dramatically improved in the past thirty years, resulting in an overall five-year survival rates of 70 - 80% , whereas little progress has been made in ALL treatment in adults, revealing a five-year survival rate of 30-50%. Recently, more attention has been given to adolescents and younger adult patients with ALL, who make about 15% of all ALL-patients. This population has not been commonly monitored so far, as they were treated in pediatric and adult (internal medicine departments) healthcare institutions.

Material and methods: With an aim of comparing presented clinical findings, response to therapy, and disease outcomes in a group of adolescents (older then 10 years) with the same parameters in younger children with ALL we conducted a research that encompassed 75 patients younger then 18, who were diagnosed and treated at the Institute for Health Protection of Children and Youth of Vojvodina in Novi Sad in the period from January 01, 2000 to July 01, 2009. The patients were distributed into two groups: Group 1 included 22 adolescents (age 10 – 18), and Group 2 consisted of 53 children under 10 years of age.

Results: Out of 75 patients encompassed by this research 53 were below 10 years of age (median age 3.5 years), and 22 were adolescents of median age 13 years. Average initial leukocyte count was significantly higher in the adolescent group, being 57.43 g/L, related to 30.16g/L in the group of younger children. Moreover, statistically significant number of adolescent patients had an initial leukocyte count equal to or higher then 50.0 g/L (p= 0.0003).

With respect to the immunophenotype, 27.2% adolescents revealed T-cell ALL, contrary to only 5.6% of younger children with the T-cell phenotype, which is considered statistically significant difference (p= 0.013).

Cytogenetic findings revealed no statistically significant differences between investigated groups; however, it should be emphasized that t(9;22) was observed in two patients, both of whom were from the group of older children, i.e. adolescents. Statistically significant differences were established neither with respect to early death, i.e. induction death, nor for the rate of complete remission; however, significantly higher rate of the relapse was established in the group of adolescents (33.3%) than in younger children (7.8%), which is considered statistically significant (p= 0.0108)

Results of OS and DFS suggest better response to therapy among younger patients, but the differences between groups are not statistically significant. At the time of study completion (July 01, 2010), 59% of adolescent patients and 81.1% younger children with ALL were in continuous complete remission (CCR). After completion of the research, 45/53 (84.0%) children under 10 years and 15/22 (68.0%) adolescent patients were alive. Differences related to CCR as well as to survival rates are not statistically significant.

Conclusion: It is still too early to reach any definite conclusions, as it is less then five years since the last patient was included in the research, but in general, we can consider our results satisfactory and similar to those reported in available large-scale studies.

Key words: acute lymphoblastic leukemia, adolescent, prognosis

Introduction

The outcome and prognosis of childhood acute lymphoblastic leukemia (ALL) has been dramatically improved in the past thirty years, resulting in an overall five-year survival rates of 70 - 80% (1,2), which can vary according to the risk group (standard, moderate, high). Survival rates range from below 70% up to over 87% in patients from high-risk and moderate-risk groups, respectively (1,2).

Contrary to the significant prognosis improvement in children with ALL, little progress has been made in ALL treatment in adults, revealing a five-year survival rate of 30-50%. There are several possible explanations for a poor treatment response in adults with ALL.

Namely, ALL is the most common type of leukemia in children and certainly the most frequent hematological malignancy that pediatric hematologists have to cope with. Large numbers of treated patients, participation in multicentric and multinational studies as well as experience in applying randomized therapy protocols contributed to creation of specific protocols that can improve the overall treatment outcomes and, at the same time, reduce the toxic side effects of the therapy.

Adult patients exhibit higher incidence of other types of leukemia and carcinoma. Besides, older ALL patients are not treated according to randomized clinical protocols, and their tolerance of cytotoxic drugs is markedly poorer than that in children. Recently, more attention has been given to adolescents and younger adult patients with ALL, who make about 15% of all ALL-patients. This population has not been commonly monitored so far, as they were treated in both pediatric and adult (internal medicine departments) healthcare institutions.

Adolescence is a transitional turbulent stage between childhood and adulthood. It is the period of functional, psychological and social maturation, which is characterized by personality development and reach for independence from the family.

Adolescents with malignant disease are also facing disturbance of physiological functions and changes of physical appearance, as well as absence from school and insecurity about the future.

All this contributes to highly complex psychosocial manifestation of the disease at this age (3,4).

It is well known that favorable prognostic indicators are more frequently observed in children up to 10 years of age, whereas higher rate of poor prognostic factors including high initial leukocyte count, T-immune phenotype and molecular genetic markers such as t (9,22) and Ph + ALL are recorded in adolescents. Thus, poorer therapy results are established in adolescents compared to the children younger than 10 years. Moreover, all studies published so far reported better therapy outcomes in adolescents subjected to pediatric therapy protocols then in those underwent protocols for adult patients.

The aim of this research was to compare presented clinical findings, the response to therapy, and disease outcomes in a group of adolescents (older then 10 years) with the same parameters in younger children with ALL.

Patients and methods

Patients

Our research encompassed 75 patients younger then 18, who were diagnosed and treated at the Institute for Child and Youth Healthcare of Vojvodina in Novi Sad in the period from January 01, 2000 to July 01, 2010. The patients were distributed into two groups:

Group 1 included 22 adolescents (age 10 – 18), and Group 2 consisted of 53 children under 10 years of age.

Diagnosis

The diagnosis was based on cytomorphologic analysis of the aspirate, i.e. bone marrow biopsy according to FAB (The French-American-British) classification.

Immunophenotyping was performed using flow cytometry, whilst cytogenetic analysis implicated high-resolution G-banding technique. In some children molecular genetic analysis was performed, i.e. determination of most common gene rearrangements such as Bcr/Abl, MLL/AF4, FEL/AML1, PBX1/ELA utilizing the RT-PCR technique.

Therapy

Patients were treated according to two therapy protocols. Until 2002, the therapy was applied according to the YU ALL 95 protocol. It is a proto-

col of Yugoslav Co-operative Group for Children's Hematology, Oncology and Immunology, which relies on the protocol AEIOP LLA 95 (L Associone Italiana ematologia ed Oncologia pediatrica), i.e. the protocol of Italian Association for Pediatric Hematology and Oncology. After 2002, the protocol ALL IC-BFM 2002 was applied. This intercontinental protocol developed by BFM (Berlin, Frankfurt, Muenster) study group has been applied in all centers for treatment of ALL in Serbia since November 2002.

Statistical analysis

The *Kaplan-Meier* survival *curve* was used to estimate the survival rate. Disease free survival (DFS) was calculated as the time between achievement of remission status until the end of first remission (relapse or death during first remission) or until analysis time. Overall survival (OS) was measured from the date of initial chemotherapy until death, or until time of analysis. Differences between the groups were assessed using χ^2 test, t-test and exact probability *test. The value* $p < 0.05$ was considered statistically significant.

Results

Basic characteristics of patients

Out of 75 patients encompassed by this research 53 were under 10 years of age (median age 3.5 years), and 22 were adolescents of median age 13 years.

Patients' characteristics within particular age groups

Comparative basic characteristics of patients are displayed in Table 1. As obvious from Table 1, both investigated groups are characterized by prevalence of male patients; however, these differences are not statistically significant.

Average initial leukocyte count was significantly higher in the adolescent group, being 57.43 g/L, related to 30.16g/L in the group of younger children. Also, high statistically significant number of adolescent patients had an initial leukocyte count equal to or higher then 50.0 g/L (p= 0.0003).

With respect to the immunophenotype, 27.2% adolescents manifested T-cell ALL, contrary to only 5.6% of younger children with the T-cell phenotype, which is considered statistically significant difference (p= 0.013).

Table 1. Basic characteristics of patients and therapy outcomes

Variable	< 10 years	10-18 years	P (< 0.05)
Number of patients	53	22	
Median age (years)	4	13	
Male patients	29 (54%)	11 (50%)	>0.05
Average initial leukocyte count (g/L)	30.6	57.43	**< 0.05**
Le =/>50.0	2/53 (3.7%)	8/22 (36.3%)	**< 0.05 (0.0003)**
Le =/> 100.0	3/53 (5.6%)	3/22 (13.6%)	>0.05
T-ALL	3/53 (5.6%)	6/22 (27.2%)	**< 0.05 (0.01)**
Cytogenetics			
Hyperploidy	4/53 (7.4%)	2/22 (9.04%)	> 0.05
Hypoploidy	1/53 (1.8%)	0/22	> 0.05
t (9,22)	0/53	2/22 (9.04%)	>0.05
Early death	2/53 (3.7%)	1/22 (4.5%)	> 0.05
CR	51 (96.2%)	21 (95.4%)	> 0.05
Relapse	4/51 (7.8%)	7/21 (33.3%)	**< 0.05**
Number of patients in CCR	43/53 (81.1%)	13/22 (59.0%)	> 0.05
Number of live patients (concluded with July 01, 2010)	45/53 (84.0%)	15/22 (68.0%)	> 0.05

Legend:
CR - complete remission
CCR - continuous complete remission

Cytogenetic findings revealed no statistically significant differences between the investigated groups; however, it should be noticed that t(9;22) was observed in two patients, both of whom were from the group of older children, i.e. adolescents.

Therapy response and outcome

Table 1 indicates an induction death in 2/53 (3.7%) younger children and in 1/22 (4.5%) adolescents, but this difference is not statistically significant.

Statistically significant differences were established neither with respect to early death, i.e. induction death, nor for the rate of complete remission. However, higher relapse incidence was established in the group of adolescents (33.3%) than in younger children (7.8%), which is considered statistically significant (p= 0.0108).

Chart 1 displays overall survival (OS) for both age categories. The *Kaplan-Meier* survival *curve indicates better survival rate in patients younger then 10 years then in the adolescents; however, the differences are not statistically significant.*

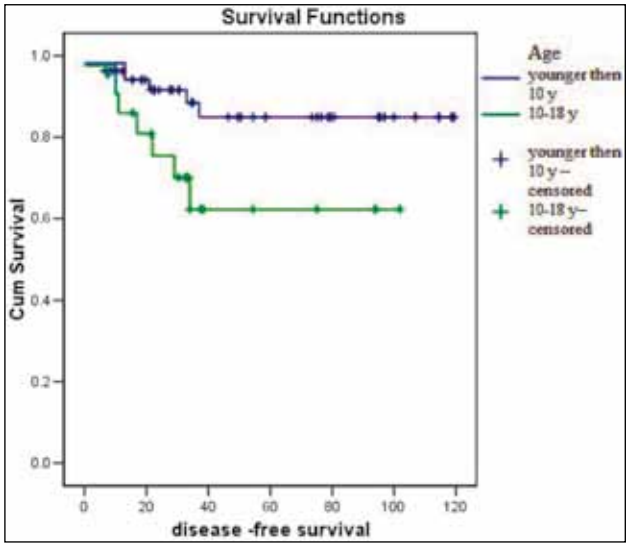

Chart 1. Overall survival (OS) according to age category

The *Kaplan-Meier* survival *curve in Chart 2 indicates better DFS in children less than 10 years of age then in adolescents; however, the difference is not statistically significant.*

Results of OS and DFS suggest better response to therapy among younger patients, but the differences between groups are not statistically significant.

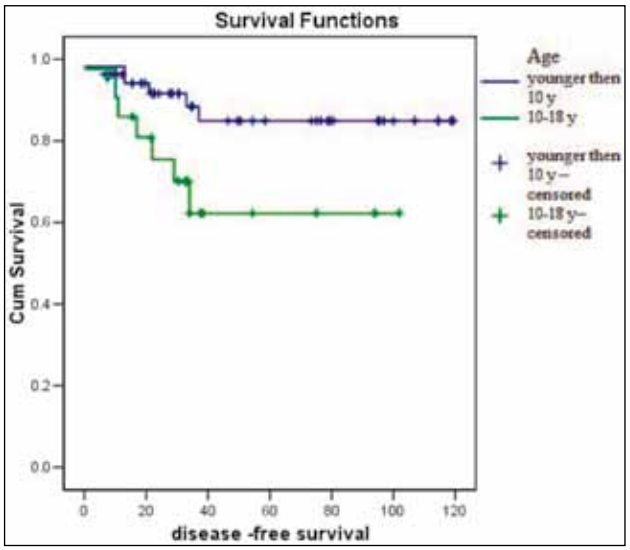

Chart 2. Disease free survival (DFS) according to age category

At the time of study completion (July 01, 2010), 59% of adolescent patients and 81.1% younger children with ALL were in continuous complete remission (CCR). After completion of the research, 45/53 (84.0%) children under 10 years and 15/22 (68.0%) adolescent patients were alive. Differences related to CCR as well as to survival rates are not statistically significant.

Discussion

Comparative studies and results of current multicentric protocols for treating ALL in children revealed poorer therapy outcomes in adolescents compared to children under age ten. The five-year DFS in children is 80.0% in contrast with the current DFS in adolescents, ranging from 46% to 68%. (5)

It is probably due to different underlying biologic features of the lymphoblasts, as well as higher incidence of unfavorable prognostic factors such as high initial leukocyte count, T-immunophenotype and molecular genetic markers: t(9;22) and Ph+ALL. Möricke A et al. (6) confirmed it in their reports on protocols ALL-BFM 86, 90, and 95. Santana VM et al. (7) as well as Rivera GK et al. (8) established higher incidence of poor prognostic features in adolescents with ALL.

Our research revealed higher incidence of unfavorable prognostic factors in the group of adolescent patients with ALL. Thus, statistically significant difference was established between the

average initial leukocyte counts in adolescents and younger children, being 57.43 g/L and 30.16 g/L, respectively. Also, significantly higher number of adolescents had initial leukocyte count equal to or higher than 50.0 g/L (p= 0.0003).

With respect to the immunophenotype, the percentage of adolescents with T-cell ALL was 27.2%, in contrast with only 5.6% of younger children, which is considered statistically significant difference (p= 0.013).

Cytogenetic findings revealed no statistically significant differences between the investigated groups; however, it is to be emphasized that t(9;22) was detected in two patients, both of whom belonged to the group of older children, i.e. adolescents.

There is a limited number of studies on outcomes and treatment of different age groups of children with ALL, but majority of them confirmed poor results in adolescent patients. Thus, a five-year DFSs in adolescents reached 24% and 46% in the protocols of St. Jude Total Study Group 10 and St. Jude Total Study Group 11, respectively (9,10,11).

Report of Schrappe et al. on the protocol ALL BFM 90 revealed a six-year DFS in adolescents of 64%, whereas the same parameter in younger children reached 83% in the age group 1-5 years and 74% in the age group 6-9 years. (12, 13)

Experiences of Italian Association of Pediatric Hematology-Oncology (AIEOP) with the protocol AIEOP 91 revealed 5-DFSs of 75% and 53% in younger children and adolescents, respectively. (14, 15)

In a research of prognostic value of age in children with acute lymphoblastic leukemia, pertaining to studies ALL-BFM 86, 90 and 95, Möricke et al. established eight-year DFS values of 82%, 75% and 63% for children aged 1-5 years, 6-9 years and older, respectively.(6)

As obvious from the Charts 1 and 2, our research revealed no statistically significant differences with regard to DFS and OS between the investigated age groups, yet higher survival rate and longer period from remission until relapse were established in children under the age of 10 years. It is still too early to reach any definite conclusions, as it is less then five years since the last patient was included in the research, but we can consider our results satisfactory and in compliance with those reported in available large-scale studies.

In spite of high rate of remission, some 20% children with ALL still have to deal with relapse of their disease, which is mostly highly resistant to therapy and presents a major challenge in the treatment of ALL in children.

In our study, an isolated bone marrow relapse occurred in 21% patients who established CR, with a statistically significant relapse incidence of 33.3% in the group of adolescents, compared with only 7.8% in the group of younger children.

Our results are similar to those of the study ALL BFM 90 published by Schrappe et al., as well as to research of Möricke et al., who reported results of studies ALL- BFM 86, 90, and 95 with a relapse occurring in 18% children with ALL.(6,13)

In our patients, early death was observed in 3/75 patients (4.0%). The rate was higher in the group of adolescents, being 4.5%, then in younger children, being 3.7%. Comparison of our results with similar studies (Schrappe et al.) revealed that rate of early death was somewhat higher in our patients, mainly as a consequence of infection associated with neutropenia during an intensive induction phase of treatment.

Conclusion

Comparative results of multicentric and multinational protocols and studies on treatment of ALL in children, as well as the results of this research, indicated poorer therapy outcomes in adolescents as compared to the children below 10 years of age.

This may be due to more frequent occurrence of unfavorable prognostic factors in adolescent population, which was confirmed in this study.

It is still too early to reach any definite conclusions, as it is less then five years since the last patient was included in the research, but we can consider our results satisfactory and similar to those reported in available large-scale studies.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

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Influence of education on primary school students nutrition in Ankara, Turkey

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Abstract

Primary school students' knowledge on nutrition and breakfast, most important meal of the day, in Turkey is relatively weak. Studies on primary school students revealed low success and obesity problems in the country. For increasing awareness and mitigating problems said above, education programs were launched recently. The efficiency of education methods provided to students was determined in the study along with changes in breakfast habits. Research was conducted by questionnaires undertaken with 565 students from 5th and 6th grade from two primary schools in Keçiören (Ankara, Turkey) in 2007. The nutrition knowledge levels of the students were evaluated by Two Way ANOVA for Repeated Measures. The statistical data processing of the interview and variance analyses of the results were obtained by SPSS 15.0. Means were made by Duncan's multiple range test using the MSTAT-C program. The average of the nutrition knowledge increased following various education methods. However, monitoring test undertaken after a month showed a decrease in nutrition knowledge. Education on nutrition provided by audio and visual training to primary school students significantly improved their knowledge and habits. However, we suggest continuous education for maintaining nutrition knowledge particularly in primary school because the efficiency of education sharply decreases within a short period.

Key words: Primary education, nutrition, nutrition education, nutrition knowledge level.

Introduction

Nutrition habits of children and adolescents are effecting development of certain health problems in later life. Occurrence of cancer and osteoporosis, obesity, diabetes, cardiovascular diseases are related to received energy amount and nutrition composition in early ages.[1,2] Individuals should have sufficient knowledge on healthy eating habits, adequate food selection and over all for healthy life which can be only attained by education. Several studies revealed the efficiency of education courses on nutrition habits particularly at early ages.[3,4,5] However, the educational strategy should include needs and interest of students, communication on nutrition, message should be understandable by children, instead of components of foods ie protein, fat etc. names of the foods should be used, and teachers should be capable to overcome possible problems which may arise during courses such as social and cultural aspects.[6,7]

Schools provide effective and efficient environment for various segments of the population such as young people, teaching staff, families and community members.[7,8,9].However, main focus should be given to teaching staffs knowledge on nutrition prior to launching courses on education.[10]

Children should be aware of nutrients that are required for their growth and development[10,11] suggested that education would be more successful when received in primary school. Thus, for permanency and sustainability of education on nutrition, efforts should be given to students when they start to primary school.

Giovannini et al[12] indicate the importance of breakfast habit for primary school students. Authors suggested regular breakfast consumption's risk reducing of chronic diseases due to the potential impact on the composition of the overall diet, and added its association with improved learning abilities and better school performance in children. Moreover, the positive health outcomes of breakfast habit have been described by Agostoni and Brighenti[13] The efficiency of nutrition education to primary school students on their nutrition

knowledge, and changes in breakfast behaviors all developing proper nutrition habits were determined in the study.

Methods

Research was conducted by questionnaires undertaken with 565 students from 5th and 6th grade from two primary schools in Keçiören (Ankara, Turkey). Data were obtained by interactive interviews from 09.04.2007 to 15.06.2007. Interviews were repeated three times as pre-test (PT) prior to nutrition education, last test (LT) after completion of nutrition education. And a monitoring test (MT) was carried for evaluating persistency of the education after one month.

Students are grouped into four classes; Class I (CI): 40mn/4 weeks oral – visual training, only educational printed material was provided to Class II (CII), Class III (CIII) had 40 m/4 weeks oral – visual training + printed material (brochures), and control group which was Class IV (CIV), did not receive any training or printed material.

The body mass indexes (BMI) of the students were evaluated according to WHO[14] standards.

The food preferences of students were evaluated by using the equation $T= 3T_1+2T_2+T_3$. In the equation T is total score, T1 is first choice, T2 is second choice, T3 is Third choice. Students asked to sort their preferences from 1 to 3 ie most preferred to less preferred. The first choice, sorted as 1 is valued by 3, 2 was given to second choice and 1 was for third choice.

Statistical analysis

The nutrition knowledge levels of the students were evaluated by Two Way ANOVA for Repeated Measures. The statistical data processing of the interview and variance analyses of the results were obtained by SPSS 15.0. Means were made by Duncan's multiple range test using the MSTAT-C program.

Results

Majority of the students aged 12-13 years with an average of 11.6 years. The BMI of 4.4% was underweight, 92.0% was in normal range and 3.6% was pre-obese. The average BMI for all students was 17.951 kg/m^2 (Table 1).

Table 1. Demographic properties of students

	N	%
Age (years)		
10-11	244	43,2
12-13	321	56,8
Gender		
Male	253	44,8
Female	312	55,2
BMI (kg/m^2)		
Underweight	25	4,4
Normal Range	520	92
Pre-obese	20	3,6
Total	**565**	**100**

Only 29.1% of the students had education on nutrition while 70.9% did not receive any training which was also reported by previous studies.[15,16,17] The low figure on nutrition knowledge manifested insufficiency of education on nutrition in the country. Students of the study obtained knowledge on nutrition from books (26.2%), teachers (22.6%) and via media sources in which TV being major source (20.1%) (Table 2). Similar results were determined by Abdollahi et al.[18] stated primary schools students receive nutrition information from their families, TVs, books, teachers and friends. Students expressed that their preference for nutrition education with 63,1% nutritionist, with 17.6% teachers, with 11.2% health staff, and 8.0% was uncertain (Table 2).

The mean knowledge level of students, obtained by scoring, prior to nutrition education was 11.0, and increased to 18.6 at the end of training and decreased to 15.9 after one month of education. Highest knowledge level was obtained at Class II with 21.6 in last test (Table 3). MT revealed that knowledge marks are decreased at all Classes. This showed the necessity of education courses continuing with certain intervals for ensuring the durability of the nutrition education. Peltzer[19], determined a common low level of nutrition knowledge, however university students have better level than high school students in South Africa. Eboh and Boye[20], provided 40 minutes of education 4 days a week for 3 weeks to 6th grade students and they determined a significant development at trained group showed on nutrition knowledge.

Table 2. The acquisition of nutrition education

	N	%
Nutrition education		
Received	164	29.1
Not received	401	70.9
Total	565	100.0
Source of information		
School	30	18.3
Television	33	20.1
Books	43	26.2
Teachers	37	22.6
Newspaper and Journal	17	10.4
Internet	4	2.4
Total	164	100.0
Source of preferred education		
Nutritionist	315	63.1
Medical personnel	56	11.2
Teacher	88	17.6
Indecisive	40	8.0
Total	**565**	**100.0**

Table 3. Means and standard deviations (SD) of tests

	PT		LT		MT	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
Class I	11,4	3,5	19,1	5,6	17,5	4,6
Class II	11,0	3,2	17,2	5,3	15,7	4,6
Class III	11,1	3,0	21,6	5,4	18,3	3,5
Class IV	10,5	3,5	16,8	6,2	12,3	3,6

The frequency of answers, false or true to questions was provided in Figure 1. The relatively low level of knowledge which was 31,8% on nutrition was determined at PT (Figure 1). Highest rate of true answers with a mean of 57.7% at all Classes in LT (Figure 1) presented the efficiency of education course on student. However, the 14.2% (Figure 1) decrease from 57,7% to 45.7% of true answers in MT undertaken after one month of education course revealed the importance of continuation of repeating of courses.

The highest true answer frequency with 74.2% was determined at CIII followed by CI and CII (Figure 2). Similarly, although significantly lower than LT, highest true answers were observed again at CIII of MT with 52,3% (Figure 3).

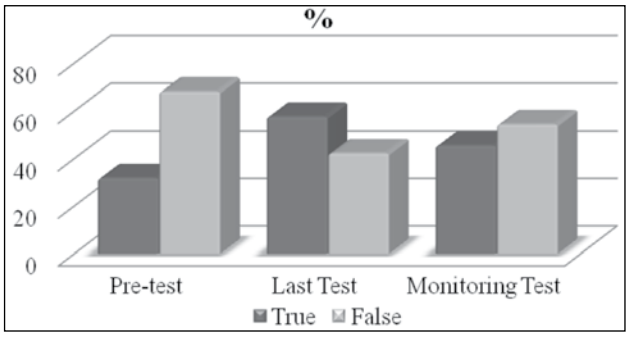

Figure 1. The answers given by students to questionnaires at all Classes

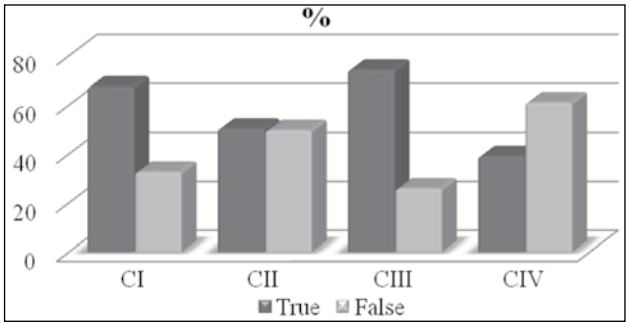

Figure 2. The frequency of true and false answers in LT

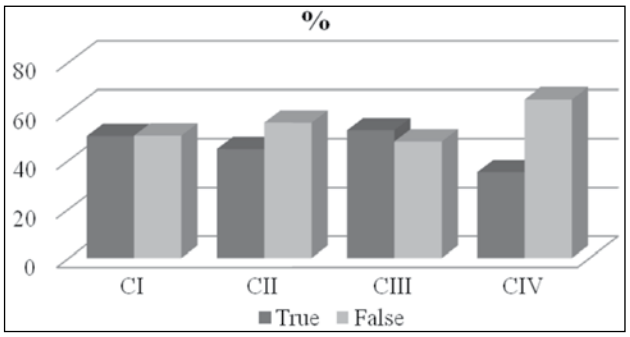

Figure 3. The frequency of true and false answers in MT

The highest true answer at LT with 75.6% was given by all classes were on question 33 that was on the nutrition value of milk, lowest with 33,1% was determined via question 34, that was on fats rich in saturated oil. At MT, highest true answer was given to question 6 with 66.9% on unhealthy food, whereas lowest was recorded on question 13 with 20% which was on vitamin A containing food. The significant decrease at MT (Figure 1, 3) manifested the need of continuation of education courses at specified intervals.

The regular breakfast habit was increased at CI and CIII following education highest was in former (Table 4) with 64.4%. However, MT showed

a decrease in the breakfast habit (Table 4). Changes in food selection of students at breakfast were determined as well (Table 5) which were tea and hazelnut paste. LT and MT revealed that milk and cheese become major consumed foods at breakfast following and later on education (Table 5).

Discussion

Proper eating habit is based on appropriate food choices and sufficient knowledge on nutrition. Thus, education is indispensible for gaining healthy nutrition habits particularly nutrition training in childhood continues ensuing years of life. However, for establishing sustainable success on nutrition habits courses and/or training practices should be employed in continuing manner especially for elementary school students in Turkey.

Education on special topics such as nutrition, traffic and vocational training necessitates specific approaches. In this study training was provided via direct education, leaflets, and education + printed material. The high success in terms of change in food selection and proper eating particularly for breakfast was obtained at CI and CII in LT undertaken at the end of the course, which revealed the effici-

Table 4. Students daily breakfast habits

	CI			CII			CIII			CIV			Total		
	PT	LT	MT	PT	LT	MT	PT	LT	MT	PT	LT	MT	PT	LT	MT
Has	34.0	64.6	60.3	28	61.5	55.9	46.3	64	60.5	67.9	32.9	37.3	43.7	56.1	53.8
Does not	5.7	1.4	1.4	6.3	2.8	2.1	4,8	2	1.4	13.4	11.9	3.7	4.8	1.8	2.1
Sometimes	60.3	34	38.3	65.7	35.7	42	48.9	34	38.1	18.7	55.2	59	51.5	42.1	44.1
Total (%)	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
X^2	33.179			38.006			12.409			58.771			26.188		
SD	4			4			4			4			4		
P	0.000			0.000			0.015			0.000			0.000		

Table 5. Food and drink preferences of students at breakfast before, following and later on education

Food/Drink	PT	LT	MT	PT-LT t test		LT-MT t test	
	X±SD	X±SD	X±SD	t	p	t	p
Tea	1.64±1.22	1.52±1.27	1.24±1.22	1.97	0.049*	4.79	0.000*
Milk	0.98±1.18	1.65±1.16	1.93±1.12	-9.57	0.000*	-5.72	0.000*
Fresh fruit juice	1.60±1.01	1.55±1.07	1.65±1.00	0.86	0.392**	-2.38	0.018*
fruit juice	1.30±1.12	0.88±0.97	0.84±0.92	6.75	0.000*	0.94	0.347**
Instant coffee	0.38±0.75	0.39±0.77	0.25±0.65	0.04	0.969**	4.13	0.000*
Cheese	1.46±1.32	1.81±1.28	2.09±1.21	4.54	0.000*	-5.01	0.000*
Egg	0.49±0.91	0.66±1.01	0.51±0.91	3.06	0.002*	3.50	0.001*
Olive	0.49±0.83	0.55±0.86	0.57±0.85	1.24	0.217**	-0.62	0.538**
Sausage	1.08±1.13	0.78±1.11	0.60±0.98	4.51	0.000*	3.85	0.000*
Salami	0.95±1.17	0.60±0.97	0.48±0.86	5.55	0.000*	2.78	0.006*
Butter	0.05±0.29	0.08±0.40	0.06±0.34	1.20	0.229**	1.10	0.274**
Cornflakes	0.70±1.10	0.62±1.03	0.78±1.14	1.23	0.219**	-3.39	0.001*
Toast	0.45±0.83	0.60±0.89	0.71±0.88	2.80	0.005*	-2.57	0.011*
Tuck	0.17±0.58	0.27±0.73	0.15±0.52	2.57	0.010*	3.90	0.000*
Honey	1.55±1.07	1.80±1.24	1.90±1.25	2.41	0.016*	-1.89	0.059**
Jam	1.24±1.05	1.43±1.06	1.48±1.04	2.99	0.003*	-1.14	0.253**
Molasses	0.55±0.89	0.71±0.95	0.60±0.92	2.97	0.003*	2.71	0.007*
Marmalade	0.67±0.98	0.41±0.83	0.40±0.79	4.87	0.000*	0.27	0.790**
Hazelnut paste	1.61±1.23	1.36±1.16	1.33±1.12	3.51	0.000*	0.70	0.486**
Molasse-Tahini	0.21±0.62	0.24±0.64	0.18±0.51	0.80	0.423**	2.38	0.017**

*$p<0.01$, **$p>0.05$, (n: 565)*

ency of education in 4 weeks, relatively short period. But, the level of penetration for proper nutrition decreases with time which was determined at MT carried after one month. Thus, continuous training should be provided to elementary school students on issues related to nutrition which will prevent them unhealthful nutrition habits leading to obesity, the biggest public health challenge of the 21st century.

Several studies revealed the importance of education for improving students' knowledge. Lack of knowledge on nutrition decreases students overall success and increase health problems, obesity being major issue. Today, the high obesity rate within children necessitates urgent actions. One time education courses on nutrition may create awareness on nutrition. However, primary school students have a short memory on nutrition due to external influences alike media. Thus, we suggest a continuing education on nutrition for primary school students for mitigating obesity and increasing success in school life since obesity in elementary school is still not an alarming state.

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Urinary Tract Infections caused by Group B streptococcus in adult women: Survey of 11800 urine culture results

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Abstract

Background and Objective: Symptomatic or asymptomatic urinary tract infections can still subject the pregnancy and recur in considerable of pregnant women. Early diagnosis of Streptococcus agalactiae remains difficult, since symptoms are very nonspecific. Its frequency has not been completely investigated in urinary tract pathogen of pregnant ant and non pregnant women in Iran. In the present study, we were going to evaluate the frequency of Group B Streptococci in female patients.

Materials and Methods: A Total 11800 urine specimens were received from female out-patients and admitted during June till December 2010. All specimens were cultured routinely on Blood and EMB agar. All Group B Streptococci isolates were confirmed by typical colony morphology, and identified by differential tests as well as by the growth characteristics in chromoagar. A provisional urinary tract infection diagnosis was defined by the presence of single organism Group B Streptococci bacteriuria (>10 CFU/liter) with at least one of urinary tract infection symptom. Susceptibility testing was carried out by disk diffusion method.

Results: Of all specimens processed during this study, 498 specimens (4.22%) yielded significant bacteriuria caused by Group B Streptococci. The mean age of these patients were 26.6 + 19.37 that were mostly in young and middle ages. Pregnant patients were 3.82% and the rest were non-pregnant. Antibiotic susceptibility test revealed that vancomycin, clindamycin and cefazolin had the lowest resistant rate and Penicillin showed the highest resistant rate.

Conclusion: Analyzed data revealed frequency of Group B Streptococci was low in this study. Pattern on antibiotic susceptibility test showed high resistant rate to some antibiotic that made it difficult for pregnant patients. It was not possible to compare the antibiotic susceptibility pattern of pregnant women with non-pregnant because of the low number of pregnant women registered in this study

Key words: Group B Streptococcus, Urinary Tract Infection, Susceptibility test, Urine specimens

Introduction

Group B Streptococcus (GBS) is a leading cause of serious infections in newborns, pregnant women and even in adults with chronic medical illness (1-3). It is reported recently as an important infectious agent of invasive disease in non-pregnant adults especially those underlying conditions such as diabetes mellitus, malignancy, or liver disease (4-5).

Recent studies revealed that rate of infection by GBS in adults continue to increase (6). GBS is a common normal flora of bowel and vaginal cavity and found some what less frequently in the urethra and throat (7), however colonization of GBS is highly prevalent among pregnant women and varying between 4% to 30%. The variation in rate of colonization may due to the sample collection site, the culture medium used for isolation of organism, the ethic group geographical location, immunological factors and the age of population studied (7).

In addition to maternal cervicovaginal colonization and neonatal meningitides and septicemia that results from vertical transmission of organism from mother to newborn. CBS is a causative agent of urinary tract infections. The UTI caused by GBS in pregnant women may be asymptomatic bacteriuria or serious infection like cystitis, pyelonephritis urethritis and urosepsis. Clinically UTI caused by

GBS in many times may be indistinguishable from those caused by other organisms. However recent studies suggest that microbiological and clinical features may be different depending causative agent. Various rate of GBS UTI rate have been reported in pregnant and non-pregnant adults. GBS TUI rate have been different from 1% to up to one third and even more in these studies (8-12).

In this study we were going to evaluate the frequency of GBS in suspected female to UTI and to assess the activity of commonly used antibiotics.

Materials and Methods

Study period: This is a cross–sectional study carried out in Department of microbiology of Milad hospital. A total 11800 urine specimens were received from female patients during June till December 2010.

Specimens: The specimens were belonging to out-patients, those patients that evaluated in the emergency department, and hospitalized in different wards of hospital. Urine samples were obtained as clean catch voided or catheterized samples from all patients who were subjected for assessment for UTI.

Isolation and Identification: All specimens were cultured routinely in Microbiology laboratory and cultured routinely on Blood and EMB agar and were incubated at 35oC for 24h (all media were provided from Merck Co., Germany). All significant isolates were identified using conventional microbiological methods (13). All GBS isolates were confirmed by typical colony morphology, Type of hemolysis, Gram stain, Catalse test, CAMP reaction1, Hipourate hydrolysis and growth characteristics in chromoagar (Chromagar Co, France) (Picture 1).

In case where GBS cultured from urine with any count the medical record of each patient were reviewed for symptoms, demography data and pregnancy. A provisional UTI diagnosis was defined by the presence of single organism GBS bacteriuria (>10 CFU/liter) with at least one symptom that include dysuria, increase urine frequency and or urgency, fever >38oC flank pain and/or lumber tenderness. In cases where urine analysis was performed, UTI was confirmed on the basis of positive urinary leukocyte esterase and pyruria.

