

THE EFFECT OF ORTHODONTIC TREATMENT ON pH, BUFFER CAPACITY AND LEVELS OF STREPTOCOCCUS MUTANS AND LACTOBACILLUS

EFEKTI ORTODONTSKOG TRETMANA NA pH, PUFERSKI KAPACITET I NIVO STREPTOCOCCUS MUTANSA I LACTOBACILLUSA

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SAŽETAK

Cilj: Cilj ove studije bio je da se utvrde promjene salivarnog pH i puferski kapacitet uspoređen sa salivarnim nivoima *S. mutans* i *Lactobacillus* spp kod zdravih osoba prije i u toku fiksnog ortodontskog tretmana.

Materijal i metode: U ovom istraživanju bilo je uključeno 30 ispitanika, uzrasta od 14 do 18 godina, kojima je bio potreban ortodontski tretman fiksnim aparatom. Uzorci pljuvačke su sakupljeni dvije sedmice prije početka fiksnog ortodontskog tretmana, uzimajući ih kao bazične vrijednosti. Zatim su uzorci pljuvačke sakupljeni nakon jedne sedmice, zatim nakon četiri sedmice, te dvanaest i osamnaest sedmica nakon postavljanja fiksnog ortodontskog aparata. CRT Bacteria Test (Ivoclar, Vivadent) korišten je za određivanje salivarnih vrijednosti *S. mutans* i *Lactobacillus* spp, pomoću selektivnog kultiviranja u mediju. Za analizu pljuvačke koristili smo digitalni pH metar (Piccolo plus ATC pH meter, Hannah Instruments).

Rezultati: Ortodontski tretman mijenja oralne faktore usne sredine: pH i puferski kapacitet. Nivo salivarnih bakterija ima znatno povećane vrijednosti tokom prvog mjeseca nakon ortodontskog tretmana.

Zaključak: Neophodno je održati ravnotežu između faktora koji imaju zaštitnu funkciju i karijes rizičnih faktora tokom ortodontskog tretmana, uključujući kućni program njege, pravilnom oralnom higijenom uz neophodnu kontrolu nagomilavanja plaka, što su procedure koje su neophodne za prevenciju nastanka karijesa i parodontalne bolesti.

Ključne riječi: ortodontski aparati, salivarni pH, *Streptococcus mutans*, *Lactobacillus* spp

ABSTRACT

The objective: The aim of this study was to determine the changes of salivary pH, and buffer capacity in relation to salivary levels of *S. mutans* and *Lactobacillus* spp in healthy patients before and during therapy with fixed orthodontic appliances.

Material and methods: The subjects of this study were 30 children between the ages of 14 and 18 years who were need fixed orthodontic treatment. Salivary samples were collected 2 weeks before fixed appliance treatment a baseline assessment. Then salivary samples were taken at week, 4, 12 and 18 weeks after placement of fixed orthodontic appliances. The CRT Bacteria Test (Ivoclar, Vivadent) was used to determine the measured the *Streptococcus mutans* and *Lactobacillus* spp count in saliva by means of selective culture media. For salivary analysis we used a handheld digital pH meter, (Piccolo plus ATC pH meter, Hannah Instruments).

Results: Orthodontic treatment changes the oral environmental factors: was a decrease in salivary pH and buffering capacity. The bacterial levels increase significantly in the first month of the orthodontic treatment.

Conclusion: It is necessary to maintain the balance between the protective factors and the caries risk factors during the orthodontic treatment with a home care program toward correct oral hygiene procedures necessary to control plaque accumulation for caries and periodontal disease prevention.

Key words: orthodontic appliances, salivary pH, *Streptococcus mutans*, *Lactobacillus* spp.

