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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Effects of the fixed orthodontic therapy on biochemical and microbiological parameters of saliva

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SUMMARY

Introduction/Objective Malocclusions are one of the most frequent disorders in dentistry, and pose a risk for the onset of caries and periodontal diseases. Fixed orthodontic treatment solves the problem of malocclusions; however, it requires simultaneous cooperation of the patients, parents, and dentists involved.

The objective of this study is to examine the effects of fixed orthodontic therapy on the *Streptococcus mutans* and *Lactobacillus spp.* bacteria in saliva, the pH value, and buffering capacity of saliva.

Methods The research was carried out at the Faculty of Medicine in Foča, Department of Dentistry. The study included 100 respondents, aged 13 to 17 years. The respondents were divided into two groups: the study group (respondents wearing fixed braces) and the control group (respondents not subjected to fixed braces therapy). Saliva samples were taken from the respondents four, 12, and 18 weeks after the start of the orthodontic therapy. The study used the bacteria caries risk test (CRT) and CRT buffer (Ivoclar Vivadent).

Results The study showed an increased number of bacteria in saliva of the respondents during all three follow-up periods (χ^2 test, $p = 0.001$). The largest numbers of the *Streptococcus mutans* and *Lactobacillus spp.* bacteria were found in week 12 of the therapy. Saliva pH value and buffering capacity of saliva increased statistically significantly in week 12 of the therapy (χ^2 test, $p = 0.001$).

Conclusion Oral conditions in patients changed during the fixed orthodontic therapy: the number of bacteria increased, the pH value and buffering capacity of saliva changed. It was necessary to use preventive measures in order to avoid complications during the fixed orthodontic appliances therapy.

Keywords: malocclusion; *Streptococcus mutans*; bacteria

INTRODUCTION

Saliva is the main defense mechanism in the oral cavity and is a major factor for preserving and maintaining the health of oral tissue. Chemical properties of saliva are affected by local factors in the oral environment and by the general health of the individual. The physico-chemical properties of saliva determine the progress of orthodontic treatment and its adverse effects in an orthodontic patient.

Malocclusion is one of the most frequent dental disorders and increases the risk of the onset of caries and periodontal diseases [1]. The placement of orthodontic brackets and bands may compromise oral hygiene, because new retentive places are formed resulting in increased accumulation of dental plaque leading to gingival inflammation [2]. Orthodontic treatment may solve the problem of malocclusion, but it increases the risk of caries onset and the severity of this lesion may range from a white spot on a tooth or demineralization to the loss of integrity of the enamel surface and the onset of a cavity [3]. Some studies report that the prevalence of white spot lesions during orthodontic treatments ranges 30–70% [3]. The changes in salivary parameters such as the decline in the pH level and buffering capacity of saliva may contribute to the demineralization of enamel and increase the susceptibility of teeth to the onset of caries [4]. Amongst various microflora in the mouth, the *Streptococcus mutans* bacterium is the main culprit for the onset of caries. The presence of these microorganisms at high levels indicates an increased risk of caries. Caries is an undesirable side effect of treatment with fixed orthodontic appliances [5, 6]. Patients undergoing orthodontic therapy experience oral ecological changes that lead to an increased number of mutans streptococci in the saliva and dental plaque [7]. At the same time, the presence of the *Lactobacillus spp.* bacteria increases the severity and incidence of caries [8]. *Lactobacillus spp.* is a secondary invasive bacterium and is responsible for the progression of the caries lesions.

The side effects of the fixed orthodontic therapy on oral health are described in numerous studies [9, 10, 11]. The studies report that caries as a complication of fixed orthodontic

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therapy is present in 2–96% of patients [12, 13]. Chang et al. [14] were amongst the first who had examined the impact of the fixed orthodontic therapy on the salivary microbiological parameters, pH value, and buffering capacity of saliva. Other authors also examined the impact of the fixed orthodontic therapy on the salivary microbiological parameters, pH value, and buffering capacity of saliva, which is the objective of the present study as well [15, 16].

METHODS

The present study was designed as a prospective cohort one. The research included 100 respondents who reported to the Faculty of Medicine in Foča, the study program in Dentistry during the years 2015 and 2016, who then underwent dental check-ups and were diagnosed with malocclusions. The respondents were divided into two groups. The exposure group (the study group) consisted of 50 respondents who underwent the treatment for malocclusion. The non-exposed group (the control group) consisted of 50 respondents, who were also diagnosed with malocclusion, but did not receive treatment (financial means, durability of treatment, patients satisfied with the current positions of teeth). The criteria for the inclusion in the study were the following: the presence of permanent dentition with other permanent molars in place, good overall health condition of the patient, patients indicated as requiring the upper and lower fixed orthodontic appliances, without dental caries. The criteria for the exclusion from the study were the following: periodontal diseases, patients with clefts, syndromes, and orofacial diseases, and patients who suffer from a chronic disease and receive a form of therapy (diabetes mellitus, autoimmune diseases, epilepsy).

