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

Утицај фиксне ортодонтске терапије на биохемијске и микробиолошке параметре пљувачке

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SUMMARY

Introduction/Objective Malocclusions are one of the most frequent disorders in dentistry. They pose a risk for the onset of caries and periodontal diseases. Fixed orthodontic treatment solves the problem of malocclusions; however, it requires the cooperation of patients, parents and dentists at the same time. The goal of this study is to examine the effects of fixed orthodontic therapy on the *Streptococcus mutans* and *Lactobacillus spp* bacteria in saliva, the pH 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. 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 respondents 4, 12 and 18 weeks respectively after the start of the orthodontic therapy. The study used the CRT bacteria test and CRT buffer (Ivoclar vivadent).

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

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

Keywords: malocclusion, *Streptococcus mutans*, bacteria

САЖЕТАК

Увод/циљ Малоклузије представљају један од најчешћих поремећаја у стоматологији, а уједно представљају и ризик за настанак каријеса и пародонталних оболења. 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, одсек стоматологија. 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 након 4, 12, и 18 недеља од почетка ортодонтске терапије.У студији је кориштен *CRT* бактерија тест и *CRT* пуфер (Ивоклар вивадент).

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

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 factor in the oral environment and general health of 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]. Studies report that the prevalence of white spot lesions during orthodontic treatments ranges from 30 to 70% [3, 4]. The changes in salivary parameters such as the decline in the pH and buffering capacity of saliva may contribute to the demineralization of enamel and increase the susceptibility of teeth to the onset of caries [5].Amongst various microflora in the mouth the *Streptococcus mutans* bacteria 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 [6, 7].Patient undergoing orthodontic therapy have oral ecological changes that lead to increased number of mutans Streptococci in the saliva and dental plaque [8]. At the same time, the presence of the *Lactobacillus spp* bacteria increases the severity and incidence of caries [9]. *Lactobacillus spp* is a secondary invasive bacteria 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 [10, 11, 12]. The studies report that caries as a complication of fixed orthodontic therapy is present in 2-96% of patients [13, 14]. Chang et al. [15] were amongst the first who had examined the impact of the fixed orthodontic therapy on the salivary microbiological parameters, pH and buffering capacity of saliva. Other authors too examined the impact of the fixed orthodontic therapy on the salivary microbiological parameters, pH and buffering capacity of saliva [16, 17].

The goal of this study was to examine the impact of the fixed orthodontic therapy on the salivary microbiological parameters, pH and buffering capacity of saliva.

METHODS

The study was designed as a prospective cohort study. The research included 100 respondents who reported to the Faculty of Medicine in Foča, the study programme 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 of malocclusion. The control group (the non-exposed group) consisted of 50 respondents, who were also diagnosed with malocclusion, but did not receive treatment (financial means, durability of treatment, patients are satisfied with the current positions of teeth). The criteria for the inclusion in the study were: the presence of permanent dentition with other permanent molars in place, good overall health condition of a patient, patients indicated as requiring the upper and lower fixed orthodontic appliances, without dental caries. The criteria for the exclusion from the study were periodontal diseases, patients with clefts, syndromes and orofacial diseases, patients who suffer from a chronic disease and receive a form of therapy (diabetes mellitus, autoimmune diseases, and 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, twelve and eighteen weeks (T1, T2, T3) after the attachment of the fixed orthodontic appliances. The saliva pH, buffering capacity, and quantitative presence of the *Streptococcus mutans* and *Lactobacillus spp* bacteria were determined for every single 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 slightly leaning forward and were chewing bilaterally a paraffin ball for five minutes to stimulate saliva. Saliva was collected in sterile plastic cups for each patient. A CRT bacteria test (Ivoclar vivadent) 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 370C over 48 hours. Thereafter the presence of the grown colonies on both seeded bases (CFU/ml of saliva) was measured by way of comparison with the standardised scheme specified by the manufacturer. The buffering capacity of saliva was determined using CRT buffer (Ivoclar vivadent, Liechtenstein). The saliva pH value was determined using a digital pH meter (Funke Gerber Germany, 4440 pH Type). The research is in compliance with the Helsinki Declaration. For the purpose of the conduct of this study, the 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. $\chi 2$ test and Fischer's 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 to 17. The average age was 14.88+/-1.35. The age group of 13 to 14 years old included 40% of respondents, 24% of respondents were 15 years old, while the remaining 36% of respondents were in the age group of 16 to 17 years old. Male respondents made up 48%, while female respondents made up 52% of the total number of respondents.