Introduction

In orthodontics caries usually occurs on smooth surfaces, affecting 2 to 96% of all orthodontic patients [1]. Increase in caries risk during such treatment is due to several factors, lesions are difficult to locate, lowering of resting pH, increased volume of dental plaque and rapid shift in bacterial flora [2]. Maxillary lateral incisors, maxillary canines and mandibular premolars are the most commonly affected teeth [3]. During treatment, retentive areas are created that favor biofilm accumulation and bacterial growth. One of the greatest challenges in orthodontics consists in maintaining proper oral hygiene during treatment. Brackets, bands and other accessories further aggravate these conditions by retaining dental plaque, which can lead to gingivitis and enamel demineralization, causing white spots and caries [4]. Microbiological studies have established that, after placement of a fixed orthodontic appliance, the number of bacteria raises significantly, particularly streptococci and lactobacilli, subjecting the oral environment to an imbalance and enabling the emergence of diseases. Although dental biofilm is composed of numerous species of bacteria, it is believed that *Streptococcus mutans* is involved in the early development of carious lesions [5].

Li et al. [6] found that during the first month of fixed orthodontic treatment, the whole saliva flow rate and concentrations of some saliva ions increased significantly, but were at normal levels after 3 months. Sanpei et al. [7] found changes in salivary *Lactobacillus spp* levels but no changes in salivary flow rates, buffer capacity, and *S. mutans* levels during and after active orthodontic treatment.

Some *in vitro* studies have demonstrated significant differences in bacterial adhesion and biofilm formation between brackets made from different materials. Initial adhesion of *S. mutans* was higher on plastic brackets than on stainless steel and ceramic brackets, on which adhesion was lowest. In contrast, another *in vitro* study showed no significant differences of *S. mutans* adhesion to stainless steel, ceramic or plastic brackets [8]. However, the difference between metallic and ceramic brackets in the adhesion of *S. mutans* could not be confirmed *in vivo*, neither was there a difference in adhesion of *Lactobacillus* species [9]. *In vivo*, maxillary brackets harvested more *S. mutans* and *S. sobrinus* than mandibular brackets [10], while labial brackets harvested more biofilm than lingual brackets. Also a split-mouth study indicated more anaerobic and aerobic organisms in self-ligating than in conventional bracket

sites [11]. Combined with the observation that the occurrence of white spot enamel lesions and gingival inflammation was similar in patients with self-ligating and conventional brackets, this may indicate that biofilm formation on the brackets themselves is less harmful than when formed at the bracket-enamel-adhesive junction [12].

The aim of this study was to determine the changes of salivary pH, and buffer capacity in relation to salivary levels of *S. mutans* and *Lactobacillus spp* in healthy patients before and during therapy with fixed orthodontic appliances.

Material and Methods

The subjects of this study were 30 children between the ages of 14 and 18 years who were need fixed orthodontic treatment. Salivary samples were collected 2 weeks before fixed appliance treatment a baseline assessment. Then salivary samples were taken at week, 4, 12 and 18 weeks after placement of fixed orthodontic appliances.

Saliva sampling and microbial evaluation

The CRT Bacteria Test (Ivoclar, Vivadent) was used to determine the measured the *Streptococcus mutans* and *Lactobacillus spp* count in saliva by means of selective culture media. Before collecting saliva for the CRT Bacteria Test, the patients were asked not to eat or drink for at least an hour. Salivation was stimulated by having the children chew a paraffin pellet for 5 minutes. The saliva from each patient was collected in a calibrated container. The agar carrier was removed from the test vial, and a NaHCO_3 tablet was placed at the bottom of the vial. The protective foils were removed carefully from the agar surface. Using a pipette, agar surface were wetted with saliva and excess was allowed to drip off. The agar carrier was placed back into the vial, which was closed tightly. The vials were incubated at 37°C for 48 hours. After that all of the samples were evaluated as product company directions by its scale.