The samples of saliva in the control group were collected several days after the dental check-up (T0). The samples of saliva in the study group were taken four, 12, and 18 weeks (T1, T2, T3) after the attachment of the fixed orthodontic appliances. The saliva pH level, buffering capacity, and quantitative presence of the *Streptococcus mutans* and *Lactobacillus spp.* bacteria were determined for each patient. The respondents were instructed not to consume any food and drinks for at least one hour before giving a sample of saliva and to brush their teeth once, in the morning, on the day of giving saliva samples. The respondents were sitting in a dental chair leaning slightly forward and were chewing bilaterally a paraffin ball for five minutes to stimulate salivation. Saliva was collected in sterile plastic cups for each patient. A bacteria caries risk test (CRT) (Ivoclar Vivadent, Schaan, Liechtenstein) was used to determine the quantitative presence of bacteria. The agar carrier of the mentioned test was removed from the test vial and $NaHCO_3$ tablet (for the purpose of ensuring anaerobic conditions) was placed at the bottom of the vial. The nutrient base was moistened with a thin layer of saliva using a pipette, to the amount of 1 ml. The agar carrier (the base) was carefully returned to the vial, which was then firmly closed. The vials with the seeded base were incubated at 37°C over 48 hours. Thereafter the presence of the grown colonies on both seeded bases (colony-forming unit – per ml of saliva) was measured by way of comparison with the standardized scheme specified by the manufacturer. The buffering capacity of the saliva was determined using a CRT buffer (Ivoclar Vivadent). The saliva pH value was determined using a digital pH meter (4440 pH Type; Funke-Dr.N.Gerber Labortechnik GmbH, Berlin, Germany).

The research complies with the Helsinki Declaration. For the purpose of this study, Approval No. 01-1142 was obtained from the Ethics Committee of the Faculty of Medicine in Foča.

The data were analyzed using descriptive and analytical statistics methods; χ^2 test and Fisher's exact test, for qualitative variables, and Student's t-test, for quantitative variables, were used to determine the statistical significance between the two groups of respondents.

RESULTS

The study included 100 respondents, aged 13–17 years. The average age was 14.88 ± 1.35. The age group of 13–14 years made up 40% of the respondents, 24% of the respondents were 15 years old, while the remaining 36% of the respondents were in the age group of 16–17 years. Male respondents made up 48%, while female respondents made up 52% of the total number of respondents.

The numbers of the *Streptococcus mutans* were statistically significantly increased during all three periods of research when compared to the control group of respondents. The largest numbers of the *Streptococcus mutans* bacterium were found after 12 weeks of therapy (p = 0.001). The number of bacteria was reduced after 18 weeks when compared to week 12 of the treatment; however, a statistically significant difference still existed when compared to the control group of the respondents (Table 1). The numbers of the *Lactobacillus spp.* bacteria were statistically significantly increased during all three periods of research when compared to the control group of the respondents. The largest number of the *Lactobacillus spp.* bacteria was found after 12 weeks of therapy (p = 0.001). The number of the bacteria was reduced after 18 weeks when compared to week 12 of the treatment; however, a statistically significant difference still existed when compared to the control group of the respondents (Table 2).

Table 1. Data obtained by saliva analysis on the amount of *Streptococcus mutans* bacterium in subjects undergoing orthodontic treatment after four, 12, and 18 weeks and in the control group

Time (weeks)	Level of bacteria	Group of respondents number (%)		Total number (%)	χ^2; F	p
		Study group	Control group			
4	Low (< 10^5) High (≥ 10^5)	6 (12) 44 (88)	22 (44) 28 (56)	28 (28) 72 (72)	12.698	0.001
12	Low (< 10^5) High (≥ 10^5)	5 (10) 45 (90)	22 (40) 28 (56)	27 (27) 73 (73)	14.663	0.001
18	Low (< 10^5) High (≥ 10^5)	10 (20) 40 (80)	22 (44) 28 (56)	32 (32) 68 (68)	6.618	0.010