Picture 1. Streptococcus agalactiae on Chromagar (Cromagar Co.)
Strptococcus agalactiae (GBS) appears in mauve and the other bacteria will be observed in blue color

Antibiotic susceptibility Testing: Susceptibility testing was carried out by disk diffusion method as recommended by CLSI (Antibiotic disks were provided from Mast Co.) (14). All GBS isolates were tested for resistance against penicillin, ampicillin vancomycin, ceftriaxone, cefotaxime, clindamycin and erythromycin

Quality Control and Standard Species: Briefly for the quality control of susceptibility tests E.coli (ATCC 25922), Pseudomona aeruginosa (ATCC 27853), Staphylococcus aureus (ATCC 25923) and Enterococcus faecalis (ATCC 929212) strains were used. A deceptive statatistic were used for expression of data as a percentage using SPSS software (Version 16).

Results

Of the 11800 specimens processed during the study period, 498 (4.22%) specimens yielded significant bacteriuria that 487 were out-patients and 11 cases were of those admitted patients. The-

1 CAMP is name of first researchers (Christie, Atkins, Munch-Petersen) who used this phenomenon to observe this reaction

Table 1. Sensitivity rate of Group B streptococcus isolates to applied antibiotics (%).

	Penicillin	Ampicillin	Vancomycin	Ceftriaxone	Cefotaxime	Erythromycin	Clindamycin
Sensitive	10.6	47.8	84	56.2	53.4	49.6	65.8
Intermediate	-	-	-	-	-	26.2	17.4
Resistant	89.4	52.5	16	43.8	46.6	24.2	16.8

se cases were recorded among young and middle age with mean of 26.6 + 19.37 years old. Those, patients who were not in the age of pregnancy were 28.87%.

Nineteen patients (3.82%) were pregnant and the rest 479 patients (96.18%) were non pregnant. Hipourate hydrolysis and chromoagar were positive in 495 cases while CAMP reaction was positive in all 498 cases.

Analysis of the collected data revealed that the highest sensitive rate was observed in vancomycin (84%) and the lowest was Penicillin (10.6%) (Table 1). Sensitivity results of the rest antibiotics are presented in the table 1.

Discussion

At the present study, frequency rate of GBS was 4.22% among female patients engaging UTI. The mean age of the studied patients showed they were in mostly in pregnancy ages, although a few patients (3.82%) were registered as pregnant patients. Antibiotic susceptibility was done by disk diffusion method that is a routine procedure in all laboratories. Use of E-test to evaluate the MIC of applied antibiotics gives a accurate results, but we were not able to provide it in this study. However, quality control of the provided disks proved the reliability of all antibiotic disks. The analyzed results of antibiotic susceptibility test showed that isolated GBS was highly resistant to Penicillin and about 50 percent to Erythromycin. It is revealed that vancomycin had the lowest resistant rate among antibiotics tested. Low resistant of vancomycin had been also confirmed in the previous study (15). Analyzed result, proved resistant to cefotaxime and cefrtiaxon was observed in nearly about half the patients (46.6% and 43.8 respectively) that needs to be noted in any prescription of UTI infection (Table 1). Frequency of isolated GBS was nearly comparable in this study with other reports (16-18).

Our results showed antibiotic resistant pattern was different than other studies reported from different part of the world. Simoes (et al. 2007) reported characteristics of GBS and its antibiotic resistant pattern in female patients at Rio de Janeiro (Brazil) (19). He reported the highest resistant rate for gentamicin, while the highest sensitivity was for the penicillin, ampicillin, erythromycin and nitrofurantoin. The frequency of GBS resistance to penicillin showed 89.4 % in our study, that is remarkable very high, although the resistant rate of ampicllin was relatively lower the penicillin, but Simoes has reported higher sensitivity for the ampicillin.

In another study Sherk (et al. 2009) evaluated the antibiotic sensitivity for UTI agent including GBS. They reported cephalexin was the most commonly administered drug in empirical therapy for acute uncomplicated cystitis, recurrent cystitis and urethritis in women. However, co-amoxiclav was suitable for acute uncomplicated pyelonephritis and complicated UTIs (20). Although Sherk isolated GBS in his study, but he did not specify the antibiotic resistant pattern for different bacterial agents. Therefore his results might not gives similar sensitivity results for various isolated microorganisms from urine.

In a study performed at the Taiwan, the antibiotic susceptibility was performed for a range of antibiotics. GBS rate was 15% in the studied patients that is more than three times higher than our study. Reported antibiotic susceptibility test showed azithromycin, clindamycin, erythromycin, ofloxacin, penicillin G, tetracycline, trimethoprim/sulfamethoxazole and vancomycin were sensitive 44.6%, 66.1%, 70.5%, 70.5%, 60.7%, 39.3%, 35.7%, and 100% respectively (21).

As it is obvious from the results of these above reports, antibiotic resistant rates shows different pattern comparison with our study. It indicates performance of antibiotic susceptibility test is necessary before any antibiotic prescriptions. According to the CLSI (N100 S21 version 2011 tables

2H1 page 100), specific antibiotic has not been recommended for urine isolates of non-pyogents Streptococci. However, it is mentioned ampicillin and cefazolin is recommended for the pregnant women in this table. Clindamycin and erythromycin can also be used if the pregnant patients who have allergic reaction to penicillin.

In this study number of pregnant women entered in this study was very few. Hence, it was not possible to compare the antibiotic susceptibility pattern of pregnant women with non-pregnant. Therefore a separate study for pregnant patients is required.

Unfortunately, as we understand from other reported, results of antibiotic susceptibility test underlined high resistant to Penicillin that is important in those female with the UTI as noted in other studies (20-22). In our study Penicillin was highly resistant to the isolated GBS from studied patients. Penicillin is the first choice for intrapartum prophylaxis, with Erythromycin according to the recommendation of CDC and Clindamycin as alternatives for Penicillin-allergic patients. The high resistant rate for Penicillin makes the physician to prescribe other antibiotic.

Conclusion

Frequency of GBS in female patient was low, although pattern on antibiotic susceptibility test showed high resistant rate to some antibiotic that is expected to be increase in future too. Non-pregnant patients were not facing with treatment failure because of available different susceptible antibiotics, but those who were pregnant may find some problem. However some other sensitive antibiotics are still available that can be prescribe for pregnant female patients such as Clindamycin.

Acknowledgements

We are pleased for all provided opportunity to access the patients' data and re-examinations in some necessary cases by Milad Hospital staff.

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Importance of Tranexamic acid in pharmacological taking care of bleeding in urological patients

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Abstract

Introduction – aim: Bleeding in urology is very important problem and it participates in mortality and morbidity of urological patients. It can be linked with empty system – hematuria, and also it can be developed outside of the empty system in retroperitoneal space. The bleeding requests quick diagnostic and therapy without delaying. The aim of work is to show positive output of pharmacological ageneses of antifibrinolytics in increased bloody drainage. Antifibrinolytics administration and useful effects are based on inhibition of proteolyses activity and prevention of lyses, actually on melting fibrinogen and fibrin, which makes possible reduction of bleeding level. Researches involved 50 patients who were under surgery treatment in Urological Clinic in Clinical Center Nis. All patients were prepared before intervention and anesthesiological risk was estimated according to ASA classification. Because of comorbidity according to the estimation of the risk factor they were given prophylactic doses of low molecular Heparin, two hours before surgical intervention together with monitoring screening of coagulation and D dimmer. All patients, who received Ugorol in intravenous way, were operatively treated. Postoperative bleeding in urology as an operative complication can be treated with antifibrinolytics. Short-term treatment with tranexamic acid is effective in reduction of operative and postoperative loss of blood. Decreasing of bleeding helps recovery, because the results of a better drainage lessen the risk of postoperative infection.

Key words: Antifibrinolytics, tranexamic acid, bleeding, urologic surgical procedures urological patients

Introduction

Bleeding in urology is a very significant problem and participates in great measure in morbidity and mortality of urology patients. Bleeding can be massive, visible by naked eye and microscopic, occulted. Bleeding can be connected with empty system of urotract and is described as hematuria micro and macro. It can occur outside of empty urotact system in tissues and retroperitoneal space, while it doesn't have to be visible although it can be massive. Taking into account duration, bleeding can be acute, which becomes clinically significant if it is massive, or chronic, which can be significant because it causes sideropenic anemia.

Bleeding is a dramatic syndrome which requires quick diagnostics and therapy without delay. The most common type of bleeding is hematuria- the presence of blood in urine. Blood in urine is mostly liquid because urine contains urinokinase which activate fibrinolysin from plasma so that newly formed small clots are melted immediately. Nevertheless, if hematuria is massive, the amount of present urinokinases will not be sufficient for fibrinolysis, so clots are formed that are able to cause renal colic s if they originate from upper parts of urotract. The most important causes of chemotury are:

1. pathological conditions
2. surgical procedures
3. disturbances of blood coagulation and hemostasis.

Albrehsten reports that the prostate is one of human tissues with the greatest concentration of plasminogen activators.[1] Distribution of these activators in tissues was studied in more detail by Todd, Pandolfi, Nilsson and others.[2] Plasminogen activator in urine was discovered by Williams and

fibrinolytic activator was found in other bodily fluids.[1,2] Due to al this, in urologic surgery of the prostate there are considerations of medicament application of antifibrinolytic therapy which has its just place in the treatment of hemorrhage (postoperative drainage or massive hematuria).[2,3] The purpose of this therapy is above all to reduce fibrinolytic activity of prostate frame, and at the same time to reduce the risk of thromboembolic occurrences through correct systemic administrating of the medicine.[3,4]

The action of trenaxemic acid is base on inhibition of proteolyse activity of plasmin after conversion of plasminogen into plasmin. Plasmin inhibition prevents lysing of the fibrinogene and fibrin. When fibrin is formed the reverse action stimulate the activation of tissue plasminogene and its transformation from an inactive into active form, into natural plasmin enzyme. Apart from the activators in the organism inhibitors are also created Fibrinolysis is activated in urinary tract, so antifibrinolytics in this way have their place in the treatment of urinary tract bleeding.[4,5]

Material and methods

The research included 50 urological, surgically treated patients. Among them there were 17(34%) women and 33(66%) men aged 42-71 years. All patients were preoperatively treated by means of appropriate urological procedures and anesthesiologicly with estimated system of risk according to ASA classification. They mostly belonged to higher risk level according to ASA classification and because of it, prophylactic measures for prevention of thromboembolism were performed. The patients received preoperatively a prophylactic dose of low molecular heparin (LMH). All patients preoperatively developed increased blood drainage, in the form of hemorrhage or in the form of pronounced hematuria. In 10 patients endoscopic surgical procedure was performed and bleeding was partly controlled. As immediately after the operation blood drainage increased in all patients, and the surgeon was reluctant to initiate intervention, in order to regulate homeostasis and prevent the bleeding first measure after urological control was initiated. All patients after general measures of compensation of circulatory volume received also tranexamic acid (Ugurol) in daily doses of 1.5 to 2 g in 500 ml of physiological solution by means of an infusion pump, by speed of 140 ml/h (administration longer than 3h during 24h). The length of administration of tranexamic acid was 1-3 days u depending on clinical symptoms. During that time, apart from clinical control biochemical monitoring of hematological profile and coagulation was performed. It is necessary due to blood products therapy as well as due to assessment of possible thromboembolic complications.

In this way, all patients, by means of administration of pharmacological agent (Ugurol) for bleeding reduction, we achieved bleeding control and stopping of bleeding. In 20 patients, we administrated 2 units of allogeneic blood transfusion. This is done because biochemical monitoring revealed low hemoglobin values. There were no thromboembolic complication.

Statistic methodology

In order to describe the groups of subjects we used standard descriptive, statistic parameters: arithmetic mean (Xsr.), standard deviation (SD), amplitude deviation (min-max), structure index (%), 95% trust interval (95%CI).

In order to test regularity of distribution of the researched marks we used Shapiro-Wilk test.

Student t– test for two small dependent samples (Paired Samples Test) compared the hemoglobin level before and after the operation, that is, UGUROL administration (tranexamic acid).

The difference in anemia frequency (hemoglobin<125gr/l) before and after surgery, that is, UGUROL administration (tranexamic acid) was researched by use of McNemar Chi Square Test.

Results

All subjects (n=50) were the patients of Urology Clinic, KC Niš and all received low molecular heparin. Among them there were 17 (34%) women and 33 (66%) men, aged 42-71.

In order to stop the bleeding all patients received tranexamic acid (Ugurol) in dose 1.5gr in 500ml of physiological solution by means of infusion pump, by 140mL/h (administration longer than 3^h) during 24^h.

Out of total of 50 patients, 10 (20,0%) received Ugurol intraoperatively, and 40 (80,0%) perioperatively.

Hemoglobin values, varied before the operation at 122.0-152.0gr/l with an average of 135,40±8,51gr/l. After the surgery there was a drop in hemoglobin. The first day after surgery the values decreased, in average, for 28,30±15,08gr/l and this decrease is statistically highly significant (p<0,0001). (Table 1)

Table 1. Hemoglobin values in subject's blood (Xsr. ± SD) before and after first day of surgery (n=50)

Hemoglobin (gr/l)	Before surgery	1. day after surgery
Min – max	122,0-152,0	75,0-134,0
Xsr. ± SD	135,40±8,51	107,10±16,90
95%CI	131,4-139,4	99,2-115,0
Change (Δ)	28,30±15,08	
T – test	8,391	
P	0,00000008 p<0,0001	

On the third day after surgery, hemoglobin values were averagely, at least at 97,70±12,46gr/l. Compared to pre-surgery hemoglobin level there was a statistically significant reduction (p<0,0001). (Table 2)

Table 2. Hemoglobin value in subject' s blood (Xsr. ± SD) before and on the third day after surgery (n=50)

Hemoglobin (gr/l)	Before the surgery	3. day after surgery
Min – max	122,0-152,0	74,0-136,0
Xsr. ± SD	135,40±8,51	97,70±12,46
95%CI	131,4-139,4	91,9-103,5
Change (Δ)	37,70±11,06	
T – test	15,250	
P	0,0000000001 p<0,0001	

In the period from the first to the third day after surgery there was a further drop in hemoglobin level, but significantly smaller. Averagely, hemoglobin value was reduced by 9,40±15,26gr/l. This drop was statistically significant for level p<0,05. (Table 3)

The first day after surgery out of total of 50 subjects, 40 or 80,0% had anemia. On the third day after surgery the number of anemic patients increased to 45 (95,0%). The prevalence of anemic patients was increased for only 15,0% and this difference was not statistically significant (McNemar Test; p=0,25; p>0,05 or NS). (Table 4)

Table 3. Hemoglobin values in subject s blood (Xsr. ± SD) on the first and third day after surgery (n=50)

Hemoglobin (gr/l)	1. day after surgery	3. day after surgery
Min – max	75,0-134,0	74,0-136,0
Xsr. ± SD	107,10±16,90	97,70±12,46
95%CI	99,2-115,0	91,9-103,5
Change (Δ)	9,40±15,26	
T – test	2,755	
P	0,013 p<0,05	

Table 4. Presence of anemia (hemoglobin<125gr/l) in subject's blood on the 1. and 3 . day after surgery. (n=50)

1. day after surgery	3. day after surgery		TOTAL
	Anemia NO	Anemia YES	
Anemia NO	5	5	10
Anemia YES	0	40	40
TOTAL	5	45	50

** McNemar Test*

The separate group which received Ugurol on the day zero or the first day (in those who received on the second or third day Hb is reduced both in group and in the whole) ant its values before the surgery oscillated from 126,0-148,0gr/l with an average of 136,20±7,21gr/l. After the surgery there was a drop in hemoglobin values. After the first day of operation the values were reduced averagely for 32,40±17,88gr/l and this drop is statistically highly significant (p<0,001). (Table 5)

Table 5. Hemoglobin values (Xsr. ± SD) before and after the first day after the operation in the group of patients receiving UGUROL on the day zero or on the first post-surgery day.(n=25)

Hemoglobin (gr/l)	Before surgery	1. day after surgery
Min – max	126,0-148,0	75,0-132,0
Xsr. ± SD	136,20±7,21	103,80±19,53
95%CI	131,0-141,4	89,8-117,8
Change (Δ)	32,40±17,88	
T – test	5,729	
P	0,0003 p<0,001	

** T-test for two small dependant samples*

In the period from the first to the third day after surgery there was a slight increase in hemoglobin level. On the first post-surgery day the average value was 103,80±19,53gr/l, a and after the administration of UGUROL and perhaps transfusions 4 patients received transfusions on the third day and the average value was 104,40±10,98gr/l. Hemoglobin level, in the group of patients who on the day zero or on the first day received UGUROL, was slightly increased until the third post-surgery day, although not statistically significantly (p>0,05 or NS).

Table 6. Hemoglobin values (Xsr. ± SD on the first and the third day after surgery in the group of subjects who received UGUROL on the zero or the first post-surgery day (n=25)

Hemoglobin (gr/l)	1. day after operation	3. day after operation
Min – max	75,0-132,0	90,0-126,0
Xsr. ± SD	103,80±19,53	104,40±10,98
95%CI	89,8-117,8	96,5-112,2
Change (Δ)	-0,60±13,73	
T – test	0,138	
P	0,893 p>0,05 NS	

** T-test for two small independent samples*

Twenty patients or 40% je received two units of allogeneic blood transfusion each, because biochemical hemoglobin values were significantly below the physiological values. (Table 7)

Discussion

Bleeding in urology – heamturia, blood drainage is present as a symptom which because of its scope presents a significant factor which can often be unpredictable.[5,6] This is why the preparation of an urological patients must have two aspects. On the one hand the patient's general condition is often damaged by other accompanying illnesses. On the other hand, renal insufficiency is present. It can have a significant influence on the perioperative period so a minor hemorrhage level can more than anything else in some other cases, significantly endanger the patient. Renal insufficiency is followed by constant anemia (patients are well adapted to it, relative hypovolemia and hypoproteinemia and even small bleedings in these conditions can significantly damage homeostasis and seriously endanger the patient [5,6,7] Correction of the coagulation status is of great importance. The problem can be the patients who are on anticoagulant therapy for other reasons, but are in present renal insufficiency[,7,]. These patients retain uremic toxins (methylguanidine, creatinin, guanidinosuccinic acid, mionoitol) and other middle molecules from 500 to 12000 daltons.[8]

This toxic effect of middle molecules can be seen in inhibition of hemoglobin synthesis, glucose use, reduction of nerve conductivity, phagocyte activity and lymphoblast transformation. The loss of vascular volume in these patients can very quickly lead to hypovolemia, that is hemorrhagic shock. Clinical picture is more pronounced in acute bleedings, and leading etiological factors for development of shock in urology are hypovolemia and infection.[8,9]

Because of all these reasons pharmacological agents are used which reduce the bleeding, reduce the drainage of blood loss and reduce the need for allogeneic transfusion. Tranexamic acid was frequently used in the 70s during open prostatectomy, and then in the beginning of the 80s and 90s. It is equally used today prophylacticly, preoperatively, intraoperatively and postoperatively in reduction of the drainage of the blood losses. [8,9,10]

Aniti Raniko analyzed group of patients who received prophylactic tranexamic acid in the dose of 2g intravenously and those who did not.[8,9,10] In the group where tranexemic acid was admini-

Table 7. Ugurol administration time and transfusion

Transfusion		Day zero	1. day	2. day	3. day	zero and 1. day	zero and 2. day
NO	number	2	10	12	0	3	2
	%	50,0	100,0	60	0,0	33,3	100,0
YES	number	2	0	8	4	7	0
	%	50,0	0,0	40	100,0	66,6	0,0
TOTAL	number	4	10	20	4	10	2
	%	100,0	100,0	100,0	100,0	100,0	100,0

strated, and transurethal prostatectomy was performed during surgery, the level of hemorrhage per gram of resected tissue was 8ml. In the group which did not receive tranexamic acid prophylacticly, the losses were 13ml of blood per gram of the resected material.[8,11] We must emphasize that excessive bleeding after surgery can be excepted due to insufficient surgical hemostasis.[10,12,13]

McNikoli et al report that antifibrinolytic therapy administrated during the first 12 hours after surgery, in case of increased blood drainage, reduces postoperative blood loss by 50% in suprapubic prostatectomy, and even for 75% after TUR-a. [1,2,14]

Vanikombe and Shutlewort report that postoperative hemorrhage in randomized series of patients in retropubic prostatectomy receiving antifibrinlytic, aproximately reduces hemorrhage for one third compared to the number of patients which in their therapy did not have administration of antifibriolytic.[2,12,15,16]

On our material with intraoperative losses which were estimated below 10% of blood volume, in zero and the first postoperative day there was a significant drop of hemoglobin which is statistically very significant. Administration of tranexamic acid began immediately with increased blood drainage and lasted until it was significantly reduced[17]. The number of patients requiring blood transfusion was 8 or 37,5%.

Conclusion

Postoperative bleeding in urology as complication can successfully be treated by antifibrinolytic medicines. Short-term treatment by tranexmic acid is effective in reduction of surgery and postsurgery blood loss. It must be said that excessive bleeding after surgery is most commonly expected duet o insufficient surgical hemostatsis.

There are no medicines that can replace surgical technique but antifibrinolytics are complementary to surgical procedures.

The reduction of bleeding helps recovery because the results of the better drainage reduce the risk of postoperative infection.

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Determination of anthropometric measurements and nutritional status of children with Phenylketonuria

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Abstract

Background: Phenylketonuria (PKU) is a metabolic disease genetically transited from carrier parents to child occurs in 1 of 4000-4500 children in Turkey.

Objectives: The aim of this study was determine nutrition, growth and malnutrition status in children with classical PKU which receive regular diet therapy.

Methods: The study was conducted on 70 children aged between 1-6 years. Information about children and families were collected with face to face interview. The determination of nutritional status asked for recording foods they consumed for at least 3 months during diet therapy. Anthropometric measurements were applied by researchers.

Results: According to body weight %22.7 of children aged 1-3 years and 46.2% of children aged 4-6 years were chronically malnourished. Also, daily energy, protein, phenylalanine, niacin, Vitamin B12, Vitamin D and fiber consumption were under recommendations ($p<0.05$).

Conclusions: Children and their families should be educated about adequate and balanced nutrition for enhancing their nutritional habits and maintaining their health in the future life. Growth and development of children with PKU who received restricted diet therapy should be evaluated tightly and their adequate and balanced nutrition should be provided by routine biochemical evaluation.

Key words: Phenylketonuria, anthropometric measurement, nutritional status, children.

Introduction

Phenylketonuria (PKU) is a newborn metabolic disease which caused serious brain damage and microcephalia by elevating phenylalanine (phe) in blood(1,2). While the prevalence of PKU is 1/15.000 in USA, 1/10.000 in UK, 1/60.000 in Japan and 1/18.000 in France, it occurs per 4.500 live births in Turkey (3). Two hundred fifty- three hundred children born with PKU in every year and because one of every 20-25 person carried this disease and one of each 3 marriage is kin marriage in east of the country; caused this disease to be seen more frequently (4,5). In Turkey, the major problems encountered in management of PKU are the late onset of treatment, socio economic conditions, complicated demographic and geographic conditions, lack of dietary compliance due to illiteracy and financial limitations and lack of regular blood phe monitoring (6, 7).

Dietary phe restriction is the most effective way of reducing its excess in blood and is the only efficient method for treating PKU [7]. The aim of the early onset of diet therapy is to control blood phe concentration with restricted phe diet and preserve normal plasma phe concentrations, prevent mental retardation and neurological complications. Child's protein, energy, vitamin and mineral requirements needed for growth and development must be meeted with lifelong diet therapy. The acceptability of dietary taste variety, hindering the uniformity of diet and saturating of the patient are also important (3, 7, 8). But, application of the diet therapy inadequate and unbalanced can cause anemia, hypoproteinemia, changes in bone and malnutrition in early period of life and retardation in growth and development can also occur (8). Diets that cannot saturated children adequately and lack of balance between fluid and energy and vomiting, diarrhea and inflammatory disease can cause excessive elevation or falling phe in blood. This situation affects the quality of life unfavorably by causing brain damage and malnutrition (9).

Success of diet therapy in children with PKU depend on the parents ability, understanding diet, motivation, organization ability, converting diet therapy to behavior changes discipline and application of phe restricted diet (10). Parent education about nutrition of children with PKU is important and effective in blood phe control and preventing quality of life. Families taking part in active education will facilitate communication between health professionals and person (11).

This study was conducted to determine growth and nutritional status of children with classical PKU and received regular diet therapy.

Methods

Subjects

The study was conducted on 70 children (44 boys, 26 girls) with PKU, aged between 1-6 years, at Hacettepe University Ihsan Dogramaci Children's Hospital, Nutrition and Metabolism Unit between February-July 2009.

Research Design

Health controls of children with PKU were performed at the beginning of the study in H.U Nutrition and Metabolism Unit and they have been referred to diet consultation. The aim, subject and content of the study has been explained to families of children. Participants signed a voluntary participation form which adhered to Declaration of Helsinki protocols (World Medical Association). A questionnaire about child and family's descriptive information and nutritional status were conducted to mothers.

Anthropometric Measurements

For evaluation of children's growth, anthropometric measurements were performed by researchers. Measurement of body weight was performed with light clothes and with a scale nearest to 0.1 kg. Height was measured while foots are collateral and head was in Frankfort plane. In children who aged less than 2 years the height measurements were performed with infantometer in laying position. Mid upper arm circumference, was measured at the mid-point between the tip of the shoulder and the tip of the elbow (olecranon process and the acromium) while the arm was bent with 90 °C angles (12). BMI was calculated with Body weight (kg)/height^2 (m^2) formula. Head circumferences was measured the distance from above the eyebrows and ears (occipital – frontal process) and around the back of the head (13). Percentile values were used to evaluate of the data's. $\leq 5^{th}$ percentile was evaluated as under nutrition, $\geq 5^{th}$ -$\leq 15^{th}$ as underweight, $\geq 15^{th}$ -$<85^{th}$ as normal, $\geq 85^{th}$ -$<95^{th}$ mild obese and $\geq 95^{th}$ as obese (14).

Biochemical Measurements

Children's blood phe level were measured monthly in Laboratory of Metabolism Unit of H.U. Ihsan Dogramaci Children's Hospital with HPLC method. These data of children's were obtained from patients.

Food Consumption Records

Mother's asked for recording the foods and drinks their children consumed in 1 weekend day and 2 week days consequently to determine the consumption of foods by children. They were requested to bring these records with in respectively 3 monthly controls. The daily average energy, macro and micro nutrient intake of children were calculated from these records by BeBiS computer program which was developed for Turkey. Data obtained from this study were compared with daily recommended consumption amounts (15).

Statistical Analyses

Data were evaluated with SPSS 11.5 Statistical Package Program. Number and percent of descriptive data were calculated. Mean ($\bar{x}$), standard deviation (SD), minimum, maximum values were received in tables. Statistical significance was established at a p value of <0.01 and < 0.05.

Results

Demographic information about children are shown in Table 1.

Table 1. Demographic information about children with PKU

Demographic information	$\bar{x} \pm$ SD
The age of the children	3.14 ± 1.48
The number of people living at home	5.68 ± 2.67
The total number of mothers pregnancies	2.87 ± 2.00
The number of sibling with PKU	0.15 ± 0.43
The number of living children	2.28 ± 1.47

Forty four of children participated this study was boy and 26 was girl. The mean age was 3.14 ± 1.48 years. Children lived 48.6% in city and 51.4% in town or village and while 32.9 % (n=23) of children's parents were not relative, 67.1 % (n=47) were relative. The mean of total pregnancy num

While height for age was under 5th percentile in %32.0 of boys and %22.7 of girls aged between 1-3 years, this value were %52.9 and %33.3 in boys and girls aged between 4-6 years, respectively. According to weight, while 40.0% of boys aged between 1-3 years were under the 5th percentile accumulation was seen more between ≥15-<85th percentile (%48.0 of boys and %47.4 of girls aged between 1-3 years and 52.9 % of boys and 66.7% of girls aged between 4-6 years). When BMI percentile were evaluated, while in 1-3 years aged group 12.0 % of boys and 10.5% of girls have BMI under 5th percentile , in 4-6 years aged group this value was 11.8% in boys and girls didn't have BMI under 5th percentile. Mid arm circumference values were accumulated between ≥15-<85th percentile in both 2 age and gender groups.

15.6% of 64 children's aged between 1-5 years head circumference were determined under 5th percentile. 12.5% percentile of children had head circumference ≥95th percentile and 71.9% ≥5-95 percentile.

While before the onset of the diet therapy the mean blood phe value was 26.93 ± 7.88 mg/dL, after the diet therapy it was 6.67 ± 3.39 mg/dL in children aged between 1-3 years and 9.17 ± 4.63 mg/dL in children aged between 4-6 years. But, daily phe, protein and energy values recommended with diet were not consumed by children ($p<0.01$, Table 3).

When energy, fiber, vitamin B12, folic acid, calcium and iron consumption of children aged 1-3 years with PKU were under recommendations, protein, carbohydrate, thiamine, riboflavin and vitamin C intake were high ($p<0.01$; $p<0.05$). Although daily energy, fiber, niacin, vitamin B12, folic acid,

Table 2. The distribution of children's height for age, body weight, BMI and upper mid-arm circumference according to the percentiles (n:70)

		≤ 5.	≥ 5.-<15.	≥ 15.-<85	≥ 85.-< 95	≥ 95
		n (%)	n (%)	n (%)	n (%)	n (%)
Height (cm)						
1-3 years	Boys	8(32.0)	3(12.0)	12(48.0)	- -	2(8.0)
	Girl	10(22.7)	10(22.7)	21(47.7)	- -	3(6.8)
4-6 years	Boys	9(52.9)	2(11.8)	5(29.4)	1(5.9)	- -
	Girls	3(33.3)	3(33.3)	3(33.3)	- -	- -
Weight (kg)						
1-3 years	Boys	10(40.0)	3(12.0)	12(48.0)	- -	- -
	Girls	2(10.5)	5(26.3)	9(47.4)	3(15.8)	- -
4-6 years	Boys	5(29.4)	3(17.6)	9(52.9)	- -	- -
	Girls	2(22.2)	1(11.1)	6(66.7)	- -	- -
Body Mass Index (kg/m²)						
1-3 years	Boys	3(12.0)	4(16.0)	13(52.0)	2(80)	3(12.0)
	Girls	2(10.5)	- -	12(63.2)	2(10.5)	3(15.8)
4-6 years	Boys	2(11.8)	1(5.9)	10(58.8)	4(23.5)	- -
	Girls	- -	2(22.2)	7(77.8)	- -	- -
Upper Mid-Arm Circumference (cm)						
1-3 years	Boys	- -	5(20.0)	19(76.0)	- -	1(4.0)
	Girls	- -	1(5.3)	11(57.9)	5(26.3)	2(10.5)
4-6 years	Boys	2(11.8)	- -	13(76.5)	1(5.9)	1(5.9)
	Girls	2(22.2)	1(11.1)	5(55.6)	1(11.1)	- -
Head Circumference (cm)						
1-5 years	Boys	6(15.8)	2(5.3)	24(63.1)	- -	6(15.8)
	Girls	4(15.4)	- -	18(69.2)	2(7.7)	2(7.7)

Table 3. The amount of daily consumption and recommended phe, protein and energy after educated children with PKU

	Recommended $\bar{x}$ ± SD	Daily Intake $\bar{x}$ ± SD	p value
Phe (mg/gün)	423.2 ± 107.07	330.0 ± 204.23	0.000*
Protein (g/gün)	24.0 ± 4.80	19.8 ± 5.67	0.000*
Enerji (kkal/gün)	1342.9 ± 191.97	992.10 ± 323.81	0.000*

* p<0.01, Wilcoxon test was applied.

Table 4. The daily energy and nutrient intakes of educated children compared with the levels of the DRI

Daily energy and nutrient intake	Age (years)	$\bar{x}$ ± SD	DRI levels	t test	p
Energy (kcal)	1 – 3	930.4 ± 321.2	1250	6.59	0.000**
	4 – 6	1096.4± 306.5	1650	9.21	0.000**
Protein (g)	1 – 3	18.2 ± 5.1	13	6.77	0.000**
	4 – 6	22.3 ± 5.7	19	3.00	0.000*
Carbohydrate (g)	1 – 3	140.6 ± 48.7	108.1	1.44	0.156
	4 – 6	178.4 ± 48.6	137.3	5.08	0.000**
Fiber (g)	1 – 3	7.1 ± 3.14	19	24.98	0.000**
	4 – 6	7.9 ± 3.36	25	25.82	0.000**
Tiamin (mg)	1 – 3	0.56 ± 0.18	0.5	2.16	0.036*
	4 – 6	0.57 ± 0.12	0.6	0.89	0.379
Riboflavin (mg)	1 – 3	0.65 ± 0.25	0.5	3.86	0.000**
	4 – 6	0.62 ± 0.14	0.6	0.82	0.417
Niasin	1 - 3	5.90 ± 1.83	6.0	0.32	0.744
	4 – 6	6.79 ± 1.45	8.0	4.20	0.000**
Vitamin C (mg)	1 – 3	85.10 ± 46.89	15	9.91	0.000**
	4 – 6	81.79 ± 48.69	25	5.94	0.000**
Folic acid (mcg)	1 – 3	96.79 ± 34.68	150	10.17	0.000**
	4 – 6	112.39± 28.11	200	15.89	0.000**
Calcium (mg)	1 – 3	385.30± 51.47	500	5.02	0.000**
	4 – 6	364.70± 91.83	800	24.16	0.000**
Iron (mg)	1 – 3	6.10 ± 2.28	7	2.45	0.018*
	4 – 6	6.10 ± 1.26	10	15.56	0.000**
Zinc (mg)	1 – 3	3.30 ± 1.71	3	1.51	0.137
	4 – 6	3.20 ± 0.68	5	13.34	0.000**

**p<0.05; **p<0.01*

calcium, iron, zinc intake of children aged between 4-6 years were lower according to DRI, protein, carbohydrate, vitamin C intake were high (p<0.01; p<0.05) (Table 4). Children with PKU complied their diet therapy 18.6% completely, 57.1% usually, 21.4% sometimes and %2.1 never. When the reasons to not comply with the diet were examined, mothers reported that 33.3% their children were anorectic, 17.5% cannot prepare foods according to diet, 15.8% their children didn't like foods they gave, 23.6% didn't saturated with foods take part in diet, 8.8% eaten inhibited foods secretly.

Discussion

The most important finding occurs in cases with untreated hyperphenilalaninemia is mental retardation. Even in patients partially treated and have intelligence level approximately at normal, it occurs deficiency of arithmetic and reading abilities, impairment of motor functions, learning and behavior problems. With appropriate diet therapy findings of disease caused permanent damages may prevent and intelligence of children with PKU may be similar to their peers (9, 16, 17).

Taking precaution to before development of malnutrition and especially measuring height and head circumference routinely is very important. It is emphasized that there is an association between cognitive functions and head circumference in early childhood period. Holm and Knox (18) reported that brain development was impaired and microcephalia was occurred in 220 untreated PKU patients. In this study 15.6% of 64 children aged between 1-5 years head circumferences were below 5th percentile (Table 2). Children with low head circumference should be evaluated in terms of brain development.

In patients treated with phe restricted diet growth retardation is seen in first year of life. Defaults in application of restricted diet therapy may affect growth and development of children. In a study, height/weight values of PKU patients were found markedly below the values of reference population (19). Dhondt et al. (20) reported that mean height *z* scores between 2-8 years of age for French children treated for PKU were often -0.5 or below. Schaefer et al. (21) reported that of 82 German children with PKU, the standard score for height was not significantly different from zero at study entry but had dropped to a nadir of -0.78 for boys and -0.54 for girls by two-and-a-half years of age. Although the girls had some recovery toward zero during the next three-and-a-half years, boys had no recovery. They reported poor linear and head circumference growth in children with PKU undergoing nutrition management. Fisberg et al. (22) reported that the height/weight Z-score means for children with PKU were 0.47 for those under 7 years and 1.86 for 7 years-old and over; in normal children the means were 0.97 <7 years and 1.54 ≥7 years, with no statistically significant difference. The height/age Z-score means were significantly lower in the PKU children <7 years (-1.23) than in the normal controls (0.91)**.** The present study pointed out that according to height for age values 74.7% of boys and 66.6% of girls were below 15th percentile in children aged between 4-6 years. Also, BMI for age values of 12.0% of boys and 15.8% of girls aged 1-3 years were above the 95th percentile. But, nobody had BMI for age values above the 95th percentile in children aged 4-6 years.