To estimate the number of colony-forming units of *Streptococcus mutans* counts (SM) per milliliter of saliva (CFU/mL) was used Dentocult® SM. Counts were categorized as follows score: 0 = negative or $<10^4$ colony-forming units (CFU)/mL, 1 = $<10^5$ CFU/mL, 2 = $>10^5$ to $<10^6$ CFU/mL, and 3 = $>10^6$ CFU/mL. The number of *Lactobacillus* was obtained by the use of Dentocult® LB (Orion Diagnostica). The growth densities were categorized as follows: 0 = negative or 10^3 CFU/mL, 1 = 10^4 CFU/mL, 2 = 10^5 CFU/mL, and 3 = 10^6 CFU/mL.

	baseline	4 weeks	12 weeks	18 weeks
mean (SD)	7.18	6.77(0.96)	6.66(0.77)	6.67(0.65)
significance		**	**	**

** Significant $p < 0.01$

Table 1.

Summary data for salivary pH before (week 0, baseline) and 4, 12, 18 weeks after insertion of orthodontic appliances

	baseline	4 weeks	12 weeks	18 weeks
mean (SD)	7.18	7.84(2.31)	7.23(1.53)	7.67(2.24)
significance			**	**

** Significant $p < 0.01$

Table 2.

Summary data for buffer capacity of saliva before (week 0, baseline) and 4, 12, 18 weeks after insertion of orthodontic appliances

Test Score (CFU/mL)						
week	0	1(<10 ⁵)	2(>10 ⁵)	3(>10 ⁶)	mean (SD)	p
0	2	23	3	2	1.39 (0.54)	*b
4	0	19	7	4	2.34 (0.81)	*b
12	0	14	6	10	2.53 (1.04)	*b
18	0	19	5	6	2.04 (0.9)	*b

** Fridmans analysis of variance test a significant result ($p < 0.05$)
^b Wilcoxon test was significant when baseline value (0) was compared with the 4, 12 and 18 week data points

Table 3.

Summary data for salivary *Streptococcus mutans* in milliliters of saliva before (week 0, baseline), 4, 12, and 18 weeks after insertion of orthodontic appliances

Test Score (CFU/mL)						
week	0	1(<10 ⁵)	2(>10 ⁵)	3(>10 ⁶)	mean (SD)	p
0	2	20	5	5	1.59 (0.84)	*b
4	0	16	9	5	2.74 (0.91)	*b
12	0	12	8	10	2.23 (1.08)	*b
18	0	17	6	7	2.09 (0.85)	*b

** Fridmans analysis of variance test a significant result ($p < 0.05$)
^b Wilcoxon test was significant when baseline value (0) was compared with the 4, 12 and 18 week data points

Table 4.

Summary data for Salivary *Lactobacillus* spp in milliliters of saliva before (week 0, baseline), 4, 12, and 18 weeks after insertion of orthodontic appliances

For salivary analysis we used a handheld digital pH meter, (Piccolo plus ATC pH meter, Hannah Instruments) and a CRT bacteria test kit, including mitis-salivarius with bacitracin agar, Rogosa agar, NaHCO₃ tablets, pipettes, and vials (Ivoclar Vivadent, Schaan Liechtenstein).

Results

A total of 30 subjects met the criteria for inclusion, 14 males (46.6 %) and 16 females (53.4%); mean age was 14.04 ± 1.62 (range 14-18 years).

Salivary pH significantly decreased after first 4 weeks, continued to decreased during the next 8 weeks ($p > 0.05$), and between week 12 and week 18 increased toward baseline (**Table 1**). A significant decrease in salivary buffer capacity at week 12 of orthodontic therapy with fixed appliances preceded an increase of salivary flow at week 18 (**Table 2**).

Salivary *Streptococcus mutans* and *Lactobacillus* spp counts before and during of orthodontic therapy are presented in Tables 3 and 4. The overall level of significance for salivary *Streptococcus mutans* and *Lactobacillus* spp data was analyzed using a Friedman procedure. Salivary *Streptococcus mutans* increased in 23 of 30 subjects. A Wilcoxon test for salivary *S. mutans* in milliliters of saliva before and after the placement of orthodontic appliances, over 12 weeks, revealed a significant increase after first 4 weeks of appliance placement (**Table 3**). In the first sample 20/30 subjects had high levels ($>10^5$) of *Lactobacillus*, in the second stage we found 9/30 subjects in these same level, although statistically significant differences were observed in this distribution (**Table 4**).

