

## SALIVA LYSOZYME LEVELS AND ANTIOXIDANT ENZYME ACTIVITY IN PATIENTS WITH FIXED ORTHODONTIC APPLIANCES

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**ÖZET: SABİT ORTODONTİK APAREYLERLE TEDAVİ GÖREN HASTALARIN TÜKÜRÜĞÜNDEKİ LİZOZİM SEVİYELERİ VE ANTİOKSİDAN ENZİM KAPASİTESİ.** Bu çalışmanın amacı, ortodontik aparatların tükürükteki antimikrobiyal ve antioksidan bazı ajanlar üzerine etkilerinin incelenmesidir. Çalışma için, yaşları 12-20 arasında değişen, herhangi bir sistemik hastalığı olmayan 17 birey (11 kız, 6 erkek) seçilmiştir. Her hastadan ortodontik tedavi öncesinde stimüle edilmemiş tükürük örnekleri toplanmıştır. Bant, braket ve ark telleri yerleştirildikten üç ay ve 6 ay sonra aynı işlem tekrarlanmıştır. Bütün tükürük örnekleri, üzerinde çalışma yapılmadan önce -35°C'de saklanmıştır. Antimikrobiyal protein lizozimin ölçümü Osserman ve Lawlor'un yöntemi ile gerçekleştirilmiştir. Antioksidan savunma enzimlerinden süperoksit dismutaz (SOD) aktivitesi, tetrazolium nitroblue yöntemi kullanılarak ölçülmüştür. Protein içeriği Lowry yöntemi ile değerlendirilmiştir. Verilerin istatistiksel incelenmesi eşleştirilmiş t-testi ile yapılmıştır. Ortodontik aparatların uygulanmasından üç ay sonra lizozim seviyelerinde değişiklik görülmezken, 6 ay sonra anlamlı bir azalma meydana gelmiştir ( $1.11 \pm 0.62$  mg/mg protein vs  $0.63 \pm 0.41$  mg/mg protein;  $p < 0.05$ ). Diğer taraftan SOD aktivitesinde tedaviyi takiben artış gözlenmiştir. Tükürükteki SOD aktivitesi tedavinin üçüncü ayında ortalama  $3.93 \pm 1.73$  U/mg protein, 6. ayında  $5.21 \pm 2.19$  U/mg protein değerleri göstermiş, her iki dönemde de başlangıç seviyesine göre anlamlı artış meydana gelmiştir. Çalışmanın her üç aşaması sırasında stimüle edilmemiş tükürükte total protein içeriğinde değişiklik olmamıştır. Sonuç olarak, tükürüğün antibakteriyel ve antioksidan kapasitesinin, sabit ortodontik aparatların ilk altı aylık uygulaması sırasında değişikliğe uğradığı gözlenmiştir.

Anahtar Kelimeler: Tükürük, lizozim, süperoksit dismutaz, sabit ortodontik aparatlar

**SUMMARY: SALIVA LYSOZYME LEVELS AND ANTIOXIDANT ENZYME ACTIVITY IN PATIENTS WITH FIXED ORTHODONTIC APPLIANCES.** The aim of this study was to examine the effect of orthodontic appliances on antimicrobial and antioxidant capacity of the saliva. For this purpose seventeen children (11 girls, 6 boys) aged between 12-20 years with no systemic disease

were selected. Unstimulated whole saliva sample was collected before orthodontic treatment of each patient. The sample collection was repeated on third and sixth month after the placement of brackets, bands and wires. All saliva samples were kept at -35°C until studied. The antimicrobial protein lysozyme assays were performed by the method of Osserman and Lawlor. The antioxidant defense enzyme superoxide dismutase (SOD) activity was measured using the nitroblue tetrazolium assay. The protein content was measured by the method of Lowry. The data were evaluated statistically with paired-t test. Three months after the placement of orthodontic appliances, lysozyme levels were unchanged; however they were significantly reduced on the sixth month of treatment ( $1.11 \pm 0.62$  mg/mg protein vs  $0.63 \pm 0.41$  mg/mg protein;  $p < 0.05$ ). On the other hand, SOD activity was continuously elevated following the treatment. Mean SOD activity in saliva was  $3.93 \pm 1.73$  U/mg protein on the third month, and  $5.21 \pm 2.19$  U/mg protein on the sixth month which were both significantly higher than the basal level ( $2.83 \pm 1.26$  U/mg protein;  $p < 0.01$ ). No significant difference in the total protein content in unstimulated saliva was observed between the three stages of the study. It is concluded that fixed orthodontic appliances may alter the antibacterial and antioxidant capacity of the saliva following a six-month treatment.