Acosta and Yannicelli (23) reported that mean phe intake of the normally growing infants with PKU was 38 % greater than that of the poorly growing infants with PKU whereas mean plasma phe concentrations and mean energy intakes of the two groups did not differ. Because nutrition is important for ensuring normal growth, the objectives of this study were to evaluate nutrient intakes and physical growth of 58 children undergoing current nutrition management for PKU. Plasma phe concentrations were also obtained because deficient concentrations of plasma phe have been associated with poor growth (24, 25). In our study it was determined that children with PKU could not consumed recommended energy, protein and phe amounts with diet therapy. This situation may arouse from anorexia of children.

The basic of PKU treatment phe restricted diet therapy. Infants with PKU should diagnose in first days of life, diet therapy must start in first 21 days and continue for a lifetime. Problems may prevent with early diagnose and appropriate diet therapy. But finding can change according to severity of disease. In children with PKU blood phe level should be remained in between 2-6 mg/dL (120-360 μmol/L) which is normal range for phe (9, 16, 17). Diet phe amount is above normal requirement although it can change from patients to patient for remaining blood phe level in normal range (8). In this study it was shown that blood phe levels after the diet therapy were significantly lower than baseline blood phe levels. This situation reveal important and necessity of diet therapy.

In children with PKU restriction of diet phe may led to malnutrition due to inadequate energy consumption. Hanley et al. (26) determined that patients blood phe remained in normal range with phe restricted diet were sever malnourished. When 16 children aged 11-15 years with PKU evaluated it was found that they consumed 16% lower energy compared with healthy matches [8]. In this study energy consumption of PKU patients were significantly lower than DRI (P<0.01). Besides, when it consider that their protein intake also lower (p<0.01), risk of malnutrition is high in a long term (Table 4).

During infancy, children ingested a mean of 3.1 g protein/kg and 98kcal/kg whereas from 1 to 6 years of age, mean protein intake declined from 2.7 g/kg to 1.8 g/kg and mean energy intake declined from 114 kcal/kg to 67 kcal/kg (27). Diets with inadequate protein may cause malnutrition

in first year of life, poor growth, anemia, hipoproteinemia and radiological bone changes. This situation may prevent by monitoring blood phe level and evaluating dietary intake frequently (28). Acosta and Yannicelli (29) reported in a study with 12 PKU patients that when patients consume 3.12 g/100kcal protein with amino acid mixture, they catch up normal growth, but in 13 patient consume 2.74 g/100kcal protein with amino acid mixture weight and knee-heel length were low. Mean protein intake of infants growth normally were 38% higher than infants' growth poor. Dobbelaere et al. (19) shown in a study with 20 PKU patients that energy and protein intake of all patients were higher than 2/3 of requirements. Energy intakes of patients were 61-128 kcal/kg/d and protein intakes were 1.2-2.1 g/kg/d.

Nutrient intakes of children with PKU met or exceeded RDIs when protocol-recommended protein intakes were ingested, suggesting that protein intakes were adequate when a major portion was obtained from an elemental medical food, indicating that dietitians should prescribe and encourage protein intakes greater than RDIs. Linear growth of children with PKU was within the normal range; however, obesity was present in a significant number of children, suggesting the need for dietitians to plot weight on NCHS growth charts and prescribe energy intakes and exercise programs to help prevent this problem (29). For control of blood phe; protein, energy and phe intakes should provide appropriate amounts.

Children with PKU are at risk of selenium, zinc, copper, iron, calcium, vitamin B2, B6, B12 and folic acid deficiency. Therefore, when tightly monitoring growth and development, this deficiency should taken into account (28, 30). In one study was found that when adolescents and adults with PKU stopped consumption of phe free amino acid mixture, their consumption calcium, folate and iron were below 60% and thiamine, riboflavin, niacin, vitamin B6 and vitamin B12 were below 30-50% (31). In another study was reported that children with PKU ingested calories, calcium, iron, zinc, and copper below the recommended values, whereas the protein intake was within the normal range (22). Hanley et al. (26) was shown that in PKU patient treated with diet therapy serum vitamin B12 levels was 65.8 pmol/L(Normal range 150-670 pmol/L) and with vitamin B 12 therapy findings were recruited. It is emphasized that due to neurological damage is a risk for PKU, patients serum vitamin B12 level should evaluate (32). Stopped taking the phe-free amino acid mixture 90 PKU patients' protein intake still met the recommendations, but thiamin, riboflavin, folate, calcium and iron levels were below 80% of the US RDA in most patients (31). In a study conducted by Dobbelaere et al. (19) when mineral intake of children with PKU compared with RDA values, while intake of iron was 79%, iodine 61%, zinc 24%, selenium intake were below the normal ranges. But it was not found relationship between growth retardation and protein and energy intake, blood phe levels and daily phe intake. This decreasing of growth rate did not depend on endocrinological causes and thought that may caused by vitamin and mineral deficiency. Growth retardation in PKU not only depend on restricted diet therapy and so clinical and biochemical parameters should evaluated routinely (19). In present study intake of energy, fiber, vitamin B 12, folic acid, calcium and iron of children with PKU was found low (Table 4). So children received restricted diet should supplemented with vitamin and mineral. When diet therapy arranges according to requirements and provides consuming of advised amounts, complication of disease may prevent and normal growth and development provide. It is important in treatment of disease that providing adequate phe (according to type of PKU, age, weight, height and blood phe level), protein (2.2-3.5 g/kg/d in infant and 1.5-2.2 g/kg/d toddler), energy, vitamin and mineral intake, acceptable for taste and diversity of diet, preventing monotony of diet with exchange list and more importantly saturating of patients (33).

One of the factors increase dietary compliance is improvement of patient and families knowledge of disease. Beside the metabolic control, family education and environmental stimulus should consider. Especially, mothers have an important role in understanding and application diet by child (34).

Conclusion

Effective and continued education of families is beneficial. Dietary compliance, family educa-

tion and growth and development of child can evaluate with tightly monitoring. Because, errors in application of restricted diets can affect children's growth and development. Especially, early definition of growth and developmental problems caused by metabolic disease and restricted diet and providing appropriate rehabilitation conditions if necessary, is important to reintegrate healthier as physical and mental persons into the society.

Acknowledgement

We would like to thank all the participants who devoted their time to participate in this study. Their helpful and wholehearted cooperation is warmly acknowledged.

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Yellow nail syndrome: recurrent pleural effusion in a patient with severe chronic obstructive pulmonary disease

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Abstract

Recurrent pleural effusion of unrevealed etiology is important problem in clinical practice. Yellow nail syndrome (YNS) is a rare disorder, which consists of the triad of deformed yellow nails, lymphedema and respiratory disorder. It is often accompanied with bronchiectasis, pleural effusion and/or chronic rhinosinusitis. Frequently, only two of three features are present. The exact mechanism of YNS remains unknown. However, it is thought to reflect functional defects in the lymphatic vasculature. We report on a case of yellow nail syndrome in a 75-year old man, heavy smoker, treated for exacerbation of chronic obstructive pulmonary disease and chronic hypercapnic respiratory failure. He had characteristic nail changes and recurrent pleural effusion of the unknown etiology until the exudation was put into connection with YNS.

Key words: yellow nail syndrome, pleural effusion, COPD, respiratory failure

Introduction

Yellow nail syndrome (YNS) is a rare disorder, first described in 1964 by Samman and White[1]. The syndrome is characterized by a classic triad of yellow dystrophic nails, lymphedema, and respiratory tract abnormalities. It is often accompanied by other features, mainly bronchiectasis, pleural effusion and/or rhinosinusitis. It can occur from birth to eight decade, but mainly in middle-aged[2]. YNS is less frequent in men than in women (female/male ratio: 1.6).[2]

We report on clinical presentation and radiographic features of YNS detected at the Teaching Hospital of Pulmonary Disease of the Clinical Centre of Serbia in Belgrade.

Case report

A 75-year-old man, long-term smoker, was admitted to respiratory Intensive Care Unit due to dyspnea, cough, and fatigue with signs of somnolence, cyanosis and generalized oedema. History taking revealed that he has been hospitalized several times during past decade and treated for chronic obstructive pulmonary disease (COPD) and recurrent pleural effusion on the right side. Thoracocentesis was performed several times, but the etiology of the effusion remained unknown. Despite treatment with diuretics, pleural effusion often reacumulated. Apart from somnolence, cyanosis and generalized oedema, on pertinent physical examination of the hands, the nails of both hands were dystrophic and thickened (Figure 1). Auscultation revealed impaired breathing sound, and decreased vocal fremitus was found over the right lung base. The rest of physical examination was normal. Peripheral blood analyses showed increased hemoglobin concentration (190 g/l), red blood count was $6.1x10^{12}$/L (normal: 4.3-5.7) Haematocrit: 0.599L and the rest was within normal range. Arterial blood gas analyses showed signs of chronic hypercapnic respiratory failure : PaO_2: 6.5 kPa, $PaCO_2$:11kPa, pH: 7. 25, SaO_2 O_2: 71%. Chest x-ray showed signs of hyperinflation and right side pleural effusion.

The patient's condition required immediate non-invasive positive pressure ventilation (NIPPV), antibiotics, bronchodilatators, corticosteroids and diuretics. Venepunction was performed several times due to haemoconcentration. About 800 ml of clear pale yellow pleural fluid was evacuated by thoracocentesis and biochemical analysis has shown it to be an exudate (glucose 4.6 mmol/l, LDH 326 IU/l, protein 48 g/l, albumin 26 g/l,

cholesterol 4.4 mmol/l, triglycerides 0.8 mmol/l); cytologic examination showed normal cell count with lymphocyte predominance without morphologically malignant cells. The results of analyses for infectious agents both in the patients sputum and effusion, including *Mycobacteria,* were negative. Tests for systemic and autoimmune disease were also negative.

Figure 1. Left hand of a patient with yellow nail syndrome. Apart from typical syndrome-related nails' presentation (yellow and thickened nails), more intensive coloration of the thumb and index finger might be also caused by long-term tobacco smoking

Under treatment, respiratory gas exchange improved, and the patient slowly started to recover. Repeated arterial gas analysis showed PaO_2 7.8 kPa, $PaCO_2$ 8.4 pH 7.35 SaO_2: 88%. During the hospitalization, pleural effusion did not reacumulate, so no further medical procedures were necessary. All the applied measures have lead to clinical improvement so patient was released to home treatment.

Discussion

We presented a case of YNS as a rare syndrome detected in a 75-year-old man with chronic respiratory failure and recurrent pleural effusion. As to our knowledge, this is the first such case of the syndrome in patient with severe chronic obstructive pulmonary disease and chronic respiratory failure, with recurrent pleural effusion presented in medical literature. Once the effusion was put into connection with YNS, its etiology seemed to be more certain. The exudation did not reoccur when respiratory failure has been improved and stabilized.

The nail abnormalities have been the first sign to be noticed on physical examination, but YNS is now known to involve multiple organ systems and its association with other diseases is well described. Although disorder is characterized by a classic triad of yellow dystrophic nails, lymphedema, and respiratory tract abnormalities, the diagnosis requires two of the three primary features, and the complete triad is observed in only 27 % of cases[2,3]. In our patient, nail and respiratory changes certainly occurred. Nail changes in YNS include slow growth, yellow and green discoloration, transverse and longitudinal over-curvature that results in a hyperconvex nail plate, onycholysis, shedding, and loss of lunulae and cuticles[4].

In some cases, YNS can be familiar or congenital[5]. Our patient has one son, one daughter and five grandchildren. Members of the family were carefully examined and there was no relatives with characteristic nail changes. Disease occurs sporadically in most adults. It has been reported in association with autoimmune diseases, such as thyroiditis, systemic lupus erythematosus, rheumatoid arthritis. YNS has also been described as a paraneoplastic syndrome in association with malignant diesases, such as lymphoma and breast, lung, larynx, endometrium, and gall bladder neoplasms[6]. None of these has been detected in our patient.

Respiratory manifestations usually include rhinosinusitis, bronchiectasis and exudative pleural effusion. Our patient presented with recurrent pleural effusion, which is considered late manifestation of YNS. In most cases, pleural effusion, unilateral or bilateral, is exudate, and that was the case in our patient. Thoracic drainage is often necessary. Further procedures such as pleurodesis are required in cases of early reccurence or in the cases of massive pleural effusion[7].

The etiology of the syndrome is not known. Pathogenesis seems to involve impaired lymphatic drainage. In patients with YNS, lymphangiography demonstrated hypoplastic, deficient, or sclerotic lymphatic vessels. Such abnormal lymphatic ducts are hypothesized to result in reduced lymphatic drainage, which leads to peripheral edema, pleural effusions, and nail changes when affecting the nail bed[8]. Some patients present with hypoalbuminemia, which is consequence of increased enteric loss of albumin[9].

The treatment of YNS is symptom based. Spontaneous improvement of nail changes is reported

in 30% cases especially if associated disease is treated[10]. Recent studies and case reports describe clinical improvement with the use of topical and oral vitamin E, oral zinc supplementation, oral anti-fungals[11,12]. Our patient was treated with NIPPV for exacerbation of chronic respiratory failure due to COPD. Hypercapnia improved, and patient was treated with oxygen therapy. Long term oxygen treatment was suggested. Pleural effusion did not reoccur under treatment during hospitalization. Thus, no further procedure was required. Patient was discharged in stable condition for home treatment.

Conclusion

Recurrent pleural effusion of unrevealed etiology is important problem in clinical practice. YNS is a rare disorder, but this diagnosis should be considered in patients with typical nail changes, unilateral or bilateral pleural effusions of unknown etiology, followed or not with other lymphedema, bronchiectasis or rhinosinusitis.

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Relations of the Congestive Cardiac Failure with Troponin I and CK-MB

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Abstract

The cardiac failure is a disease of a significant increase in incidence within the last two decades. In Turkey, like many other countries, the cardiac failure is the main reason of many deaths. In this study, the major structural characteristic of Congestive Cardiac Failure (CCF) is the myocardial cell death, and therefore the authors preferred to research the relation between the troponin I and cardiac CK-MB that are specific proteins for the cardiac necrosis.

Methods: In this study, the Troponin I and CK-MB levels were checked in 75 patients of congestive cardiac failure and a control group. The control group comprises of 60 people of comparable ages and genders with no cardiovascular or other diseases. While the ejective fraction was lower than 50% in all the 75 patients, it was over 50% in all individuals of the control group.

Results: The troponin I and CK-MB levels of the patient group were observed to be significantly higher in comparison with the control group. Furthermore, based on NYHA, the troponin I and CK-MB levels in the class III and IV patient groups were significantly higher than the levels of the class I and II groups.

Conclusion the congestive cardiac failure patients have significantly higher troponin I and CK-MB levels. The cardiac troponin I and CK-MB levels may be an appropriate and cost effective scanning test for such patients. It may however be useful to use such parameters in classification of the advanced level cardiac failure morbidity, mortality and short-long term prognosis.

Key words: Cardiac Failure, Troponin I, CK-MB, Ventricular Ejection Fraction

Introduction

The congestive cardiac failure (CCF) is the sole major cardiovascular disease of increasing incidence, prevalence and mortality throughout the world.[1,2] The cardiac failure is the final stage cardiac disease that develops after the entire myocardial reserve and compensatory mechanisms are deployed. One half of the patients die within 5 years in spite of an intensive medical treatment after emergence of the obvious signs.[3] In the advanced level CCF patients, the rate of mortality for 1 year is 40 to 50%, while it is 15 to 25% in those of slight-medium level symptoms.[4,5] The most important sign of survival in the congestive cardiac failure is the exercise tolerance, left ventricular ejective fraction (LVEF) and ventricular arrhythmia.[6,7] The cardiac troponin T and cardiac Troponin I are the highly specific and sensitive signs of the myocardial damage.[8,9,10] In particular, the cardiac troponin has become more specific for its amino acid sequence different from that of the musculoskeletal troponin.[11] Adams et al. has demonstrated that there is no cardiac troponin I release in the acute or chronic skeletal muscle damage without any cardiac muscular damage.[9]

The patient clinic and echocardiography have an important role in diagnosis of the cardiac failure. In this study, the major structural characteristic of CCF is the myocardial cell death, and therefore the authors preferred to research the relation between the troponin I and cardiac CK-MB that are specific proteins for the cardiac necrosis.

Material and Methods

Upon receipt of the ethical board approval, the subjects were chosen among the patients who were admitted in the emergency service units and

monitored in the cardiology polyclinics within the last one year.

Study Population: The study covered 95 patients of NYHA functional classes I, II, III and IV with the congestive cardiac failure diagnosis.

Criteria for Exclusion: Upon anamnesis, ECG and cardiac enzyme analysis, the patients who had acute myocardial infarction, unstable angina, and underwent surgical treatment for cardiac or non-cardiac reasons within the last 30 days before the study, and had acute and chronic pulmonary, renal or hepatic diseases, uncontrollable hypertension, and inflammatory or skeletal-muscular diseases were excluded. The remaining 75 patients were included in the study. 32 (42.7%) and 43 (57.3%) ones of the said patients had NYHA class I and II, and class III and IV symptoms respectively. The cardiac failure was associated with the ischemic cardiomyopathy, and the diagnosis was based on the clinical history, ECG and segmentary wall motion abnormality.

The control group comprised of 60 healthy people of comparable ages and genders with no cardiovascular or other disease, who were admitted to the cardiology polyclinic for check-up purpose. Written consents were obtained from all the patients who were properly informed.

Blood Sampling & Working Method: Venous blood samples were taken through 6 ml vacuum tubes from an antecubital vein for the cardiac troponin I and CK-MB determination in the patient and control groups. Hettich Zentrifugen Universal 32 centrifuge was used at 4500 rpm for the serum separation for 5 minutes.

The quantitative analysis of the cardiac Troponin I was made by immunofluorescence assay at 36°C for 15 minutes by means of Troponin I Kit (Innotrac Aio, Finland) at the Biochemical Laboratory of the Faculty of Medicine under the Cumhuriyet University. The normal value is less than 0,4 ng/ml.

The CK-MB quantitative analysis was made by immunoinhibition method by means of IL Test™CK-MB 18254540 (Instrumentation Laboratory, Italy) with ILab™ 900/1800 autoanalyser at the Biochemical Laboratory of the Faculty of Medicine under the Cumhuriyet University. The normal value of CK-MB is less than 24 U/L.

Statistical Analysis: The collected data were loaded in the SPSS Program (Ver=10.0) for comparison of the groups, where the significance test (t test) and Chi-square test (X^2) were applied for the difference between the two averages. The tables demonstrate the values as the average ± standard deviation ($-X \pm$ Se).

Results

The study covered 75 patients with congestive cardiac failure, and 60 healthy individuals of comparable ages and genders with no cardiovascular or other disease. There were 45 male (60.0%) and 30 female (40.0%) CCF patients. The control group comprised of 29 male (48.3%) and 31 female (51.7%) patients. Chi-square test demonstrated no significant difference for gender between the two groups ($X^2 = 1.83$; $p>0.05$). The CCF patients were 36 to 75 years old (62.14 ± 0.91 years), while the control group patients were 44 to 48 years old (59.26 ± 1.21 years) with the age difference between the two groups being found insignificant as a result of the t-test ($t=1.92$, $p>0.05$). The Table 1 shows the average ages and gender distribution of the patient groups.

Based on NYHA, the congestive cardiac failure patients included 32 ones (42.7%) with class I-II symptoms, and 43 ones (57.3%) with class III-IV symptom. See Table 2.

Chi-square (X^2) test demonstrated no significant statistical difference between the Class I-II and Class III-IV patients in the patient group ($p > 0.05$).

Table 1. Average ages and gender distribution of the patient and control groups.

Groups	Number of cases (n)	Average age (year)	Male/Female
Patient	75	62.14 ± 0.91	45/30
Control	60	59.26 ± 1.21	29/31

Table 2. NYHA classification of patients

Groups	Class I-II		Class III-IV		Total	
	n	%	n	%	n	%
Patient Group	32	42.7	43	57.3	75	100.0

Table 3 shows the ejective fractional distribution calculated upon echocardiography in the patient and control groups that participated in the study. For the ejective fraction, the difference between the two averages for the both groups was found statistically significant upon a significance test ($p<0.05$, $t=24.71$)

Table 3. Distribution of ejective fraction between groups

Groups	Ejective fraction (%) $X^- \pm Se$
Patient group	41.20 ± 0.67
Control group	65.10 ± 0.67
	t = 24.71 p < 0.05

Table 4 covers the distribution by cardiac troponin I and CK-MB values of patient and control groups included in the study. No significant difference was found between the groups in terms of the cardiac troponin I and CK-MB values ($p<0.05$).

CK-MB isoenzyme levels increased in the patient group of this study. CK-MB levels were higher than the upper reference limit of 24 U/L in 49 ones (65.3%) of the patients, while it was lower than 34 U/L in 26 ones (34.7%). Troponin I and CK-MB distribution between the two groups was statistically significant ($p<0.05$).

Table 5 covers the study patient group, including 32 ones (42.7%) with asymptomatic and moderate cardiac failure (NYHA class I-II, EF > 40-50%) and 43 ones (57.3%) with medium-severe cardiac failure (NYHA class III-IV, EF ≤ 40%). In these patients, the troponin I and CK-MB values in the patients of EF ≤ 40%, and the troponin I and CK-MB difference in those of EF > 40% were found significant ($p<0.05$) at the end of the significance test for the difference between the two averages.

Discussion

The congestive cardiac failure is a dynamic with a progressive rate and risk profile varying in time as to adversely affect the life quality, and significantly shorten the survival. The annual rates of mortality vary from 40% to 50% in the patients with NYHA functional class III and IV symptoms.[12,13] The symptomatic cardiac failure following an asymptomatic cardiac dysfunction is accompanied with the excessive myocardial reformation at the muscular, collagenous and vascular compartments. The advanced level cardiac failure includes a number of typical morphologic changes like the death of myocardial cells in the non-adjacent zones, which are then replaced with the fibrotic foci. [14,15] It is known that the congestive cardiac failure has an irreversible progression, but the underlying mechanisms are unclear, which is probably caused by the less sensitivity of the measuring methods deployed.[16]

The troponin proteins exist as the products of different genes in the cardiac and skeletal muscular tissue. Troponin has three separate sub-units: troponin C, troponin T and troponin I.[17] The cardiac muscular isoform of troponin I is secreted from the adult human myocardium, and the increase in circulation levels is highly associated with the

Table 4. Troponin I and CK-MB distribution between the patient and control groups

Groups	Patient group (n=75) $X^- \pm Se$	Control group (n=60) $X^- \pm Se$	Result
Troponin I (ng/ml)	0.78 ± 0.06	0.11 ± 0.01	t=10.67, p<0.05
CK-MB (U/L)	29.76 ± 1.87	10.88 ± 0.40	t=9.87, p<0.05

Table 5. Troponin I and CK-MB distribution of the CCF patients in NYHA classes I-II and III-V

Groups	Troponin I (ng/ml) $X^- \pm Se$	CK-MB (U/L) $X^- \pm Se$	N (%)
LVEF ≤ 40% Class III-IV	0.97 ± 0.07	32.92 ± 1.46	43 (57.3%)
LVEF 40%-50% Class I-II	0.60 ± 0.08	26.51 ± 3.42	32 (42.7%)
	t = 3.15 P = 0.002	t = 1.73 P = 0.017	75 (100%)

myocardial defect. While the increase in Troponin I levels was, till the recent times, used to determine the acute myocardial infarction, perioperative myocardial defect and the myocardial defect in critical patients[16], many actual studies demonstrate that the Troponin level I is associated with the degree and prognosis of the cardiac failure, while the treatment results in reducing its level, so that it could be used in treatment monitorin.[16,18-23]

The authors however found a significantly higher Troponin I levels in the congestive cardiac failure patients, compared to those of the healthy individuals. This significant high rate is compatible with the literature. [24-27]

In this study, the Troponin I and serum CK-MB levels in the cardiac failure patients were found to be significantly higher in the patient group, compared to the control group. The cardiac Troponin I and serum CK-MB levels in the cardiac failure patients resulted in important hypotheses. In particular, whether the serial measurements of cardiac Troponin I levels come with some advantages in the CCF control and in prevention of the progression of the disease is an important hypothesis. In the patients who have values higher than the normal limits, an optimal therapy would likely make it possible to normalize the cardiac Troponin I values, and some comprehensive studies are required to determine if this normalization is associated with a better CCF compensation and/or a better prognosis. The clinical methods may not always be successful in diagnosing the congestive cardiac failure, and there is a need of sensitive and specific biochemical tests that could detect the subclinical myocardial defect before a final cardiac failure appears. The authors are of the opinion that the cardiac Troponin I and serum CK-MB levels may be helpful in diagnosing the cardiac failure, and it may be an appropriate and cost effective scanning test for such patients. The utilization of sensitive biochemical indicators allows a more reliable estimation of the remodeling process rate, so that it may contribute to the development of therapeutic strategies.

This study does, to some extent, prove the increase in cardiac Troponin I and serum CK-MB levels in the CCF patients, and such indicators promise a simple way to define the suspected cardiac failure, and monitor the treatment efficiency by means of protein measurements through such indicators.

The determination of cardiac Troponin I and serum CK-MB levels may be useful in classification of the short and long term prognosis of the advanced cardiac morbidity and mortality. Furthermore, all such indicators may be suggested as a new indication for the CCF patients. Nevertheless, there is still a need for some further studies that would cover more epidemiological final points till the clinical value of cardiac Troponin I and serum CK-MB levels in the CCF patients is definitely explained.

Conclusion

When the Troponin I values for the patient group were separately compared with those of the control group, the difference was found statistically important. In this context, the CK-MB and Troponin Level I values of the patient group cases were found high. The Troponin I and CK-MB distribution based on the patient group CK-MB levels revealed a significant statistical difference between the two groups. Furthermore, the patient group cases were divided into two groups as the class III-IV (EF ≤40%) and class I-II (EF ≤40-50%), and then the Troponin I values of the two groups were compared, whereupon a statistically significant difference was found in terms of Troponin I between the two groups. The patient group cases were divided into two groups as the class III-IV (EF ≤40%) and class I-II (EF ≤40-50%), and then the CK-MB values of the two groups were compared, whereupon a statistically significant difference was found in terms of CK-MB values between the two groups.

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The role of alcohol in fatal work related injuries

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Abstract

In this retrospective autopsy study, a detailed analysis of the role of alcohol in fatal work related injuries is presented. In the period from 1996 to 2008, 16.261 forensic autopsies were carried out at IFM in Belgrade, Serbia. Out of this number, in 519 cases (3.2 %) cause of death was connected with injuries sustained at work. The fatally injured at work were most frequently male persons aged between 41 and 50. Alcohol in blood was detected in 81 cases (15.6 % of 519 or 24 % out of 337 cases in which blood alcohol testing was performed). In the majority of cases the blood alcohol concentration (BAC) ranged from 0.5 to 1.5 gram pro mille (‰). The results have shown that a great number of workers consume alcohol before and during the work hours. For this reason it is necessary to take measures for stricter control of workers both incoming to work and during the work hours.

Key words: Alcohol, fatal work related injury, forensic autopsy

Introduction

Injuries at work take high position in morbidity of workers and thus have social-medical and social-economic importance because they lead to absence from work, reduced productivity of work, disability, and sometimes even to death [1-4]. Injury at work represents an injury which is related to the particular job covered by the health insurance and which happens to the worker performing that job; the injury may be caused by mechanical, physical or chemical action, sudden change of position of the body, abrupt loading of the body or other changes of physiological condition of the organism. According to legal regulations in Serbia, work related injury includes any injury which entails inability to work in the period of at least one day. Injuries at work encompass also injuries sustained during coming to or back from work, as well as those suffered at business trips.

In the study of any accidents, particularly in work related accidents, correctly identifying high risk groups and quantifying the risk of deaths and injuries is the key to successful interventions. Among numerous factors that lead to injuries and deaths at work (age, gender, work experience, working conditions, etc.) [5-6], alcohol takes significant place [7]. Alcohol is a depressant that decreases response of the central nervous system. As little as two alcoholic beverages can impair coordination and thinking [8].

The consequences of alcoholism observed at work are mainly: absence from work, loss of interest in work, varying mood, inclination to criticism and opposition, frequent interpersonal conflicts, giving too much importance to own work and diminishing other people's work, as well as injuries at work. Alcoholics get injured at work much more frequently and become disabled more often than non-alcoholics.

The influence of alcohol in fatal accidents at work may become a great social issue, which is a potential task in forensic post-mortem investigation, about which there are few data available in the literature. Therefore, the purpose of this study was to determine the role of alcohol in fatal work related injuries in Belgrade, Serbia, and to present important medico-legal characteristics of these accidents.

Materials and methods

The analysis presented in this paper is a part of much larger study of all work related traumatic deaths that occurred in Belgrade and surrounding areas. All the cases of fatal work injuries from a 13-year-long period (1996-2008) autopsy material of the Institute of Forensic Medicine (IFM), Faculty of Medicine, University in Belgrade, were analyzed.

Blood samples were taken from femoral vein and analyzed in the toxicological laboratory of IFM using gas chromatography. A " HEWLETT PACKARD“ model 5890 series II gas chromatograph with a flame ionization detector (250° C) was interfaced with a “HEWLETT PACKARD“ headspace sampler 19395A (Column: Permabond CW20M-0,35µm, 25m, ID 0,32mm, temp. 40° C).

Statistical processing of data was performed using Chi-square test, with SPSS version 13.0 software for windows. For all tests P values less than 0.05 were considered to be statistically significant.

Results

In the period from 1996 to 2008, 16.261 forensic autopsies were carried out at IFM in Belgrade. Out of this number in 519 cases death was caused by injury at work, which represents 3.2 % of all autopsies. The average age of the persons fatally injured at work was 44.7 ± 11.6, there were 93.3 % (484) males, and 6.7 % (35) females.

Presence of alcohol in blood was investigated in 337 fatally injured persons (64.9% of 519), while in 182 (35.1 %) cases chemical analysis was not performed due to outliving period longer than 24 hours. Alcohol in blood was detected in 81 fatally injured individuals (15.6 % of 519 or 24 % out of 337 in which blood alcohol testing was performed), since 256 (49.3 % of 519 or 76 % of 337) were not under influence of alcohol in the moment of injuring (Figure 1).

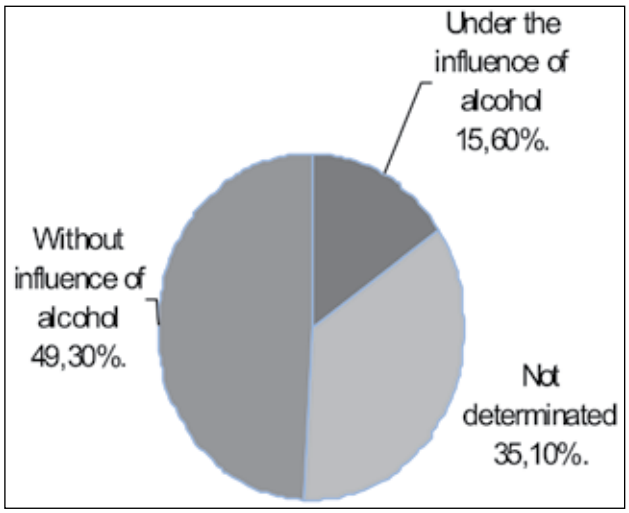

Figure 1. Distribution of 519 persons fatally injured at work according to presence of alcohol in blood

Figure 2 shows the annual percentage of persons under the influence of alcohol who were fatally injured at work in the period from 1996 to 2008 in regard to the total annual number of fatally injured.

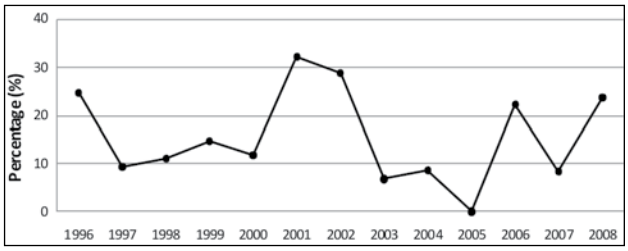

Figure 2. Annual percentage of persons under the influence of alcohol who were fatally injured at work in the period from 1996 to 2008 in regard to the total annual number of fatally injured

The average age of 81 persons fatally injured at work with proved alcohol in blood was 45.8 ± 9.8, and 79 (97.5 %) of them were males and 2 (2.5 %) females. The presented values regarding age and sex of individuals under the influence of alcohol are similar to the corresponding values in the whole group of all fatally injured at work. Out of all 484 fatally injured men, 79 (16.3 %) were under influence of alcohol, while this percentage was much lower in the female group - 5.7 % (2 out of 35).

Those fatally injured at work were most frequently persons aged between 41 and 50, both in the whole group of 519 and in the group of 81 individuals under the influence of alcohol, whereas the smallest number was in the age group above 60 (Table 1). The age distribution in the group of persons under the influence of alcohol was not significantly different comparing to the corresponding distribution in the whole sample of fatally injured at work (χ^2=3,974, p=0,553). The percentage of individuals under the influence of alcohol was highest in the age group between 41 and 50 (18.2 %) and between 51 and 60 (18.0 %).

The determined BAC in the fatally injured at work varied between 0 and 4.4 pro mille. According to the blood alcohol content (BAC), all the persons under the influence of alcohol fatally injured at work were categorized into 5 groups (Figure 3). In the majority of persons under the influence of alcohol (33 or 40.7 % of 81) BAC ranged from 0.5 to 1.5 pro mille (50-150 mg/100 ml).

Table 1. Distribution of fatal work related injuries according to age of injured and presence of alcohol in blood

Age group	A all injured		B performed BAC testing		C positive blood alcohol		% C in A	% C in B
	No	%	No	%	No	%		
18 – 30	72	13.9	48	14.2.	8	9.9	11.1	16.7
31 – 40	98	18.9	63	18.7.	12	14.8	12.2	19.1.
41 – 50	176	33.9	115	34.1	32	39.5	18.2	27.8.
51 – 60	133	25.6	84	24.9	24	29.6	18.0	28.6.
> 60	40	7.7	21	8.1.	5	6.2	12.5	23.8.
Total	519	100	337	100	81	100	15.6	24.0

Table 2. Distribution of fatal work related injuries according to the place of accident and presence of alcohol in blood

Place of accident	A all injured		B performed BAC testing		C positive blood alcohol		% C in A	% C in B
	No	%	No	%	No	%		
coming to work	46	8.9	27	8.0	5	6.2	10.9	18.5
back from work	59	11.4	38	11.3	18	22.2	30.5**	47.4
business trip	34	6.5	26	7.7	5	6.2	14.7	19.2
own workplace	325	62.6	213	63.2	46	56.8*	14.2	21.6
foreign workplace	55	10.6	33	9.8	7	8.6	12.7	21.2
Total	519	100	337	100	81	100	15.6	24.0

** p≈0.020 (Pearson chi-square test) ** p≈0.020 (Pearson chi-square test)*

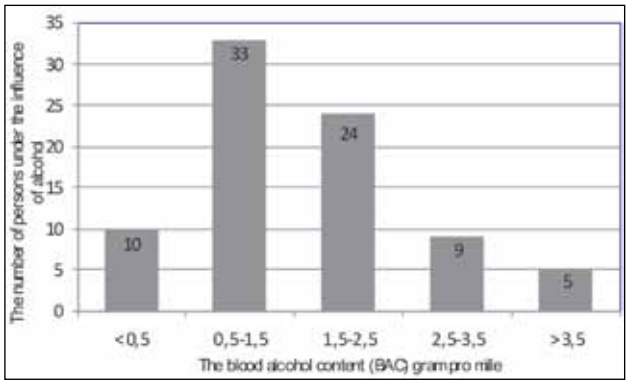

Figure 3. Distribution of 81 persons under the influence of alcohol fatally injured at work according to the blood alcohol content (BAC)

Regarding the place of injuring, the majority of fatal accidents occurred at the work place of injured person, both in the whole group of 519 cases (325 or 62.6 %), and in the group of 81 individuals under the influence of alcohol (46 or 56.8 %), and these percentages are statistically significantly higher comparing to the corresponding values for other places of accidents (χ^2=11,619, p≈0.020 in the group of people under the influence of alcohol) (Table 2). On the other hand, the highest percentage of persons under the influence of alcohol in regard to the total number of all fatally injured was found in the individuals who get injured on the way back home from work (30,5 %), which was statistically significantly higher than in other groups in which this percentage varied between 10.9 and 14.7 % (χ^2=11,619, p≈0.020).

