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A Study of MMP-1 and TGF-β Levels in Lumbar Disc Herniation Patients With and Without Traditional Medical Practices

Geleneksel Tıbbi Uygulamalar Uygulanan ve Uygulanmayan Lomber Disk Hernisi Hastalarında MMP-1 ve TGF-β Düzeylerinin İncelenmesi

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ABSTRACT

Aim: Chronic and recurrent low back pain resulting from lumbar disc disease significantly impacts patients' quality of life and psychological well-being. Patients may seek traditional treatments for pain relief. This study aimed to investigate and evaluate the effect of utilizing or not utilizing traditional medical practices (TMPs) in patients who have undergone surgery for lumbar disc herniation, by examining samples of the disc and ligamentum flavum.

Material and Methods: A total of 46 patients, 28 (60.9%) male and 18 (39.1%) female, who underwent surgical treatment for lumbar disc herniation were included in the study. The patients' data were retrieved retrospectively from the patient registry system. Immunohistochemical staining was performed and evaluated on the disk and ligamentum flavum samples removed during the surgical procedure.

Results: Of the 46 patients included in the study, 21 (45.6%) had undergone TMPs, while 25 (54.4%) had not. The levels of MMP-1 and TGF-β of patients who underwent TMPs were significantly higher in both flavum (both $p<0.001$) and disk ($p=0.014$ and $p=0.020$, respectively) samples. When analyzing the immunohistochemical staining scores, it was found that MMP-1 staining scores were higher in the flavum ($p<0.001$) and disk ($p=0.002$) samples of patients who underwent TMPs.

Conclusion: The notion that TMPs are suitable methods for all ailments is a significant misconception. This study emphasizes the importance of accurately informing the public about TMPs, given their increasing prevalence in Turkish society. There is a need for further controlled clinical studies to investigate which method can be used for which disease.

Keywords: Disc herniation; traditional therapy; MMP-1; TGF-β.

ÖZ

Amaç: Lomber disk hastalığı nedeniyle ortaya çıkan kronik ve tekrarlayıcı bel ağrısı, hastaların yaşam kalitelerini ve psikolojik durumlarını önemli ölçüde etkilemektedir. Ağrı nedeniyle hastalar geleneksel tedavilere yönelebilmektedir. Bu çalışmanın amacı, lomber disk hernisi nedeni ile cerrahi uygulanmış hastalarda, geleneksel tıp uygulamaları (GTU) kullanmış ve kullanmamış olmanın etkisini disk ve ligamentum flavum örneklerini inceleyerek araştırmak ve değerlendirmektir.

Gereç ve Yöntemler: Bu çalışmaya, lomber disk hernisi nedeni ile cerrahi tedavi uygulanmış olan 28 (%60,9) erkek ve 18 (%39,1) kadın olmak üzere toplam 46 hasta dahil edildi. Hastaların verilerine hasta kayıt sisteminden geriye dönük olarak ulaşıldı. Cerrahi işlem sırasında çıkarılan disk ve ligamentum flavum örneklerinde immünhistokimyasal boyama yapıldı ve değerlendirildi.

Bulgular: Çalışmaya dahil edilen 46 hastanın 21'i (%45,6) GTU yaptırmış, 25'i (%54,4) ise yaptırmamıştı. GTU uygulanan hastaların MMP-1 ve TGF-β düzeyleri, hem flavum (her iki $p<0,001$) ve hem de disk (sırasıyla, $p=0,014$ ve $p=0.020$) örneklerinde anlamlı düzeyde daha yüksekti. İmmunhistokimyasal boyanma skorları incelendiğinde, GTU uygulanan hastalarda flavum ($p<0,001$) ve disk ($p=0,002$) örneklerinde MMP-1 boyanma skorlarının daha yüksek olduğu saptandı.