χ^2 – Chi-squared test; F – Fisher's exact test

Table 2. Data obtained by saliva analysis on the amount of *Lactobacillus spp*. bacterium in subjects undergoing orthodontic treatment after four, 12, and 18 weeks and in the control group of subjects

Time (weeks)	Level of Bacteria	Group of respondents number (%)		Total number (%)	χ^2; F	p
		Study group	Control group			
4	Low (< 10^5) High (≥ 10^5)	23 (46) 27 (54)	38 (76) 12 (24)	61 (61) 39 (39)	9.458	0.002
12	Low (< 10^5) High (≥ 10^5)	15 (30) 35 (70)	38 (76) 12 (24)	53 (53) 47 (47)	21.236	0.001
18	Low (< 10^5) High (≥ 10^5)	18 (36) 32 (64)	38 (76) 12 (24)	56 (56) 44 (44)	16.234	0.001

χ^2 – Chi-squared test; F – Fisher's exact test

Table 3. Buffer capacity of the control and study group

Buffer capacity in saliva samples	Level of buffer capacity	Respondents number (%)		Total number (%)	χ^2; F	p
		Study group	Control group			
Study group after 4 weeks and control group	Low Medium High	2 (2) 23 (23) 25 (25)	4 (4) 26 (26) 20 (20)	6 (6) 49 (49) 45 (45)	1.406	0.495
Study group after 12 weeks and control group	Low Medium High	2 (2) 6 (6) 42 (42)	4 (4) 26 (26) 20 (20)	6 (6) 32 (32) 62 (62)	20.973	0.001
Study group after 18 weeks and control group	Low Medium High	6 (6) 23 (23) 21 (21)	4 (4) 26 (26) 20 (20)	10 (10) 49 (49) 41 (41)	0.608	0.738

χ^2 – Chi-squared test; F – Fisher's exact test

Table 4. Saliva pH values of the study and the control group

pH value in saliva samples	Respondents AM (SD)		t	p
	Study group	Control group		
Study group after 4 weeks and control group	6.76 (0.40)	6.66 (0.26)	1.482	0.141
Study group after 12 weeks and control group	6.89 (0.21)	6.66 (0.26)	4.881	0.001
Study group after 18 weeks and control group	6.84 (0.47)	6.66 (0.26)	2.345	0.210

AM – arithmetic mean; SD – standard deviation, t – Student's t-test

The pH value and buffering capacity of saliva statistically significantly increased 12 weeks after the therapy when compared to the control group of the respondents (p = 0.001). Four and 18 weeks after the orthodontic treatment no statistically significant difference was found in comparison to the control group of the respondents (Tables 3 and 4).

DISCUSSION

This research showed that a statistically significant increase in the number of *Streptoccocus mutans* bacterium occurred in the patients' saliva after all three study periods (four, 12, and 18 weeks after the orthodontic therapy). The study results show that the adverse effects of the fixed orthodontic therapy occurred as early as four weeks after the treatment. Chang et al. [14] presented similar results, according to which they also found a statistically significant increase in the number of bacteria four weeks and three months after wearing the braces. The authors detected the peak in the number of microorganisms in week four of the therapy, in contrast to our research. This study shows that in week 18 of the therapy the number of bacteria starts to decline, which is a very significant piece of information when it comes to planning preventive and prophylactic measures. The increased number of the *Streptococcus mutans* bacteria during the therapy may be explained by the increased retention locations following the attachment of the appliance, which is conducive to the accumulation of plaque, which in turn increases the number of aciduric and acidogenic bacteria, which prefer hard and uneven surfaces for their growth [17]. The results of this study are consistent with the results of other authors, who also found the increased numbers of bacteria during all three follow-up periods, whereas they followed up on patients six, 12, and 18 weeks of the therapy [8, 16]. In addition, all these studies found the largest number of bacteria in week 12 of the orthodontic therapy. When compared to our study, the difference was the sample size. Numerous authors suggest that white spots on teeth, as the start of a caries lesion, are detected as early as four weeks after the orthodontic treatment; therefore, we are of the opinion that it is justified to start monitoring the changes in saliva after four weeks [18, 19, 20]. White spot lesions on teeth, if left untreated, may progress into a lesion on a tooth and this entire process progresses rapidly in orthodontic patients [21].

