African Journal of Microbiology Research Vol. 4 (8), pp. 655-659, 18 April, 2010
Available online http://www.academicjournals.org/ajmr
ISSN 1996-0808 © 2010 Academic Journals

Full Length Research Paper

Oral colonization and boric acid susceptibility of yeast in boron mineral workers

Zafer Cetinkaya[1]*, Semsettin Karaca[2], Mustafa Kulac[2], Ihsan Hakki Ciftci[1], Gulsah Asık[1], Osman Cenet[4] and Nuri Kiraz[3]

[1]Department of Microbiology, Faculty of Medicine, Afyon Kocatepe University, Afyonkarahisar, Turkey.
[2]Department of Dermatology, Faculty of Medicine, Afyon Kocatepe University, Afyonkarahisar, Turkey.
[3]Department of Microbiology, Faculty of Medicine, Osmangazi University, Eskişehir, Turkey.
[4]Faculty of Occupation of Higher Education, Balikesir University, Balıkesir, Turkey.

Accepted 26 March, 2010

In this study, we aimed to investigate the effects of boron on *in vivo* oral yeast colonization in study groups which are exposed to boron dust in different sections of the boron mine. The study was carried out in the boron mining areas of two districts (Eskisehir and Balikesir) of Turkey. We included 184 people working in open quarry and stone milling unit, 144 people working in the factory and 150 people as control group. Specimens were taken from four oral mucosal regions and cultured onto Sabouraud dextrose agar. After incubation for 3 - 7 days at 30°C, the total number of yeast colonies on the plates was considered the relative intensity of carriage, and the total number of yeast colonies on the plates was considered the relative intensity of oral carriage. The susceptibility of *Candida* spp. to boric acid was investigated. The frequency of *Candida* colonization in boron intensive area workers was found significantly higher than automatic factory workers and control groups (p = 0.012), there were no difference between automatic factory workers and control groups in point of *Candida* colonization (p = 0.749). We observed that oral yeast colonization had increased directly proportional with boron powder exposure in boron mine (p = 0.005). Mean minimum inhibitory concentrations (MICs) of boric acid for Boron intensive area, 0.87 - 2.0% for automatic factory and 0.83 - 2.0% for control subjects. We observed that intensive exposure to boron mineral powders was strictly related to oral yeast colonization. Exposure to industrial boron mineral powder may cause important health problems by increasing *Candida* colonization in oral cavity. It may be useful to do periodical health control in boron mineral workers and population under risk.

Key words: *Candida*, boric acid, boron mineral, oral yeast colonization.

INTRODUCTION

Boron is a naturally occurring element that is widely used in several industries such as medicine, space and war industry, etc. The most important commercial borate products and minerals are borax pentahydrate, borax, sodium perborate, boric acid, colemanite and ulexite. Turkey is the largest producer of borate products in the world. Annually, 63% of the world boron mineral is supplied by Turkey (WHO, 1998; Commission, 2003).

Boron plays a role in cell division, metabolism and membrane structure and function. In humans and animals, boric acid and borate are absorbed from the gastrointestinal and respiratory tracts. Up till now, only a few human studies have been conducted to assess health effects associated with exposure to boron compounds (WHO, 1998). It has been known that at definite concentrations, boric acid has bactericidal and fungicidal effects. Bacteria are more sensitive to boric acid than yeasts (Meers and Chow, 1990; Benson, 1998). Although, the antibacterial concentrations of boron that may not have antifungal effects can cause excessive oral yeast colonization, theoretically. Oral yeast colonization

*Corresponding author. E-mail: zfcetinkaya@yahoo.com. Tel:+905325217089.

may cause opportunistic infections especially in immune suppressed persons. *Candida* spp. is the most important fungal pathogens of mucosal yeast infections in human (Edwards, 2000).

In this study, we aimed to investigate the effects of boron dust on the oral mucosa yeast colonization of subjects who were exposed to different concentrations of boron dust in boron mine sections (boron intensive area (open quarry, stone milling) and factory units).