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

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

DISCUSSION

This research showed that statistically significantly increased numbers of the *Streptococcus mutans* bacteria existed in the patients' saliva after all 3 periods of study (four,

12, and 18 weeks after the orthodontic therapy). The study results show that the adverse effects of the fixed orthodontic therapy occurred already four weeks after the treatment. Chang et al. [15] presented similar results, where they also found statistically significantly increased numbers of bacteria already in four weeks, and in three months after wearing the braces. The authors detected the peak in the number of microorganisms in week four of the therapy, unlike our research. This study shows that already in week eighteen of the therapy the numbers of bacteria start to decline, which is a very significant piece of information when it comes to planning preventive and prophylactic measures. The increased numbers 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 thus increasing the numbers of aciduric and acidogenic bacteria, which prefer hard and uneven surfaces for their growth [18]. The results of this study are consistent with the results of other authors [9, 17], who also found the increased numbers of bacteria during all three follow-up periods, whereas they followed up on patients six, twelve and eighteen weeks of the therapy. In addition, all these studies found the largest numbers of bacteria in week twelve 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 already 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 [19, 20, 21]. 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 [22].

The study showed the statistically increased numbers of the *Lactobacilus spp* bacteria in saliva in all three follow-up periods (four, twelve, and eighteen weeks after the orthodontic treatment). The greatest value of the numbers of the bacteria were found in week twelve of the therapy, while the decline in the numbers of the bacteria was found already in week eighteen, which makes for a significant piece of information when planning preventive measures. The study showed no statistically significant difference when it came to the sex and age of patients. These results are consistent with the results of other authors [9, 17] who also found the increased numbers of the *Lactobacillus spp* bacteria in all three follow-up periods, with the highest value of the numbers of the bacteria found in week twelve of the therapy. Chang and associates [15] followed the numbers of this microorganism in four weeks and three months following the therapy and they found the greatest numbers of bacteria in month three (week twelve) of the therapy, which matched the results of our study,

as well as of other studies [17]. Other authors also found statistically significantly increased numbers of this microorganism in week twelve of the therapy [23]. Eighteen weeks after the treatment, the results showed that the numbers of this microorganism were declining when compared to week 12, however the numbers were still statistically significantly increased when compared to the control group of respondents. The week twelve 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 numbers of the Lactobacillus spp bacteria in saliva six months after wearing the braces, while no statistically significant difference was detected four and twelve weeks after the therapy in the numbers of this microorganism when compared to the values before the attachment of the braces [24].

The study results show that statistically significant difference occurred between the study and control groups of respondents, (p=0.001), in week twelve of the therapy, when it comes to the saliva pH values, (Table 4), which is consistent with the results of other studies [15, 17]. Some studies found no changes in the saliva pH value during all three follow-up periods of patients with fixed orthodontic appliances [25].

The study results showed statistically significant difference when it came to the buffering capacity of saliva twelve 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 think about some other ions of the stimulated saliva, apart from the bicarbonate ones, which have the buffering effect [17]. Arab and associates [9] found a statistically significant decline in the saliva pH value already in week six of the therapy, which continued in weeks twelve and eighteen of the orthodontic treatment, unlike in our research. These results support the great risk for the onset of caries, given the weak defensive ability of the organism, while the numbers of bacteria are significantly increased. Carrillo et al. [26] found a statistically significant increase in the pH value and buffering capacity of saliva of the respondents already 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 dentist specialists, in order to observe in due time the risk factors for the onset of oral and dental diseases.

Conflict of interest: None declared.

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Table 1. Data obtained by saliva analysis on the amount of Streptococcus mutans bacteria 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 ($\geq 10^5$)	6 (12) 44 (88)	22 (44) 28 (56)	28 (28) 72 (72)	12.698	0.001
12	Low($<10^5$) High ($\geq 10^5$)	5 (10) 45 (90)	22 (40) 28 (56)	27 (27) 73 (73)	14.663	0.001
18	Low($<10^5$) High ($\geq 10^5$)	10 (20) 40 (80)	22 (44) 28 (56)	32 (32) 68 (68)	6.618	0.010

χ^2 – Chi square test; F – Fisher's test

Table 2. Data obtained by saliva analysis on the amount of *Lactobacillus spp* bacteria 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 ($\geq10^5$)	23 (46) 27 (54)	38 (76) 12 (24)	61 (61) 39 (39)	9.458	0.002
12	Low ($<10^5$) High ($\geq10^5$)	15 (30) 35 (70)	38 (76) 12 (24)	53 (53) 47 (47)	21.236	0.001
18	Low ($<10^5$) High ($\geq10^5$)	18 (36) 32 (64)	38 (76) 12 (24)	56 (56) 44 (44)	16.234	0.001

χ^2 **– C**hi square test; F – Fisher's 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 four 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 square test; F – Fisher's test

Table 4. pH values of saliva study and control group

pH value in saliva samples	Respondents AS (SD)		t	*p*
	Study group	Control group		
Study group after four 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

AS – arithmetic mean; SD – standard deviation; t – Student's t test