**Key words:** Saliva, lysozyme, superoxide dismutase, fixed orthodontic appliances

### INTRODUCTION

The placement of orthodontic bands, brackets and wires increases the risk of plaque accumulation, caries and decalcifications (1-3). Increased numbers of microorganisms in the saliva have been recorded in all patients after the insertion of fixed appliances (4,5). Gingival inflammation often is also associated with the application of orthodontic appliances (2,6). On the other hand, it has been shown that the placement of a fixed appliance changes the flow rate of saliva (3,7).

Natural antimicrobial systems of human saliva are correlated with dental health parameters. Lysozyme is an important antibacterial protein found in saliva and other mucous secretions of the body (8). The other factors, which may be altered in saliva in response to oral pathogens, are endogenously synthesized antioxidants, i.e. reduced glutathione and the enzymes superoxide dis-

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mutase (SOD), glutathione peroxidase (GPx) and catalase (Cat)(9,10).

Although there are ample reports involving the increased risk of dental caries and periodontal diseases due to the placement of orthodontic appliances (2,4-6), the salivary compositions of the antioxidant and antimicrobial agents have not been investigated in patients who underwent orthodontic therapy. Therefore, this study was designed to examine the role of orthodontic appliances on the antimicrobial and antioxidant capacity of saliva.

**MATERIALS AND METHOD**

Seventeen patients (11 girls, 6 boys) aged between 12-20 years were selected among the cases applied to the Department of Orthodontics, Faculty of Dentistry, Istanbul University. All subjects had no history of any systemic disease and had not undergone orthodontic treatment previously. The study was carried out in three stages. Unstimulated saliva samples were collected from all patients in the first appointment, after breakfast prior to the orthodontic treatment. The same procedure was repeated on third and sixth months after the placement of bands, brackets and wires. Collected saliva samples were kept at -35°C until examined. All saliva samples were centrifuged at 1800 g for 20 min at 10°C before biochemical analysis.

Lysozyme assays were performed by the method of Osserman and Lawlor (11). SOD activity was measured using the nitroblue tetrazolium (NBT) assay (12). In this method, one unit of SOD is defined as the amount of pro-

tein that inhibits the rate of NBT reduction by 50%. The protein content was measured spectrophotometrically using bovine albumin as standard (13). The values obtained from both lysozyme and SOD assays were expressed as mg per mg protein and units per mg protein, respectively.

The data were evaluated statistically with paired t test.

**RESULTS**

Mean values (± SD) for the lysozyme level, SOD activity and total protein concentration in the saliva of the patients were presented in Table 1.

Six months after the placement of orthodontic appliances, lysozyme levels per mg protein were found significantly reduced (1.11±0.62 mg/mg protein vs 0.63±0.41 mg/mg protein, p<0.05) (Table 1). A slight, but nonsignificant decrement was observed at the end of first three months. When mean lysozyme levels as mg per ml saliva were compared, a statistically significant difference was obtained between the levels of the third and the sixth months (p=0.028).

On the other hand, SOD activity was continuously elevated during the treatment. On the third month, mean SOD activity was 4.53±1.74 U/ml, which was significantly higher than the basal level (3.52±1.46 U/ml p<0.01). On the sixth month, mean SOD activity was 5.8±1.41 U/ml, which was significantly higher than the levels obtained on the third month (p<0.01). When the enzyme activity was expressed as units per mg protein, the significant difference between the levels of SOD on the third

Table 1 : Data obtained from saliva of seventeen orthodontic patients

Parameters	Baseline		3 <sup>rd</sup> Month		6 <sup>th</sup> Month		T test		
	Mean	SD	Mean	SD	Mean	SD	1-2	2-3	1-3
Lysozyme (µg/mg protein)	1.11	0.62	1.11	0.67	0.63	0.41	n.s	p=0.05	P=0.037
SOD (U/mg protein)	2.83	1.26	3.93	1.73	5.21	2.19	p=0.005	n.s	P=0.003
Protein (mg/ml)	1.35	0.64	1.24	0.48	1.23	0.42	n.s	n.s	n.s

n.s: nonsignificant.

and sixth month did not exist (Table 1). As shown on the table, no significant difference in the total protein concentration in unstimulated saliva was found between the three stages of the study.