Sonuç: GTU'nun her hastalık için uygun yöntemler olduğunun düşünülmesi büyük bir yanılgıdır. Bu çalışma, Türk toplumunda giderek kullanım sıklığı artan GTU hakkında halkın doğru bilgilendirilmesinin önemini vurgulamaktadır. Hangi yöntemin hangi hastalık için kullanılabileceğini araştırmak için çok sayıda kontrollü klinik çalışmaya ihtiyaç vardır.

Anahtar kelimeler: Disk hernisi; geleneksel tedavi; MMP-1; TGF-β.

INTRODUCTION

Lumbar disc herniation (LDH) refers to the displacement of the lumbar intervertebral disc into the vertebral canal. LDH is characterized by intermittent low back pain, lumbar stiffness, along with neurological symptoms. The herniated disc material causes ischemia in neural tissue either through direct mechanical pressure or by inducing an inflammatory response. The resulting edema leads to compression of vascular structures, nerve root ischemia, and symptoms. Chronic and recurrent low back pain can significantly impact patients' quality of life and psychological well-being (1-3). The degenerative process resulting from changes in the intervertebral disc structure can lead to microinstability and anterior or lateral displacement of the vertebrae (1).

Thickening of the ligamentum flavum is an age-related finding observed in LDH, independent of gender (4,5). In hypertrophic ligamentum flavum, increased expression of matrix metalloproteinases (MMPs) has been demonstrated. MMP-1 is a member of collagenases that degrades all collagen subtypes, particularly fibrillar collagens responsible for providing mechanical strength (6-8).

Transforming growth factor-β (TGF-β) is a multifunctional growth factor involved in cellular proliferation, differentiation, and synthesis of extracellular matrix proteins. It is also believed to play significant roles in various physiological and pathological conditions, including wound or fracture repair, ossification of the posterior longitudinal ligament of the cervical spine, and hypertrophic burn scarring. It is considered that the hypertrophy of the ligamentum flavum, observed in patients with lumbar stenosis and herniated disc disease, may be attributed to the elevated concentration of this cytokine in those regions (7).

Due to the chronic, recurrent, and discomforting nature of pain associated with LDH, patients may occasionally seek out alternative and complementary medical techniques for treatment (1-3). These treatment methods, defined as traditional medical practices (TMPs), have been frequently used in Türkiye in recent years due to advertising and the opening of centers in hospitals. Traditional methods commonly practiced in Türkiye include cupping, leech therapy, wet cupping, acupuncture etc.

This study aimed to examine and evaluate the effect of using or not using alternative and complementary medical techniques before surgery in patients who underwent surgical treatment for LDH, by analyzing samples of the disc and ligamentum flavum.

MATERIAL AND METHODS

The study included patients who underwent surgical treatment for LDH within the past two years at the Department of Neurosurgery, Faculty of Medicine, Düzce University. The patients' medical records were retrieved retrospectively from the patient registry system. The data of the patients aged 18 and above who underwent surgical treatment for LDH were reviewed. Data on the patients' age, gender, level of herniation, and whether alternative and complementary medicine techniques were used before surgical treatment were recorded. Patients were divided into two groups based on whether they used alternative and complementary medicine techniques preoperatively, as patients did not use TMPs or used TMPs.

Immunohistochemical staining was performed on the disk and ligamentum flavum samples removed during the surgical procedure. The paraffin-embedded tissue blocks were obtained from the archives of the Medical Pathology Department at Düzce University Faculty of Medicine. Immunohistochemical studies were conducted on formalin-fixed, paraffin-embedded tissue blocks to determine the expression levels of MMP-1 and TGF-β. All specimens were cut into 3-4-µm-thick sections using a fully automated assay according to the manufacturer's instructions. MMP-1 and TGF-β expressions were graded semi-quantitatively on a scale from 0 to 3 points, based on staining intensity. Staining was categorized as follows, no staining scored as 0, light staining scored as 1, moderate staining scored as 2, and strong staining scored as 3. The percentage of staining was scored for each slide.

