

## PAPER DETAILS

TITLE: The level of two trace elements in carious, non-carious, primary, and permanent teeth

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# The level of two trace elements in carious, non-carious, primary, and permanent teeth\*

## Purpose

The boron and fluoride mainly accumulate in the bones and teeth of the human body. The purpose of this study is to determine boron or fluoride levels in the whole tooth, to evaluate the correlation between their levels and to compare these levels in primary/permanent, carious, and non-carious groups.

## Materials and Methods

The boron and fluoride levels of thirty-six teeth, separated such as primary carious (n=9) and non-carious (n=9), permanent carious (n=9) and non-carious (n=9), were determined by ICP-MS and ion-selective electrode, respectively.

## Results

While boron levels were between 0.001 and 5.88 ppm, the fluoride levels were between 21.24 and 449.22 ppm. The boron level of non-carious teeth was higher than those of carious teeth in primary and permanent tooth groups. However, this difference was not statistically significant ( $p>0.05$ ). The fluoride level of non-carious teeth was higher than those of carious teeth in primary ( $p=0.062$ ) and permanent teeth groups ( $p=0.046$ ). Negative correlation, found between boron and fluoride in all groups, was significant only in non-carious teeth group ( $r=-0.488$ ,  $p=0.040$ ).

## Conclusion








The results of our study proved the importance of fluoride as a protective factor for dental caries once more. The boron levels in non-carious teeth were also higher than carious teeth. However, it was not significant. Moreover, there was negative correlation between teeth boron and fluoride levels. Therefore, it is necessary to conduct more detailed studies on the tooth boron level and its relation with caries formation and with fluoride levels.

**Keywords:** Boron, Fluoride, Teeth, Trace elements, Dental health

## Introduction

Dental caries, the most common oral disease and the major cause of tooth loss, are also considered as the third among non-communicable disease that endangers human health (1). Studies have shown that some of the trace elements such as F, Al, Fe, Se, Sr, Mn, Cu, and Cd are closely related to dental caries; some prevent dental caries while others accelerate dental caries (2). Amount of trace elements in teeth can provide information on environmental factors, eating habits, and oral health (3).

Boron is a trace element found in a daily diet and it is as a potentially essential element for humans (4-8). It is known that distributed throughout the human body with the highest concentration in the bones and teeth (9,10). It has been shown in various studies that boron plays important roles, especially in mineral metabolism and bone development (11,12). It has been shown that boric acid reduced alveolar bone loss in

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rats with experimental periodontitis and osteoporosis (13). In a study performed with pre-osteoblastic cells, boron has been found to affect mRNA expression of collagen-I, bone sialoproteins, osteocalcin, osteopontin, and extracellular matrix proteins (14). Recent studies on the development of boron-containing dental composites due to their antibacterial properties are being carried out in order to prevent secondary caries formation (15). However, in the literature, it is unclear whether the boron has a cariogenic, anticariogenic or cariostatic effect on the teeth. Moreover, studies on the tooth boron level are very limited (3,16).

Fluoride is found mostly in bones and teeth because of its affinity to calcium. It prevents caries in adults and children by making the external surface of teeth more durable to the acid attacks (17). However, it is also associated with dental fluorosis and if consumed in excessively, it has potential health risks such as bone fragility. Fluoride can be incorporated into the structure of the teeth by means of nutrients and drinking water as well as by the use of agents such as toothpaste, mouthwashes (18).

This study is important in terms of being the first study that determines boron levels of human teeth in Turkey. The purpose of this study is to determine boron or fluoride levels in the whole tooth, to evaluate the correlation between their levels and to compare these levels in primary/permanent, carious, and non-carious groups.

