CHANGES IN BIOCHEMICAL PARAMETERS OF ORAL FLUID IN PATIENTS DURING THE ORTHODONTIC TREATMENT WITH A BRACKET SYSTEM UNDER THE ACTION OF A DEVELOPED MUCOSAL GEL WITH PROBIOTIC

ZMIANY PARAMETRÓW BIOCHEMICZNYCH ŚLINY POD WPŁYWEM ŻELU Z PROBIOTYKAMI DO STOSOWANIA NA BŁONY ŚLUZOWE U PACJENTÓW LECZONYCH PRZY POMOCY ZAMKÓW ORTODONTYCZNYCH

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ABSTRACT

Introduction: Many research studies involving orthodontic patients focus on changes in levels of oral microbiocenosis after bracket placement. Based upon this the objective of the current study was to determine the effect of the developed mucosal gel with probiotics on the biochemical parameters of the oral fluid of patients during the orthodontic treatment with a bracket system.

The aim: Aim of our study is to determine the effect of the developed mucosal gel with probiotics on the biochemical parameters of the oral fluid of patients during the orthodontic treatment with a bracket system

Materials and methods: 45 patients at the age of 18-24, with 15 people in each group (control, main and comparison group) were examined. The main group was presented by patients who, in order to prevent dysbiosis of the oral cavity during orthodontic treatment, were prescribed local use of the developed mucosal gel with probiotic. The statistical processing of the results of the study was carried out using methods of variation statistics using the EXCEL program (the standard package of Microsoft Office).

Results: According to the results of biochemical studies, it was found that the use of orthodontic treatment of mucosal gel with probiotic in patients with crowded teeth contributes to the strengthening of antioxidant protection, an increase in nonspecific resistance, decrease in inflammation and normalization of microbiocenosis of the oral cavity.

Conclusion: These studies indicated that the use of the developed mucosal gel with probiotic in patients with maxillofacial anomalies from the first day after fixation, as indicated by the level of biochemical markers of inflammation.

KEY WORDS: bracket system, microbiocenosis, prevention, treatment, dysbiotic disorder, mucosal gel, probiotics

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INTRODUCTION

Modern orthodontics is no longer possible to be associated with the treatment of bracket system. This method of treatment is effective and is widely used in orthodontic practice. According to a number of scientists, the presence of a permanent orthodontic device in the patient's oral cavity worsens the conditions for self-cleaning, complicates the oral hygiene, which leads to high microbial contamination of the teeth surfaces and orthodontic devices, changes the microflora of the oral cavity, which increases the number of pathogenic and opportunistic microorganisms and the phenomena of dysbiosis in general [1-3]. These factors contribute to increased prevalence of tooth decay and inflammatory diseases of the tissues of the oral cavity. Therefore, the diagnosis of disorders of the biological system and the use of agents that affect this link of pathogenesis are of great importance for the prevention and treatment of complications that occur in dentofacial anomalies and their orthodontic treatment [4].

In connection with the above presented, for the prevention and treatment of complications that develop in patients with dentofacial anomalies and for those who are receiving an orthodontic treatment, it is advisable to use drugs for the restoration of an optimal species ratio of bacteria such as probiotics [5].

THE AIM

Aim of our study is to determine the effect of the developed mucosal gel with probiotics on the biochemical parameters of the oral fluid of patients during the orthodontic treatment with a bracket system.