Statistical Analysis

The distribution of the data was analyzed using the Shapiro-Wilk test, and the homogeneity of variance was assessed using the Levene test. An independent samples t-test or Mann-Whitney U test was employed for group comparisons involving numerical variables. Categorical variables were analyzed using the Pearson chi-square test or the Fisher-Freeman-Halton test. Descriptive statistics were reported as mean±standard deviation or median with interquartile range and minimum-maximum values for numerical variables, and as frequency and percentage for categorical variables. Statistical analyses were conducted using the IBM SPSS v.22 software package, and a significance level of 0.05 was utilized.

RESULTS

The study included 46 LDH patients with a mean age of 46.35±8.29 years. Of the patients, 28 (60.9%) were male and 18 (39.1%) were female. The number of patients who received TMPs was 21 (45.6%), while the number of patients who did not was 25 (54.4%). The mean age of patients who underwent TMPs was 46.05±7.37 years, compared to 46.60±9.14 years for those who did not. Of the patients who underwent TMPs, 13 (61.9%) were male and 8 (38.1%) were female, and 15 (60.0%) of the patients who did not undergo TMPs were male and 10 (40.0%) were female (Table 1). There was no statistically significant difference between the groups in terms of age (p=0.825) and gender (p=0.895). The mean body mass index (BMI) was 30.03±3.42 in patients who underwent TMPs and 29.39±2.68 in patients who did not, with no statistically significant difference between the groups (p=0.485).

There were significant differences between the groups for all staining methods in flavum and disc (Table 2). The staining percentage of the flavum for both MMP-1 and TGF-β was higher in patients who used TMPs compared to those who did not (both p<0.001). Furthermore, the staining percentage of the disc for both MMP-1 (p=0.014) and TGF-β (p=0.020) was higher in patients who used TMPs compared to those who did not (Figures 1 and 2).

When examining the staining scores, no significant difference was found in staining scores for flavum (p=0.064) and disc (p=0.215) with TGF-β between the groups. However, significant differences were observed between the groups in terms of staining scores with MMP-1 for both flavum (p<0.001) and disc (p=0.002). The post hoc tests

revealed that the ratio of score 2 was significantly higher in the group that used TMPs (66.7% vs. 8.0%), while the ratio of score 1 was significantly lower (19.0% vs. 88.0%) compared to the group that did not use TMPs. Likewise, the ratio of MMP-1 score 2 for the disc was significantly higher in the group that used TMPs compared to the group that did not (52.4% vs. 12.0%), while the ratio of score 1 was significantly lower (23.8% vs. 76.0%). The ratio of MMP-1 staining score 3 was similar in both groups for both flavum and disc (Table 3).

DISCUSION

Lumbar disc disease is a significant health issue affecting many individuals, impacting their quality of life. Before the introduction of scientific treatment options into medical literature, certain traditional methods appeared as treatment alternatives and have been tried throughout human history (1). Despite advancements in modern medicine, the use of multiple drugs in the treatment of chronic diseases, along with the numerous side effects of drugs and medical interventions, as well as patients' desires to strengthen their immunity and stay fit, along with the frustration caused by the inadequate response expected from treatments, have led patients to seek new approaches (9,10).

The prevalence of the use of TMPs is reported to be 33% in the UK, 42.1% in the United States, 48.2% in Australia, 49.3% in France, and 70.4% in Canada. While in developing countries, the use of TMPs reaches 71% in Chile, 70% in China, 40% in Colombia, and it can be as high as 80% in African countries (11). The lifetime prevalence of TMP usage ranges from 35% to 69% (12,13). China stands out among the leading countries where TMP usage is most prevalent. Traditional Chinese medicine has been particularly used in the treatment of LDH, with reported therapeutic effects on LDH-related low back pain (14). It is reported that the primary goal of traditional Chinese medicine in the treatment of disc herniation and disc degeneration is to reduce the release of inflammatory mediators and reduce oxidative stress and anti-apoptosis in nucleus pulposus cells by regulating the expression of proteins in relevant pathways. In traditional Chinese medicine, acupuncture, Chinese manipulations, and other