## Materials and Methods

### Samples

Thirty-six teeth of thirty-six patients, who admitted to the Marmara University Faculty of Dentistry Department of Oral and Maxillofacial Surgery between May 2017 and July 2017, were included in this study. The non-carious permanent teeth were extracted for orthodontic purposes or because of periodontal diseases, the non-carious primary teeth were extracted due to delayed physiologic root resorption. The carious primary and permanent teeth were extracted because of excessive caries. The collected thirty-six teeth were divided mainly into two groups such as primary (n=18) and permanent (n=18). Each group has two sub-groups as carious (n=9) and non-carious (n=9) teeth. Enamel, dentin, and other parts of teeth were not separated and the decayed tissue of carious teeth was not removed. The whole tooth was used for boron and fluoride determinations. This study has been reviewed and approved by the Ethical Committee of Marmara University Health Sciences Institute (03.04.2017-108). Informed consent was obtained from all individual participants included in this study.

### ICP-MS method for the boron assay

Each whole tooth was first washed and cleaned in the saline solution (0.9% NaCl). Then they were ground and weighed. Five mL of concentrated HCl (Merck, Darmstadt, Germany) per gram of tooth was used to dissolve them and they were filtered later by using the syringe filter (0.2 µm). One mL of them was taken into the falcon tube. Then, it was diluted to 5 mL with ultrapure water. Boron levels were determined in all diluted samples by using an inductively coupled plasma mass spectrometry (ICP-MS) (Thermo Scientific X Series 2, nebulizer gas, 1.2/min; cooling gas, 13/min; power, 1051 W; auxilia-

ry gas, 0.9/min). Calibration solutions (Chem Lab, Zedelgem, Belgium) at different boron concentrations as 0.002, 0.02, 0.2, 2, 20, 200, 2000 and 20000 ppb were used in the experiment.

### Ion-selective electrode method for the assay of fluoride levels

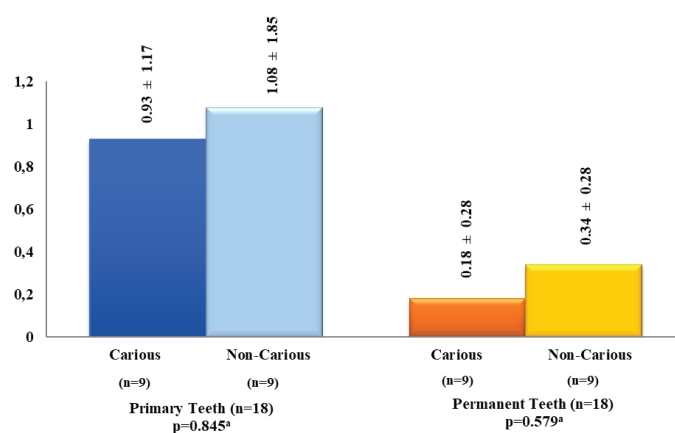
Sodium acetate solution (15 %, 900 µL) was added to increase the pH of each 100 µL of dissolved tooth samples in concentrated HCl. Then, to adjust the total ionic strength, 900 µL of this was taken and 100 µL of TISAB-III buffer solution (Orion 940911) was added. The fluoride level of these tooth solutions was then determined using an ion-selective electrode (Orion-96-09). The electrode was calibrated with six standard fluoride solutions ranging from 1 to 10<sup>6</sup> µM. Electrode potentials of standard solutions are measured and plotted on the linear axis against their concentrations on the log axis. The fluoride levels of the tooth solutions were calculated using the slope of the calibration curve.

### Statistical analysis

Statistical Package for the Social Sciences for Windows software, version 24.0 (SPSS Inc., Chicago, IL, USA) was used for the statistical analysis. The Shapiro-Wilk test was used to evaluate the normal distribution of continuous variables. Student t-test was used for two independent group comparisons for normally distributed variables which were given as mean ± standard deviation. Mann-Whitney U test was used for two independent group comparisons for non-normally distributed variables which were given as median values. Correlation of normally and non-normally distributed variables were used Pearson and Spearman correlation, respectively. The confidence interval was set to 95% and p < 0.05 was considered statistically significant.