In regards to the way of getting injured at work, all fatal accidents were categorized into 9 groups (Table 3). The most of fatal injuries occurred in traffic accidents, both in the whole group of 519 fatally injured at work (185 or 35.6 %) and in the group of individuals under the influence of alcohol (39 or 48.1 % out of 81). The determined percentages are statistically significantly higher comparing to the corresponding values for other types of accidents (χ^2=20.982, p=0,007 in the group of people under the influence of alcohol). The highest percentage of persons under the influence of alcohol in regard to the total number of all fatally injured individuals was found in the group of fatal poisonings at work (36.8 %), which was statistically significantly higher than in other group of accidents.

Table 3. Distribution of fatal work related injuries according to the type of accident and presence of alcohol in blood

Type of accident	A all injured		B performed BAC testing		C positive blood alcohol		% C in A	% C in B
	No	%	No	%	No	%		
traffic accidents	185	35.6	128	38.0	39	48.1*	21.1	30.5
falls	117	22.5	40	11.9	16	19.8	13.7	40.0
falling objects	60	11.6	25	7.4	3	3.8	5.0	12.0
powered machinery	54	10.4	30	8.9	9	11.1	16.7	30.0
electrocution	36	6.9	11	3.3	4	4.9	11.1	36.4
explosion	32	6.2	4	1.2	1	1.2	3.1	25.0
poisoning	19	3.7	60	17.8.	7	8.6	36.8	11.7
fire	11	2.1	36	10.7	1	1.2	9.1	2.8
non-classified	5	1.0	3	0.9.	1	1.2	20.0	33.3
Total	519	100	337	100	81	100	15.6	24.0

** p≈0.007 (Pearson chi-square test)*

Table 4. Distribution of fatal work related injuries according to working field and presence of alcohol

Working field	Positive blood alcohol		Negative blood alcohol		Not performed BAC testing	
	No	%	No	%	No	%
Agriculture, forestry	8	9.9	30	11.7	28	15.4
Building industry	28	34.6	63	24.6	65	35.7
Trade	10	12.3	26	10.2	11	6.0
Electricity, gas and water	5	6.2	18	7.0	7	3.8
Transport storage and comunication	11	13.6	33	12.9	21	11.5
Health and social work	0	0	3	1.2	1	0.6
Public administration	2	2.5	11	4.3	4	2.2
Manufacturing	10	12.3	36	14.1	29	15.9
Community services	1	1.2	15	5.8	7	3.8
Mining and quarrying	2	2.5	12	4.7	3	1.7
Hotels and restaurants	0	0	4	1.6	3	1.7
Financial business	4	4.9	5	1.9	3	1.7
Total	81	100	256	100	182	100

Table 4 shows the influence of alcohol in fatal injury to workers by working fields. 34.6% of fatal injury to workers has been in the building industry, while in other branches of activities, the influence of alcohol has been significantly lower.

Discussion

Injuries at work present a serious problem, both for employees and for employers, as well as for the society as a whole. Defining accidental factors which lead to injuries at work makes better organization of safety at work and prevention of these injuries possible. One of the potential accidental factors is certainly alcohol. It is well known that alcohol increases self-confidence, reduces attention and criticism, slows down reflexes, induces disturbances in the sphere of sight, hearing, balance, causes drowsiness and other disorders, all being preconditions for accidental injuring at work.

On the territory of the city of Belgrade, in the period from 1996 to 2008, 16.261 forensic autopsies were carried out, and in 519 cases fatal accidents at work were registered. The presence of alcohol in blood was toxicologically proved in 81 cases (15.6 % of all 519 fatal accidents or 24 % out of 337 in which blood alcohol testing was performed). In 182 (35.1 %) persons fatally injured

at work toxicological analysis was not performed as the injured individual outlived injury longer than 24 hours, which enabled eventually present alcohol to be metabolized and eliminated from the body. Therefore, a real number and percentage of persons fatally injured at work under influence of alcohol could have even been larger than the above presented. On the basis of the obtained results, it could be concluded that determination of alcohol must be performed as soon as possible in all individuals that are admitted to hospital to be treated for injuries sustained at work.

The average age of the persons fatally injured at work was 44.7, in the group of people under the influence of alcohol 45.8, and most of them belong to the age group ranging from 41 to 50. The similar results were obtained by other researchers [6, 9-10]. This is the age when a worker is in the best physical condition and capable of achieving the best productive effects. Taking into account the active work life of employees covered by health insurance, it comes out that each worker would have done his job for about 20 years more if he had not been killed at his work place. In the same age group (between 41 and 50) the highest percentage of individuals under the influence of alcohol was determined (18.2 %) comparing to other age groups.

The results of our study show obvious dominance of males (93.3 %) among victims of fatal accidents at work. This was even more expressed in the group of 81 people under the influence of alcohol, in which 97.5 % of individuals were males. It has been demonstrated in other countries too that men are more injury-prone at work places than women [11-12]. As we have expected, there was a higher percentage of men fatally injured at work under influence of alcohol comparing to women (16.3 % vs. 5.7%). All the above mentioned results point to conclusion that alcohol consumption represents far more important accidental factor for injuring at work in males than in females. However, these results should be interpreted with some caution, since it is known that in some high-risk jobs most employees are usually men, while in some others, often less dangerous, we can find mostly women [11-12].

In the majority of persons under the influence of alcohol the BAC ranged from 0.5 to 1.5 gram pro mille (50 - 150 mg/100 ml). It is well known that this alcohol level in blood leads to very expressive psychic changes characterized by subjectivity, weakened criticism, thoughtlessness and elevated self-confidence [13]. Furthermore, this BAC is connected with incremental loss of motor response. All these pathophysiological changes influenced by alcohol consumption may significantly contribute to fatal injuring at work. It is of high concern to point out that in 6.2 % of the persons under the influence of alcohol fatally injured at work the BAC was higher than 3.5 gram pro mille (350 mg/100 ml). This stadium of drunkenness, which is characterized by stupor or coma [14-15], completely diminishes working abilities leading to high risk of sustaining injury at work.

The obtained results show that the majority of fatal accidents occurred at the work place of injured person, which was noticed as very significant both in the whole group of fatal injuries at work and in the group of 81 individuals under the influence of alcohol. All these data indicate the dramatic fact that a great number of workers consume alcohol during work hours. It was also noticed that high risk for sustaining injuries exists on the way back home from work. Namely, the highest percentage of individuals under the influence of alcohol (30.5 %) was registered in the group of individuals who were fatally injured on the way back home from work, comparing to 14.2 % in the group of workers that were injured on their own work place.

The most frequent type of injuries at work were traffic accidents, both in the whole group of 519 fatally injured at work (35.6 %) and in the group of 81 individuals under the influence of alcohol (48.1 %). The high percentage of persons under the influence of alcohol fatally injured in traffic accidents confirms the negative effects of alcohol upon people in traffic [8, 16]. The fatal falls are on the second place regarding frequency of fatal cases. In the whole group of fatal accidents at work they account for 22.5 %, and in the group of individuals under the influence of alcohol for 19.8 % of cases.

Regarding different type of fatal accidents at work, the strongest influence of alcohol consumption was detected in the group of poisonings and traffic accidents, since in these two groups the highest percentage of individuals under the influence of alcohol was determined (36.8 % and 21.1 % respectively). In 7 out of 19 fatal intoxications at

work, the poisoning by methanol was diagnosed, and it was caused by drinking of impure alcoholic beverages at work place. A similar study has been made in Turkey, where in a 12-year-period from 1992 to 2003 there were 18 cases of methanol poisoning recorded [17].

The results of our investigation indicate a significant influence of drunken state on fatal injuring at work. However, it could be assumed that drunken state as an accidental factor cannot be observed only trough its influence on the injured worker under the influence of alcohol. Furthermore, other workers under the influence of alcohol could have caused the accident with resultant injuring of workers not under the influence of alcohol, while they irrespective of their drunken state may remain uninjured. In our study, in more than one third of cases the blood alcohol analysis was not performed due to the outliving period longer than 24 hours. Therefore, in these cases eventual accidental influence of alcohol could be neither confirmed nor excluded. All these facts point to necessity of thorough investigation of all fatal accidents at work, including taking of blood samples as soon as possible not only from the injured person but also from those in their work surrounding who could have induced or contributed to the accident.

All the above presented tells us that there is an urgent necessity to start taking as radical measures as possible to prevent consumption of alcoholic beverages before and during working hours, which could be completely or at least to great extent eliminated thanks to efforts of the society and awareness of individuals.

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Evaluation of Mandibular Fractures in Children during Five years' in a Dental School

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Abstract

Mandibular fractures are rarely encountered among the pediatric population, and when they do present, their clinical features differ from those in adults. This retrospective study looked at the age, sex, type and cause of fracture, treatment method and associated injuries and complications in 260 cases of mandibular fracture in children under age 16. Among the most significant findings: Fracture incidence had a 3:5 male:female ratio; 52% of all mandibular fractures involved condyle/subcondyle fractures and 50.7% involved symphysis/parasymphysis fractures; the most common treatment method was intermaxillary fixation; in no cases were severe complications observed during the healing period.

Key words: Mandibular fractures, children, intermaxillary fixation.

Introduction

Facial fractures in general and mandibular fractures in particular are rarely encountered among the pediatric population. Among the reasons for this are the soft and elastic structure of the developing mandible, the relatively small facial area when compared to the size of the skull, limited facial bone pneumatization and the greater protection against trauma provided to children under age five.[1-3] After this age, a relative increase in the frequency of mandibular fractures can be observed, largely in connection with the start of school attendance and participation in social activities.

The main concern in treating mandibular fractures is achieving anatomical stabilization of bone segments so as to provide maximum function. In view of continuing facial and dental development during childhood, treating mandibular fractures in children requires a different strategy than treating adults. Appropriate treatment of mandibular fractures in children has long been the subject of debate. Treatment may consist of either immobilization using intra-osseous fixation or maxillomandibular fixation (MMF) or therapy that emphasizes early movement and normal eating habits.[4] [The most popular treatment method is 10-14 days of immobilization with MMF, followed by one week or 10 days of careful eating habits.

In view of the continuing growth of the alveolar process and mandible, as well as possible tooth damage, conservative, non-invasive treatment using a splint is recommended over surgical treatment.[5] However, open reduction may be indicated in children in extreme situations in order to restore ramus length and prevent progressive deformity[6] Recently, biodegradable bone fixation has become a viable treatment option, particularly for pediatric patients. This treatment method offers several advantages, including faster mobilization and avoidance of secondary surgery.[7]

This retrospective study reviewed the treatment of pediatric mandible fractures in children in terms of age, sex, type and cause of fracture, treatment method and associated injuries and complications.

Materials and Methods

This retrospective study examined the records of all children under age 16 who presented with mandibular fractures at the Dicle University Faculty of Dentistry's Department of Oral and Maxillofacial Surgery in Diyarbakir, Turkey between January 2000 and December 2005. Fractures were diagnosed through clinical examination, conventional radiographs and computerized tomography. Factors examined included age, sex, etiology, anatomic location, associated injuries, treatment methods and complications.

Results

Of a total of 260 patients under age 16 presenting with mandibular fractures, 156 (60%) were female and 104 (40%) were male. The majority (28%) of mandibular fractures occurred in children aged 7-9 years, followed by children aged 4-6 (22%), children aged 10-12 (21%), children aged 13-15 (15%) and children aged 0-3 (14%) **(Table 1).** The most common cause of mandibular fracture was falling from heights (n=175, 67%), followed by traffic accidents (n=52, 19.9%), animal kicks (n=10, 3.9%), assaults (n=9, 3.5%), sports injuries (n=6, 2.3%) and epileptic seizures (n=6, 2.3%). In addition, 2 patients (0.7%) sustained avulsive mandibular fractures as a result of gunshot injuries **(Table 2).**The most frequent fracture sites were the condyle/subcondyle (n=135, 52%) and the symphysis/parasymphysis (n=132, 50.7%) **(Table 3).** Whereas 212 patients (81%) suffered isolated mandibular fractures, in 48 patients (19%), fractures were accompanied by other injuries **(Table 4).**

Table 1. Age distribution of pediatric patients with mandibular fractures

Age (Years)	Number	%
0-3	37	14
4-6	57	22
7-9	72	28
10-12	54	21
13-15	40	15
TOTAL	**260**	**100%**

Table 2. Etiology of mandibular fractures in children under age 16

Cause of Fracture	Number	%
Fall from Height	175	67.0
Traffic Accident	52	20.0
Animal Kick	10	3.9
Assaults	9	3.5
Sports Injury	6	2.3
Epileptic Seizure	6	2.3
Gunshot Injury	2	1.0
TOTAL	**260**	**100%**

Conservative treatment methods such as arch bars were used in the majority of cases (n=220, 84.4%). In 28 patients (11%) who were diagnosed with stable, non-dislocated fractures, treatment consisted of dietary/oral hygiene recommendations and periodic examination only, 10 patients (3.8%) were treated using mini-plate osteosynthesis (MPO) and reconstruction plates and 2 patients (0.8%) who underwent surgery for gunshot wounds received autogenous bone grafts **(Table 5).** In all cases, fractures healed successfully, with no complications observed during the healing period. Notably, no facial asymmetry or temporomandibular ankylosis was observed in patients with condyle/subcondyle fractures, although minimal occlusal disharmony was observed in patients with mixed dentition.

Table 3. Distribution of fracture sites

Fracture Location	Number	%
Condyle/subcondyle	135	52.0
Symphysis/parasymphysis	132	50.7
Alveolar process	45	17.3
Body	26	10.0
Angle	21	8.0
Ramus	1	0.4
Coronoid	0	0.0

Table 4. Other fractures/injuries associated with mandibular fractures

Injury Type	Number	%
Maxillofacial injury		
Maxilla	9	3.5
Zygoma	2	0.8
Nasal	8	3.0
Orthopedic injury	14	5.4
Cranial injury	5	2.0
Other injuries		
Tongue laceration	5	2.0
Scalp laceration	3	1.2
Liver contusion	2	0.8

Discussion

This retrospective study looked at the age, sex, type and cause of fracture, treatment method and associated injuries and complications in 260 cases of mandibular fracture in children under age 16. While previous studies[8-11] have reported that mandibular fractures occur more frequently in males than females in all age groups, our study found a male: female ratio of 3:5. Differences in fracture incidence between males and females may be related to educational and socio-economic environments

Table 5. Distribution of mandibular fractures by treatment type/method

Type of Treatment	Treatment Method	Number	%
Observation (no treatment)	Recommendations for soft diet and oral hygiene	28	11
Conservative treatment	Arch bar + MMF	176	68
	Circummandibular wires + occlusal splint	23	8.4
	Inferior arch bar	11	4.2
	Interdental cerclage	9	3.4
	IVY Loops	1	0.4
Open reduction	MPO	10	3.8
	Reconstruction plate + graft	2	0.8

(e.g., non-school attendance of girls, differences in work/play patterns between boys and girls).

The incidence of mandibular fracture has been reported to be lower among pre-school-age children than among adults[12-13]. Earlier studies[14-15] have found fracture incidence to be close to 5% among children under age 12 and only 1 percent among children under age five. In our study, mandibular fracture was observed most frequently among children aged 7-9. Once children reached the age of 9, incidence levels began to decrease.

Thoren[16] found the condylar region to be the most common fracture site among all pediatric age groups, accounting for 60% of all fractures. Posnick[17] also reported that most mandibular fractures occurred in the condyle region (55%), followed by the parasymphyseal region (27%), body (9%) and angle (8%). Our study also found the most common mandibular fracture sites to be the condyle/subcondyle (52.0%) and parasymphyseal/symphyseal (50.7%) regions.

Several studies[18-20] have cited motor vehicle accidents and falls as the most common causes of pediatric mandibular fractures. Our study also found the most common etiological factor in mandibular fractures among children to be falling from heights. However, in contrast to other studies, our series also reported mandibular fractures resulting from animal kicks related to living in a rural area, from a lack of awareness of appropriate intervention during epileptic seizures, and from gunshot wounds resulting from children playing with firearms in the home.

Limited sinus pneumatization and the greater amount of cancellous bone compared to cortical bone increase the elastic stability of the mandible during the pediatric period, so that more powerful trauma is required for the mandible to fracture in children in comparison to adults. As a result, cervical vertebrae and cranial injuries are often encountered in conjunction with pediatric mandibular fractures. Our study found that mandibular fractures were accompanied by other injuries in 20.3% of cases. With the exception of one case of avulsive mandibular fracture resulting from a gunshot injury (Figure 1,A,B,C), no airway obstruction was observed in any patient in our study.

Figure 1. A Preoperative view

Figure 1. B Postoperative view

Figure 1. C Postoperative panoramic view

In treating mandibular fractures, the objective is to ensure the integrity of the mandible without causing asymmetry, malocclusion or functional deficiency. Conservative methods are usually used to treat mandibular fractures in pediatric patients because the high level of cancellous bone makes it difficult to apply internal fixation. However, in the case of severely displaced and unstable fractures, open reduction must be considered.

The location of the fracture is also an important factor in treatment selection, with closed reduction techniques preferable in condyle fractures. Although surgery can be avoided in non-dislocated fractures when the anatomical basis for function has remained largely intact, displaced and dislocated condyle fractures treated with open reduction have lower incidences of facial asymmetry, locking and occlusal imbalance.[21] In view of the continuing growth of the condyle until adulthood, post-treatment follow-up is important; a pediatric patient who has not been well treated may eventually suffer progressive impairment of mandibular and facial development[22-23]

In recent years, biodegradable bone fixation has become an attractive alternative to open-reduction techniques. The use of resorbable fixation materials offers many advantages that are of particular benefit for the pediatric population, including shorter immobilization periods and elimination of painful procedures associated with implant removal. However, potential problems. Such as applying mixed dentition, the possibility of tooth germs damage, for this reason, urgency of monocortical implement and in displaced fractures sufficiently rigidity can not be provided.

In conclusion, in line with the literature and our clinical experience[24] suggests that conservative (closed reduction) protocols are preferable in treating mandibular pediatric fractures. However, open reduction and internal fixation with screws or plates is required in cases where mandibular fractures are unstable, highly displaced or malunited.

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Designing productivity indicators in healthcare department: A case of pediatric orthopedics

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Abstract

Objective: To define productivity indicators in healthcare department which reflect department functioning and enable productivity measurements over years.

Methods: A combination of two methodologies, the Seven Steps of Determining Productivity Indices and the Objectives Matrix Method, was applied at the Department for Pediatric Orthopedics and Traumatology of the Institute for Orthopedic Surgery "Banjica" in Belgrade in order to determine and analyze productivity indicators during the period from 2005 to 2010.

Results: Based on Departments' measurable inputs and outputs, productivity indicators were defined, divided in three categories: department, surgeons and nurses. By observing single indicators in Objectives Matrix, the score increased for average length of hospital days which shortened over the observed six years. The relationship between the number of surgeries and the number of working hours in the operating theater increased, as well as the indicators of the productivity of nurses. Productivity indicators of the Department regarding ambulatory working hours were constant in case of ultrasound exams, while those regarding emergency and consultative exams were decreased.

Conclusion: Although the methods of the Seven Steps of Determining Productivity Indices and Objectives Matrix have been developed for the needs of industrial organizations, the paper shows that their application is possible in healthcare services as well. Productivity follow-up using indicators and total weighted score during years, enables healthcare managers to analyze low-productivity fields where improvements are needed.

Key words: Productivity, Indicators, Healthcare Department, Method

Introduction

In the business world productivity is viewed as a key factor of success. The concept of productivity has existed for a long time and has been analyzed for over two hundred years (Chew, 1988; Rutkauskas et al., 2005). There is no universal definition of productivity. It is a multidimensional concept and its meaning depends on the context (Tanagen, 2005). The productivity concepts can be viewed in relation to different fields and dominant aspects, i.e. the technological, engineering and the economic aspects (Ghobadian et al., 1990; Zhang, 2011).

Productivity is generally defined as the ratio between output and input (Rutkauskas et al., 2005). This traditional general definition has, from the economic standpoint, been described as a measure of resource utilization to achieve specific results (Bain, 1982). Also, productivity, efficiency and effectiveness were used in combination and sometimes as alternatives of each other. However, productivity requires both efficiency and effectiveness (Rutkauskas et al., 2005). Productivity is the effective realization of activities in an efficient manner (Benefield, 1996). Quality is an integral part of productivity and they should have a positive relationship which is based on logical reasoning and some mathematical models (Langford et al., 2000, Sahay, 2005). Moreover, productivity is a component of performance and it can be measured as a tool for improving performance of the system (Sink, 1983).

In cases of manufacturing organizations productivity measurement is most often clearly defined by various quantitative indicators. Productivity in the service sector has become more important at the end of the 20th century demanding a specific approach (Hollingsworth, 1999). This becomes particularly significant in terms of healthcare services and healthcare industry, which is

further contributed by the intangible nature of output services due to the limited knowledge of what we are measuring and what is the product - visits or health (Linn et al, 1984).

Productivity indicators are to be regarded as measures in the field of healthcare services providing useful information to management when planning future activities and in decision-making in the process of resource allocation (Harris, 2010). However, the field of healthcare service productivity evinces some characteristics that make its measurement more difficult than in other fields. There are usually several inputs, as well as difficulties in the determination and measurement of outcome, i.e. improved health or improved quality of life (Kooreman, 1994). As the result of recognizing the problem of outcome measurement, output has been measured in units of service or units of measure, e.g. by patient days, discharge, procedures, cases, satisfactory, i.e. healthier state or unsatisfactory, i.e. morbidity or mortality, or in terms of patient's age, type of treatment (Chilingerian, 1993; Batten, 1984). Capital as measure of input has been used most often. It has been presented by the number of hospital beds, occupancy rate, or depreciation and interest expenses per bed (Worthington, 2004). The essential part of productivity is also the effective use of time (Harris, 2010).

Productivity measurement can be performed at two levels, namely, at the level of single activity or unit and at the level of the whole organization (Mammone, 1980). It is necessary to measure and improve productivity in all areas so as to achieve the overall productivity improvement of the entire organization, which is reflected in organization mission statement and clients' demands for services. The measurement of total productivity is needed in order to improve internal efficiency and the competitiveness of a business unit. (Hannula, 2002)

Techniques for measuring productivity in healthcare services

Today, the number of studies which seek to measure health service efficiency and productivity continues to increase quite dramatically (Hollingsworth, 2008). According to Hollingsworth (2008) there is still a large disparity between the number of the studies published and the actual use of efficiency measures in this area in practice. In the healthcare literature, different analyses as means for measuring productivity could be found. Data Envelopment Analysis (DEA) is a technique particularly appropriate when multiple outputs are produced from multiple inputs. It is one of the most used methods of analysis (Hollingsworth, 2003; Nayar and Ozcan, 2008; Zuckerman et al., 1994). The next analytic tool is Stochastic Frontier Analysis (SFA) where output is a function of a set of inputs, inefficiency and random error (Rosko and Mutter, 2008). Free Disposal Hull (FDH) analysis was designed as an alternative to DEA models, where only the strong (free) disposability of inputs and outputs is assumed (Podinovski, 2004). Malmquist Productivity Indices (MI) is used to measure change in efficiency and productivity over time (Maniadakis and Thanassoulis, 2000). Reinhardt developed the production function model where the physician's output is a function of personal labor inputs (i.e. hours worked), capital inputs, other labour inputs and practice characteristics (Reinhardt, 1972). Some authors (Gaynor and Pauly, 1990; Conrad et al., 2002) have extended this framework to a behavioral setting in which physician characteristics, organizational design features, market characteristics and financial incentives are included in the production function.

The application of methods for productivity measurement in manufacturing has a long-standing tradition. One of the methods is the Objective Matrix for productivity measurement developed by Riggs (Riggs, 1984). Productivity indicators in the Objective Matrix can contain the ratio between output and input, or it can be given as a value in original units, e.g. average costs, demanded time for operation, etc. Simplicity and generality of the method allow its application in various kinds of businesses, in specific business situations and cases. According to Felix and Riggs (1983), an Objectives Matrix model enables the management of an organization to combine all important productivity criteria into easily communicated format. The method suggested as a guideline to researchers in designing their own productivity measurement is the Seven Steps of Determining Productivity Indices (Brinkerhoff and Dressler, 1990). At each step specific actions should be performed in order to construct productivity measures relevant for

an organization or a department. Compatibility of these two methods was implemented by Rahman and Ismail (2004) to determine productivity measures for individual workers in maintenance work environment. According to inspected literature, information on relevant practices and available knowledge sources, these methods have not found application in healthcare systems.

Characteristics of healthcare in Serbia

The Serbian healthcare system has slowly changed over the past years due to a politically unstable environment and insufficient economic development. The healthcare system and especially hospital healthcare, is over-dimensioned, inefficient, and expensive (Tosic, 2002; Matovic Miljanovic and Jankovic, 2006). Serbian hospitals are characterized by excessive physical capacity, cumbersome service delivery systems with high levels of medical interventions inconsistent in clinical quality, staff surpluses, and human resource management problems. In Serbia, hospital healthcare is provided by 128 inpatient facilities, of which 37 are special hospitals. The implementation of reforms has gradually resulted in the changes of indicators for healthcare utilization, especially in inpatient health facilities. In 2005, the total number of standard hospital beds was 44.142 or 6 beds per 1000 population, the rate which was gradually decreased over years, so that in 2009 it was 5.60 beds per 1000 population. Also, the average length of hospital stay per patient over a year was reducing, whence the average bed occupancy rate was growing. In 2005, the average length of hospital stay per patient was 10.6 days, and the average bed occupancy rate was 71.87% (Health Statistical Yearbook of the Republic of Serbia, 2007). In 2009, the average length of hospital stay per patient was 8.8 days, and the average bed occupancy rate was 74.50%. (Health Statistical Yearbook of the Republic of Serbia, 2007). As in other countries with non-profit hospitals, in Serbia the efforts have also been made to maximize the quantity and quality of provided care (Gruca and Nath, 2001). In 2004, the first act was passed in the field of healthcare quality improvement, and in 2005, by Health Care Law continual improvement of healthcare quality was mandatory. Thus, the conditions were created for the formulation of quality indicators and their continual follow-up (Regulation on Healthcare Quality Indicators, 2005). This enables a follow-up of single productivity component, i.e. quality (McCarthy, 2007), but in many cases, hospital performance and productivity are not yet at a satisfactory level. This was a step made with the goal to reform the healthcare system in Serbia and to adapt it to the European standards. Also, the Serbian Government, the Ministry of Health and the Institute of Public Health brought numerous strategies and strategy documents to support the current reform (Ministry of Health of the Republic of Serbia, 2011).

In accordance with healthcare system changes in the European countries (Kastberg and Siverbo, 2007; Garcia-Lacalle, 2010), hospitals in Serbia are also forced to adapt to new requirements and pressures which are being imposed. Productivity measurement in healthcare services is a new approach in Serbia which could ensure a more realistic framework for policy-makers. It would present a support to decision-making and application of new technologies, particularly in order to provide higher level of services offered to clients (Batten, 1984). Also, productivity measurement would provide information that could assist in personnel projections, staff effectiveness improvement, new programs creation, and containing or reducing costs as well benefits for clients and customers.

With the objective to evaluate productivity measurements in healthcare services, and having in mind the specific nature of healthcare and difficulties in finding relevant productivity measures, in this paper we examine the application possibilities of compatible methods, the Seven Steps of Determining Productivity Indices (Brinkerhoff and Dressler, 1990) and the Objectives Matrix Method (Riggs, 1984). The aim of the research was to develop the representative list of indicators for productivity measurement and calculate and monitor productivity indicators in a healthcare department.

Methods

A combination of two methodologies, the Seven Steps of Determining Productivity Indices and the Objectives Matrix Method, was applied at the Department for Pediatric Orthopedics and Traumatology of the Institute for Orthopedic Sur-

gery "Banjica" in Belgrade during the period from 2005 to 2010.

Description of the Institute of Orthopedic Surgery "Banjica"

The Institute of Orthopedic Surgery "Banjica" in Belgrade is a typical example of a tertiary healthcare institution in Serbia which provides both secondary healthcare services to the local community, and highly specialized services to inhabitants of several districts. It covers the entire orthopedic and trauma pathology, with a capacity of 520 beds and 797 employees. The Institute of Orthopedic Surgery "Banjica" provides care and treatment of the osteoarticular system of various pathologies and for patients of all ages, especially in primary and reintervention joint aloarthroplasty, surgery of foot and ankle, pediatric orthopedic surgery, spinal surgery, using the Ilizarov's method, treatment of bone tumors, and sport injuries.

In nine operating theaters, which satisfy all up-to-date demands, over 30 surgical procedures are carried out daily. With modern equipment for physical therapy, the Institute also provides complementary medical treatment. The Institute of Orthopedic Surgery "Banjica" is specific and important by its centers: Department for Surgery and Treatment of Spinal Column (Spinal Center), Department for Adult Orthopedics and Traumatology, Department for Pediatric Orthopedics and Traumatology, Department for Oncoorthopedics. The Institute is considered to be the institution of special national significance.

The Department for Pediatric Orthopedics and Traumatology is the leading center for the treatment of children with orthopedic problems and diseases. At the beginning of 2011, the medical personnel of the Department constituted of seven surgeons and physicians (five specialists of pediatric orthopedics, a pediatrician and a physiatrist), 18 nurses and medical technicians and three physiotherapists. Beside fully employed personnel, three pedagogues and a great number of university professors are also engaged at the Department. The average age of the employees is 39.5 years, and the average length of full-time employment is 16 years. The spectrum of services of the Department also involves the application, with a significant experience, of the Ilizarov's method. The Department for Pediatric Orthopedics and Traumatology is the referent center for the education in the field of orthopedic ultrasound diagnostics.

Description of applied methodology

The Seven Steps of Determining Productivity Indices starts with **Step 1** which defines department mission identifying the major goals and customers of the unit. The mission statement must be complete and compatible with the mission of the larger organization. **Step 2** defines expectations, identifying the unit's services for each customer. The expectations must be clearly identified by explaining quality needs and expectations held by each major customer group for the department services. In **Step 3** key outputs are recognized, important for the department mission, responsive to the customer needs and expectations and which account for the majority of the expenditures of department resources. **Step 4** identifies and describes the major functions of the department that must clearly represent department operations and inputs and explain how the key outputs are produced. **Step 5** is output measurement selection to construct measurement techniques for one or more key outputs that will produce most practical and useful quality and productivity information. **Step 6** involves input measurement selection to construct measurement techniques for one or more key inputs that are critical to the production of the outputs selected in Step 5. **Step 7** is index construction of one or more productivity indicators to incorporate the output and input measures into a sensitive, practical and useful index. The Seven Steps are applied by consultations with employees of the Department for Pediatric Orthopedics and Traumatology, so as to obtain the necessary data for each step and assess measurement inputs and outputs chosen as significant to be under follow-up. The starting point for the assessment of indicators under follow-up in healthcare facilities included proposed indicators for evaluating the quality of healthcare (Regulation on Healthcare Quality Indicators, 2005/2009). Since these indicators do not take into consideration the specificities of a healthcare facility or its departments, we wanted to find new and specific indicators that would have a potential to be practical and useful productivity measures for the Department.

When creating the Objectives Matrix the first step defines possible indicators for productivity measurements, which contain the ratio of output into input (Output/Input). In this case the indicators were identified by the Seven Steps of Determining Productivity Indices method. In the next step, by consultations with the management of the department with relevant data and experience, score range 0 – 10 is determined, which is the base for the quantification of actual value of the indicator within a period of time. The lowest value level tolerable in an organization is scored by value 0, and is determined according to past experience. Value 10 refers to optimistic expectations of future indicator value. Average values are calculated based on the data from previous years and are scored by value 3. Score range is based on three key values: the lowest, average and optimistic. After the score-range formation, indicators' weights are determined by Delphi method. The Delphi panel consisted of 10 experts from different institutions in Serbia in the field of orthopedics and pediatric orthopedics. The sum of indicators' weights in the matrix equals 100. By defining all values, the basis for matrix scoring is created.

At the Institute for Orthopedic Surgery "Banjica" the data sources related to input and output measures indispensable for the formation of the matrix and calculation of indicators actual values for the period 2005-2010 were obtained from medical documentation, records, and reports. Based on the actual value, a corresponding score in the matrix, which could be further multiplied with the indicator weight, was obtained:

Weighted score = Score * Weight

Total weighted score, i.e. overall productivity value was calculated by formula:

Total weighted score = Sum of weighted scores in the matrix

After calculating productivity for the observed period, analysis of total annual productivity changes was determined by formula:

Productivity change = (Total weighted score for current year - Total weighted score for previous year) / Total weighted score for previous year * 100 (%)

Results

The Seven Steps of Determining Productivity Indices was implemented at the Department for Pediatric Orthopedics and Traumatology of the Institute for Orthopedic Surgery "Banjica".

Step 1 defined the Department mission statement. The mission of the Department for Pediatric Orthopedics and Traumatology is the treatment of children with orthopedic problems and diseases, the patient's recovery and return to community; with a special accent on surgical methods, providing modern, efficient and evidence based treatment, with high ethical standards and respect for the child's rights, maximal professional engagement and continual education of personnel, all implemented in a safe environment adopted to the child's needs that always come first. In Step 2 relating to expectations two groups of service users were defined at the Department for Pediatric Orthopedics and Traumatology. The most important group consisted of children, aged 1-18 years, with orthopedic problems and diseases, while the second group was composed of escort (parents). Department services involve surgery, ambulatory exams (ultrasound, x-ray, etc.), and services of postoperative care and rehabilitation. A special service offered by the Department is childhood and adolescent hip pathology, and the application of the Ilizarov's method in childhood in which the department presents a nation-wide leading center. The Department has been continually engaged in the assessment of users' satisfaction, with fully transparent results based on which the expectations were defined. Patients expect efficient service that is ensured by absence of waiting lists and preoperative preparation lasting for 3.2 days on average. The expectations are also associated with the quality of obtained service, as well as with as short as possible hospital stay, with adequate information about surgery and postoperative course.

Step 3 determined key outputs, which are important to meet Department's mission statement and to represent satisfactory fulfillment of users' needs and expectations. The following outputs were defined: the number of performed examinations in a specific category (ambulatory examinations, ambulatory check-ups, emergency examinations, ultrasound imaging); the number of surgeries performed in surgical theaters, the number of

operated patients under general, regional and local anesthesia performed in surgical theaters, and the total number of patients discharged annually.

In Step 4 the Departments' functioning was analyzed explaining how the key outputs are produced. The Department is performing the activities through surgery and ambulatory exams, which are supported by services related to bandaging and postoperative care of patients. On this basis inputs are defined, primarily those which are measurable and can be later included into the productivity indicators. The key inputs indispensable in service provision involve: the number of surgeons included in the Department's operative program, the number of nurses engaged in the care of patients at the Department, the number of annual working hours for surgeons, nurses and other personnel, the number of available beds, the number of the Department's working hours (admission, bandaging, etc.), the number of Department's hospitalizations, total number of days per month available for specialist/consultant exam appointments, and the total number of afternoon shifts per week. By the analysis of Department functioning, throughputs were recognized as potentially usable for productivity analysis; duration of hospital treatment, duration of preoperative preparation, and bed occupancy rate (average bed occupancy rate at the Department).

In Step 5 measurable outputs were defined which could be included into the productivity indicators. For the observed department, after having analyzed a possible output, the following outputs and throughputs were chosen:

- Total number of hospital days
- Average hospital bed occupancy rate
- Total number of preoperative preparation
- Total number of surgeries done under general, regional and local anesthesia
- Number of patients operated on under general, regional and local anesthesia
- Number of ultrasound exams
- Number of emergency outpatient ambulatory exams
- Number of exams by a specialist/consultant

In Step 6, inputs measurements for the Department were selected as follows:

- Number of hospitalizations
- Total working hours in operative theater
- Total number of ambulatory working hours
- Number of surgeons included in operative program
- Number of nurses engaged in patients' care
- Total number of days per year available for specialist/consultant exam appointments

In step 7, index construction, based on the chosen inputs and outputs, the productivity indicators for the Department, surgeons and nurses were defined (Table 1).

Results of applying the method

The indicators which refer to personnel productivity scored higher weights in relation to others. The indicators of surgeons' productivity measured by the indicators I-6 and I-7 scored 3-4 times higher weight than the indicators referring to Department productivity (Table 2). Weights for the productivity of nurses were slightly lower than those of surgeons. The actual values of indicators were calculated and scored by the Objective Matrix for each year separately for the period of 2005-2010. Example for the matrix for 2010 is shown in Table 2.