The study showed a statistically increased number of the *Lactobacilus spp.* bacterium in saliva in all three follow-up periods (four, 12, and 18 weeks after the orthodontic treatment). The greatest value of the number of the bacteria were found in week 12 of the therapy, while the decline in the number of the bacteria was found in week 18, which is a significant piece of information when planning preventive measures. The study showed no statistically significant difference in regard to the sex and age of patients. These results are consistent with the results of other authors, who also found an increase in the number of the *Lactobacillus spp*. bacteria in all three follow-up periods, with the highest number found in week 12 of the therapy [8, 16]. Chang et al. [14] studied the numbers of this microorganism four weeks and three months following the therapy and they found the greatest number of the bacterium in the third month (week 12) of the therapy, which matches the results of our study, as well as of other studies [16]. Other authors also found statistically significant increase of the number of this microorganism in week 12 of the therapy [22]. Eighteen weeks after the treatment, the results showed that the number of this microorganism was declining when compared to week 12; however, the number was still statistically significantly increased when compared to the control group of the respondents. Week 12 may be regarded as the period of the most intense growth of the *Lactobacillus spp*.

bacteria in the respondents' saliva. However, some authors found the statistically increased number of the *Lactobacillus spp*. bacterium in saliva six months after wearing the braces, while no statistically significant difference in the number of this microorganism was detected four and 12 weeks after the therapy when compared to the values before the attachment of the braces [23].

The study results show that statistically significant difference occurred between the study and control groups of respondents ($p = 0.001$) in week 12 of the therapy, in regard to the saliva pH values (Table 4), which is consistent with results of some other studies [14, 16]. Some studies found no changes in the saliva pH values during all three follow-up periods of patients with fixed orthodontic appliances [24].

The study results showed a statistically significant difference regarding the buffering capacity of saliva 12 weeks after the therapy ($p = 0.001$) (Table 3). Some studies reported the decline in the buffering capacity of saliva with the increase in the pH value, which is not consistent with the results of this study and compels one to consider other ions of the stimulated saliva which have the buffering effect apart from the bicarbonate ones [16]. Arab et al. [8] found a statistically significant decline in the saliva pH value as early as week six of the therapy, which continued in weeks 12 and 18 of the orthodontic treatment, which is in contrast to our research. These results support the notion of great risk for the onset of caries, given the weak defensive ability of the organism, while the number of bacteria is significantly increased. Lara-Carrillo et al. [25] found a statistically significant increase in the pH value and buffering capacity of saliva of the respondents four weeks after the orthodontic treatment.

CONCLUSION

The research showed the changes in biochemical and microbiological parameters during the fixed orthodontic appliances therapy. The patients wearing fixed orthodontic appliances require regular dental check-ups to be performed by orthodontists and other dental medicine specialists, in order to detect in due time the risk factors for the onset of oral and dental diseases.

Conflict of interest: None declared.

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Утицај фиксне ортодонтске терапије на биохемијске и микробиолошке параметре пљувачке

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САЖЕТАК

Увод/Циљ Малоклузије представљају један од најчешћих поремећаја у стоматологији, а уједно представљају и ризик за настанак каријеса и пародонталних обољења. Фиксна ортодонтска терапија решава проблем малоклузије, али истовремено захтева сарадњу како пацијента, тако и родитеља и стоматолога.

Циљ овог рада био је да се испита утицај фиксне ортодонтске терапије на количину бактерија *Streptococcus mutans* и *Lactobacillus spp.* у пљувачки, *pH* и пуферски капацитет пљувачке.

Методе Истраживање је рађено на Медицинском факултету у Фочи, на одсеку за стоматологију. У студији је учествовало 100 испитаника, узраста 13–17 година. Испитаници су подељени на студијску групу (испитаници који носе фиксни ортодонтски апарат) и контролну групу (испитаници без фиксне терапије). Испитаницима су узимани узорци пљувачке после четири, 12 и 18 недеља од почетка ортодонтске терапије. У студији су коришћени *CRT* тест на бактерије и *CRT* пуфер (*Ivoclar Vivadent*).

Резултати Студија је показала повећану количину бактерија у пљувачки испитаника у сва три пратећа периода (χ^2, p = 0,001). Највећа количина бактерија *Streptococcus mutans* и *Lactobacillus spp.* нађена је у 12. недељи терапије; *pH* вредност пљувачке и пуферски капацитет пљувачке су статистички значајно повећани у 12. недељи терапије (χ^2, p = 0,001).

Закључак Током фиксне ортодонтске терапије мењају се услови у устима пацијента: повећана је количина бактерија, долази до промене *pH* вредности и пуферског капацитета пљувачке. Неопходно је користити превентивне мере како би се спречиле компликације током терапије фиксним ортодонтским апаратима.

Кључне речи: малоклузија; *Streptococcus mutans*; бактерија