## Results

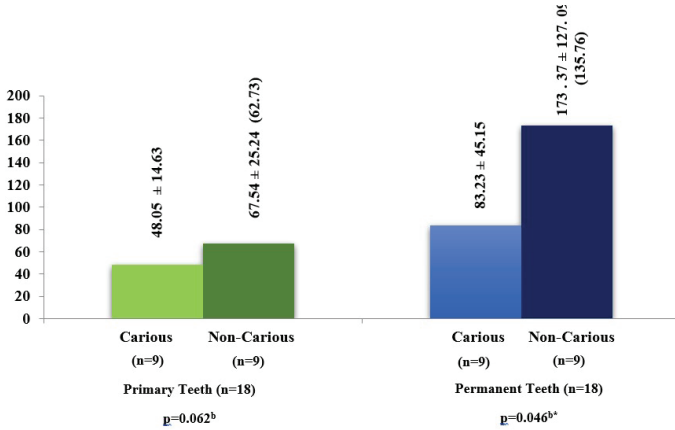
According to the results of boron analysis, the average boron level of all teeth (n=36) was 0.63 ± 1.19 ppm. The boron level of non-carious teeth was higher than those of carious teeth in primary and permanent tooth groups. However, these differences were not statistically significant (p>0.5) (Fig. 1). As seen in Figure 1, boron levels of primary teeth were higher than those of permanent teeth (p<0.001).



**Figure 1.** Boron levels (ppm) of teeth.

Values are given as Mean ± SD. SD: Standard Deviation. <sup>a</sup>: t-Test

The fluoride level of all teeth was  $93.05 \pm 82.00$  ppm ( $n=36$ ) and it was higher than that of the boron level. The fluoride level of non-carious teeth was higher than those of carious teeth in primary ( $p=0.062$ ) and permanent teeth groups ( $p=0.046$ ) (Fig.2). As seen in Figure 2, fluoride levels of primary teeth were lower than those of permanent teeth in contrast to boron results.



**Figure 2.** Fluoride levels (ppm) of teeth.

Values are given as Mean  $\pm$  SD, SD: Standard Deviation. Median values are given in parentheses. <sup>b</sup>: Mann-Whitney U Test

Negative correlation, found between boron and fluoride in all groups, also in all teeth, was significant only in non-carious teeth group ( $r = -0.488$ ,  $p = 0.040$ ).

## Discussion

The effects of trace elements on the protection of oral health are very controversial issue. Trace elements of teeth have been investigated for various reasons, for example, there are some dental health studies where trace element concentrations have been correlated with dental caries such as Si, Se, Cd, and Pt (19). As mentioned above, studies on the evaluation of teeth boron levels and their relationship with caries are very limited in the literature. Its reason is the difficulty in standardizing environmental factors with other elements. In various studies, the levels of some trace elements in the whole tooth were determined (20-22). However, to our knowledge, there is one study to determine the levels of boron and fluoride in the whole tooth (23). Therefore, it is very difficult to compare our studies with the literature.

Similar to the present study, Riat and Sharma (23) determined boron levels in the whole teeth of 15 healthy and 15 carious permanent teeth. The reason for using the whole tooth instead of tooth parts such as enamel or dentine was explained by that tooth cutting procedure causes positive contamination with trace elements. In contrast to our study, which ICP-MS was used, the ICP-Atomic Emission Spectroscopy has been used and found that boron levels measured in carious teeth ( $49.85 \pm 18.15$  ppm) were significantly higher than those of non-carious teeth ( $39.05 \pm 8.19$  ppm). Moreover, teeth boron levels have been found higher than ours. The reason of this may be due to the differences in methods used for boron determination; sample size differences; the differences in the causes of caries formation; differences due to individuals and environmental; and geographical differences.

In another study, dental enamel boron concentration has been determined by the ICP-Atomic Absorption Spectros-

copy method and found that the amount of boron in the healthy permanent teeth was higher than that of the healthy primary teeth (24). Kumagai *et al.* (25) determined the boron level in the dentin of 121 healthy permanent teeth as about 1.63 ppm by the ICP-MS method.

In a healthy adult, dentin is composed by approximately 45% mineral, 33% organic matrix, and %10 water by volume. Enamel is composed by about 87% mineral by volume. The most mineralized part of the tooth is enamel, this is followed by dentin (26). Therefore, when the whole tooth is examined, it may be correct that we find it lower.