Discussion

Many factors have been reported to contribute to the development of enamel demineralization. Orthodontic adhesive remaining around the bracket base can be a strong predisposing factor for enamel demineralization, because the rough adhesive surface provides an ideal site for the rapid attachment and growth of oral microorganisms [13]. Orthodontic brackets can also play a role in enamel demineralization, because they provide additional adhesion sites for pathogenic bacteria. However, few studies have investigated the adhesion capacity of cariogenic streptococci to various orthodontic raw materials. Analysis of the adhesion of cariogenic streptococci to orthodontic raw materials will increase our understanding of the factors that

cause enamel demineralization. Orthodontic appliances, both fixed and removable, impede the maintenance of proper oral hygiene and result in plaque accumulation. Many studies report that changes in the dental flora occur after starting the orthodontic treatment (higher concentrations of pathogen microorganisms), while others claim the opposite [14, 15, 16].

Enamel demineralization is caused by organic acids produced mainly by cariogenic streptococci, the prime causative organisms of dental caries [17, 18, 19]. Of these, *Streptococcus mutans* and *S. sobrinus* have been identified as the main pathogens in dental caries and enamel demineralization. Adhesion and colonization of cariogenic streptococci are considered to play key roles in the development of enamel demineralization related to orthodontic materials, because these materials in the oral cavity present a unique surface that can interact with bacteria, leading to pathogenic plaque formation for enamel demineralization [20, 21]. Several studies reported that the placement of fixed orthodontic appliances leads to increases in the volume and number of cariogenic streptococci in dental plaque, and the elevated levels of streptococci return to normal after removal of the appliance [22, 23].

Our study shows the changes in salivary and bacterial markers that occur in the oral environment at the beginning of orthodontic treatment with fixed appliances. The following markers emerged as protective factors: patients without active caries injuries increased significantly buffer capacity and salivary pH, after placement orthodontic appliances.

In the group using orthodontic appliances, there was a significant decrease in salivary pH and buffering capacity ($p < 0.05$). These results are similar to those reported by Kanaya et al, who found a pH decrease in individuals that underwent orthodontic treatment [24]. On the other hand, Chang, Walsh and Freer [25] suggested an increase on the salivary buffering capacity after the placement of orthodontic appliances, but their subjects had appliances for a shorter time. The factors associated with the risk of caries, such as buffering capacity and salivary pH, were altered in the group with fixed orthodontic appliances, what suggests that orthodontic treatment may have an effect the intraoral environment. Because of the long time that fixed appliances remain in the mouth; our findings suggest that preventive measures, such as hygiene, topic fluoride application and prophylaxis should be adopted for orthodontic patients.

In contrast, the following markers were negative risk factors to the oral environment: slightly increase in the

infection levels of *Streptococcus mutans* and *Lactobacillus supp*, and of occult blood in saliva. Oral environment has the capacity of adjustment to the presence of a foreign body, increasing the salivary flow which contributes to the autolysis and modifying the salivary composition to raise the pH and buffer capacity, it prevents colonization by potentially pathogenic microorganisms by denying them optimization of environmental conditions. In our study, as in some other investigations, a significant increase in cariogenic microorganisms *Streptococcus mutans* and *Lactobacillus supp* in saliva was found after commencing fixed orthodontic therapy. In this study, the first significant increase of *Streptococcus mutans* and *Lactobacillus supp* in saliva was detected as early as 4 weeks after the placement of fixed orthodontic appliances, and the highest levels were at the 12th week of therapy. Other authors have reported a significant salivary microbial increase only at week 12, but they only followed their patients for weeks of therapy [26, 27].

Conclusion

Orthodontic treatment changes the oral environmental factors: was a decrease in salivary pH and buffering capacity. The bacterial levels increase significantly in the first month of the orthodontic treatment. It is necessary to maintain the balance between the protective factors and the caries risk factors during the orthodontic treatment with a home care program toward correct oral hygiene procedures necessary to control plaque accumulation for caries and periodontal disease prevention.

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