Changes in scores for each indicator, total weighted scores and productivity changes for the period 2005-2010 are presented in Table 3. The obtained results represent the basis for the comparison and analysis of single indicators and the overall productivity score. In the observed period productivity change showed a sudden increase of productivity in 2007; nearly 60% higher rate as compared to 2006 was detected. In 2008 productivity decreased by 19.62%, while in 2009 productivity increased overcoming the 2007 rate (418.5 vs. 395), and further on it mildly increased in 2010 rating 424. By observing single indicators per year, the score increase of some indicators could be observed, such as the average length of hospital days (I-1) which shortened over the observed six years. The relationship between the number of surgeries and the number of working hours spent in the operating theater increased (I-2), as well as the indicators of the productivity of surgeons (I-6 and I-7) and nurses (I-8 and I-9). Productivity indicators of the Department regarding ambulatory working hours were constant in case of ultrasound exams, while those regarding emergency and consultative exams were decreased.

Table 1. Productivity indicators

Productivity area	Indicator	Indicator[a] = Output/Input
Department	I-1	Total number of hospital days / Number of hospitalizations (Average length of hospital stay)
	I-2	Total number of surgeries / Total working hours in operative theatre
	I-3	Number of ultrasound exams / Total number of ambulatory working hours
	I-4	Number of emergency outpatient ambulatory exams / Total number of ambulatory working hours
	I-5	Number of exams by specialist - consultant / Total numbers of days per year available for specialist – consultant exam appointments
Surgeons	I-6	Number of patients operated on under general, regional and local anesthesia / Number of surgeons included in operative program
	I-7	Total number of surgeries / Number of surgeons included in operative program
Nurses	I-8	Total number of hospital days / Number of nurses engaged in patients' care
	I-9	Average hospital bed occupancy rate / Number of nurses engaged in patients' care

[a] *All values are on an annual basis*

Table 2. Objectives Matrix for Department for Paediatric Orthopaedics and Traumatology, 2010

Productivity in 2010		Productivity indicators								
		I-1[a]	I-2[b]	I-3[c]	I-4[d]	I-5[e]	I-6[f]	I-7[g]	I-8[h]	I-9[i]
	Actual value[j]	**24.79**	**1,26**	**1.75**	**2.05**	**71.84**	**77.40**	**193.20**	**1337.13**	**2.80**
Score range[k]	10	15.00	2.00	3.00	4.00	125.00	146.00	292.00	1095.00	1.00
	9	18.51	1.85	2.81	3.83	119.51	135.42	273.42	1157.99	1.27
	8	22.02	1.70	2.63	3.66	114.05	124.85	254.85	1220.59	1.57
	7	25.53	1.56	2.45	3.49	108.59	114.28	236.28	1283.23	1.84
	6	29.04	1.42	2.27	3.32	103.13	103.71	217.71	1345.87	2.13
	5	32.55	1.28	2.09	3.15	97.67	93.14	199.14	1408.51	2.42
	4	36.06	1.14	1.91	2.98	92.21	82.57	180.57	1471.15	2.71
	3	39.57	1.00	1.73	2.81	86.75	72.00	162.00	1533.49	3.01
	2	46.38	0.91	1.48	2.54	74.50	63.00	140.00	1630.66	3.34
	1	53.19	0.83	1.24	2.27	62.25	54.00	118.00	1727.83	3.67
	0	60.00	0.75	1.00	2.00	50.00	45.00	96.00	1825.00	4.00
Score[l]		7	5	3	0	1.5	3.5	5	5.5	4
Weights[m]		6	10	5	5	4	18	22	12	18
Weighted score[n]		42	50	15	0	6	63	110	66	72
Total weighted score[o]				424						

[a] *I-1- Total number of hospital days / Number of hospitalizations (Average length of hospital stay)*
[b] *I-2 - Total number of surgeries / Total working hours in operative theater*
[c] *I-3 Number of ultrasound exams / Total number of ambulatory working hours*
[d] *I-4 Number of emergency outpatient ambulatory exams / Total number of ambulatory working hours*
[e] *I-5 Number of exams by specialist - consultant / Total numbers of days per year available for specialist – consultant exam appointments*
[f] *I-6 Number of patients operated on under general, regional and local anesthesia / Number of surgeons included in operative program*
[g] *I-7 Total number of surgeries / Number of surgeons included in operative program*
[h] *I-8 Total number of hospital days / Number of nurses engaged in patients' care*
[i] *I-9 Average hospital bed occupancy rate / Number of nurses engaged in patients' care*
[j] *Actual value of each indicator, performance in for certain period of time*
[k] *Score range – the base for quantification of actual value proposed by management*
[l] *Score – value of a range according to actual score*
[m] *Weights - relevance of each indicator, proposed by management;*
[n] *Weighted score = (Actual score * Weight)*
[o] *Total weighted score = Sum of Weighted scores in the matrix*

Table 3. Changes in scores and total weighted score in 6 years period

Indicators of productivity	Values[a]/ Score[b]	Years					
		2005	2006	2007	2008	2009	2010
I-1	Value	44.98	50.15	43.14	34.09	25.49	24.79
	Score	2.00	1.50	2.50	4.50	7.00	7.00
I-2	Value	1.01	0.83	1.01	0.91	1.22	1.26
	Score	3.00	1.00	3.00	2.00	4.50	5.00
I-3	Value	1.54	1.89	1.77	1.72	1.72	1.75
	Score	2.00	4.00	3.00	3.00	3.00	3.00
I-4	Value	2.30	2.53	3.49	3.26	2.46	2.05
	Score	1.00	2.00	7.00	5.50	1.50	0.00
I-5	Value	107.50	84.17	80.78	93.23	68.08	71.84
	Score	8.00	3.00	2.50	4.00	1.50	1.50
I-6	Value	45.67	71.00	84.50	74.20	84.80	77.40
	Score	0.50	3.00	4.00	3.00	4.00	3.50
I-7	Value	129.83	159.50	194.00	140.00	186.80	193.20
	Score	1.50	3.00	5.00	2.00	4.50	5.00
I-8	Value	1574.47	1625.00	1564.40	1493.00	1410.60	1337.13
	Score	2.50	2.00	3.00	3.50	5.00	5.50
I-9	Value	3.33	3.33	2.80	2.80	2.80	2.80
	Score	2.00	2.00	4.00	4.00	4.00	4.00
Total weighted scores[c]		197	241	395	317.5	418.5	424
Productivity change[d]			22.33%	63.90%	-19.62%	31.81%	1.31%

[a] *Actual value of each indicator, performance in for certain period of time*
[b] *Value of a range according to actual score*
[c] *Sum of Weighted scores in the relevant matrix*
[d] *Productivity changes in total weighted scores in relation to previous year, (%)*

Discussion

The research was developed at two levels; first, the creation of a representative list of indicators for productivity measurement in the healthcare department using the Seven Steps of Determining Productivity Indices, and second, establishing the Objectives Matrix model and calculating productivity indicators using data for the six-year period.

The summary of the results, i.e. indicators value by years and their scores by the Objectives Matrix Method are presented in Table 2. The first indicator (I -1) shows that the average length of hospital treatment in the observed period was shortened from 44.98 to 24.79 days. This was necessary to do be done, so as to comply with legislative requirements and follow-up of quality in healthcare facilities in Serbia (Institute of Public Health of Serbia, 2010). However, although the number of hospital treatment days decreased by double, this number is still higher than the average for the entire facility (21.41 days). It should be kept in mind that the Institute is a unique facility in the region, where users are children with escorts often arriving from very distant places and who are of lower economical status. This could be the reason for a longer hospital stay in order to avoid traveling expenses during preoperative exams and postoperative recovery. The second indicator (I-2), referring to the number of performed surgeries and working hours in the operating theater, increased, meaning that working hours spent in the operating theater decreased. The result of acquired experience (average surgeons' age was 38.5 years) and application of new technologies in preoperative diagnostics and during surgery was partly leading to higher efficiency. There are contradictory opinions regarding efficiency improvement as the result of new technologies introduction. A small increase in productivity has been registered in UK hospitals (Ferrari, 2006), even with inefficiency increases. In contrast, the efficiency improvement was registered in 10 Greek

hospitals (Lyroudi et al., 2006). What characterizes the developing countries is that the insufficiency of new technologies is compensated by the development of human resources, by team-work, improvement and transfer of knowledge from older to young personnel (McChartly, 2007).

Analyzing the work of the Department by ambulatory work indicators (I-3, I-4 and I-5) oscillation in values by years could be observed (I-3, I-4 and I-5) (Table 2). The values of indicators for ultrasound exams (I-3), after minor changes done at the initial observed period, do not show higher oscillations over the last four years. Indicator values relating to emergency (I-4) and specialist/consultation ambulatory exams (I-5) showed more extensive changes and had lower values in 2010 in comparison to 2005. This was also influenced by the program of shifts in 2010 when the number of physicians was reduced by one. According to the manager's assessment, the indicators relating to ambulatory work (I-3, I-4 and I-5), had a lower significance for the overall productivity of the Department and were scored with lower weights values. During the observed period, as the result of changes in healthcare policy, Serbia experienced decentralization and wider local autonomy, with the transfer of a part of healthcare center activities from larger cities to smaller centers, as well as the implementation of contracts between public health institutions and the private sector. Country inland facilities and departments specialized in pediatric orthopedics educated medical staff for specific exams utilizing advanced equipment in diagnostics, and the results of these facilities were accepted by the Institute "Banjica". The outcome was a reduced number of patients referred for diagnostic exams to the Institute, but also to the Department for Pediatric Orthopedics and Traumatology. The application of legislative regulation (Legislation on Healthcare Insurance, 2005) also had an impact on the reduced number of ambulatory exams, because hospital software limited the maximal number of patients per day that a physician could receive. Besides, the oscillations were also influenced by two physicians having left during 2006 and 2007, while new ones were employed only after several months. The literature also reports that many confounding factors can influence productivity (Hollingsworth, 2008).

The values of surgeon's productivity indicators (I-6 and I-7) were higher in the 2009-2010 periods than at the initial observed period (Table 2). In 2010, the number of operated patients per surgeon (I-6) was increased nearly by 70%, as well as the number of operations per surgeon (I-7) by 49% in relation to 2005. The Department deals with overall pediatric orthopedic issues, and it is renowned for successful treatment of the so-called childhood and adolescent hip treatment and the use of the Ilizarov's method. Bearing in mind the specificities of the rendered services, the Department is directed toward the development of surgical interventions, which is also associated with Department's mission that underlines surgical methods, by providing advanced, efficient and evidence-based treatment. Decentralization and redistribution of ambulatory activities enables surgeons to have more time for acquiring knowledge and develop surgical skills. Passing the Regulation on closer conditions for the implementation of continual education of healthcare personnel and healthcare co-workers by the end of 2007, also contributed to the continual development of knowledge, and so did the introduction of licensed healthcare workers by the authorized chambers (Regulation on Closer Conditions for the Implementation of Continual Education of Healthcare Workers and Healthcare Co-workers, 2007).

The first productivity indicator for nurses (I-8) shows that the number of hospital treatment day in relation to the number of nurses is decreasing. This is in accordance with the desire to shorten the duration of hospital treatment. The value of the second indicator (I-9), the number of occupied beds in relation to the number of nurses also decreased (Table 3). The aim of the Department management is to further decrease these indicators as well, with optimistic expectation that the relation between the number of beds and nurses reach score 1 (Table 3). There is a need for more nurses at the Department due to its specificity, and also because the patients are children who require increased check-up for general safety and good treatment results (avoidance or shortening of the duration of immobilization and rehabilitation). This would be the way to improve working quality, but which would also decrease productivity, as reported by some authors (Fizel and Nunnikhoven, 1992; Chilingerian,

1993). However, the increased number of nurses also increases expenditure of the institution. On the other hand, the fact is that nurses are an indispensable part of the physician-nurse team, department or intuitional management team (Roussel, 2006). They are considered as a critical component od health care systems. In the observed Department the significance of nurses is recognized by attributing to nurses a relatively high value of weight productive indicators. At the beginning of 2011 the Department employed three more nurses, so that now there are 18 in total.

By having observed the indicators over the last two years (Table 3) it can be detected that working results of the Department are much improved in relation to 2005. Most indicators (I-1, I-2, I-3, I-6, I-7, I-8 and I-9) show results which are above average, which is reflected by their scores in the Objectives Matrix rating from 3 to 7. The best result was achieved in the decrease of the number of hospital treatment days (I-1), score 7, which was also triggered by legal regulations (Legislation on Healthcare, 2005). Other indicators value scores from 3 to 5 (I-2, I-3, I-6, I-7, I-8 and I-9). Such a result can be considered satisfactory. However, two indicators related to ambulatory work (I-4 and I-5) have scores below average, i.e. 3. It is possible that the process of decentralization and the transfer of ambulatory check-up exams to other institutions dealing with pediatrics, pediatric surgery and pediatric physiatrist reflected negatively on the Department productivity. Department management should reconsider whether such a mode of carrying out the work can be sustainable in the future as well, and how it will reflect on productivity. The current priorities and mission of the Department attach a lower significance to ambulatory exams. Since they have lower weights in the Objective Matrix, their lower scores do not have much influence on the overall productivity scoring of the Department, i.e. total weighted score. The total weighted score, with the exception of the 2007-2008 periods has been continually rising (Table 3), with the highest productivity change in the 2006-2007 period. The total weighted score in 2010 was by 115% higher compared to the initial period.

The analysis of single productivity indicators and total weighted score enable the management to view productivity fields as single and united entities, and to reach conclusions on achieved results and accordance of activities between the Department and its mission. In addition, the reached conclusions are significant for bringing decisions on the engagement of already present resources (resources allocation), securing new resources, award presentation, etc.

The presented approach, the Seven Steps of Determining Productivity Indices and Objectives Matrix, can find its application in productivity measurement of the productivity of various departments. It has been recommended that each department should develop its own objective matrix and weighted against criteria which employees feel are important, taking into account local differences or the particular views of a group of professionals (Harvey, 1987). The list of indicators taken into consideration, significance of certain indicators for rating productivity will change in specific situations during work and according to the line of business (Noory, 1990). Halachmi (1996) reported that "an interesting way of linking productivity measurement tools would be to let each department develop its own objective matrix, with the important constraint that the indices used by the funding agency be included and weighted against other specific criteria which the group felt were important". The development of this approach is directed toward the connection of health insurance requirements and specific needs and healthcare institution/department functioning, which represents one of the most urgent issues in Serbia.

Further application approach is suggested at different institutional levels, in the function of the connection between department indicators and institution indicators. In addition, the utilization of such an approach can be taken into consideration to develop indicators and comparison among different departments within an institution or benchmarking of different institutions. When making comparison, it should be kept in mind that productivity indicators of various institutions/departments are not always comparable, so that inadequate explanations could lead to false decisions on the distribution of resources. This is important when considering to include the approaches to follow-up of productivity in policy context, for which there are realistic conditions (Hadley and Zuckerman, 1994).

Generally, healthcare productivity is difficult to measure, because it is doubtful how to define the actual output. Most researches published so far have used some variant of intermediate outputs, in terms of numbers of treated patients (Hollingsworth, 2008). The dilemma remains open whether the patient's health has been improved (Jacobs, 2006), particularly when children as a vulnerable group are involved.

Using methods based on management's subjective evaluation about what presents input and output of the department, as well as when choosing input and output to design productivity indicators, could be the limitations of applied approach. Subjective expert assessments are also used when defining the lower and upper limits forming the basis of the scoring scale of Objectives Matrix. Subjectivity is reduced for indicator weights by Delphi method implementation. The next limitation is that different surgical interventions and their complexity were not considered. Also, the availability of data from medical data records significantly influenced the selection of indicators.

Conclusion

The main conclusion of this paper indicates the significance of productivity indicators formulation at the level of healthcare departments of the institution, which reflect department functioning and enable productivity measurements over years. Although the methods of the Seven Steps of Determining Productivity Indices and Objectives Matrix have been developed for the needs of industrial organizations, the paper shows that their application is possible in healthcare services as well. Productivity follow-up using indicators and total weighted score during years, enables healthcare managers to analyze low-productivity fields where improvements are needed. The approach applied at the Department for Pediatric Orthopedics and Traumatology can be also utilized in other departments and healthcare institutions. In addition, this paper opens the way for further research on the possibilities of department's productivity follow-up, but taking into consideration their specificities and missions. The next field of research should involve the connection of department productivity indicators with institution indicators and their needs in policy terms. A special research subject would be related to the possibilities and justification of indicator comparison among different departments and institutions.

Acknowledgments

This work was supported by Ministry of Science, Republic of Serbia (Grant No. 41004).

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Importance of Health Workers' Communication in Immunisation Programmes

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Abstract

Introduction: Immunisation represents the simplest and most cost efficient investment in health, whilst providing better health standards for children by protecting persons and the community from occurrence and spread of disease. In Bosnia and Herzegovina (B&H) immunisation against ten infectious diseases is compulsory and free. Health workers are expected to promote vaccination and provide up-to-date, understandable information not only on the topic of benefits but also potential risks of vaccination so that children can be vaccinated with an informed consent of their parents.

The purpose of this paper is to show results of research about knowledge, attitude and practice among health workers that directly take part in administration of routine immunisation, with a special focus on importance of communication between health workers and parents.

Methodology: cross-sectional study, conducted by means of semi-structured questionnaire with 49 questions, conducted in field on a sample of 103 subjects (52 teams working on immunisation) at 20 selected locations.

Results: Health workers believe they inform parents enough about benefits and possible side-effects of vaccination, but not also about possible complications of the process. They judge their knowledge to be satisfactory, but emphasise the need for additional education.

Key words: immunisation, health workers, parents, communication

Introduction

In the public health sector, immunisation is mainly perceived in terms of availability and cost of vaccines, their storage and handling, and possibilities of prevention, control and monitoring of mortality from vaccine-preventable diseases. Diseases population is vaccinated against occur rarely, if at all, therefore brining up a question among parents and health workers whether vaccination is justifiable, effective and safe (1, 2). A significant number of parents are especially concerned about post-vaccination reactions and complications. After repeated negative media campaigns on the subject of children's immunisation programme in Federation of Bosnia and Herzegovina (FB&H), a research was conducted in 2010 to look into knowledge, attitude and practices of health workers. Apart from administering vaccination process in a professional manner, health workers taking part in the vaccination programme are an important source of information about possible side-effects, complications and development of diseases population is vaccinated against. For an immunisation programme to succeed, it is therefore important for health workers to be well informed, to provide a good source of authoritative, scientifically justified advice, and to openly talk about benefits and risks of vaccination so that parents could understand both possibilities, benefits and risks of vaccinating their children (4, 5).

Purpose

The purpose of this paper is to show the knowledge, attitude and practice of health workers that take an immediate part in administering routine vaccination in FB&H as part of the Vaccination Programme, looking at aspects of health communication and promotion of health, also aiming to point out possible directions of education for professionals in the area of communication between health professionals and parents.

Methodology

This paper presents results of research conducted as a cross-sectional study using a semi-structured questionnaire with 49 questions, carried out in-field on a sample of 103 examinees (52 teams working on immunisation) at 20 selected localities/municipalities.

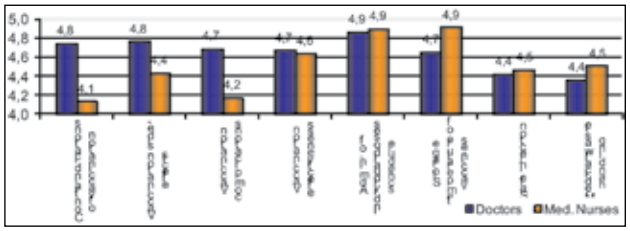
Results

Sociodemographic characteristics

Research encompasses 103 examinees, of which 52 doctors and 51 medical nurses. Average age of respondents in the total sample is 46 years (doctors 51, medical nurses 42 years of age).

Average work experience in their profession is 24 years among doctors, of which 18 years experience in vaccination. Average experience among medical nurses is 19 years of work, of which 12 working with immunisation.

Knowledge questions



Graph 1. Self-measured knowledge of examinee on a scaleof 1 to 5

Majority of examinees marked highest their knowledge of schedule of individual vaccines administration. Doctors assess their knowledge about side-effects and contraindications as good, while medical nurses cite their knowledge about storage temperature for vaccines and medical waste handling as excellent. Four percent of nurses consider not to know enough about safe injection and effectiveness of vaccination.

Answering the question about possible reactions after vaccination, majority of examinees (98-100%) answered correctly that swelling at area of injection, as well as higher body temperature may occur, but are less confident in answers about other possible side-effects (rash, persistent tearful cry, loss of appetite). Majority of examinees are aware of other, new vaccinations used in developed countries and most frequently cite pneumococcal and meningococcal vaccines believing it would be justifiable to introduce both in the Vaccination Programme in FB&H.

Questions about attitude

Largest percentage of examinees (87%), believe vaccinating children by the Compulsory Vaccination Programme is justified, and this percentage grows with the years of professional experience.

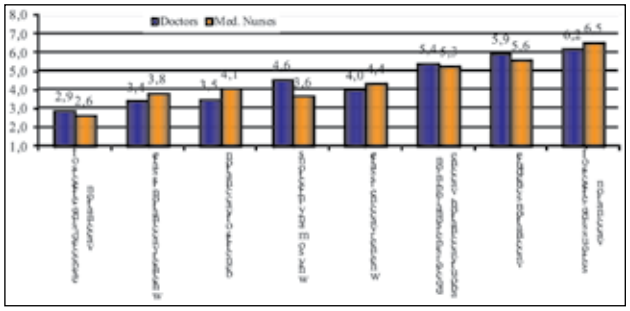
Large majority of all examinees (96%), regard vaccines within this Programme as safe.

With regards to the need for information provision to parents, examinees believe that parents should primarily be informed about benefits of vaccination, the expected side-effects, followed by information about timescale when they should contact the doctor regarding vaccination. **(Position of Table 1)**

Examinees from both sub-samples do not regard as a priority for parents to know about serious side-effects of vaccination.

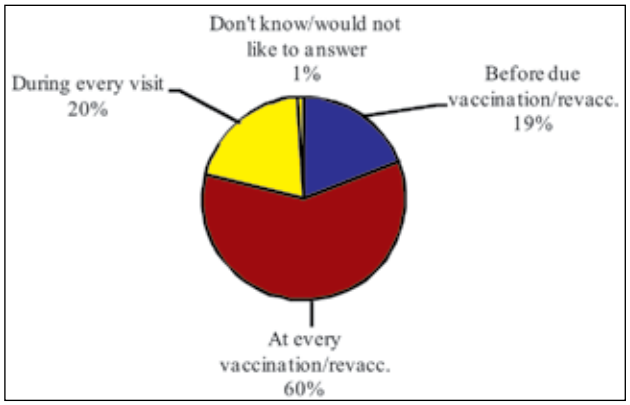
Table 1. What should, by examinees opinion, parents know about vaccination

Parents should know	Doctors		Medical nurses		Total	
	order	X	order	X	order	X
Benefits of vaccination	1	1,6	1	1,8	1	1,7
Expected side-effects of vaccination	2	2,3	2	2,6	2	2,5
When to contact a doctor about vaccination	3	3,1	3	3,1	3	3,1
Vaccination schedule	4	4,9	4	4,7	4	4,8
Contraindications	5	5,4	5	4,9	5	5,2
Serious side-effects of vaccination	6	5,5	6	6,3	6	5,9
Meaning of abbreviations (DTP, MRP…)	7	6,7	8	6,6	7	6,7
New vaccines	8	7,1	7	6,5	8	6,8
Country of production for vaccine being administered to the child	9	8,4	9	8,2	9	8,3



Graph 2. By your opinion, what would parents like to know about vaccination? Please rank the following by priority

Asked about what they believe parents would like to know about vaccination, examinees by large believe parents would like to know what are the side-effects of vaccination, followed by „whether vaccination is safe“ (doctors), whilst medical nurses believe parents would like to know „why so many injections“. Majority believes parents would not like to know what the serious side-effects of vaccination are.

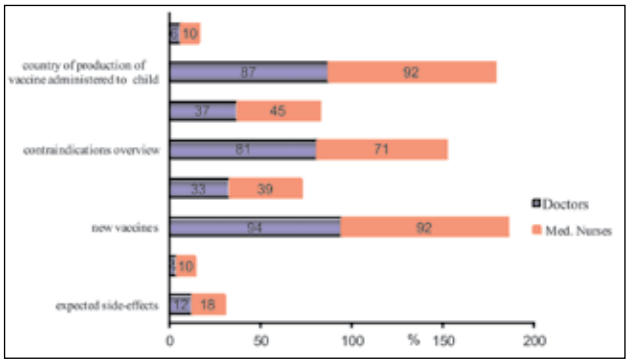


Graph 3. When should the topics of risk/benefits of vaccination be discussed with parents?

Over 90% of examinees answered they inform parents what to do in case of side-effects and/or complications, and what side-effects can be expected.

From a selection of educational/promotional material examinees found most useful to health workers, they chose material about new vaccines, followed by data on country of production of vaccine administered to the child, and finally educational material summarising contraindications as most useful to health workers.

Examinees hold that educational promo material dealing with benefits of vaccination, expected side-effects and recommended measures to take in case of side-effects would be most useful to parents.



Graph 4. Most useful type of educational/promotional material for health workers, by examinees' opinion

Most respondents think that they need additional education about advancements and development of new vaccines, followed by vaccination risks. Health professionals cite printed materials as most acceptable means of education on the topic of immunisation, followed by workshops, i.e. group discussions.

Asked what would help most in the process of vaccination in FBiH, responders point out regular purchase of vaccines as a priority, followed by introduction of vaccination responsibility regulated by law, i.e. better protection of health workers, which is understandable considering vaccination is compulsory, and the doctor upon examination decides on whether vaccination should take place after considering possible contraindications.

In general, 57% of all subjects of this research believe that the topic of children's vaccination in FB&H is well regulated by Law. However, they also believe that vaccination rate among especially vulnerable, hard-to-reach groups of children would be increased by more frequent visits of health workers to field, by a vaccination campaign or some other approach.

Over 90% of examinees believe that all health workers working on vaccination are educated sufficiently to successfully conduct vaccination procedure, although only 24% of them received any form of training on the topic in the last 12 months.

Respondents showed relatively good knowledge of procedure of verifying quality of the vaccine, followed by cold chain check (more familiar to medical nurses than to doctors, explained by the

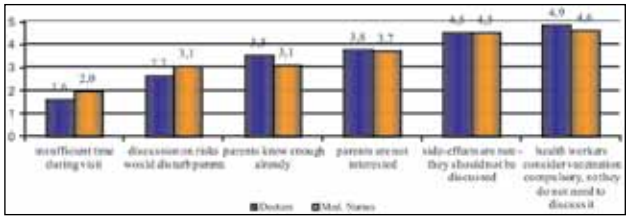
fact that it is primarily nurses who handle vaccines). When asked about the quality of vaccines, a relatively high percentage of responders answered that „they trust public health institutes“, regardless of the fact that final responsibility prior to administration is theirs, namely that of doctors.

Communication with parents

As most frequent problems in their relationship with parents of children taking part in compulsory vaccination, respondents to this research cite fear of possible side-effects occurrence, failure to attend due child vaccine by schedule, followed by refusal by parents to vaccinate children due to failure to understand the importance of vaccination.

Insufficient appointment length, succeeded by „discussion about vaccination would disturb parents“ and „side-effects are anyways rare, so should not be discussed“ are three main reasons respondents of this research cite as largest obstacles in communication with parents. Examinees also indicate that health workers should not spend a lot of time explaining possible risks if vaccination is compulsory – which is the case!?

Over 90% of respondents believe they inform parents sufficiently about vaccination, although largest percentage of (58%) cite that, on average, they spend 5-10 minutes talking to parents, whilst a third spends more than 10 minutes.



Graph 5. By your opinion, what are the largest obstacles in communication with parents on the topic of vaccination risks? Rank your answers by priority

During vaccination of children, most frequent form of getting information about vaccination is verbal, followed by handing over of vaccination schedule/calendar and health promo material.

Largest percentage of examinees (58% doctors, 59% medical nurses) cite that on average, they spend 5 to 10 minutes in their discussion with parents on the topics of their children's development, appropriate diet, communication and early detection of delayed development. A third spend more than 10 minutes, whilst one doctor replied that he „does not have enough time for that kind of counselling“.

Respondents record information about administered vaccines, together with the date of next due dose to be administered: in the patient's vaccination card (doctors 96%, nurses 100%), followed by patient's medical record (doctors 92%, nurses 100%), and in patient's personal medical ID card (doctors 71%, nurses 73%).

Discussion

Participants taking part in this research have sufficient experience in the vaccination programme, especially doctors. As a result, their answers are a reflection of their knowledge and practical experience, besides less than a quarter of participants attending any form of additional education or a seminar in the last 12 months. Majority of examinees mark their knowledge „satisfactory“, but also emphasise that they need additional education about advancements and development of new vaccines and risks of vaccination.

Through self-assessed grading of knowledge among doctors and medical nurses, highest marks were awarded for knowledge about the timing schedule of administration of individual doses. Doctors are well knowledgeable about side-effects and contraindications of vaccination, while nurses graded high their knowledge about storage temperature. This is understandable, considering the “division of duties”, although doctors must also be aware of other components of safe handling of vaccines, considering they are directly responsible for the safety of vaccination.

Largest percentage of respondents believe it is justifiable to vaccinate children in line with Compulsory Vaccination Programme, with 11% of them noting the Programme should be expanded with introduction of new vaccines, most frequently citing pneumococcal and meningococcal vaccines they believe should be introduced in the Compulsory Vaccination Programme in FB&H.

Large majority of assessors believe vaccines are safe, which is very important considering the role of doctors who have an obligation to build

trust in immunisation as an effective measure for prevention of disease (6, 8).

Although vaccination against certain infectious diseases is compulsory in Bosnia and Herzegovina (B&H), due to experiences of other countries and the principle of ethical approach to the „rights of patient", vaccination should not be administered without provision of rightful information to parents as they have a right to know about benefits, but also for eventual risks of vaccination (9, 13). Health workers answered that they inform parents sufficiently about the vaccine they are about to administer to the child, as well as about what to do in case of side-effects, vaccination complications, expected side-effects, but not about possible complications. Respondents are of a general opinion that a discussion about possible complications would disturb parents because „ parents already know enough about it" or are not interested for a discussion about vaccination risks. Furthermore, they answer that „side-effects are anyways rare and therefore should not be discussed", finally marking that - if vaccination is compulsory, as it is – then they do not need to explain much the possible risks!? Such results point to a need for effective communication about immunisation, raising awareness of health professionals about the importance of inclusion of parents in protection of their children through vaccination.

Health workers working on immunisation are in a large percentage interested in success of the immunisation programme and, by their opinion, the quality of immunisation programme in FB&H would be improved though regular acquisition of vaccines, better information provision for parents (information campaigns), but also regulation of vaccination process responsibility by law, i.e. better protection of health workers, which is understandable considering vaccination is compulsory, and the doctor upon examination decides on whether vaccination should take place after considering possible contraindications.

Conclusion

Results of research show that health workers working on immunisation programme are aware of the importance of immunisation, that they have enough knowledge and confidence about vaccines they administer routinely through the Vaccination Programme, but are also aware of a need for better communication towards parents about benefits as well as risks of vaccination. Persons administering the vaccine should know technical information about the vaccine (administration, schedule, cold chain, vaccine safety, safe disposal, etc) and the disease, because for a successful immunisation programme it is important that health workers are well informed and provide a good resource of up-to-date information and scientifically proven advice. Regardless of the limited time they have for a discussion with parents, health workers should clearly speak about advantages and risks of vaccination so that parents understand in full the benefits and possible unwanted outcome of vaccination.

It is therefore important to educate health workers to cover these communication needs, key messages and questions related to vaccines in case of instances of unwanted events after vaccination or when questions on the safety of vaccines arise.

Acknowledgement

We give an especially distinguished acknowledgment to all health professionals who work in the immunisation programme and who participated in this study. Also, we thank UNICEF for their support for the implementation of this research. Special thanks to all the parents who co-operate with health professionals and immunize their children.

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Ethiopatogenesis and frequency of atrial fibrilation in students population of Sarajevo university

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Abstract

Introduction: Atrial fibrillation presents arrhythmia with an irregular, disorganized electrical activation of atria. Such electrical activation of atria prevents any effective atrial contraction. Atrial fibrillation is one of the most common and most important arrhythmias and usually cause of the onset of fibrillation is an organic disease. Atrial fibrillation usually occurs in acquired heart defects of rheumatic etiology. In addition to hemodynamic disturbances caused by atrial fibrillation other clinically significant consequence of heart rhythm disorders is the occurence of peripheral thromboembolism.

The aim of the investigation: To establish frequency and ethio-pathogenesis of atrial fibrillation in students of University of Sarajevo.

Material and methods: Investigation was conducted as prospective, retrospective, clinical, descriptive and analytical. The sample consisted of University of Sarajevo students. The sample included students at regular and scheduled examinations at the Institute for Health Protection of Students University of Sarajevo in period of 2006–2009. The analysis was done on 109 students. Work methods included the analysis and presentation of personal history data for assessing cardiac status of the patient and family history, analysis and presentation of auscultatory data on cardiac rhythm and regularity, intensity and quality of heart tones, systolic and diastolic sounds and cardiac noise, analysis and presentation of ECG findings; analysis and presentation of a 24-h Holter monitoring; analysis and presentation of echocardiographic findings, analysis of the administered therapeutic procedures.

Results: Atrial fibrillation is most frequent in patients with congenital and acquired heart defects-37 patients (33.94%), 7 with systemic and metabolic disorders (6.42), 6 with foci (5.50%), 5 with hypertension (4.59 %), 2 with endocrinological diseases (1.83%), 2 cases of athlete's heart (1.83%), 1 case of hypotension (0.92), 1 case of neurological disorder (0.92%), 1 case owith other disorders (0.92%).

Key words: Atrial fibrillation, University of Sarajevo students, congenital and acquired heart defects, rheumatic heart disease, mitral valve prolapse.

Introduction

Arrhythmia in which there is an irregular, disorganized electrical activation of atria, is one of the most common and most important arrhythmias. There are also a number of persons who have this condition and in whom there are no clinical signs of heart disease. Atrial fibrillation in people with heart diseases occurs:

- In acquired valvular defects of rheumatic etiology, most commonly mitral defects;
- The damaged mitral valve due to the other causes as well as in mitral valve prolapse, with lesser or greater regurgitation;
- In cardiomyopathy of different origin;
- Of ischemic origin;
- Hypertensive origin;
- Endocrine origin;
- Infiltrative origin;
- Chronic disease pericardium;
- In sinus node disease;
- Cardiac insufficiency;
- Pulmonary embolism.

Atrial fibrillation can exist as a stable, chronic disorder of rhythm, or as intermittent paroxysmal arrhythmia. Sooner or later, atrial fibrillation becomes stabilized. Subjective problems depend on the number of impulses conducted in the chambers

and functional capacity of left atria. During physical exertion the subjective difficulties as shortness of breath, tiredness with palpitations are increased. Tachyarrhitmia is cardiac action that occurs at the beginning of atrial fibrillation, is accompanied by signs of scarcity in the central nervous system, i.e., instability, dizziness and visual disturbances.

Objective examination demonstrates a completely arrhythmic heart action, in which may not be observed the regularity of the ventricular rhythm (irregularly irregular). Therefore, this type of arrhythmia is called arrhythmia absolute. Subjective symptoms are manifested as palpitations, tiredness and shortness of breath. Are there may be also possible signs of blood shortage in the central nervous system, the emergence of dizziness, instability in walking and vision disorders.In addition to hemodynamic disorders caused by, other clinically significant consequence of this disorder is the onset of peripheral embolism. It was found that in chronic atrial fibrillation the risk of embolism is five times higher, and in patients with rheumatic mitral defect and atrial fibrillation even 17 times by patients who have atrial fibrillation. Particularly is frequent cerebrovascular embolism. During the existence of atrial fibrillation, systolic pressure in the pulmonary artery and the right chamber increases. It also increases the pressure in the left atrium, while the systemic blood pressure decreases which is consistent with reduced efficiency of cardiac work. Taken into account the fact that in atrial fibrillation the efficiency of cardiac work is reduced, as well as that clinically significant consequences of the emergence of peripheral embolism occurs, with particularly high risk in rheumatic heart defects, we make conclusion that this disorder of heart rhythm needs attention to be paid to.