MATERIAL AND METHODS

The study was carried out in the boron mining areas of two districts (Eskisehir and Balikesir) of Turkey. We included 184 people working in open quarry and stone milling unit, 144 people working in the factory and 150 people as a control group (The control group (from the same area) consisted of volunteer office male workers). Dust concentrations of boron intensive area (open quarry, stone milling), and factory were detected as 5.55 – 8.33 mg/m^3 and 0.93 - 1.85 mg/m, respectively.

The subjects were informed about the nature of the study and written consent was obtained. Smoking attitudes, hygiene of mouth (i.e. prosthesis, oral erosive and bullous lesions) and chronic diseases (Diabetes mellitus, Asthma) was questioned in all groups. Respirable dust mask usage was also questioned in study groups. Persons with chronic diseases, dental prosthesis and oral erosive, bullous lesions were excluded. Specimens were taken from four oral locales: the buccal mucosa, the floor of the mouth, the dorsal surface of the tongue and gum. Samples were collected only by one investigator using method in the literature (Kleinegger et al., 1996). Briefly, each sample was collected by passing a sterile cotton swab (Copan, Brescia, Italy) several times across the particular oral surface. Immediately after sampling, each swab was replaced in its sterile containment tube and was moistened with sterile salt solution by crushing the glass ampoule in the tube. The containment tubes were transported within 2 h of sampling from the place of collection to the laboratory. The cotton end of each swab was inserted into 0.5 ml of sterile water in a microcentrifuge tube, the tube was rigorously mixed for 30 s with a laboratory vortex mixer, and 0.15 ml of the wash was spread onto Sabouraud dextrose agar (Oxoid, Basingstoke, Hampshire, United Kingdom) plates. The plates were incubated for 3 - 7 days at 30°C. The total number of yeast colonies on the plates was considered the relative intensity of carriage and the total number of yeast colonies on the plates was considered the relative intensity of oral carriage.

The identification of growing yeast colony was made according to germ tube formation, microscopic appearance on Cornmeal Tween 80 Agar (Oxoid, Basingstoke, Hampshire, United Kingdom), and API ID32 C (bioMerieux, Marcy l' Etoile, France) carbohydrates fermentation results. In the study, yeast differentiation was made by combined use of the tests of germ tube formation, microscopic appearance on Cornmeal Tween 80 Agar, and API ID32 C. The susceptibility of 117 *Candida* spp. to boric acid (116 strains isolated and 1 control strain) was investigated.

Standard antifungal powder of boric acid was purchased from the manufacturer firm (Sigma, Steinheim, Germany). Stock solutions were prepared in distilled water as 16% boric acid solution. Serial twofold dilutions were prepared exactly as outlined in NCCLS document M27-A (National Committee for Clinical Laboratory Standards, 2002). Final dilutions were made in RPMI 1640 medium (L-glutamine without $NaHCO_3$) (Sigma, Steinheim, Germany) buffered to pH 7.0 with 0.165 M morpholinepropanesulfonic acid buffer (Merc, Darmstadt, Germany). Broth micro dilution testing was performed in accordance with the guidelines in NCCLS document M27-A2 (National Committee for Clinical Laboratory Standards, 2002). The inoculums suspension was prepared by the spectrophotometric method of inoculums preparation and with final inoculums of (1.5 ± 1.0) X 10^3 cells per ml. A 100 µl yeast inoculums was added to each well of the micro dilution trays. The final concentrations of the boric acid were 8 to 0.125%. The trays were incubated at 35°C, and MIC endpoints were read after 48 h of incubation. Following incubation, the minimum inhibitory concentrations (MICs) of boric acid were read as the lowest concentration at 100% inhibition of growth. Quality control was ensured *Candida albicans* ATCC 90028.