MATERIALS AND METHODS

45 patients at the age of 18-24, 15 people in each group (control, main and comparison group) were examined. The study group included 30 people who had been shown the idea of orthodontic treatment with a bracket system. The selection criteria for the groups were as follows: 1) men or women from 18 to 24 years old; 2) Angle I class of anomalies of bite with a crowded position of frontal teeth; 3) absence of systemic diseases; 2) absence of active carious lesions; 3) satisfactory condition of oral hygiene (oral hygiene index (OHI-S) no higher than 3.0); 4) the absence of antibacterial therapy for 6 months. The control group consisted of 15 people who had physiological bite and intact periodontium. The main group was presented by 15 people who were assigned local use of the developed mucosal gel with probiotic as an accompanying orthodontic treatment. The comparison group consisted of 15 people who did not receive any additional therapeutic and prophylactic drugs during the stages of orthodontic treatment (for the accuracy of the experiment). All patients before the beginning of orthodontic treatment underwent professional oral hygiene (scaling and polishing of teeth in 1-2 visits), training in hygiene care for teeth and oral cavity with the appointment of hygiene products (toothbrushes, superflosses, interdental brushes). Orthodontic treatment was carried out with metal braces Roth Mini, groove 0,22, 3M Unitek Corporation. The developed mucosal gel was applied as an application to the gums two times a day (in the morning and in the evening after eating and after the oral hygiene) for 30 minutes daily. All examinees signed an informed consent about the use of their data in the study. All people were asked to refrain from eating, brushing their teeth or rinsing the cavity in less than 2 hours before sampling the biomaterial. The effectiveness of the proposed gel with probiotic was evaluated based on the dynamics of biochemical parameters of the examination of the patients of the main group when compared with the patients of the control and comparison group before the beginning of orthodontic treatment, in 2 weeks and in 2 months after the fixation of the non-removable appliance.

The oral fluid in patients was sampled in the morning on an empty stomach using measuring centrifuge tubes with a funnel. The test tube was immersed in a glass with ice. The patient pre-rinsed his mouth with tap water and after 3 minutes began to spit the oral fluid into the test tube. The oral fluid was being collected for 5 minutes. After that, it was centrifuged at 3000 rpm./min. for 5 minutes, the volume of unstimulated oral fluid was measured, a transparent layer of supernatant fluid was taken into clean penicillin bottles, which were sealed, frozen at t (-10°C.) and transported to the laboratory in a thermos with ice.

The activity of elastase was determined by Visse method. The principle of the method is based on the fact that the activity of the enzyme was evaluated by the degree of hydrolysis of the synthetic substrate N-t-BOC-L-alanine-p-nitrophenyl ester (BOC) ("Sigma", the USA). Under the action of elastase from the substrate, n-nitrophenol, which has a yellow color, is cleaved. The intensity of the color is proportional to the activity of elastase [7].

The content of MDA in the oral fluid was determined by the color reaction with thiobarbituric acid according to I. D. Stalna and T.H. Harishvili method. The principle of the method is based on the fact that at high temperature in the acidic environment malonic dialdehyde reacts with 2-thiobarbituric acid, forming a colored trimethyl complex, with a maximum absorption at 532 nm. The molar extinction coefficient of this complex is E = 1.56X105CMIM'1. Density of 532 nm is measured on a spectrometer in comparison with a control sample [7].

The activity of catalase was determined by M. O. Koroliuk and L.I. Ivanova method, based on the ability of hydrogen peroxide to form a stable orange complex with molybdenum salts. The intensity of the staining of the substrate-buffer mixture depends on the concentration of H2Cl which is in the solution, that is, inversely from the activity of catalase [7].

Antioxidant-prooxidant index (API). In a normal tissue, the balance between prooxidant systems (activity of oxygenase, xanthine oxidase, NADPH-2 oxidase, etc.) and antioxidant systems (activity of superoxide dismutase, catalase, glutathione peroxidase, tocopherol, glutathione, taurine, ascorbic acid, etc.) is always maintained. During the inflammatory process, this balance is disrupted in the direction of increasing the level of prooxidant factors. API clearly responds to the change in the status of antioxidant-prooxidant systems. The API index is the ratio of the antioxidant system index – the activity of catalase, to the prooxidant system index – MDA concentration [7].

To test lysozyme in the oral fluid, the bacteriolytic method by Horin in A.P. Levytsky modification was used. The principle of the method is based on the ability of lysozyme to dissolve a number of bacteria and, in particular, the cells of Micrococcus Iysodeikticus (standard strain 2665) [7].