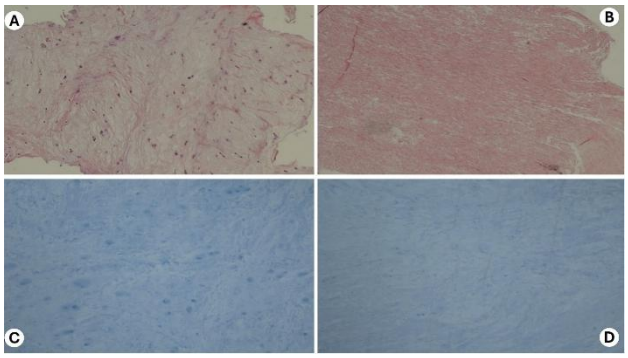

Figure 1. In the group not receiving traditional medical treatment, no immune staining with TGF-β and MMP-1 was shown in both disc and flavum materials. **A)** disc, H&E x4, **B)** flavum, H&E x4, **C)** TGF-β x10, **D)** MMP-1 x10

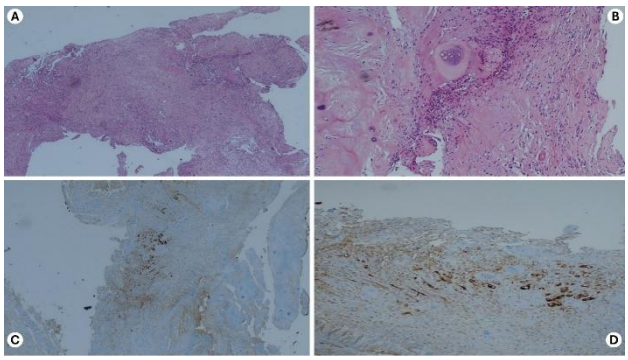

Figure 2. In the group receiving traditional medical treatment, immune staining with TGF-β and MMP-1 was shown in both disc and flavum materials. **A)** disc, H&E x4, **B)** flavum, H&E x4, **C)** TGF-β x10, **D)** MMP-1 x10

Table 1. Demographic characteristics in groups

	TMPs + (n=21)	TMPs – (n=25)	p
Age (years)	46.05±7.37	46.60±9.14	0.825
Gender, n (%)			
Male	13 (61.9)	15 (60.0)	0.895
Female	8 (38.1)	10 (40.0)	
BMI (kg/m^2)	30.03±3.42	29.39±2.68	0.485

TMPs: traditional medical practices, BMI: body mass index

Table 2. Comparison of staining percentages of flavum and disc with MMP-1 and TGF-β

	TMPs + (n=21)	TMPs – (n=25)	p
MMP-1			
Flavum	20 (20-20) [10-50]	10 (10-10) [10-30]	**<0.001**
Disc	20 (10-30) [10-50]	10 (10-20) [10-50]	**0.014**
TGF-β			
Flavum	20 (20-30) [10-50]	10 (10-10) [10-30]	**<0.001**
Disc	20 (10-30) [10-50]	10 (10-20) [10-30]	**0.020**

MMP-1: matrix metalloproteinase 1, TGF-β: transforming growth factor-β, TMPs: traditional medical practices, descriptive statistics were presented as median (25th-75th percentile) [minimum-maximum]