Statistical analysis

All parametric results were expressed as mean ± standard deviation for each group. Statistical analysis was performed using chi-square test, to independent samples test and one way ANOVA tests. The chi-square test was used to compare selected categorical variables. Chi-square test and one way ANOVA test were used with yeasts growing percent and density on the media associated with working area. Independent samples test was used to determinate *in vitro* activity of *Candida* species isolated from boron intensive area and automatic factory workers and control group against boric acid. A p-value less than 0.05 was considered to be statistically significant.

RESULTS

The mean age ± S.D., of our samples were (open quarry and stone milling unit workers, factory workers and control group) 35.1 ± 4.9, 36.9 ± 4.8, 34.1 ± 4.9, respectively. They were aged between 24 and 56 years. There were no significant differences in yeast colonization between smokers and nonsmokers (p = 0.635) (Table 1). None of the workers in boron intensive area and factory were using respirable dust mask.

Yeasts growing in percent and density on the media associated with working area is shown in Table 2 and Figure 1. Although, the frequency of *Candida* colonization in boron intensive area workers was found significantly higher than automatic factory workers and control groups (p = 0.012), there were no difference between automatic factory workers and control groups in point of *Candida* colonization (p = 0.749).

The yeasts isolated in specimens of workers in boron intensive area, factory and control group were 72/184 (39.1%), 28/144 (19.4%) and 16/150 (10.6%), respectively. The distribution of isolated species of *Candida* spp. were 75 (64.6%) *C. albicans,* 21 (18.1%) *C. glabrata,* 16 (13.8) *C. krusei and* 4 (3.4%) *C. tropicalis* (Table 3).

In vitro activity of *Candida* species isolated from workers of boron intensive area, automatic factory and control group against boric acid is shown in Table 3. Boric acid MICs for the *Candida* spp. strains ranged between 0.88 - 2.11% for boron intensive area, 0.87 - 2.0 % for automatic factory and 0.83 - 2.0% for control subjects. *In vitro* activities of *C. albicans* and Non-*C. albicans* (*C. tropicalis, C. krusei and C. glabrata)* were not different among the groups (p = 0463). There was a statistically significant difference in the antifungal susceptibilities of *C. albicans* and non-*C. albicans* strains to boric acid (p = 0.001) (Table 3). The susceptibility of *C. albicans* ATCC

Table 1. Yeast colonization between smokers and nonsmokers.

		no	Colonization (no)	Colonization %	p value*
Boron intensive area	**Smoking**				
	Yes	63	26	41.3	>0.05
	No	121	46	38.0	
Automatic factory	**Smoking**				
	Yes	51	11	21.6	>0.05
	No	93	17	18.3	
Control	**Smoking**				
	Yes	47	6	12.8	>0.05
	No	103	10	9.7	

*Chi-square test

Table 2. Yeasts frequency and intensity of carriage in the study groups*.

	n	Yeast growing %	Mean**
Boron intensive area	184	39.1[a]	43.41±51.05[c]
Automatic factory	144	19.4[b]	20.64±20.74[d]
Control	150	10.6[b]	11.12±11.01[d]

*One way ANOVA test. ** Mean colony number in yeast growing group
[a]P<0.01, Boron intensive area versus Automatic factory and Control group.
[b]P>0.05, Automatic factory versus Control group.
[c]P<0.001, Boron intensive area versus Automatic factory and Control group.
[d]P>0.05, Automatic factory versus Control group.