It has been suggested that fluoride could prevent caries in permanent and primary teeth (24,27,28). Our results support this suggestion. Because the fluoride level in non-carious teeth was found to be higher than that of carious teeth in primary or permanent groups. Although it was non-significant, boron levels in non-carious teeth were also higher than those of carious teeth. This may show their cariostatic effect while boron and fluoride are together. However, negative correlation was found between fluoride and boron levels in all teeth and groups. The significant negative correlation was present only in non-carious teeth group. In Lius' study (29), when boron and fluoride are added together to drinking water in rats fed a cariogenic diet, boron has been shown to reduce the caries protection effect of fluoride. It has been suggested that boron may inhibit the fluoride absorption from the gastrointestinal system.

## Conclusion

The results of our study also proved the importance of fluoride as a protective factor for dental caries once more. Although not statistically significant, our results showed that non-carious teeth have contained more boron than carious teeth. Moreover, there was negative correlation between teeth boron and fluoride levels. The further detailed studies about tooth boron level and its relation with caries formation and with fluoride levels should be done using large samples, comparing different geographic areas. It is also necessary to identify the boron and fluoride level in water or food samples and their cariogenic effects.

**Türkçe Özet:** Çürüklü, çürüksüz süt ve daimi dişlerde iki eser elementin düzeyleri. Amaç: Bor ve florür, insan vücudunda başlıca kemik ve dişlerde birikir. Bu çalışmanın amacı, dişin tamamında bor ve florür düzeylerini belirlemek, bu düzeyler arasındaki korelasyonu değerlendirmek ve çürüklü, çürüksüz süt ve daimi dişlerde karşılaştırmaktır. Gereç ve Yöntem: Çürüklü süt dişler ( $n = 9$ ) ve çürüksüz süt dişler ( $n = 9$ ) ile çürüklü daimi dişler ( $n = 9$ ) ve çürüksüz daimi dişler ( $n = 9$ ) olarak ayrılan otuz altı dişin bor ve florür seviyeleri sırasıyla ICP-MS ve iyon seçici elektrot ile belirlendi. Bulgular: Bor seviyeleri 0.001 ile 5.88 ppm arasında iken, florür seviyeleri 21.24 ile 449.22 ppm arasındaydı. Süt ve daimi dişlerde, çürüksüz dişlerin bor düzeyleri çürüklü dişlerden daha yüksekti. Ancak bu fark istatistiksel olarak anlamlı değil ( $p > 0.05$ ). Süt dişlerinde ( $p = 0.062$ ) ve daimi dişlerde ( $p = 0.046$ ), çürüksüz dişlerin florür düzeyleri çürüklü dişlerden daha yüksekti. Tüm gruplarda bor ve florür düzeyleri arasında bulunan negatif korelasyon sadece çürüksüz olmayan diş grubunda anlamlıydı ( $r = -0.488$ ,  $p = 0.040$ ). Sonuç: Çalışmamızın sonuçları, diş çürüğü için koruyucu faktör olan florürün önemini bir kez daha kanıtlamıştır. Çürüksüz dişlerdeki bor seviyeleri çürüklü dişlerden daha yüksekti. Ancak, anlamlı değildi. Hatta diş bor ve florür düzeyleri arasında negatif korelasyon vardı. Bu nedenle, diş bor düzeyi, çürük oluşumu ve florür düzeyi ile ilişkisi hakkında daha ayrıntılı çalışmalar yapmak gerekmektedir. Anahtar Kelimeler: Bor; florür; diş; eser elementler; diş sağlığı

**Ethics Committee Approval:** This study has been reviewed and approved by the Ethical Committee of Marmara University Health Sciences Institute (03.04.2017-108).

**Informed Consent:** Informed consent was obtained from all individual participants included in this study.

**Peer-review:** Externally peer-reviewed.

**Author contributions:** SA, AY and FS designed the study. RK, GB, SY and PNT participated in generating the data for the study. RK, GB, SY and PNT participated in gathering the data for the study. SA, AY and FS participated in the analysis of the data. RK and AY wrote the majority of the original draft of the paper. RK and AY participated in writing the paper. All authors approved the final version of this paper.

**Conflict of Interest:** The authors had no conflict of interest to declare.

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