Material and methods of investigation

The study was conducted as prospective, retrospective, clinical, descriptive and analytical. The sample consisted of the University of Sarajevo students. The survey sample included students in regular and systematic scheduled exams for the Department of Public Health students at the University of Sarajevo in the period 2006 - 2009 ye-

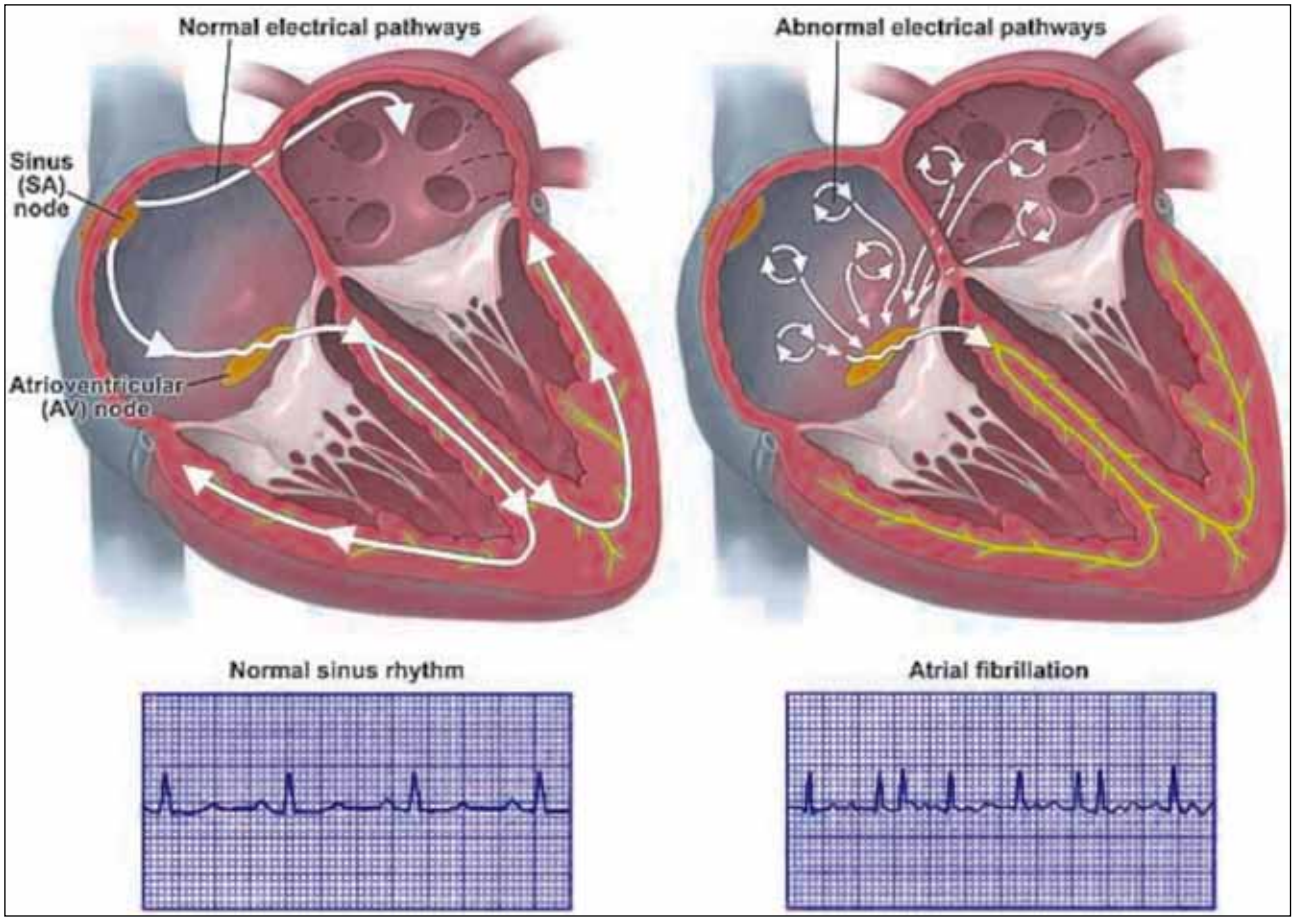

Figure 1. Atrial fibrillation: atrial rhytm rate 350-600/min

ars. The analysis was done on 109 students. Our intention is to continue to study and include as many students of all ages and to make a comparative study with similar studies from the region.

Methods of investigation

a) Analysis and presentation of personal history data for assessing cardiac status of the patient and family history;
b) Analysis and presentation of auscultatory data on cardiac rhythm and its regularity, intensity and quality of heart tones, systolic and diastolic sounds, heart noises;
c) Analysis and presentation of ECG findings;
d) Analysis and presentation of a 24-h Holter heart monitoring;
e) Analysis and presentation of echocardiography findings;

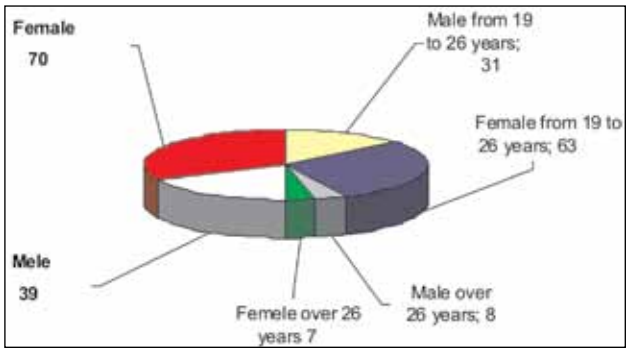

Figure 2. The majority of patients were in age group

The majority of patients were in age group:
- from 19 to 26 -**63 female patients or 90% and male patients 31 or 79,49%**
- over 26 - **7 female patients or 10% and male patients 8 or 20,51%**
- relatively total **94 (86,24%)** all examined patients.

The difference is highly significant on level p<0,0005 for male population with t=3,68, for female population on level p<0,0000 with t=6,69, and for total sample on level p<0,0000 with t=7,57.

Total women**: 70 or 64,22% men 39 or 35,78%**

Results of the investigation

Display of presence of atrial fibrilation in male patient population

Table 1. Review in male patients

Type	Number	%
Hypertensio art.	3	7,69
Systemic and metabolical disorders	1	2,56
Athlete's heart	2	5,13
Congenital and acquired heart deffects	9	23,08

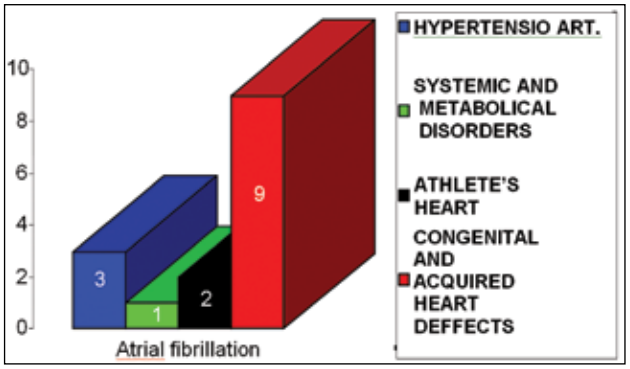

Figure 3. Frequency relation of atrial fibrilation in male patient population

Display of presence of atrial fibrilation in female patient population

Table 2. Review in female patients

Type	Number	%
Hypertensio art.	2	2,86
Congenital and acquired heart deffects	28	40,00
Other	1	1,43
Foci	6	8,57
Hypotensio art.	1	1,43
Endokrinological disorders	2	2,86
Systemic and metabolical disorders	6	8,57
Neurological disorders	1	1,43

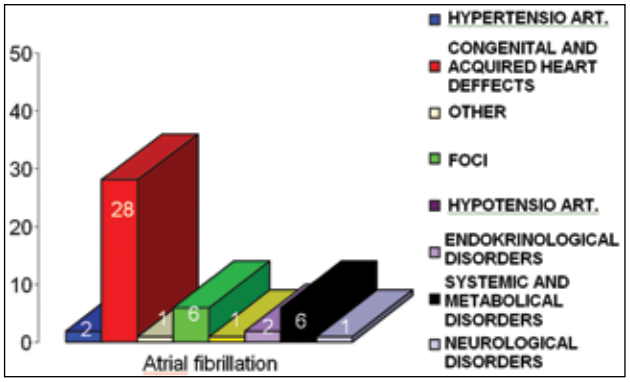

Figure 4. Frequency relation of atrial fibrilation in female patient population

Display of atrial fibrillation in all patients

Table 3. Review in all patients

Type	Number	%
Other	1	0,92
Foci	6	5,50
Hypertensio art.	5	4,59
Hypotensio art.	1	0,92
Endokrinological conditions	2	1,83
Systemic and metabolical conditions	7	6,42
Neurological conditions	1	0,92
Athlete heart	2	1,83
Congenital and acquired heart deffects	37	33,94

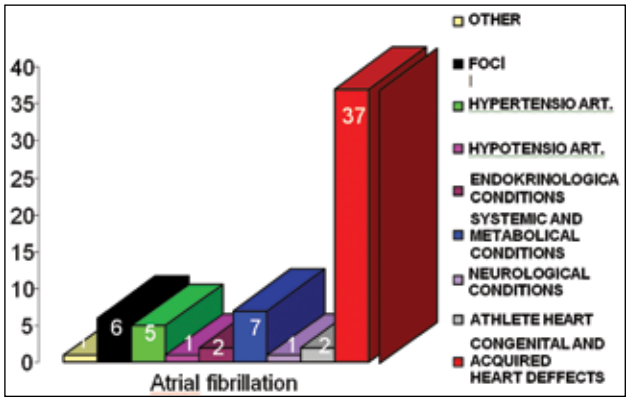

Figure 5. Atrial fibrillation in all patients

Relation of frequency of atrial fibrillation in all patients

There is a significant difference on level of $p<0,01$ compared to congenital and acquired heart defects with $t=2,68$ and $t=2,62$ and less significant on level $p<0,05$ for $t=2,41$ and $t=2,27$

Discussion

Electrocardiogram diagnosis of atrial fibrillation is easily made and is based on the absence of P-waves, the existence of numerous wavelets of fibrillation and an irregular schedule of QRS. It is believed that the dissociated electrical activity of atria is a consequence of the existence of more or less circular orbits in the atria in which impulse duration is continuous and activates adjacent parts of myocardium. In clinical terms, patients can develop an attack of paroxysmal atrial fibrillation due to a cause, a fear, excitement and physical exertion. However, if there are causes in the atria, which alters the electrical properties of myocardium, damaged areas, then the atrial fibrillation can sustain by themselves even the original cause is removed. According to clinical observations, disorders in the transfer of impulses in the atria that arise if there are damaged areas in the myocardium, which are hyperpolarized contribute to the creation and maintenance of atrial fibrillation.

Extending impulse path in the atria occurs when the atria is dilated due to excess blood in it, especially left one, and shortening the refractory time under the influence of some drugs and an increase in vagal tone. It happens that there is not the same rhythm in the entire atrial myocardium. It is considered that there may be a atrial triple rhythm, sinus rhythm, fibrillation, and in some parts flutter of atria. Atrial fibrillation is one of the most common and important arrhythmias.

The cause is in most cases, organic heart disease, but there are also a number of cases without

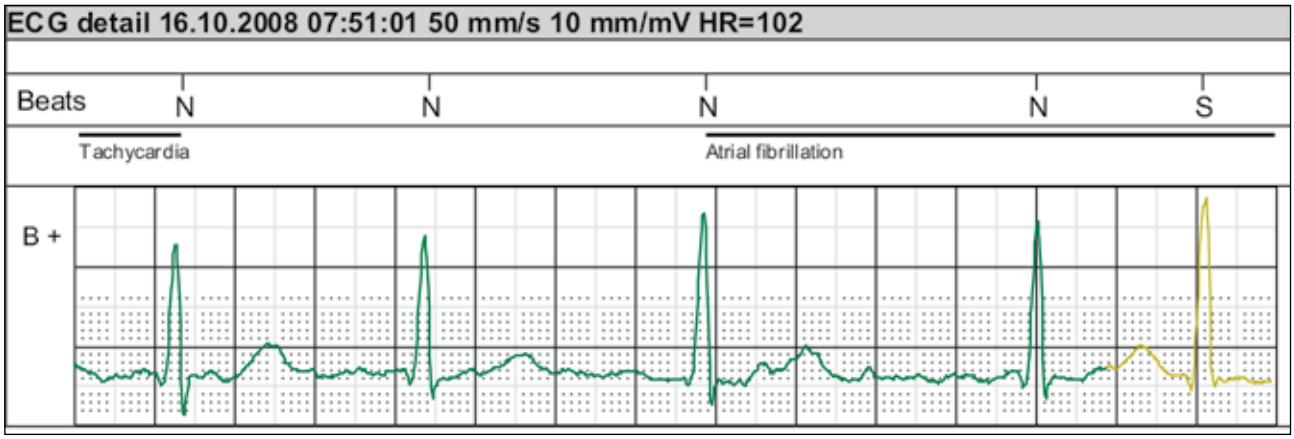

Figure 6. Display of segments from report 24 h Holter-monitoring in investigated students: atrial fibrillation

heart disease. It occurs in people with heart disease in acquired heart defects, defects in the mitral valve: mitral valve prolapse, cardiomyopathy of different origin, hypertensive origin infiltrative, endocrinology and other. Experts say there are many cases where the cause of atrial fibrillation has never been found. Alcohol - regular, excessive, long-term alcohol consumption is closely associated with an increased risk of developing atrial fibrillation.

A study by scientists at Beth Israel Medical Center showed that the risk of atrial fibrillation is as much 45 percent higher among heavy drinkers than abstainers. Performing your original search, Beth Israel Medical Center in atrial fibrillation and alcohol, in PubMed will retrieve 3 records. Am Heart J. 2007

Smoking is associated with several heart problems, including atrial fibrillation. Excessive consumption of caffeine, which may involve too much coffee, energy drinks, and / or carbonated drinks. Atrial fibrillation is often discovered during routine medical care because many people have no symptoms. Other people may notice an irregular pulse, but without the presence of other signs of illness. Instead of maintaining the proper rhythm, the atrium is shaking, and in this way, blood can accumulate and merge. Clots can be created and travel to the brain and cause a stroke (it is estimated that atrial fibrillation was the cause in 15 percent of strokes). Doctors have long known that atrial fibrillation is inherited, however, failed to assess how it affects the risk itself.

American experts recruited more than 4,000 people in their research, which did not have atrial fibrillation at the very beginning. Each participant had at least one parent or sibling who were also involved in the study. It was found that atrial fibrillation occurred in 40 percent more time in subjects who had a family member with atrial fibrillation. "This is another proof which asserts that knowledge of family history may be helpful in assessing the risk for various conditions," optimistic was Dr. Emelia Benjamin, one of the leading persons of research.

Research Focus: A heart rhythm disorder called atrial fibrillation affects more than two million Americans. Doctors lack a reliable way to identify people whose lifestyles, genetics, or health conditions put them at high risk. A team of scientists funded by an NHLBI Recovery Act grant seeks to fill this knowledge gap by developing a more precise atrial fibrillation risk assessment tool. The tool will build on a model created last year by researchers in the landmark Framingham Heart Study. Since that model was based on people living in Framingham, Massachusetts, the new study will test whether it applies to the diverse population of the United States. The researchers will pool existing data from five population studies conducted across the U.S. to create a sample of more than 30,000 people with over 3,000 cases of atrial fibrillation. Then they will examine the data for biological markers of atrial fibrillation. Rotterdam Study T Benjamin EJ. 2009. Development of a risk score for atrial fibrillation (Framingham Heart Study): a community-based cohort Lancet; hese groups already collaborate as part of the ongoing Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) consortium The Framingham Heart Study Section of Preventive Medicine Section of Cardiology Whitaker Cardiovascular Institute Epidemiology Department, Boston University.

Conclusion

Atrial fibrillation is the most frequent in patients with congenital and acquired heart defects-37 congenital and acquired heart defects (33.94%), 7 systemic and metabolic disorders (6.42), 6 foci (5.50%), 5 hypertension (4.59%), 2 endocrinological disease (1.83%), 2 athlete's heart (1.83%), 1 hypotension (0.92), a neurological disorder (0.92%), 1 with other disorders (0 , 92%). Taking into account the fact that the efficiency of cardiac activity is reduced in atrial fibrillation, as well as that clinically significant result occurs formation of peripheral embolism, with particularly high risk in case of rheumatic heart defects, we come to conclusion that the disorder of heart rhythm in students needs attention. It is necessary to perform a complete cardiac evaluation: ECG, X-ray of the heart and lungs and TEE. Medicamentous treatment is mandatory: Propranolol and Digoxin, are administered in order to slow down ventricular rate. Cardioversion may be indicated. It is necessary to regularly monitor the patient.

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Hyperprolactinemia: Medication induced hyperprolactinemia or phenomena due to pathological causes such as Prolactinomas?

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Abstract

Introduction: Hyperprolactinemia is a common condition that can result from a number of causes, including medication use or pathological causes, such as prolactinomas. This article critically evaluates the literature regarding the frequencies at which such medications can cause hyperprolactinemia. Following clinical consequences of hyperprolactinemia, medications that most commonly cause hyperprolactinemia are antipsychotic (neuroleptic) agents.

Aims: The aim of this study was to make an analysis of hyperprolactinemia in patients hospitalized at Psychiatric Clinic of Clinical Centre University of Sarajevo (KCUS) during 2010 and to present hyperprolactinemia in patients according to the therapy they used.

Material and methods: This prospective, clinical study was conducted during six month period at the Department of Psychiatry of Clinical Centre University of Sarajevo over a period June 2010 - January 2011. Our study included 16 examinees with hyperprolactinemia.

Results: A one-way repeated measures ANOVA was conducted to compare scores on the Level of Prolactine across the five time periods There was a significant effect for Level of Prolactine [$F(4, 20)=24.61$, $p<.0001$]. The results of Friedman nonparametric test suggests that there are significant differences in the Level of Prolactine scores across the five time periods [$\chi^2(4)=34.960$, $p<.0001$]. Comparing the ranks for the five sets of scores, it appears that there was a steady decrease in Level of Prolactine scores over time.

Conclusion: Clinical consequences of hyperprolactinemia have received limited attention. We hope that hyperprolactinemia may be prevented or minimized with current management approaches, clinicians understanding of the short and long-term consequences of hyperprolactinemia

Key words: Atypical antipsychotics, Risperidone, Amysulpirid, hyperprolactinemia

Introduction

Hyperprolactinemia is a common condition that can result from a number of causes, including medication use or pathologic causes, such as prolactinomas. This article critically evaluates the literature regarding the frequencies at which such medications can cause hyperprolactinemia.

The main physiological control of prolactine (PRL) secretion is exerted by the inhibiting action of dopamine. Endocrinology investigation in schizophrenic patients showed that the patients reported reduced levels of luteinizing hormone (LH) and follicule-stimulating hormone (FSH), including reduced release of prolactine and the growth hormone. By reducing the levels of dopamine, antipsychotics increase their synthesis and release of prolactine, particularly in women. Normal level of prolactine, two hours after waking up, is 20 ng/ml in males and 25 ng/ml in females (1). Antipsychotics also affect hypophysis, adrenocortical, thyroidal and gonadal activity. In general population, prevalence of hyperprolactinemia is low (0.4%) (2). Antipsychotics are the most common cause of pharmacologic hyperprolactinemia (3). Blockage of dopaminergic receptors of the tuberoinfundibular system results in development of hyperprolactinemia induced by the use of certain antipsychotics. Specifically, D_2 receptors in hypophysis tonicaly prevent release of prolactine. Recent research showed that hyperprolactinemia reported significantly higher prevalence in use of antipsychotics of the first generation (such as Sulpirid); newer second-generation

of antipsychotics with prevalence rate (89%) compared to third generation of antipsychotics (4). Zyprasidon is known to cause temporary increase of serum prolactine levels. Other than antipsychotics, antidepressants, anti-hypertension agents and medications that increase bowel motility can also cause hyperprolactinemia. Cocaine abuse has also been associated with chronic mild hyperprolactinemia (5,6). Morphine and morphine analogues increase PRL release both in short-term period (7,8,9,10). It remains unclear if amisulpiride can cause pituitary adenomas and if this process is reversible.

Hyperprolactinemia can also be compounded by alterations in behavior and mood (depression, anxiety, hostility, attention disorders and psychosis) (11). Also, there is a possibility of immunologic depression to be developed (1).

Typical antipsychotics may determine a significant increase in prolactine serum concentration that is frequently associated with symptoms. In comparison to other antipsychotics that are infrequently and only temporarily associated with increase of prolactine levels, Risperidone is most likely to induce hyperprolactinemia. Atypical antipsychotics such as clozapine, quetiapine and olanzapine have shown limited or temporary effects on serum prolactine levels. This could be scientifically accepted because their actions at other receptors sites result in relatively less dopamine blockade or in central distribution due to lower peripheral (4,11,12,13). Quetiapine is used as a first-line treatment in psychotic patient with concurrent prolactine-related disturbance, such as a prolactinoma (14).

Materials and methods

This prospective study was conducted during the six month period at the Department of Psychiatry of Clinical Centre University of Sarajevo over a period June 2010 - January 2011. Our study included 16 examinees with hyperprolactinemia.

Inclusion Criteria were:

- Female and male schizophrenic patients,
- Antipsychotic treatment with Risperidon,
- Diagnosis of a clinically relevant hyperprolactinemia,
- No indication of disturbance of the somatocortico or thyreotropic hypophysis-axis (cortisol, ACTH,TSH, FT3, FT4).

Exclusion Criteria were:

- Severe somatic disease, especially coronary disease,
- Pregnancy.

We excluded medication that cause hyperprolactinemia as antipsychotic agents, and followed levels of prolactine in time period: Time 2 (14 days Post- intervention), Time 3 (28 days follow-up), Time 4 (two months follow-up) and Time 5 (six months follow-up)

Patients' early morning (maximum two hours after waking up) values of serum prolactine level were tested and the positive results of the tested levels came back. Normal levels of prolactine in that time of measuring in KCUS was (78 nIU/ml–380 nIU/ml) in males, and (64 nIU/ml-590 nIU/ml) in females.

Results are expressed as mean value and standard deviation in case of normal distributed continue variables**,** as median and IQR in case of non-normal distributed continue variables. The Kolmogorov–Smirnov statistic with a Lilliefors significance level was used for testing normality. One-way repeated measures ANOVA was conducted to compare scores across the five time periods for continue normal distributed variables and Friedman nonparametric test was conducted to compare scores across the five time periods for continue non-normal distributed variables. A p-value <0.05 was considered as significant. Statistical analysis was performed by using the Statistical Package for the Social Sciences (SPSS Release 16.0; SPSS Inc., Chicago, Illinois, United States of America) software.

Results

We followed clinical consequences of hyperprolactinemia, the medications that most commonly cause hyperprolactinemia as antipsychotic (neuroleptic) agents. During the six month period, we followed patient admitted to the Unite of Intensive Care of Department of Psychiatry of KCUS and found 16 patients with hyperprolactinemia. Among them we detected three cases with prolactinomas and administration of amisulpiride (long-term period) with high doses (300-400 mg); three cases with hyperprolactinemia following administration of amisulpiride (long-term period) with doses (100-

150 mg) and 10 cases following administration of an atypical antipsychotic (risperidone) with hyperprolactinemia. We also suggested a therapeutic and a management plan for our patients.

a) Three cases showed microadenomas on MRI before the diagnostic method (4.5 mm; 2.5 mm and 4.0 mm). They were treated with different antipsychotics (haloperidol, amisulpiride, chlorpromazine). More than one year these three cases were on a long-term period of high doses administration (300-400 mg) amisulpiride, (a high dose of amisulpiride is effective in treatment of positive symptoms of schizophrenia).

b) Three patients reported hyperprolactinemia, two cases after long-term period of administration amisulpiride (a dose of amisulpiride were 150 mg daily), one case after being on amisulpiride for short period of time (a low dose of amisulpiride is effective in treatment of negative symptoms of schizophrenia as proved by our patient who was on 100-150 mg amisulpiride daily).
Amisulpiride is a highly selective D_2/D_3 receptor antagonist (15). Our three patients were on high dosages of amisulpiride over a long-term period. The therapy antagonized the postsynaptic D_2/D_3 receptors which resulted in reduced dopamine transmission. In our patients, amisulpiride determined occupation of D_2/D_3 receptors at the pituitary level due to poorer permeability of the brain barrier as shown in the study (16).

c) Ten cases were treated with Risperidone
Ten cases of early detection of hyperprolactinemia following administration of risperidone were not detected immediately after 12-24 hours of administration but after a few days following administration of risperidone, the same was detected. Specifically, those 10 cases of hyperprolactinemia treated at out ward involved a temporary blocking of D_2 using atypical neuroleptics with early effects of hyperprolactinemia following the acute administration. We tested prolactine levels in the serum a few days after the administration rather than after 12-24 hours (a variable mean peak time, 120 minutes for risperidone)

A one-way repeated measures ANOVA was conducted to compare scores on the Level of Prolactine with Statistics test at Time 1 (prior to the intervention), Time 2 (following the intervention), Time 3 (28 days follow-up), Time 4 (two months follow-up) and Time 5 (six months follow-up). The means and standard deviations are presented in Table 1.

Mauchly's test indicated that the assumption of sphericity had not been violated, $\chi^2(9)=10.6$ There was a significant effect for Level of Prolactine [$F(4, 20)=24.61$, $p<.0001$].

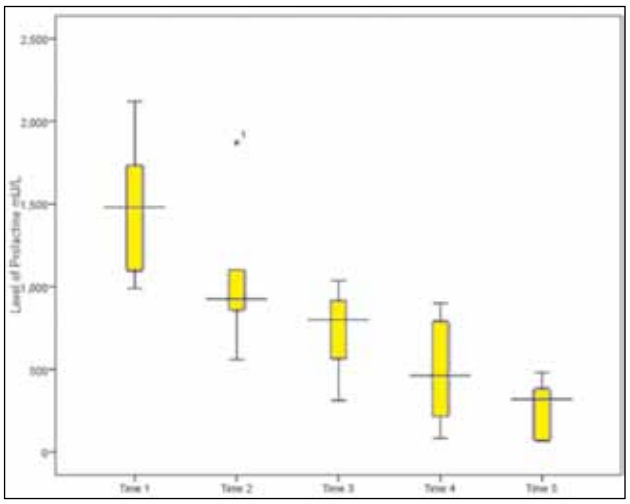

Figure 1. Box plot of Level of Prolactine for different times periods (n=6)

Table 1. Descriptive Statistics for Level of Prolactine (nIU/ml) with Statistics Test Scores for Time 1, Time 2, Time 3, Time 4, and Time 5 (n=6)

Time period	n	Mean	Standard deviation
Time 1 (Pre-intervention)	6	1483.7	442.5
Time 2 (14 days Post-intervention)	6	1040.3	444.4
Time 3 (28 days follow-up)	6	738.0	268.7
Time 4 (two months follow-up)	6	484.5	343.7
Time 5 (six months follow-up)	6	272.3	168.6

Table 2. Descriptive Statistics for Level of Prolactine (nIU/ml) with Statistics Test Scores for Time 1, Time 2, Time 3, Time 4, and Time 5 (n=10)

Time period	n	Minimum	Maximum	Median	IQR
Time 1 (Pre-intervention)	10	796	1422	1009.50	219
Time 2 (14 days Post-intervention)	10	215	987	700.00	564
Time 3 (28 days follow-up)	10	74	621	453.50	366
Time 4 (two months follow-up)	10	65	340	215.00	258
Time 5 (six months follow-up)	10	63	490	79.00	154

A Friedman nonparametric test was conducted to compare scores on the Level of Prolactine with Statistics test at Time 1 (prior to the intervention), Time 2 (following the intervention), Time 3 (28 days follow-up), Time 4 (two months follow-up) and Time 5 (six months follow-up). The medians and IQR (Interquartile range) are presented in Table 2.

The results of this test suggest that there are significant differences in the Level of Prolactine scores across the five time periods [$\chi^2(4)=34.960$, $p<.0001$]. Comparing the ranks for the five sets of scores, it appears that there was a steady decrease in Level of Prolactine scores over time.

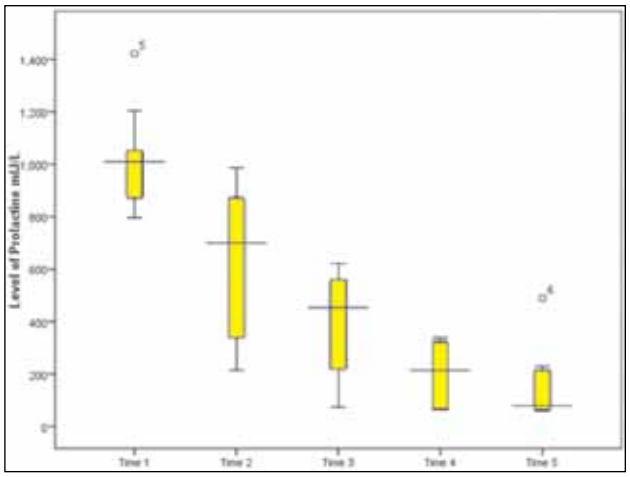

Figure 2. Box plot of Level of Prolactine for different times periods (n=10)

Discussion

During six month monitoring of the patients at the Unit of Intensive Care of Department of Psychiatry of KCUS, we noticed changes in the prolactine levels in 16 patients. In three patients changes on the MRI scans were reported in the form of microadenoma of the hypophysis whose dimensions were less than five mm. Three patients displayed hyperprolactinemia symptoms as a result of a long-term amisulpiride therapy, while 10 patients displayed such symptoms following administration of atypical neuroleptic risperidone. These patients were already using typical antipsychotics (haloperidol, chlorpromazine) for sustained period of time. Upon admission to the Unite of Intensive Care of Department of Psychiatry, clinical symptoms in women were dominated by anxiety, sleeping disorder, imposition of forced thoughts, hostility, hallucinations, headache, lack of menstruation, including galactorrhoea that was reported in three female patients (these patients were previously put on a protracted therapy with amisulpiride for a year).

Risperidone is one of the atipical neuroleptics that is most likely to induce hyperprolactinemia (it does not fully cross the blood-brain barrier). Endocrinal side effects of risperidone (amenorrhea, galactorrhoea in women, gynecomastia, loss of libido and erectile dysfunction in men) ranged from 1% - 10%. In six cases prolactine levels dropped immediately following discontinuation of risperidone but four patients continued to report slightly higher levels of prolactine within three months following the discontinuation. This period can be extended up to 54 weeks for risperidone and up to 52 weeks for haloperidol (17, 18). Treatment for our six patients using dopamine agonists in risperidone-induced hyperprolactinemia was successful as shown in Tollin's research (19).

In case of a clear symptomatic, neuroleptic-induced hyperprolactinemia, patients were medicated in four cases with bromocriptine (dopamine agonist) and in two cases were treated with cabergoline, 0.125-0.250/week for 8 weeks. After cabergoline treatment, the mean decrease in plasma prolactin levels was statistically significant ($p<0.05$) for the sample, and patients showed remission of clinical signs with prolactin values within the normal range (5).

In Tollin's research, patients were medicated with bromocriptin, and some of them were treated with cabergoline, 0,125/week for 8 weeks. After cabergoline treatment, the mean decrease in plasma prolactin levels was statistically significant ($p<0.05$) for the sample, and patients showed remission of clinical signs with prolactin values within the normal range (19).

Clinical monitoring of the patients focusing on the dominating psyhopathology and also other symptoms showed manifestations of hyperprolactinemia in three groups of patients. One group included patients with hyperprolactinemia induced by administration of typical neuroleptics, the second group of patients were put on atypical neuroleptics and the third group of patients, whose MRI scans showed microadenoma which we could not confirm as for timely adjusted diagnostics were not applied, have been a result of administration of neuroleptics and their effects at the pituitary level or the change of the same was simply solitary.

In our study three patients with the microadenoma were on long-term therapy of amysulpiride. As in our study, it remains unclear if amysulpiride can cause a pituitary adenomas and if this process is reversible after switching medication off (18,20,21,22).

Conclusion

Neuroleptic-induced hyperprolactinemia has been a „cost" of traditional antipsychotic therapy. Clinical consequences of hyperprolactinemia have received limited attention. It is very important to ensure that hyperprolactinemia in an individual patient is due to medication and not to a structural lesion in the hypothalamic-pituitary area. Low-dose cabergoline treatment of risperidon-induced hyperprolactinemia may be safe and clinically effective in a relevant number of patients. We hope that hyperprolactinemia may be prevented or minimized with current management approaches and clinicians understanding the short and long-term consequences of hyperprolactinemia. Treatment strategies include switching to an alternative medication that does not cause hyperprolactinemia (Quetiapin as first-line treatment for a psychotic patient with a concurrent prolactin-related disturbance, such as a prolactinoma) or cautiously adding a dopamine agonist. We would like to draw attention to the fact that pituitary tumors may exist co-incidentally in patients on neuroleptic medications and contribute to hyperprolactinemia.

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Chlamydia Trachomatis and female infertility

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Abstract

Chlamydia trachomatis infection is very important etiological factor of female infertility. It is prevalent sexually transmited infection that can lead to serious reproductive morbidity. Management and control of Chlamydia trachomatis is challenge lagely due to its asimptomatic nature.

Aim: The purpose of this study was to evaluate the frequency of Chlamydia infection in unselected infertile women.

Material and methods: A method used to detect Chlamydia trachomatis in the cervical swabs of infertile women was direct imunofloroscent test. If it was a suspect, then we used enzyme-immuno assay ELISA test. The study included 807 patients who were divided into two groups. The first group consisted of those patients treated for primary (493) infertility, and second group of secondary infertility (314). Patients treated for secondary infertility were further divided regarding the infertility after abortion or after delivery. Also, patients were divided into two age groups: below 35 and above 35.

Results: Cervical smear positive findings on Chlamydia trachomatis was in 75 (9.3%) patients, out of which in 40 (5%) treated for primary and 35 (4.3%) treated for secondary infertility. Positive findings on Chlamydia trachomatis in patients treated for secondary infertility after abortion was in 20 (2.5%) patients and 15 (1.8%) of those following childbirth.

Conclusion: In patients treated for infertility cervical smear on Chlamydia trachomatis should be performed.

Key words: Chlamydia trachomatis, Infection, Infertility

Introduction

A Chlamydia trachomatis serological type of D and K is the most common cause of sexually transmitted diseases. It has a special affinity for the cells of cylindrical and transitional epithelium which penetrates so it binds to receptors on the cell wall. Surrounded by vesicles, settles in the cytoplasm and destroys the host cell. It can be found in women on the vulva, vagina, endocervix and urethra. From the endocervix, where mostly resides, penetrates vertically through the endometrium, and then to the uterine tube and ovaries. Besides such vertical canalicular spreading, infection can spread to the adnexes via lymph.

Untreated Chlamydia trachomatis infection in women can result in disease sequele such as salpingitis and pelvic inflammatory disease, ultimately culminating in tubal occlusion and infertility. In some cases, perihepatitis and perisplenitis may arise as the late complications. During the presence of Chlamydia trachomatis infection (particularly in case of prolonged infection) immune system of women produces antibodies against Chlamydia proteins - “heat shock protein”. Woman's body defense cells "remember" that they should attack the heat shock protein of Chlamydia trachomatis. During pregnancy, one of the first proteins that produce an embryo is a protein similar to heat shock protein of Chlamydia, and the woman's immune system may react against the embryos resulting in abortion (1).

There is a strong evidence that the infection with Chlamydia trachomatis presents one of the leading preventable causes of infertility and many infertile patients have serologic confirmation of Chlamydia trachomatis infection in the past .(2)

The number of Chlamydia trachomatis genital tract infections is steadily increasing wordwide, with approximately 50%-70% of asymptomatic infections. 3)

The diagnosis of infection with Chlamydia trachomatis in women can be made on the swabs of the urethra, cervix, endometrial samples, or blood and urine samples. Valid samples are those that contain the cells with pathogens. Material to be taken for laboratory diagnosis of Chlamydia trachomatis infec-

tion should be submitted as soon as possible to the laboratory and 2SP (2-sucrose phosphate transport medium) used as a transport medium. Following laboratory diagnostic methods are used: direct microscopic examination, isolation of pathogens, serological tests and detection of antigens and nucleic acid with immunological methods.

Material and methods

In this study we had 807 patients aged 21-43 years who were treated for infertility at the Gynecology and Obstetrics Clinic in Sarajevo. Patients were divided into two groups: the first group comprised of patients treated for primary infertility (493) and another group of those who were treated for secondary infertility (314). Patients treated for secondary infertility were further divided regarding the infertility after abortion or after childbirth. In all of our patients cervical smears were submitted for test on Chlamydia trachomatis.