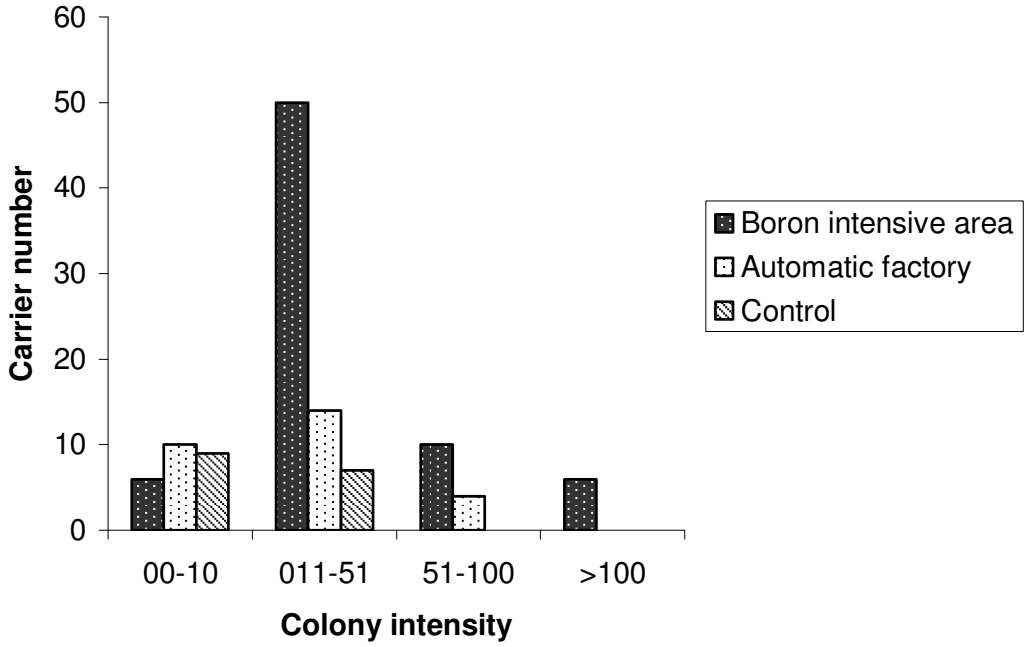

Figure 1. Histograms of the colony intensity of carriage in the working groups. Intensities are separated into 0 to 10, 11 to 50, 51 to 100 and >100 colonies.

Table 3. *In vitro* activities of boric acid against Candida species*.

	Boron intensive area				Automatic factory				Control			
	n	Mean	MIC 50	MIC 90	n	Mean	MIC 50	MIC 90	n	Mean	MIC 50	MIC 90
C. albicans	48	0.88±0.69a	0.5	2	16	0.87±0.50a	0.5	1	11	0.84±0.62a	0.5	2
*Non-*C. albicans*	24	1.58±0.76b	2	2	12	1.33±0.71b	1	2	5	1.3±0.67b	1	2
**C. glabrata*	12	1.25±0.58 b	1	2	6	1.0±0.77 b	0.5	2	3	0.83±0.29 b	1	1
**C. krusei*	10	2.11±0.74 b	2	4	4	2.0±0.0 b	2	2	2	2.0±0.0 b	2	2
**C. tropicalis*	2	1.0±0.0 b	0.5	0.5	2	1.0±0.0 b	1	1	0	-	-	0

*One way ANOVA test. *Non-*C. albicans* . [a]P>0.05, Boron intensive area versus automatic factory and control group. [b]P>0.05, Automatic factory control group. P<0.001, *C. albicans* versus non-*C. albicans*.

90028 strain used as control, to boric acid was found to be 0.5%.

DISCUSSION

Boron is a naturally occurring element found combined with other elements (primarily oxygen) throughout the environment. It is not present in the atmosphere at significant levels, but the total amount in the air is very significant owing to the huge volume of the atmosphere. Occupational exposures to boron compounds may be significant. Inhalation of dusts is the most significant route of exposure in occupational settings (Commission, 2003). The microbiostatic and microbicidal effect of boric acid against bacteria and fungi in some dilution have been known. The effective concentration of boric acid on bacteria is lower than fungi (Meers and Chow, 1990; Benson, 1998).

The signifant difference in yeast oral colonization was detected between workers of boron intensive area and factory in comparison to control group. This difference was found about two times (39.1 versus 19.4%) more frequent in workers of boron intensive area. In addition, oral bacterial flora was decreased in open quarry and stone milling unit workers as part of factory workers. Although, routine local treatment dose for boric acid was 2%, the MICs value of two yeast strains isolated in boron intensive area workers was above 2%.