Table 3. Comparison of staining scores of flavum and disc with MMP-1 and TGF-β

	TMPs + (n=21)	TMPs – (n=25)	p
MMP-1			
Flavum, n (%)			
1	4 (19.0)^a	22 (88.0)^b	**<0.001**
2	14 (66.7)^b	2 (8.0)^a	
3	3 (14.3)	1 (4.0)	
Disc, n (%)			
1	5 (23.8)^a	19 (76.0)^b	**0.002**
2	11 (52.4)^b	3 (12.0)^a	
3	5 (23.8)	3 (12.0)	
TGF-β			
Flavum, n (%)			
1	8 (38.1)	16 (64.0)	0.064
2	10 (47.6)	9 (36.0)	
3	3 (14.3)	0 (0.0)	
Disc, n (%)			
1	9 (42.9)	17 (68.0)	0.215
2	9 (42.9)	7 (28.0)	
3	3 (14.3)	1 (4.0)	

MMP-1: matrix metalloproteinase 1, TGF-β: transforming growth factor-β, TMPs: traditional medical practices, ^{a,b}: different superscript letters denote significant differences between column proportions according to the post hoc test results

physical therapy methods are combined to achieve a more pronounced effect. It is suggested that the combination of these treatments may produce synergistic effects and could potentially play an irreplaceable role in the future (15,16).

In recent years, patients in Türkiye have increasingly sought TMPs and solutions for their health issues, influenced by evolving health policies, a growing number of practitioners, and enhanced accessibility. However, data regarding the outcomes of more commonly practiced TMPs in Türkiye, such as cupping, leech therapy, and wet cupping, have not yet been documented in medical literature.

The discs of the lumbar spine possess a complex structure that allows for bending and twisting of the spine while safeguarding it during mechanical loading. Disc degeneration, biochemical changes, and mechanical stress lead to the outward swelling and elongation of the disc. The disc is comprised of the annulus fibrosus and nucleus pulposus, forming a structure that generates a collagen network in conjunction with proteoglycans and water. The nucleus pulposus is particularly rich in proteoglycans. Imbalances and loss in proteoglycan production result in significant pathological changes within the disc (14,15). The disc matrix molecules become disorganized due to enzymatic activity, leading to a process of fragmentation and loss that results in reduced disc height and elasticity. MMPs, particularly MMP-1 and MMP-3, are among these enzymes found at elevated levels in degenerated discs, often regarded as the likely initial events in disc changes (14,16-20). TGF-β is a multifunctional growth factor produced by fibroblasts that is involved in various functions such as cellular proliferation, differentiation, and synthesis of extracellular matrix proteins. It is held responsible for the hypertrophy of the ligamentum flavum (7,18).

In this study, the levels of MMP-1 and TGF-β in the disc and flavum materials of patients diagnosed with LDH who underwent surgical treatment were examined to investigate whether there was any difference with the use of TMPs. The levels of MMP-1 and TGF-β were found to be higher in both the disc and flavum of patients who underwent TMPs, reaching statistical significance. The higher levels of MMP-1, a molecule commonly elevated in degenerated discs in patients who underwent TMPs, suggest that the applied method either intensified the degenerative impact on the disc and flavum or prolonged the process, thereby potentially exacerbating degeneration even without a direct effect.

The significantly higher levels of TGF-β found in patients undergoing TMPs, a factor known to influence hypertrophy of the ligamentum flavum, suggest an expected increase in ligamentum flavum hypertrophy among LDH patients undergoing TMPs.

The lack of investigation into flavum hypertrophy in this study represents a limitation. Other limitations include the relatively small sample size and the inability to compare disk and flavum samples with those from healthy volunteers without LDH, which would serve as an ideal control group.

CONCLUSION

TMPs increase the levels of enzymes involved in disc degeneration and growth factors, leading to flavum hypertrophy in LDH. In the contemporary landscape of diverse scientific treatment modalities, the notion that a few TMPs can universally apply to every ailment stands out as a major misconception. TMPs should undergo scrutiny through scientific, evidence-based approaches. If proven beneficial, comprehensive data should be gathered regarding the specific diseases they can effectively address.

Ethics Committee Approval: The study was approved by the Non-invasive Clinical Research Ethics Committee of Düzce University (04.10.2021, 164).

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