Additionally, patients were divided according to age into two groups: those under 35 years of age and those over 35.

Diagnosis of Chlamydia trachomatis was made in the Institute of Microbiology, Immunology and Parasitology of the University Clinical Centre of Sarajevo. Direct immuno-florescent test (DIF - BIO-RAD® Pathfinder Chlamydia trachomatis Direct Specimen was used for detection of antigen Chlamydia trachomatis in cervical smears.

If the test was suspect, detection of IgM and IgG antibodies specific for Chlamydia trachomatis was performed by enzyme immuno assay (ELISA - Novatec Immunodiagnostica GMBH, Novalis).

The aim of this study was to determine the incidence of Chlamydia trachomatis genital tract infection in patients treated for infertility, whether there is a difference in the incidence of infection in those treated for primary or secondary infertility as well as to find if there is a difference in infection rates depending on the age of the patients.

Results

Positive microbiological findings of endocervical swab for Chlamydia trachomatis was found in 75 (9.3%) of 807 patients. Out of these there were 40 patients (5%) who were treated for primary infertility and 35 (4.3%) patients who were treated for secondary infertility. (Table 1) There is no statistically significant difference in the prevalence of Chlamydia infection among patients treated for primary and secondary infertility. (P> 0.05)

Positive endocervical swab was found in 20 (2.5%) patients treated for secondary infertility after birth and in 15 (1.8%) patients after an abortion. (Table 2.)

Incidence of positive endocervical swab for Chlamydia trachomatis in a group of patients treated for secondary infertility after the birth and after abortion was similar and there were no statistically

Table 1.

Patients	Positive Smear on Ch. trachotatis	Negative smear on Ch. trachomatis	Total
Primary infertility	40 (5%)	453 (51,4%)	493 (56,1%)
Secondary inferility	35 (4,3%)	279 (39,3%)	314 (43,9%
Total	75 (9,3%)	732 (90,7%)	807 (100%)

Table 2.

Patients	Positive smear on Chlamydia trachomatis
Secondary infertility after abortion	20 (2,5%)
Secondary infertility after birth	15 (1,8%)
Total	35 (4,3%)

Table 3.

Patients age group	Positive smear on Chlamydia trachomatis
<35	58 (7,2%)
>35	17 (2,1%
Total	75 (9,3%)

significant difference between these two groups. (X2 = 1.05 P = 0.30 0r 1.52295% CI 0677-3455)

A positive swab in Chlamydia trachomatis was in 58 (7.2%) patients under 35 years of age and in 17 (2.1%) patients older than 35 years, and there is a statistically significant difference. (P<0.05) (Table 3)

Discusion

Chlamydia trachomatis, an obligate intracellular bacterium, is responsible for the most common sexually transmitted disease. It is very important etiological factor of female infertility. Almost 80% cases of infections caused by this pathogen is asymptomatic making it difficult to recognize and treat. In general population the prevalence of genital Chlamydia trachomatis infection ranges from 2% -12% (4).

In their study, Keyhani and colleagues show a much higher incidence of Chlamydia trachomatis infection in infertile women compared to women who gave birth. (5)

We analyzed patients who were treated for secondary infertility and there were 20 (2.5%) patients after birth and 15 (1.8%) after an abortion in which we found a positive finding of endocervical swab for Chlamydia trachomatis. We found no statistically significant difference between the two groups treated for secondary infertility

Watson and colleagues report in their study that infection with Chlamydia trachomatis was found in 6% of patients with secondary infertility after the birth and in 5% of patients with secondary infertility after abortion (6).

Infertile women should be routinely tested for Chlamydia trachomatis infection. Chlamydia trachomatis infections was detected in 8,7% patients.(7)

Al-Ramahi and colleagues report on 3,9%. positive tests on Chlamydia trachomatis in the infertility group tested (8)

In our study we detected Chlamydia trchomatis in 9,3% of patients treated for infertility. There were 5% patients with primary and 4,3% with secondary infertility. Some authors detected even higher percentage of infections with Chlamydia trachomatis in patients treated for infertility. Chlamydia trachomatis was detected in 28,1% of infertile women.(9) Chlamydial pozitivity was seen in 27% women with primary infertility and in the 30,6% with secondary infertility.(9)

Screening for Chlamydial infection should be done routinely by suitable tests in sexually active symptomatic and asymptomatic women including pregnant women in order to prevent serious complications.(10)

Screening of infertile women for Chlamydia trachomatis is therefore recommended as an early therapeutic interventions. In high-risk settings, 2%-5% of untreated women developed PID within the approximately 2-week period between testing positive Chlamydia trachomatis and returning for treatment.(11)

Conclusion

Our findings suggest that Chlamydia trachomatis infection is present in 9,3% of unselected infertile women. Screening of infertile women for Chlamydial infection as part of routine investigation for infertility is recomended.

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Sex determination of the Bosnian population based on discriminant function analysis

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Abstract

Objective: The goal of this study was to determine sex of the Bosnian population males and females on their skeletal remains based on humeral and femoral head diameters.

Methods: This study included skeletal material of the humeri and femurs from left and right side of the body that belonged to the 135 people, 100 men and 35 women. All individuals were older than 25 years, and all epiphyses, on the examined bones, where closed. There have been excluded all bones with ongoing pathological process or post mortally damaged. This conditions reduced number of analyzed bones, so that at final 28 left and 25 right humeri, and 31 left and 33 right femurs of females where includes in this research. The following parameters were evaluated: maximum left and right humeral and femoral head diameters. All measurement where done directly on washed and dried bones with digital caliper and all values were expressed until the hundredth of a millimeter. Sexual dimorphism between variables was analyzed with sexual dimorphism index. All data were analyzed using descriptive statistic, t test and discriminant function analysis**.**

Results: Statistical analysis has shown all variable for males have bigger dimensions for femoral and humeral head diameters and also bigger variability of males than females. The biggest absolute difference has right humerus, and the lowest right femur. T test results shows significant difference between average lengths of humeral and femoral heads diameters, for males and females, considering $p<0.01$. The accuracy for the sex estimation using discriminant function is high, ranging from 89.06% for left humerus, 90.08% for left femur, 90.22% for right femur till 90.4% for right humerus. Accuracy is higher in females.

Conclusion: All variable shows statistically significant sexual dimorphism. The current study provides standards for the sample and further studies on bigger samples are needed to create population standards.

Key words: identification, sex determination, humeral head diameter, femoral head diameter

Introduction

When skeletonized bodies or body parts of unknown identity are recovered, it is necessary to estimate biological identity, using a variety of techniques. Sex determination is rarely based on only one skeletal feature alone. An expert is aware of the range of variation of sexual traits among skeletons and the degree of overlap that normally exists between males and females. As with the estimation of other parameters that lead toward a successful identification of the deceased, as many criteria as available are assessed before coming to a decision. It is imperative to develop rapid and easy techniques performed during autopsy to facilitate the identification procedure of skeletal remains. Morphological examination of remains is the quickest and easiest method of assessing sex in the great majority of unknown skeletal remains, and in experienced hands, it will result in 95–100% accuracy when the whole skeleton is available (1). Yet, this is rarely the case in forensic investigations. When a skeletonized bodies are recovered, very often from secondary or tertiary mass graves, parts of the bones are missing or are broken, and sometimes identification of sex is based on few components. Among the bones that are studied to define sex, some are stronger indicators—for instance pelvis—while others are less reliable. When fragmentary patterns are assumed, the identification becomes even more difficult. Several researchers, investigating complete and fragmented long bones concludes that sex assessment is possible but population affinity must be always taken

into account (2–4). Sexual dimorphism on the humerus and femur has been studied intensively and standards have been obtained for several different ethnic groups: In Asia, an Indian population, two Japanese, a Thai, and a Chinese population were studied (5–7). In South Africa, both African Whites and Blacks were studied and compared (8). Standards for Northern Americans included humeral dimensions, among others, (9) while a modern Guatemalan sample represents Latin America (10). In Europe, among the few published studies one should refer to the work of Mall et al. (11) on a German forensic ample, as well as the standards obtained from the Coimbra collection in Portugal (12). Some of studies are based on archaeological material, as is the case of sex determination of prehistoric central California skeletal remains using discriminator analysis of the femur and humerus (13) and of the sample from Beneville, Canada (14). In France, Olivier (15) represented standards for France population. Stewart (1979) established standards for white Northern American population (16). Šlaus and all represented standards for modern Croatian population using 195 femurs of war victims, with the accuracy of 94.4 % (17). Trancho and all examined 132 femurs of adult Spanish population. They proved that among 5 variables vertical femoral head diameter determined sex with accuracy of 91.23%, transversal diameter with the accuracy of 93.86% (18).

Materials and Methods

Skeletonized remains used in this research belong to war victim from northwest Bosnia who were exhumed and identified with the help of DNA analysis. Skeletal material was humeri and femurs from left and right side of the body that belonged to the 135 people, 100 men and 35 women. All individuals were older than 25 years, and all epiphyses, on the examined bones, where closed. There have been excluded all bones with ongoing pathological process or post mortally damaged. This conditions reduced number of analyzed bones so that at final 28 left and 25 right humeri, and 31 left and 33 right femurs of females where includes in this research.

The following parameters were evaluated: maximum left humeral head diameter (LHHD), maximum right humeral head diameter (RHHD), maximum left femoral head diameter (LFHD) and maximum right femoral head diameter (RFHD) (19). All measurement where done directly on washed and dried bones with digital caliper and all values were expressed until the hundredth of a millimeter. Sexual dimorphism between variables was analyzed with sexual dimorphism index (mean of the variable for male/mean of the variable for female x 100). The index shows degree of difference between sexes: the value around 100 show weak sexual differences. The difference between sexes is growing with the distance from 100. All data were analyzed using descriptive statistic, t test and discriminating function analysis. *P*-value for the statistic test is significant if $p< 0.01$, respectively 99% significance. Discriminate analysis is used to obtain:

- Model for determination discriminate points
- Point of separation, according to the model of discriminate analysis, for sex estimation.

Multifactor statistical analysis was done with computer program SPSS 14.0. Significance and relevance of the discriminate analysis model is observed with Eugen-value, Canonical correlation, Wilks' Lambda and Hi-square tests, as well as through values of standardized and non standardized coefficients, structural matrix and accuracy of derived functions.

Results

Table 1. shows that all variable for males have bigger dimensions for femoral and humeral head diameters. Standard deviation shows bigger values for males than for females, which shows bigger variability of males than females.

The biggest absolute difference has right humerus, and the lowest right femur. There is significant difference between average lengths of humeral and femoral heads diameters, for males and females, considering $p<0.01$ (**Table 2**.).

Eugen-value, canonic correlation (which has values from 0.724 to 0.736 shows significant relationship between groups and discriminate function I-IV, function considering that it is closer to 1 than 0). Chi-square with P=0.000 shows statistical significance of discriminate functions I-IV (**Table 3**.).

Table 1. Descriptive statistic parameters for the sample, separated by gender

Variable	N	Minimum	Maximum	Mean	SD
LHHD Males	100	43.76	55.21	49.29	2.54
RHHD Males	100	44.83	54.92	49.5	2.62
LFHD Males	100	43.86	55.39	49.63	2.59
RFHD Males	100	43.19	56.03	49.7	2.66
LHHD Females	28	38.00	47.96	43.09	2.13
RHHD females	25	37.77	48.82	42.65	2.37
LFHD Females	31	39.24	46.76	43.45	1.86
RFHD Females	33	39.82	46.96	43.62	1.83

N: Number of samples; SD-Standard deviation; All values are presented in millimeters.

Table 2. Comparison between sexes (sexual dimorphism)

Variable	Males		Females		Sexual dimorphism			
	N	Mean ± SD	N	Mean ± SD	Absolute difference	Index	t	p
LHHD[1]	100	49.29 ± 2.54	28	43.09 ± 2.13	6.2	114.39	11.79	< 0.01
RHHD[2]	100	49.50 ± 2,62	25	42.65 ± 2.37	6.85	116.06	11.89	< 0.01
LFHD[3]	100	49.63 ± 2,59	31	43.45 ± 1.86	6.18	114.22	14.64	< 0.01
RFHD[4]	100	49.70 ± 2,66	33	43.62 ± 1.83	6.08	113.94	14.67	< 0.01

[1]Left humeral head diameter (LHHD), [2] Right humeral head diameter (RHHD), [3]Left femoral head diameter (LFHD) and [4]Right femoral head diameter (RFHD)

Table 3. Discriminant analysis results for left and right humerus and femur

Variable included	Eugen-value	Canonical correlation	Wilks' Lambda	Chi-square	df	P
I diskriminant function for LH	1.104	0.724	0.475	93.356	1	0.000
II diskriminant function for RH	1.151	0.731	0.465	93.815	1	0.000
III discriminant function for LF	1.180	0.736	0.459	100.168	1	0.000
IV diskriminant function for RF	1.139	0.730	0.468	99.209	1	0.000

Table 4. Analyze of discrimintant functions I-IV

Function	Variable included	Non standardized coefficient
I Discriminant function for Left humerus	Left humeral head diameter	0.406
	Constant	-19.484
	Point of separation[1]	-0.709
	Limit value[2]	Males> 46.19>Females
II Diskriminant function for Right humerus	Right humeral head diameter	0.388
	Constant	-18.690
	Point of separation	-0.798
	Limit value	Males >46.075> Females
III Discrimintant function for left femur	Left femoral head diameter	0.410
	Constant	-19.766
	Point of separation	-0.668
	Limit value	Males >46.54>Females
IV Discriminant function for right femur	Right femoral head diameter	0.403
	Constant	-19.439
	Point of separation	-0.618
	Limit value	Males>46.66>Females

[1] Point of separation is defined with validity of discriminate points, which separate groups, by gender; [2] Limit values given in millimeters.

Discrimintant functions analysis, presented in **Table 4**., gives the limit values of head diameters in millimeters to distinguish male from female.

On the basis of discriminant analysis are derived equations to calculate discriminant scores for the sex classification, given in **Table 5**.

Table 5. Equations that calculate the discriminant scores for the sex determination

Discriminant function	Equations
I Left humerus	= -19.484 + 0.406 x LHHD[1]
II Right humerus	= -18.69 + 0.388 x RHHD[2]
III Left femur	= -19.766 + 0.41 x LFHD[3]
IV Right femur	= - 19.439 + 0.403 x RFHD[4]

[1]Maximum left humeral head diameter (LHHD), [2] maximum right humeral head diameter (RHHD), [3]maximum left femoral head diameter (LFHD) and [4]maximum right femoral head diameter (RFHD)

The accuracy for the sex estimation using discriminant function I-IV is high, ranging from 89.06% for left humerus, 90.08% for left femur, 90.22% for right femur till 90.4% for right humerus. Accuracy is higher in females.

Discussion

In such large number of missing persons in Bosnia and Herzegovina, accurate determination of sex is of great importance. Morphological approach of sexing may produce misleading results, especially when determination is performed by inexperienced persons. However methods based on measurements and morphometry are accurate and therefore can be used in determination of sex (20).

This study revealed that for all variables male have bigger dimensions of humeral and femoral heads. Standard deviations of the variables shows significant difference between two sexes that allows conclusion male are showing much bigger variation than females.

Sexual dimorphism between variables is analyzed using the index of sexual dimorphism. The biggest index of sexual dimorphism is for right humerus 16.06%, and the smallest for right femur 13.94%. All variable have statistically significant sexual dimorphism.

Sex determination was done by discriminant analysis. Accuracy of sex estimation using discriminant functions for both side of the body is high, from 89.06 – 90.4%. Accuracy in sex determination for left humeral head is 89.06%, for right humeral head 90.4%. This results are similar to the results Mall and all (11) who estimated, the accuracy on humerus is 90.41%. Dittrick and Suchey (13) claim the accuracy of 90%.

Accuracy of sex estimation using left femoral head diameters is 90.08%, and for the right side femur is 90.22% which is little lower then estimations from Šlaus and all 94.4% (17), and Trancho and all (18), who estimated accuracy for vertical femoral head diameter as 91.23% and for transversal head diameter as 93.86%.

Accuracy in all functions is bigger for females than for males, which could be caused by the difference in sample representation (100 male and 35 females), and the variability inside the group of individuals of same sex. The results show that humerus belong to the male if humeral head diameter is > 46.19 mm, and females if it is <46.19mm, for the left side of the body. For the right side, the limit value is 46.07 mm. These results are different in comparing with Olivier (15) and Stewart (16). Olivier results show that humerus belong to female if vertical diameter is less than 43 mm, and to the male if it is bigger than 48 mm, and to the results of Stewart (16) who's limiting value for females are same as Olivier's and for males is 47 mm for vertical humeral head diameter. They did not use discriminant analysis, but 95% confidence interval.

Discriminant analysis for left femur shows that femur belong to male if the head diameter is >46.54 mm, and female if the measured diameter is <46.54 mm. Limit value for right femur is 46.66 mm. These results are close to the Šlaus results for Croatian population (17), where limited value for maximal femural head diameter is 45.4 mm. Tranchos (15) results for Spanish population counted limited value for vertical femur head diameter 44.13 mm. Stewarts (16) results sows that female femoral head diameter is less than 42.5 mm. Femur probable belong to the female if the diameter is from 42.5 to 43.5 mm. Femur probable belong to the male if the diameter is from 46.5 to 47.5 and femur certainly belong to male if the head diameter is bigger than 47.5 mm (16). Although these results are not obtained by discriminant analysis, still they have great significance for the sex discrimination, because of their practicality. Comparing these results with Stewarts results it is seen that

the point of discrimination for left and right femur which determine when femur probable belong to the male, are almost the same.

Conclusion

This study confirms possibility of estimation sex using femoral and humeral head diameters for Bosnia and Herzegovina population using the discriminant functions analyses with the high accuracy. The left humerus is the bone that allows the best distinction between genders with accuracy of 90.4%. Accuracy is higher for females. However, it is necessary to keep in mind individual biological variations that can produce incorrect sex determination in certain cases. To establish standards for sex estimation for the population of Bosnia and Herzegovina, further research on larger samples, taking in account the other measurements, is needed.

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New Ruthenium Complexes with Schiff Bases as Mediators for the Low Potential Amperometric Determination of Ascorbic Acid, Part I: Voltametric and Amperometric evidence of mediation with Tetraethylamonium dichloro-bis[N-phenyl-5-hloro-salicylideniminato-N,O]ruthenat (III)

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Abstract

A bulk modified screen printed electrode was prepared with an aqueous insoluble redox mediator *Tetraethylamonium dichloro-bis[N-phenyl-5-chloro-salicylideniminato-N,O]ruthenat (III)* using carbon ink. The electrode was cyclovoltammetrically scanned between -0.4 V to + 0.4 V *vs*. Ag/AgCl in phosphate buffer, pH 7.4. The modified electrode demonstrates fast response in oxidizing of ascorbic acid. Hydrodynamic and Flow injection amperograms were also recorded for electrocatalytic oxidation of ascorbic acid by new sensor. The amperometric measurements were performed at an applied potential of 0.11 V *vs*. Ag/AgCl. Low working potential of this sensor is promising to reduce impact from numerous interferences present in real samples.

Key words: Ru (III) complexes, Schiff Bases, Amperometry, Ascorbic acid, Modified electrode, Carbon ink.

Introduction

L-Ascorbic acid (Vitamin C), hereafter referred to as AA, a water soluble vitamin, is an extremely important substance which plays a unique redox and electrochemical role (1,2). This vitamin is present or added to a wide variety of food to prevent the oxidation process (3). The ascorbic acid is determined using many analytical methods such as chromatographic, spectrophotometric, titrimetric, enzymatic and electrochemical methods (4,5). In recent times, many electrode modification strategies have been developed for improving the determination of AA in sensitivity and selectivity using electrode modifiers such as conducting polymers, nanoparticles, carbon nanotubes and special carbon materials and other inorganic and organic compounds.

Many inorganic compounds, such as metal oxides and salts (6,9), as well as complex compounds (10,11), including some ruthenium complexes (12,13) were used as electron-transfer mediators for determination of various analytes. Ruthenium occurs in a wide range of oxidation states, from 0 to +8 with the most stable state of +3. Many ruthenium complexes have been synthetized for diferent purposes due to anti-tumor activities (14,16), catalytic properies and electron-transfer mediator ability.

Within the research of ruthenium complexes and sensors and biosensors in our laboratory, we started development of new sensors modified with Ruthenium(III) compounds and here we report on Tetraethylamonium dichloro-bis [N-phenyl-5-chloro-salicylideniminato-N, O] ruthenat (III), hereafter referred to as $Et_4N[Ru(SB^I)_2Cl_2]$, where SB^I is the anionic form of Schiff base, 5-chloro-salicylideneaniline (HSB^I), as an electron transfer mediator for the determination of vitamin C.

Experimental

*Synthesis of $Et_4N[Ru(SB^I)_2Cl_2]$.*5-chlorosalicylideneaniline (HSB^I) and $Et_4N[Ru(SB^I)_2Cl_2]$

were prepared according to published procedures (17). The solution of Schiff base (2 mmol ; 0.46 g HSB^{I},) in 30 mL ethanol absolute was added to the solution of $RuCl_3$ x $3H_2O$ (1 mmol, 0.26 g) in absolute alcohol (10 mL). The mixture was refluxed for 3 hours at 65-70 °C after which the volume was reduced in rotary evaporator to the half of the initial volume. The precipitation was performed by adding of tetraethylammonium bromide (2 mmol, 0.32 g) dissolved in water (10 mL). The dark green solid was washed with diethyl ether and dried over P_2O_5.

Apparatus

Cyclovoltametry (CV), Hydrodynamic amperometry (HA) and Flow injection analyses (FIA) were performed with an electrochemical workstation Autolab potentiostat/galvanostat (PGSTAT 12). The convective transport in HA was provided by magnetic stirrer and a Teflon-coated stirring bar (approx. 300 rpm). As a counter electrode a platinum wire was used and an Ag/AgCl electrode (Model 6.0733.100; Metrohm, Switzerland) as a reference electrode.

Reagents and solution

All chemical for synthesis were purchased from commercial sources and used without further purification. Analytical reagents for electrochemical measurements were analytical grade; double-distilled water were used. Phosphate buffer, pH=7.4 was prepared by mixing appropriate volumes of 0.05M Na_2HPO_4 and 0.05M NaH_2PO_4. A buffer stock standard solution containing 10 g /L ascorbic acid was prepared freshly each day. Working standard solutions used in measurements were prepared instantly before use. Analytical grade L-ascorbic acid was obtained from Fluka. All other compounds were obtained from Merck.

Fabrication of the electrodes

$Et_4N[Ru(SB^{I})_2Cl_2]$ bulk-modified carbon ink (5%) was prepared by thoroughly mixing Ru (III) compound with carbon ink (C50905DI, Gwent, Pontypol, UK). Hand made working electrodes were screen printed on inert laser pre-etched ceramic supports (Coors Ceramic GmBh, Chattanooga, TN, USA). The resulting plates were dried at 60^0C for 1 h. For amperometric measurements in HA an insulating layer of colorless nail slick was brushed on the electrodes surfaces leaving an area of 10 mm^2 uninsulated. Electric contact was achieved with a crocodile clamp. Unmodified screen printed electrodes were prepared in the same way but without modification with $Et_4N[Ru(SB^{I})_2Cl_2]$.

Procedures

Cyclic voltammetry. CV measurements were performed in the range of potential of -400 mV to +400 mV *vs* Ag/AgCl reference electrode with a scan rate of 50 mVs^{-1} after an equilibrium period of 5 s with the initial potential applied. Hydrodynamic amperometry. Fresh measuring solutions were purged via a Teflon tube for at least 20 min with argon (99.995%, Messer Griesheim, Gumpoldskirchen, Austria). The hydrodynamic amperogram was recorded in the stirred measuring solution at an applied potential of 1.1V vs. Ag/AgCl reference electrodes.

Flow injection analysis. FIA measurement with screen printed $Et_4N[Ru(SB^{I})_2Cl_2]$ modified electrode was performed with applied potential of 0.11V *vs*. Ag/AgCl reference electrode. Flow rate of carrier solution was 0.4 mL/min and injection volumes were 100 μL. Peak height was used to evaluate the results.

Results and discussion

Voltammetric behavior of $Et_4N[Ru(SB^{I})_2Cl_2]$-modified carbon ink electrodes. Cyclic voltammograms of $Et_4N[Ru(SB^{I})_2Cl_2]$ bulk-modified SPE and unmodified SPE with and without ascorbic acid in phosphate buffer (pH=7.4) demonstrate that modification of SPE with $Et_4N[Ru(SB^{I})_2Cl_2]$ multiplies current response more than three times (Fig. 1-3).

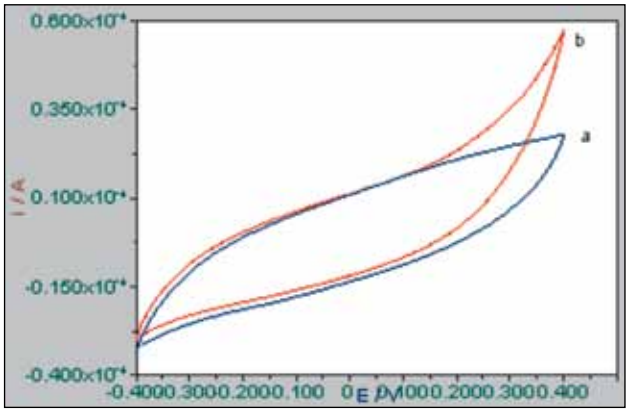

Figure 1. Cyclic voltamograms: a–bare SP Carbon electrode; b- L-ascorbic acid at unmodified electrode

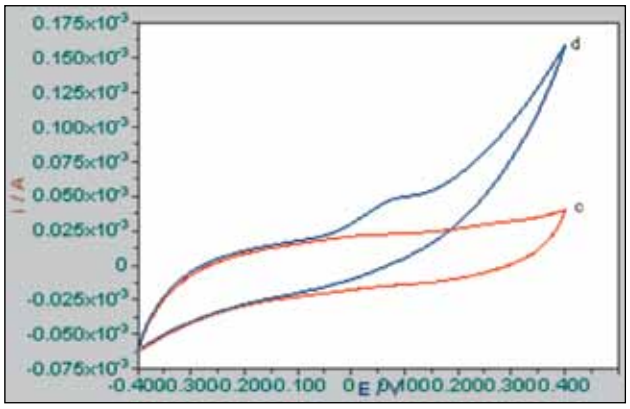

Figure 2. Cyclic voltamograms: c-$Et_4N[Ru(SB^I)_2Cl_2]$ modified SP Carbon electrode; d- L-ascorbic acid at $Et_4N[Ru(SB^I)_2Cl_2]$ modified electrode

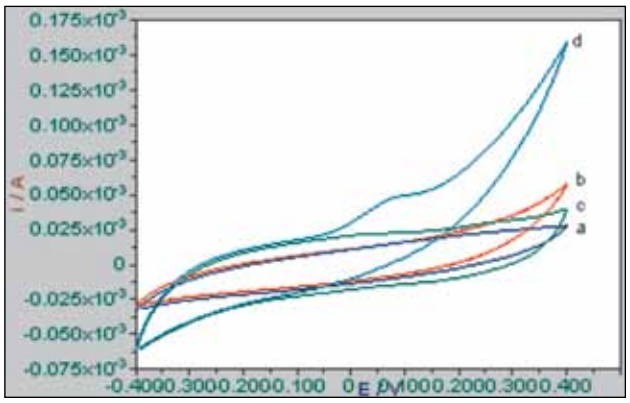

Figure 3. Comparative Cyclic voltamograms of ascorbic acid at $Et_4N[Ru(SB^I)_2Cl_2]$ modified and unmodified SPE (a-d correspond to the Fig. 1-2)

Hydrodynamic amperometry. $Et_4N[Ru(SB^I)_2Cl_2]$ modified SPE was used as an amperometric detector in an electrochemical cell. This electrode quickly response to dynamic changes in the ascorbic acid concentration in phosphate buffer and reach electrochemical equilibrium in few seconds (Fig. 4). By addition of new amount of ascorbic acid in each step, an increase of current response of about 2.5 μA is unquestionable evidence that complex modified SPE can be used as a new amperometric detector for ascorbic acid.

Flow injection analysis with an amperometric detection. Flow injection analysis (FIA) for $Et_4N[Ru(SB^I)_2Cl_2]$ modified SPE was performed with applied potential of 0.11 V *vs.* Ag/AgCl reference electrode. Current peak heights corresponding to the different ascorbic acid concentrations clearly demonstrate fast response and reproducibility of new sensor for ascorbic acid (Fig.5.)

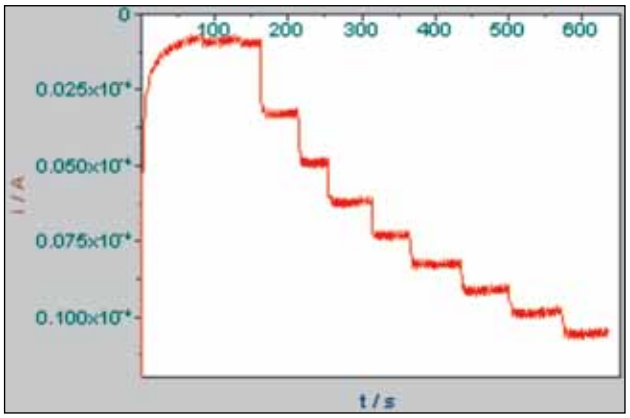

Figure 4. Hydrodynamic amperogram of ascorbic acid with $Et_4N[Ru(SB^I)_2Cl_2]$ modified SPE under bath conditions; operating potential 0.11 V vs Ag/AgCl electrode; supporting electrolyte phosphate buffer, pH 7.4. Steps correspond to the addition of 1mL ascorbic acid concentration of 1000 ppm

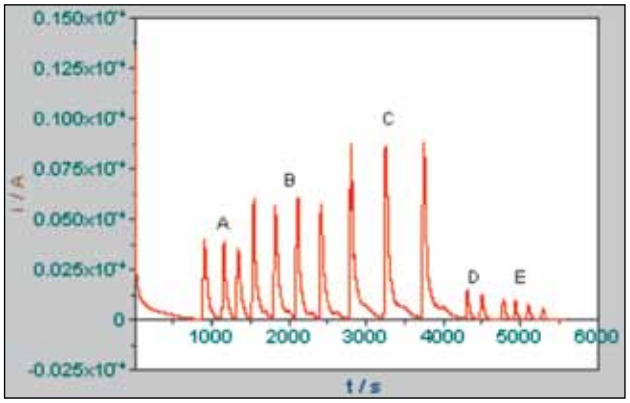

Figure 5. FIA current responses $Et_4N[Ru(SB^I)_2Cl_2]$ modified SPE; A-50 , B-100, C-250, D-25, E-20 (ppm)

Conclusion

This work offers evidence that $Et_4N[Ru(SB^I)_2Cl_2]$ modified SPE can be used as a new amperometric sensor for ascorbic acid determination. Further research will be focused on optimization of measurement parameters, linear range and detection limit and sensor testing with real samples.

Acknowledgements

This work was supported by Ministry of Education and Science of Sarajevo Canton, Bosnia and Herzegovina (project 02-05-16280-9.18/07). The authors are also grateful to Bosnalijek Pharmaceutical and Chemistry Industry, Sarajevo, Bosnia and Herzegovina.

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Human mature placenta in relation to cigarettes smoking during pregnancy

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Abstract

Introduction: Cigarettes smoking during pregnancy is understood as ante partum (maternal) cause of fetal hypoxia and fetoplacental respiratory insufficiency. It is linked to a number of complications of the pregnancy and increased perinatal morbidity and mortality. The objective of the study was to research whether morphological changes, which could cause disorder of the pregnancy course and fetal growth and development, occur in the placenta of the pregnant women who smoked cigarettes during the entire pregnancy.

Patients and methods: Prospective research was carried out in the Gynecology-obstetrics Clinic of the University Clinical Centre Tuzla and Histology and Embryology Institute of the Medical School of the University in Tuzla. Sixty (60) human mature placentas were analyzed by histo-morphological and quantitative stereological methods. The placentas were divided into two groups, depending on the cigarettes smoking in pregnancy: a) 30 placentas of the pregnant women who smoked more that 15 cigarettes a day during the entire pregnancy (examinee group) and b) 30 placentas of the pregnant women who did not smoke during pregnancy (control group). Placenta volume (V_p) and body weight of the newborn were measured at birth. Volume density (V_v) and total volume (V) of the terminal villi, other villi, perivillous fibrinoid and intervillous space were calculated and compared in both examinee groups.

Results. In the placentas of pregnant smokers absolute volume of fibrinoid ($\overline{x}$=30,15 cm^3; $\overline{x}$ =18,61 cm^3; p<0,01) and other villi ($\overline{x}$=110,62 cm^3; $\overline{x}$=86,82 cm^3; p<0,05) is significantly higher; and significantly smaller body weight of the newborn at birth ($\overline{x}$=3010,01 g; $\overline{x}$=3424,33 g; p<0,05). Total volume of the placentas, terminal villi and intervillous space is not significantly different in relation to cigarettes smoking during pregnancy.

Conclusion. The result of our quantitative analysis shows that in one cm^3 of the placenta of the pregnant women smokers changes of volume and spatial distribution of the placental parenchyma occur. Because of that fetomaternal exchange of substances is insufficient and body weight of the newborn is decreased, so the placentas of pregnant smokers functionally do not satisfy the needs of normal growth and development of fetus.

Key words: human mature placenta, cigarettes smoking during pregnancy, stereology.

Introduction

Smoking is the enjoyment of tobacco smoke by inhaling burning tobacco leaves in a cigarette, cigar or pipe. Active smoking is consuming more than one cigarette a day for at least one year (1). Tobacco and its ingredients are identified as the main cause of morbidity and mortality of people staring from the conception to the late age (2). In the world today around 1,1 milliard people smoke, and it is expected that the number of smokers in 2025. will be higher than 1,6 milliard and that more than two million people will be dying of smoking per year (3). According to the data of the Regional Office of the World Health Organisation for Europe the highest prevalence of smoking is in Hungary (41,75%) and in the countries of western Europe it is around 30% (4). Women have become a mass–smokers during the twentieth century, although tobacco is smoked more than 500 years. In Europe 20–30% of women in the reproductive age smoke, and only one fifth stops tobacco smoking during pregnancy and breastfeeding (5).

Tobacco smoke contains around 4000 chemical ingredients of which some thirty is connected with different detrimental effects on human health and four ingredients: nicotine, carbon monoxide, cyanide and cadmium are particularly harmful for

pregnant women and their children. Effects of nicotine on the heart and vascular system are caused by direct stimulus of acetylcholine receptors in ganglia of the autonomic nervous system, adrenal glands, and in neuromuscular junctions (6). Vasospasms and resultant degenerative processes (necrosis of the media) on the blood vessels of the placenta lead to its insufficiency (7).

Carbon monoxide through inhibition of intracellular enzyme processes disturbs oxidative processes and causes chronic hypoxia of cells, tissue and organs, which could cause deterioration of the inner layer of the arterial wall (7). Cadmium (as an important ingredient of tobacco) suppresses zinc in zinc-dependent enzyme reactions and reduces the transfer of zinc through the placenta, which is necessary for many aspects of growth and development of the fetus. Van der Velde found that the vaso-sincitio membrane is significantly thicker in the placenta of animals exposed to cadmium and volume density of the placental vascular system is decreased (8). Smoking during pregnancy is understood as antepartal (maternal) cause of fetal hypoxia and fetoplacental respiratory insufficiency. Smoking has multiple influences on the reproductive health. Tobacco smoking during pregnancy is connected to increased number of miscarriages, preterm birth, more frequent bleeding during pregnancy, higher percentage of placenta previa and placenta ablation, horionamnionitis and preterm membrane rupture (9, 10). Smoking during pregnancy influences fetus growth retardation which results in birth of children with an average less weight and length (11). Hypoglycemia, reduced regulation of body heat is very frequent and because of the sensitivity of blood vessels, cerebral hemorrhage and subsequent neurological failures are more frequent in those children (12). Children of mother smokers have in their early age more frequent respiratory and neurological complications, more frequently they are hospitalized and placed into the intensive care unit (13). The difference in physical, intellectual and emotional development could be found in children until 10 years of age (14).