## DISCUSSION

Patients who undergo orthodontic therapy have oral ecologic changes such as increased retentive sites for streptococcus mutans, and increased retention of food particles, which may lead to increased proportions and absolute numbers of salivary *S.mutans* (2-5,14-16). The placement of orthodontic bands and brackets increases the risk of plaque accumulation and the patients with orthodontic appliances have greater salivary flow rate (3,15). Human saliva contains a number of agents that protect oral tissues against hazardous compounds, mostly produced by various microorganism (17). A steady supply of saliva provides the presence of protective factors in the mouth. Therefore, any change in salivary flow rate may affect this protective network. If salivary flow rate were substantially reduced, this protective network would be collapsed (17). However, the quality of saliva is as important as its quantity for the maintenance of oral health. In previous studies, it has been shown that living in air polluted area and occupational hazards cause a significant decrease in the amount of salivary lysozyme (18,19). Controversely, Richter and Pelech (20) have published increased levels of salivary lysozyme in children living in the polluted area. Previous studies (3-6,14-16) have documented that the insertion of a fixed orthodontic appliance affects the oral environment in a way that favours development of anaerobic lactobacilli in particular. Thus, natural antimicrobial systems of human saliva should be expected to alter by the ecological changes due to orthodontic treatment.

In our study, decreased level of salivary lysozyme per amount of protein was observed 6 months after the placement of fixed appliances. It is known that protein concentration is not markedly affected by the stimulation of the saliva (21). Therefore, the decrement of the lysozyme concentration could not be explained by the chronic stimulation of saliva by the orthodontic appliances. Although it has previously been observed that the orthodontic treatment causes an increment in the salivary flow rate (15), the changes in the quantity of antimicrobial

agents may still influence the protection of oral environment.

It is well known that free radicals are continuously produced in the body and removed by the antioxidant defense mechanisms (22). When the balance between the oxidant and antioxidant system is disturbed, free radicals increase and cause cellular injury. This condition is called "oxidative stress" and may lead to many diseases. Antioxidants are the molecules that can act upon important reactive oxygen species as biological scavengers (23,24). The antioxidant enzymes superoxide dismutase (SOD), glutathione peroxidase (GPx) and reductase (GSR) are substantial defensive factors against oxidative stress (25). Although the substantial role for free radicals or reactive oxygen species is clearly defined in periodontal diseases few research has been performed in this area (26). Our report is first in which SOD activity in whole saliva was examined following an orthodontic treatment. Jacoby et al. have demonstrated the presence of SOD activity in PDL collagen surface and have reported that salivary SOD activity had not been studied by that time (27). They have also claimed that the association of the SOD with the surface of collagen fibrils affords biological protection from the oxygen radicals. Thus, any local inflammation is expected to lead to the increased activity of SOD.

Another endogenous antioxidant, uric acid, was measured and reported to be responsible for more than %70 of the total antioxidant activity (28). In another study, it has been shown that tissue glutathione depletion correlates with the extent of periodontal damage (29), but there is few data given for SOD activity. Therefore, it is difficult to evaluate the importance of SOD activity in saliva in regard to the maintenance of oral health. In a study of Pereslegina et al., a decrement in the activity of SOD and an imbalance of the glutathione redox system were found in the saliva and the other digestive secretions of children with gastroduodenal pathology (30).

Our study, revealed increased levels of SOD activity following the placement of fixed orthodontic appliances. This increase in salivary SOD activity may be due to the increment of superoxide generation, which is possibly related to the alteration in the oral environment.

## CONCLUSION

Fixed orthodontic appliances seem to have an effect on both the antibacterial and antioxidant capacity of the saliva. The enhancement in the antioxidant defense mechanism is generally indicative to sudden increases in oxygen metabolites. Therefore, mechanism(s) underlying the enhanced free radical generation needs to be elucidated by further studies.

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