It has been reported that the MIC values of *Pseudomonas, Staphylococcus intermedius* and *Candida* spp. are 0.5, 2.0 and 5.0%, respectively (Benson, 1998). Meers and Chow (1990) have found that, 10% solution of boric acid had bactericidal activity against *Acinetobacter calcoaceticus*, *Pseudomonas aeruginosa* and Group B streptococcus strains. Furthermore, use of 10% boric acid at the patient with vaginal candidiasis provided success in the treatment of vaginal candidiasis (Meers and Chow, 1990). It has been reported that yeasts were more resistant to boric acid than bacteria except some species of bacteria such as *Staphylococcus warnerii*. This resistance may give rise to *Candida* colonization in oral cavity. We considered that the increase of oral yeast colonization in our study might be caused by more susceptibility of bacteria than yeast to boric acid, as reported by other studies (Meers and Chow, 1990; Benson, 1998).

Shubair and Larsen (1990) have reported that 10% boric acid solution was sufficient to eliminate *C. albicans* strains that colonized on vaginal mucosa. They have observed that minimal inhibitory concentration of boric acid against yeasts included on their study was 0.4% (Shubair and Larsen, 1990). Sobel et al., 2003 has shown that the use of topical boric acid as 600 mg/dl provided usefulness (64%) in the treatment of 141 patients with vaginitis due to *Candida glabrata* (Sobel et al., 2003). We detected that the (MICs) of boric acid for *Candida* spp. strains ranged between 0.88 - 2.11% for boron intensive area, 0.87 - 2.0% for automatic factory and 0.83 - 2.0% for control subjects. All of *Candida* spp. except two strains isolated from workers in boron intensive area were found to be susceptible to boric acid (for concentrations below 2%).

As a conclusion, we observed that intensive exposure to boron mineral powders was strictly related to oral yeast colonization. Boron mineral may cause important health problems by increasing *Candida* colonization in oral cavity. It may be useful to do periodical health control in boron mineral workers and population under risk.

REFERENCES

Benson CE (1998). Susceptibility of selected otitis externa pathogens to individual and mixtures of acetic and boric acid. 14th ed. ACVD Proceedings of AAVD meeting, p.1692.

Commission (2003). Boron mine. (Chamber of mining engineers of Turkey). Min. Bull. 66: 3-10.

Edwards JE (2000). *Candida* Species. In: Mandell GL, Dolin R, Bennett JE (eds) Principles and Practice of Infectious Diseases, 5th ed., New York: Churchill-Livingstone. pp. 2656-74.

Kleinegger CL, Lockhart SR, Vargas K, Soll DR (1996). Frequency, intensity, species, and strains of oral *Candida* vary as a function of host age. J. Clin. Microbiol. 34: 2246-54.

Meers PD, Chow CK (1990). Bacteriostatic and bactericidal actions of boric acid against bacteria and fungi commonly found in urine. J. Clin. Path. 43: 484-7.

National Committee for Clinical Laboratory Standards (NCCLS) (2002). Reference method for broth dilution antifungal susceptibility testing of yeasts. Approved Standard Second Edition M27-A2 Wayne,

Pennsylvania, USA.
Shubair M, Larsen B (1990). Growth inhibition of *Candida albicans* and other medically important yeasts by vaginal contraceptive products. Gynecol. Obstet. Invest. 29: 67-70.
Sobel JD, Chaim W, Nagappan V, Leaman D (2003). Treatment of vaginitis caused by *Candida glabrata:* use of topical boric acid and flucytosine. Am. J. Obstet. Gynecol. 189: 1297-300.
WHO (1998). Environmental Health Criteria. No. 204: Boron, xviii+ 201 pages. ISBN 92 4157204 3 World Health Organization - CH-1211.