Researches on the morpho-metric analysis of parenechyma part of the placenta, as the bearer of the synthetic activity of the organ in pregnant women who smoke during pregnancy, was not done in BH until now. Due to the great importance which is given in the world to the detrimental impact of the tobacco smoking during pregnancy, and due to the lack of mentioned parameters, this research was carried out in order to numerically determine values of fetomaternal exchange of substances. Since the risk for children of smoking pregnant women grows, knowledge on the size of areas available for the transport of the substances is important for estimating quantities of nutrients which could be transferred to the fetus for its normal growth and development. Therefore, the objective of this study is histo-morphological and quantitative analysis of placentas of the pregnant women who smoked cigarettes during pregnancy and examination whether there is statistically important difference in their structure, in comparison to placentas of the pregant women who did not smoke during pregnancy.

Material and methods

Prospective research was carried out at the Gynecology – obstetrics Clinic of the University Clinical Centre Tuzla and the Histology and Embryology Institute of the Medical School of the University in Tuzla. The research was caddied out on randomly collected 60 human mature placentas of normally carried and born newborns. The criteria for selection of the sample of placenta for morpho-metric analysis were: singleton pregnancy; age of the pregnant women from 20 to 35 years; spontaneous vaginal delivery; gestation age from 37^{th} to 42^{nd} week; healthy pregnant women and pregnancies without certain pathological conditions which could influence its course and outcome.

In relation to tobacco consumption i.e. cigarettes smoking during pregnancy the placentas were divided into two groups: a) examinee group consisted of 30 placentas of pregnant women who smoked more than 15 cigarettes a day during the whole pregnancy, b) the control group consisted of 30 placentas of pregnant women who did not smoke cigarettes during pregnancy. Both groups of pregnant women had an equal number of primiparous women and multiparous women.

Immediately after the delivery birth weight of the newborn was measured by the mechanical scale «Maxima» Celje, and the weight was rounded to the nearest 10-gram value. From each exami-

ned placenta firstly membranes and the cord were removed and their volume measured, indirectly by measuring pushed out liquid. For histological examination samples of tissue were taken through the entire thickness of the organ (from chorionic to basal plate), fixed in 10%- aqueous formaldehyde, embedded in paraffin. Cuts thick 8 μm were colored by hemalaun and eosin.

The quantitative analysis of histological cuts was done by multipurpose test system M_{42}, with an increase in ocular ×10 and lens ×10. Stereological analysis included relative (refer only to a part of tissue – one cubic centimeter) and absolute (refer to the entire organ) variables. By the method of scoring we determined relative variables: volume density of the terminal villi, other villi, perivillous fibrinoid and intervillous space. Absolute volume of the mentioned structural components of the placenta was calculated from its volume density and the volume of the entire organ (15).

The obtained results were statistically processed: mean was calculated–$\bar{x}$, standard deviation–SD and standard error–SE. The significance of the different results of the examined groups was determined by F-test and Student t-test, on the significance level $p<0,05$.

Results

A case study of histo-morphological examination was parenchyma part of the placenta which consisted of terminal and other villi with all their parts, intervillous area and fibrinoid. Terminal villi are the smallest branches of villi tree. They are built of stroma in which blood capillaries are placed, and the surface of terminal villi is covered by sinciciotrophoblast. The characteristics of other villi are stroma rich with fiber connective tissue, underdeveloped fetal capillaries and more towards centre located bigger blood vessels. Perivillous fibrinoid deposits in placentas of pregnant women smokers most frequently occur on the surface of the terminal villi, although they are present in other parts of villi tree.

Relative variables

Volume density of terminal villi, other villi, perivillous fibrinoid and intervillous space of both groups of placentas are shown in the Table 1.

Comparing the volume units of the structural components of the placenta in relation to cigarettes smoking in pregnancy it was determined that:

- in the group of pregnant smokers in 1 cm^3 of placenta terminal villi make 31%, other villi 25%, fibrinoid 6% and intervillous space 38%;
- in the group of pregnant nonsmokers in 1 cm^3 of placenta terminal villi make 32%, other villi 17%, fibrinoid 3% and intervillous space 48%.

There is no statistically significant difference between volume density of terminal villi of the examined groups of the placentas. The placentas of pregnant smokers in relation to placentas of nonsmokers have statistically significantly less

Table 1. Volume density of terminal villi, other villi, perivillous fibrinoid and intervillous space of placentas of pregnant smokers and nonsmokers (mm°)

Volume density		$\bar{x}$	SD	SE	Significance
Terminal Vili	Smokers	0,31	0,05	0,007	p>0,05
	Nonsmokers	0,32	0,05	0,011	
Other Villi	Smokers	0,25	0,05	0,010	p<0,05
	Nonsmokers	0,17	0,04	0,006	
Fibrinoid	Smokers	0,06	0,03	0,002	p<0,01
	Nonsmokers	0,03	0,01	0,0	
Intervillous Area	Smokers	0,38	0,06	0,019	p<0,05
	Nonsmokers	0,48	0,06	0,011	

$\bar{x}$ – mean
SD – standard deviation
SE – standard error
p – significance

Table 2. Total volume of placentas, terminal villi, other villi, perivillous fibrinoid intervillous space (cm³) and birth weight of the newborns (g) of the pregnant smokers and non-smokers

			SD	SE	Significance
Total Volume Placenta	Smokers Nonsmokers	460,80 455,0	76,04 75,30	21,01 19,15	p>0,05
Terminal Villi	Smokers Nonsmokers	146,73 150,63	35,72 40,81	8,89 9,46	p>0,05
Other Villi	Smokers Nonsmokers	110,62 86,82	30,17 24,63	2,26	p<0,05
Fibrinoid	Smokers Nonsmokers	30,15 18,61	18,66 8,99	2,26 1,66	p<0,01
Intervillous Space	Smokers Nonsmokers	172,90 199,21	48,68 66,91	8,82 9,788	p>0,05
Body weight	Smokers Nonsmokers	3010,01 3424,33	673,67 411,26	101,28 106,35	p<0,05

volume part of intervillous space (p<0,05) and significantly bigger volume part of other villi (p<0,05) and perivillous fibrinoid (p< 0,01).

Absolute variables

Total volume of placentas, terminal villi, other villi, perivillous fibrinoid, intervillous space and birth weight of the newborns in relation to cigarettes smoking during pregnancy is shown in the Table 2.

Mean of total volume of placentas, terminal villi and intervillous space of the examinee groups of placentas statistically do not differ significantly (p>0,05). In the placentas of the pregnant smokers total volume of other villi is 27,41% bigger (p<0,05); 62% fibrinoid volume is bigger (p<0,01); and 12,1% body weight of the newborn is lower (p<0,05) than in the control group.

Discussion

Architecturally the placenta is very complex organ, which particularly refers to its parenchyma part. Detail quantitative researches of its structure began only after introduction of stereological methods. Stereology with its efficient and effective methods enables objective, quantitative description of morphology. Application on the placenta of normal and pathological pregnancy proved its great importance in better interpretation of the growth process, morphogenesis, adaptation and functioning on the level of the whole organ. This explains the very essence of the stereological approach which identifies the useful structural quantities and provides samples which could be used in the experiments of different nature (16). Chorionic villi, fingerlike chorionic outgrowths make the elementary functional unit of the placenta. These are, given the diameter size the smallest branches of terminal villi (17). Kaufman and associates (18) classified different parts of the peripheral chorionic tree depending on: 1) position of the individual villi inside the villi tree, 2) type of fetal blood vessels and 3) structure of connective tissue. According to these researches the peripheral parts of villi tree are: the main villi, immature intermediate villi, terminal (resorption) villi.

The chorionic villi consists of two parts: stromas of mesenchyme origin in which blood capillaries are places and epithelium (trophoblast) on the surface, which until the half of the third month has two clearly separated layers – sinciciotrophoblast and citotrophoblast. Thinned part of the sinciciotrophoblast without the nucleus, closely skin tightened to the dilated capillary represent so called vascular-syncitial membrane, which is metabolic barrier between mother and fetal organism. Some structural parts of the placenta take part in the adjustment of the placenta to different factors which change the course of the pregnancy. The changes of the placental villi and intervillous area during the adjustment could be anisomorphic and isomorphic. Anisomorphic changes of villi (the surface of the villi changes disproportionately to the change of their volume) and intervillous space occur in normal pregnancy and in mother anemia.

Isomorphic changes occur in the placenta of pregnant women who smoke during pregnancy (19).

Maturity of placenta as well as the volume of the placenta is individually different (19). During pregnancy the placenta changes its weight, form, structure and function. Normal development of fetus is enabled by the proportional growth of placenta and fetus. The weight of the placenta is bad indicator of its functional capacity because its non-parenchyma part, and space between villi make one third of its weight. Volume of placenta in comparison to its weight is more frequently determined, because the knowledge of its volume is necessary for calculating absolute values of some stereologic variables.

Our results show that placenta volume values in pregnant smokers are from 251 to 589 cm^3, while in the control group from 380 to 530 cm^3. We did not find statistically significant difference which is in accordance with the results of Larsen (20) and Ramić (21).

Volume density of the terminal villi of placenta is researched by many authors (20, 21, 22). Our research established mean of volume density value of terminal villi of parenchyma part of placenta between chorionic and basal plate which is 31% in pregnant smokers in comparison to 32% in the control group. The volume density of terminal villi in pregnant smokers is for 2,4% less in comparison to the control group, but that difference is not statistically significant. Most of the authors stereologically researched the entire surface of terminal villi and trophoblast. Mayhew (22), and Larsen (20) found that in placentas of pregnant smokers surface of villi changes proportionally to the change of its volume. Data on the total volume of the terinal villi in pregnant smokers are rare. Dockery (23) reports that in placentas of pregnant smokers there is decrease in diameter of terminal villi and decrease of absolute tissue of villi to 19% in comparison to 45% in nonsmokers. Grbeša (24) found that the total volume of terminal villi in parenchyma part of placenta is average 337 cm^3. Total volume of terminal villi in examined placentas of pregnant smokers do not statistically differ significantly in relation to the control group. Chorion villi proliferate during all phases of placental growth. Thus the surface of feto-maternal exchange permanently increases. Lack of difference in the volume density of the terminal villi between pregnant smokers and non-smokers shows that the essence of pathological mechanism of smoking is not in the total volume of villi, but in the structural and functional quality of the very villi, which influences feto-maternal and metabolic exchange of substances.

Comparative quantitative researches of other villi in placentas of pregnant smokers were not carried out until now, because almost all researches referred to terminal villi. Only a few authors did qualitative researches of these villi (20, 25). Our comparative research shows that the volume density of other villi in pregnant smokers is bigger for 47,05% in relation to the control group. We did not find data on the total volume of other villi of any placental space in the available literature, except Ramić (25) data who found significantly bigger total volume of other villi of placentas in younger pregnant women (32,56 cm^3) in comparison to placentas of older pregnant women (21,52 cm^3). Mean of the total volume of other villi in pregnant smokers (110,62 cm^3) is statistically significantly bigger in relation to the control group (86,82 cm^3). In placentas of the pregnant smokers with increase of volume of other villi volume and circulation in intervillous space is decreased, which could lead to fetoplacental circulation disorder and disturbs normal growth and development of the fetus.

Intervillous space is cavernous space which is in all three planes separated by chorion villi. Mother's blood circulate in it, which by principle of hemochorial placentation directly washes fetal structures of the placenta. Hemodynamic of mother's blood and maternal-fetal transmission in the placenta depends on the relation of placental villi and intervillous space (26). The comparative research in our study shows that the average volume density of intervillous space in the placentas of the pregnant smokers is 38%, and in the pregnant nonsmokers 48% in the volume unit of the parenchyma part of the placenta. Smaller volume share of intervillous space in placentas of the smokers (for 22, 40% in relation to non-smokers) we can explain by disturbance of spatial distribution of villous tree in one cm^3 of placental volume (since the share of other villi and share of fibrinoid is significantly bigger in the pregnant smokers). Mayhew also states that the changes of intervillous space in smokers are isomorphic and that the average vol-

ume of intervillous space is decreased, and total volume is reduced to 74% of the normal. In such conditions circulation of blood in intervillous area is reduced for 30% of normal (26).

Data on the absolute volume of intervillous space stated in the literature are 110 cm (26) to 210 cm (27). In our research although the volume share of intervillous space in the control group is significantly higher, total volume of intervillous space do not differ significantly. Today it is generally accepted opinion that the changes in intervillous space are discyclic changes and most frequent are: infarct, collapse of the villi and intervillous thrombosis (26). On the surface of terminal villi eosinophilic plaque is described, fibrinoid. Placental fibrinoid is extracellular disposed material which appears in normal and pathologically changed placentas, in all stages of pregnancy. Thus for example in early pregnancy it occurs in different parts of placenta and fetal membrane, so depending on the place of its precipitation it is variously called: perivillous, Langhans, Rohr, Nitabuch fibrinoid (28).

However, the analysis of human placentas with increased quantity of perivillous fibrinoid showed higher frequency of intrauterine fetal death, intrauterine growth retardation of fetus and premature birth. By origin, structure and function there are two types of placental fibrinoid (29). Fibrin-fibrinoid type consisting of fibrin combined with other molecules which originate from clotted blood or degenerative processes. Its function is regulating circulation of mother's blood through intervillous space and supporting adjustment of that space to hemodynamic. At the same time it controls growth and development of placental villi and their branches, which could cause changes in hemodynamic (stasis or acceleration of circulation of mother's blood). In addition fibrinoid is degenerative sinciciotrophoblast in maternal-fetal surface and behaves as the kind of substitution barrier.

Until now many authors determined volume density of fibrinoid and they found that it is 2,5% (17), 6,1% (28), 10% (26).Volume density of fibrinoid can be expressed as percentage of volume of parenchyma part of placenta (30) or as the part of intervillous area (29). With our research we established that the mean volume density of placental fibrinoid of pregnant smokers is 6%, and in nonsmokers 3%. Volume density values of fibrinoid in pregnant smokers were on an average 77% higher than in the control group. Larsen (20), Mayhew (19), and Vogt Isaksen (31) got the similar results on increased pervillous fibrin deposits in pregnant smokers. Significantly higher volume share of perivillous fibrinoid in smokers, indicate stronger rejection worn-out parts of the trophoblast i.e. apoptosis which prevails over proliferation and differentiation of trophoblast. Total fibrinoid volume (refers to the entire organ) in placentas of smokers is 30,15 cm^3, and in the control group 18,61 cm^3. Higher values of the total fibrinoid volume for 62% in relation to the control group are significant on the level of probability of 1%. Functionally speaking it is certain that fibrinoid settled down on the free space of terminal villi and intervillous area decreases the size of the space responsible for feto-maternal exchange of substances and acts like substitution barrier. Thus we explain results of less birth weight of the newborns of pregnant smokers in relation to nonsmokers. An average body weight of those newborns is 414,32 grams less than in newborns of the control group. A number of authors who were engaged in the study of influence of smoking on the pregnancy and newborns came to common conclusion that mother smokers give birth to children with less birth weight for the gestation age and lower average body weight at birth (10, 2, 11). In literature different data on decreased average birth weight of the newborns of the pregnant smokers in comparison to the newborns of the non-smokers are stated and they are in a wide range from 90 – 475 g (2, 4, 32). Carboxichemoglobinemia and chronic hypoxemia during smoking affects chronic histoarchitectonics of the placenta, vasoconstriction of fetoplacental circulation and disorder of intermedial metabolism which leads to disturbance of intrauterine growth and development. Deviation of fetal growth significantly increases perinatal and early neonatal morbidity (33).

Results of our quantitative analysis show that in one cm^3 of the placenta of the pregnant smoker changes in volume and spatial distribution of placental parenchyma occur. Similar values of the total placental volume, terminal villi and intervillous space, and increased values of fibrinoid volume and other villi together with decreased body weight of the newborn at birth confirm the opinion

that cigarettes smoking during pregnancy changes maternal-fetal exchange of substances necessary for normal growth and development of fetus.

This research on the human placentas of the pregnant women who smoked cigarettes during pregnancy contributed to the first results of quantitative placentology in BiH, which represents parameters for quantitative evaluation of normal and pathologically changed structures. The results of research confirmed changes which endanger pregnancy and they will contribute to the implementation of the prevention measures for decrease of smoking and its negative influence on growth and development of fetus.

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Is a multi-disciplinary approach in the delivery of care for patients with Diabetes mellitus Type 2 cost effective? A systematic review

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Abstract

Background: Diabetes is a chronic disease with serious short-term and long-term consequences. The prevalence of Diabetes Mellitus Type 2 is increasing all over the world, as well as in Slovenia. Therefore, new ways of managing diabetes care need to be found.

Objective: The objective of this systematic review was to evaluate if multidisciplinary teams in delivery of care for Diabetes mellitus are cost effective compared to usual care.

Methods: We carried out searches in NHS Economic Evaluation Database (NHS EED), Health Technology Assessment Database (HTA), Ovid MEDLINE, MEDLINE in Process and Other Non-Indexed Citations and EMBASE for studies published from January 1, 2000 to August 26, 2010. The searches were repeated in October 2011. Abstracts were reviewed and, for those studies meeting the eligibility criteria, full-text articles were obtained. Reference lists were also examined for any additional relevant studies not identified through the searches. Quality was assessed by using the Evers CHEC- list.

Results: Four cost-effectiveness decision analytic models were found. The quality of the studies, according the CHEC-list, was acceptable. All of the models performed sensitivity analysis. The highest ICER/QALY was 69,587 USD, and the highest life years' gain was 1.1.

Conclusions: Multidisciplinary care seems a promising option to deliver treatment for diabetic patients and can considered as a way of diabetes care management in Slovenia. However, the economic information in the literature is insufficient for policy decisions. The number of studies on cost-effectiveness of multidisciplinary care is still limited.

Key words: Diabetes Mellitus type 2, multidisciplinary teams, cost-effectiveness, systematic review

Introduction

Diabetes is a chronic disease with serious short-term and long-term consequences for the afflicted. The total number of people with diabetes in the world is projected to rise from 171 million in 2000 to 366 million in 2030. The most important demographic change to diabetes prevalence across the world appears to be the increase in the proportion of people >65 years of age (1). Slovenia does not differ significantly from the comparable developed countries with regard to prevalence of diabetes. The increasing prevalence of Diabetes Mellitus Type 2 and the different regional differences in prevalence in Slovenia suggest that new ways of managing diabetes care need to be found.

In Slovenia, diabetes care is currently managed in different organisational structures with important regional differences. In the past, diabetes was managed by diabetologists in health dispensaries. However, in 1992 the dispensaries for diabetes were no longer defined by the Law (2), and no definite organisational structure to replace them was provided. In the next decades, different shared-care models between primary and secondary level care evolved. They range from predominantly primary care to predominantly diabetologic secondary care. In some districts, even persons with pre-diabetes are managed at secondary level.

Nevertheless, a process of redesigning the primary care by piloting model practices started in 2011. Type 2 diabetes is the first one of the three chronic diseases to be implemented. According to the proposed model of care, a uniform shared-care model will be piloted with strictly defined patient subpopulation being managed at the secondary level.

Though the treatment was at all times in line with the recommendations and guidelines of the profession and the constant introduction of new, more effective approaches, the quality of care indicators of patients with diabetes are not satisfactory (3). When planning health policy for diabetic patients one needs to consider both, the progress of the profession and technologies, and the increasing incidence of disease, aging populations, limited resources, changes in economic conditions and variability in the organization and quality treatment of diabetic patients. On the other hand, the healthcare system is adapted to the care of acute illnesses and should be adapted to fit better the patients with chronic, progressive, complex disease as type 2 diabetes certainly is.

In 2010, the National Diabetes Prevention and Care Development Programme (National Diabetes Programme)(3) has been adopted and the main principles of the National Diabetes Programme are complementarity, equal access, comprehensive treatment- patient centeredness, quality, efficiency and safety, patient empowerment, partnership, continuous monitoring and taking into account evidence based medicine (EBM) and ensuring progress by investing in research and development of the profession. Based on these main principles, further research on diabetes care management and care organisation is required.

Literature shows that there are many different programs of chronic disease management, and the multidisciplinary approach is one of the possible models of tackling the health provision in diabetes care, which has been much promoted in the recent years (4-7). Several studies have identified the multidisciplinary team approach to provide effective and efficient care to populations that are affected by various chronic health conditions, such as diabetes, however evidence that multidisciplinary teams are cost effective is still very limited (7-14). Even though the economic implications of specific interventions should not be the main guide for health policy decisions, one of the purposes of the intervention should be that it improves health more cost-effectively (10). As cost-effectiveness analyses are used to illustrate the economic value of new programs and technologies, they can inform resource allocation decisions. Decision-analytic models can quantify outcomes in terms of mean life years or quality adjusted life years which can be gained from an intervention and can stimulate the impact of alternative interventions (11,12).

Objective

The objective of this systematic review was to critically appraise current evidence of analytic decision models and to summarize cost-effective studies of multidisciplinary teams in delivery of diabetes care.

Methods

Search methods

We conducted literature searches in NHS Economic Evaluation Database (NHS EED), Health Technology Assessment Database (HTA), Ovid MEDLINE, MEDLINE in Process and Other Non-Indexed Citations and EMBASE for economic evaluations and health technology assessment reports published from January 1, 2000 to August 26, 2010. The search strategies incorporated subject headings (for example MeSH terms in MEDLINE) and text words (in title and abstract) relating to diabetes mellitus type 2 and multidisciplinary care. Search filters to identify economic evaluations were applied in the Ovid databases. All searches were repeated in October 2011. Reference lists of included studies were also examined for any additional relevant studies not identified through the searches.

Mulidisciplinary team

According to Codispodi et al. (7) the multidisciplinary team approach ensures cooperation and coordination between disciplines to increase efficient use of resources and to improve outcomes for the patient through continuity of care. This can be achieved by ongoing, planned, community-based and patient-centered care, which consists of physicians, pharmacists, nurses, dietitians and

health educators. For this systematic review the multidisciplinary team was defined at least a nurse and a physician.

Inclusion criteria

Studies included in this review were restricted to cost effectiveness analyses (decision-analytic models) and health technology assessment (HTA) reports.

Inclusion and exclusion criteria were set for reviewing titles and abstracts. Further inclusion criteria were reporting in English and dealing with adult (18 and over) patients with Diabetes Mellitus. Titles were excluded if they did not deal with adult Diabetes Mellitus, if the population was pediatric, or were dealing with pharmacologic interventions. Abstracts were rejected if they did not report costs, economic outcomes or health related quality of life measurement of diabetes management.

Outcomes

Life Years gained is a modified mortality measure where remaining life expectancy is taken into account. Life years are calculated as the remaining life expectancy at the point of each averted death (15).

Quality adjusted life years- QALY

QALY is a generic, widely used measure of health improvement, which captures both the quality and the quantity gains. QALY is used to guide health-care resource allocation decisions. It was originally developed as a measure of health effectiveness for cost-effectiveness analysis, a method intended to aid decision-makers charged with allocating scarce resources across competing health-care programs (16,17). One can then determine the cost per QALY and compare various interventions based on their relative costs for providing an added QALY.

Incremental cost-effectiveness ratio - ICER

The results in this review are also expressed by means of an ICER is defined as the ratio of the change in costs of an intervention (compared to the alternative) to the change in effects of the intervention (16).

$$\textit{Incremental cost-effectiveness ratio (ICER)} = \frac{(C1 - C0)}{(E1 - E0)}$$

Where C1 means the cost of the intervention; C0 is the cost of the control; E1 and E0 are the health effects, LY and QALYs, respectively.

Data extraction and analysis

The articles were selected based on the title, abstract and full text. Abstracts were reviewed and assessed by ET and for those studies meeting the eligibility criteria, full-text articles were obtained. The main reasons for rejections were that the intervention was not multidisciplinary and the outcomes did not include Quality adjusted life years (QALY), or Incremental cost-effectiveness ratio (ICER), or life years (LY) gained.

Quality assessment

The abstracts of selected articles, which appeared relevant for this review, were retrieved and read through. Based on the inclusion criteria, the eligible articles were selected. The CHEC-list (18, 19) was used to assess the quality of the cost-effectiveness measurements. In the CHEC-list, 19 points can be reached when aspects are addressed accurately in the article. The criteria are mainly about research question, right population definition and perspective from which the costs and effects are measured. Moreover, it measures if the relevant outcomes were measured, if a sensitivity analysis was performed etc. A high quality economic evaluation adequately addresses all 19 items of the CHEC-list.

Results

Study selection

The initial search strategy identified 2505 references- 1st search.. After screening for general subject matter and duplication check in Reference Manager, a total of 1767 abstracts were accepted for further screening. An update search for studies in October 2011- 2nd search, identified 787 references. 553 references were left after duplicates had been removed. No additional articles in the 2nd search met the inclusion criteria for further analysis. 54 articles were obtained and read in full (Figure 1).

Finally, 4 met inclusion criteria when the articles were reviewed, which means that they determined outcomes in terms of quality adjusted life years/expectancy (12,14,20,21). The studies

used health economic modeling techniques (cost-effectiveness decision models) to assess long term clinical benefit and cost outcomes.

As shown in table 1, a maximum of 14 out of 19 points was scored on the 19-item CHEC-list (18) on quality of performed economic evaluations.

Figure 1. Flow chart of search process and results

Description of included studies

The population in included studies was general adult population with Diabetes Type 2. The mean age reported varied from 47.1 to 62.3 years. Two studies were performed in USA (14,20), one in Australia (21) and one in Canada (12). One study investigated the quality improvement programme conducted in community health centers. The components have included community health center collaboration, patient self-management support and delivery system redesign (eg. group visits) (14). One study (20) evaluated a culturally specific diabetes case management and self-management training program in four cohorts defined by insurance status. These were the uninsured, patients who are covered by Country Medical Services (CMS), those who have California's Medicaid Programme (Medi-Cal) and those with commercial insurance.

The studies included a percentage of women ranging from 49% to 67%. All but one study reported the average duration of diabetes, which was at shortest 0.1 years and 9.2 years the longest. Mean A1c (level of glycosolated hemoglobin in blood) varied from 6.9 to 9.4 and mean systolic blood pressure varied from 122.6 to 138.6mmHg.

Table 1 describes the characteristics of the included studies.

Effects

The change in health effects is measured in terms of the number of life-years (LY) gained/saved and quality-adjusted life years (QALY) gained by the intervention. These are presented in table 2.

Life Years gained varied from 0.1 years to 1.1 years, whereas the QALYs gained varied from 0.1 to 0.9.

Costs

One study (12) included diabetes related health care costs as well as program implementation costs such as patient education costs (newsletters, posters, pamphlets, promotional material and patient reminder cards), provider education costs, speciality nurse liaison costs, diabetes related medications. The average cost per patient of the implementation of the programme without complications amounted to 544 USD (664 can$), which amounts to 218,144 USD (266,236 can $) for the 401 patients included in the model.

Most studies took into account only direct medical costs (12,20,21). However, the costs are pictured in various ways. While one study estimates only the program costs under the societal perspective (14), another (20) gives mean direct lifetime costs, from payer perspective. The costs varied for the different cohort groups in the study from 57,530 USD to 82,225 USD per patient. In the same manner, the program was associated with higher lifetime costs than control in all cohorts. The lifetime incremental costs between the program and the control vary from 8,991 USD to 12,368 USD. One study (21) included costs of hospital services and pharmaceutical costs. The study shows that the program reduces hospitalisation costs saved per patient/year (- 37.1 USD (-44 AUD) and reduces anti-diabetic prescribing cost per patient/year 33.7 USD (-40 AUD).

Cost-effectiveness

All studies claimed that the program under consideration will be cost-effective.

As mentioned above, the studies included Life years gained and QALYs gained as an outcome.

Table 1. Characteristics of included studies

Reference	O'Reilly D, et al.	McRae IS,et al	Huang ES, et al	Gilmer TP, et al
Country (year)	Canada (2007)	Australia (2008)	USA (2007)	USA(2007)
Study type	Cost utility analysis	Cost effectiveness analysis (decision analytic model)	Cost effectiveness analysis	Cost effectiveness analysis (Simulation model)
Perspective	NR*	Health system	societal	Third-party payer
Population	Diabetic patients over 18	Type 2 diabetes mellitus adult patients	Non-pregnant adult patients with diabetes	4 cohorts of diabetic patients: Uninsured (N=760) CMS (N=1,345) Medi-Cal (N=1,213) Commercial (N=575)
Number of included patients	401	80	80	3893
Intervention	Multidisciplinary primary care diabetes management program	Integrated approach to assist general practitioners with diabetes management	National collaborative quality improvement program conducted in community health centers	Culturally specific diabetes management and self-management program
Comparator	NR*	Normal treatment	NR*	NR*
Effectiveness Data	UK Diabetes prospective study outcome model	UK Diabetes prospective study outcome model	NR*	CORE Model
Outcome	Surrogate clinical outcomes Life years, QALY, ICER per LY; ICER per QALY	Life years, quality adjusted life expectancy Estimation of costs of diabetes caused complications	ICER	Life Expectancy quality adjusted life expectancy
Included costs/ patient *	*544 USD	* 165 USD	*USD Year 1- 1879 Year 2-638 Year 3- 485 Year 4-378	*USD 584
Time horizon	40 years	40 years	4 years	40 years
Discount rate	3%	5%	3%	3%
Sensitivity analysis	Yes	Yes	Yes	Yes
CHEC list	11	14	13	12

N.R.-not reported
** caluculated in 2007 USD*

Moreover, incremental cost effectiveness ratio (ICER) per QALY and per LY could be calculated from the studies if it was not explicitly mentioned. ICER per QALY varied a lot. The highest ICER/QALY was shown by Gilmer et al, namely 69,587 USD for the commercial cohort. In their study they show that there is a 100 percent likelihood of being cost-effective versus control in the uninsured cohort, 92 percent probability in the CMS cohort, 57 percent probability in the Medi-Cal cohort and only 31 percent probability in the commercial cohort. The results are summarized in Table 2. Huang et al (14) showed that the difference in life time costs was 11,685 USD. According

Table 2. Presentation of study results

	O'Reilly D, Hopkins R, Blackhouse G, Clarke P, Hux J, Tarride J-E, et al. D	McRae IS, Butler JRG, Sibthorpe BM, Ruscoe W, Snow J, Rubiano D, et al. C	Huang ES, Zhang Q, Brown SE, Drum ML, Meltzer DO, Chin MH. B	Gilmer TP, Roze S, Valentine WJ, Emy-Albrecht K, Ray JA, Cobden D, et al A
Country (year)	Canada (2007)	Australia(2008)	USA (2007)	USA (2007)
Life years,	0.1142	0.36	0.3	Uninsured: 1.1 CMS:0.6 Medi-Cal:0.3 Commercial:0.2
QALY, QALE gained	0.1075	0.30	0.35	Uninsured:0.9 CMS:0.4 Medi-Cal:0.3 Commercial:0.2
ICER per LY (*USD)	*4,619	*9,162 per life year saved	38,950	USD Uninsured: 7933 CMS:18,976 Medi-Cal:36,056 Commercial:57,587
ICER per QALY (*USD)	*4,907	*10,995 per QALE gained	33,386	USD: Uninsured: 10,141 CMS: 24,584 Medi-Cal: 44,941 Commercial: 69,587
Time horizon	Lifetime (40 years)	Lifetime (40 years)	4 years	Lifetime (40 years)
Discount rate	3%	5%	3%	3%
Sensitivity analysis	Yes	Yes	Yes	Yes

** calculated USD 2007*

to this, the ICER/QALY was calculated as 33,386 USD and ICER/LY 38,950 USD. The results of the McRae study are also within the generally acceptable limits applied to health care interventions in Australia. They show the cost-effectiveness ratio of 9,162 USD (8,108 AUD) per LY saved and 10,995 USD (9,370 AUD) per year increase in QALE. O'Reilly et al (12) argue that primary care diabetes program, with 4,907 USD ICER/QALY or 4,619 USD ICER/LY, respectively, represents good value for money according to commonly quoted thresholds.

Discussion

As Diabetes mellitus Type 2 is a growing chronic disease across the world, there has been increased interest in determining cost-effectiveness of various designs of the provision of care. The underlying literature review provides some evidence that multidisciplinary care can be cost-effective. With regard to feasibility of the chosen economic outcomes it can be argued that due to the complexity of health systems across the world it is difficult to find "one size fits all" disease management program. Moreover, in contrast to clinical outcomes, QALYs depend on many variables and are more difficult to measure. Whenever a program is addressing long term chronic diseases, i.e. diabetes, it is necessary to use modeling approaches to estimate long term outcomes and costs (22).

The studies in the current review vary in the designs, and as cost-effectiveness analysis demands considerable data, a straight forward conclusion cannot be made. Nevertheless, the studies included in the current literature review scored a maximum of 14 points out of 19 on the CHEC-list, which indicated that the quality of the studies

is acceptable, but could be improved. The results of the studies show weak cost-effectiveness. The studies express the limitations and the results were taken into account.

The financial resources in health care are limited across the world. Therefore, effective and cost-effective interventions are needed in health care provision. Especially in the case of growing chronic diseases, such as diabetes, new methods of disease management that provide cost-effective resource spending are needed. Economic outcomes show a meaningful outcome, as to have an overview how the financial and also human resources are allocated. The results of the studies included in the current literature review show weak improvements in economic outcomes.

Economic outcomes

Although not included in the current review, as they did not meet the inclusion criteria, several other studies deal with economic outcomes. These are mainly defined as direct medical costs during the same time periods, and health care utilization (hospitalizations, and hospital admissions) (23-26). Four literature reviews (5, 13, 27, 28) were looking at various disease management programs and their influence on economic outcomes. While some studies (30) show that initial costs of introducing new approaches might lead to higher costs in the first years of the program, however will occasionally start saving costs when hospitalization rates begin to decrease, and (28) found reduced hospital expenditures, although statistically insignificant. The authors argue that these findings are clinically and economically important and suggest that combined stepped-care diabetes nurse case management program might provide an immediate benefit for a high-risk population.

Similarly, there is limited evidence of the effect of disease management on healthcare utilization and costs (4), however on the other hand disease management programs were associated with positive direct economic outcomes (13). Cobden, et al. (27) on the other hand found strong correlations between patient behaviors, perspectives of care, health outcomes and costs in Diabetes mellitus Type 2. In his research, Qari (25) showed cost-effectiveness and time saving in achieving glycemic control when teamwork approach of physician, dietitian and diabetic educator is involved. With regard to costs, two studies (24, 29) examined insurance claims. They came to the conclusion that participants in a managed-care sponsored diabetes disease management program experienced lower overall paid insurance for health care compared to those not in disease management and concerning the change in direct medical costs. They found out that most of the decrease in total costs was accounted for by a shift from insurance claims for emergency department, inpatient and physician office visits to prescription claims. In addition to the support of community pharmacists, the use of financial incentives and support from the medical community were positive factors in improving clinical and economic outcomes among patients with diabetes.

Limitations of the study

The current systematic review has its limitations. Because of the search filters we used in the Ovid databases we may have missed some relevant studies. The articles were appraised by a single person; however after the second search all the abstracts were reviewed and checked for inclusion criteria again. When the outcome of the search is limited to economic outcomes only, a more detailed definition of those should be in place. As a consequence, there is a potential for improvement in the outcome as evidenced by variations of economic outcome achievement. Moreover, the definition of multidisciplinary care is very broad and in this systematic review, for the purpose of clarity we chose the definition that includes at least a nurse and a physician.

All of the studies are conducted outside of Europe, which may affect the generalisability of the studies to other health care settings.

Furthermore, most publications identified in the literature search were dealing with cost-effectiveness of pharmaceuticals or medical interventions, which were outside of the scope of the present study, however might indirectly deal with care management of diabetes.

Conclusion

Multidisciplinary approach in health care seems a promising option to deliver treatment for diabetic patients. However, the economic infor-

mation of the current systematic review is insufficient for policy decisions making. The number of studies on cost-effectiveness of multidisciplinary care is still limited and the quality of these economic evaluations should be improved. Moreover, is it almost impossible to apply different models directly to the national setting. Slovenia has so far not found an optimal way of managing diabetes care and this study is a step closer to consideration if introducing multidisciplinary approach. However, in order to come closer to a political decision, a decision analysis model with local direct and indirect cost data needs to be prepared.

We need organizational solutions that will achieve professional and financial sustainable supply of all patients with diabetes and improve their health state. Changes to the organization of care should be introduced already at the level of the health team.

Acknowledgements

We are grateful to Assistant professor Gro Jamtvedt and Espen Movik, both from the Norwegian Knowledge Center for the Health Services, Norway and, professor Kåre Birger Hagen from University of Oslo, Norway for their consultancy and support.

Authors' contributions

ET analysed data and wrote the article. JZ, DMT and AI supervised and reviewed the article. SSO performed the literature search and reviewed the article.

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