For the quantitative assessment of microorganisms in the oral cavity, the index of activity of the urease enzyme, which is not produced by somatic cells, but is synthesized by a number of opportunistic and pathogenic microorganisms was determined. Test on urease activity was carried out by L. M. Havrilova and I. T. Sehen method with Nessler's reagent. The method is based on the ability of the urease in the oral fluid to split urea into ammonia, which gives a yellow color to the Nessler's reagent. The intensity of the sample color is directly proportional to the activity of urease in the oral fluid [7].

Dysbiosis stage (DS) was assessed by determining the ratio of relative activities of salivary enzymes – urease and lysozyme according to A.P. Levitsky. Because due to the evaluation of specific enzyme activities that are absent in the cells of macroorganism, but are produced by microorganisms, it is possible to analyze the specific features of the microflora and its amount [8].

Calculation of DS was carried out according to the formula:

$U_{rel.} / L_{rel.}$ = DS where DS is dysbiosis stage, U rel. is relative activity of urease and L relative is relative activity of lysozyme.

Normally, in healthy individuals, this coefficient is equal to one. When the microbiocenosis of the oral cavity is disturbed and the dysbiosis develops in patients, this index

Table I. The effect of the mucosal gel with probiotic on the level of inflammatory markers, the activity of urease, catalase, lysozyme, and the API index in the oral fluid of patients with crowded teeth during the orthodontic treatment

Groups	Duration of the Study	MDA, mmol/l	Elastase, mkat/l	Urease, mkat/l	Lysozyme, u/l	Catalase, mkat/l	API, units
Control Group, n=15		0,21±0,02	0,27±0,03	0,18±0,03	72±11	0,18±0,02	8,6±0,08
Comparison Group n=15	before the treatment	0,37±0,04 p<0,01	0,42±0,04 p<0,05	0,49±0,07 p<0,01	60±9 p>0,3	0,12±0,02 p<0,05	3,2±0,4 p<0,01
	in 2 weeks	0,46±0,07 p<0,01 p_1>0,1	0,49±0,05 p<0,01 p_1>0,3	0,43±0,06 p<0,01 p_1>0,3	63±8 p>0,3 p_1>0,1	0,14±0,02 p>0,1 p_1>0,3	3,0±0,3 p<0,01 p_1>0,5
	in 2 months	0,40±0,05 p<0,01 p_1>0,3	0,42±0,04 p<0,05 p_1=1,0	0,40±0,06 p<0,05 p_1>0,3	68±7 p>0,5 p_1>0,3	0,15±0,02 p>0,3 p_1>0,3	3,7±0,4 p<0,01 p_1>0,3
Main Group n=15	before the treatment	0,38±0,03 p<0,01 p_2 > 0,05	0,44±0,04 p<0,05 p_2 > 0,05	0,50±0,07 p<0,01 p_2 > 0,05	57±8 p>0,1 p_2 > 0,05	0,11±0,02 p<0,05 p_2 > 0,05	2,9±0,3 p<0,01 p_2 > 0,05
	in 2 weeks	0,30±0,03 p<0,05 p_1>0,05 p_2 < 0,05	0,33±0,03 p>0,1 p_1<0,05 p_2 < 0,05	0,29±0,04 p<0,05 p_1<0,05 p_2 < 0,05	74±8 p>0,8 p_1>0,05 p_2 < 0,05	0,17±0,03 p>0,6 p_1>0,05 p_2 < 0,05	5,7±0,5 p<0,05 p_1<0,05 p_2 < 0,05
	in 2 months	0,24±0,03 p>0,3 p_1<0,05 p_2<0,05	0,30±0,03 p>0,3 p_1<0,05 p_2<0,05	0,23±0,03 p>0,3 p_1<0,01 p_2<0,05	85±7 p>0,3 p_1<0,05 p_2>0,05	0,21±0,03 p>0,3 p_1<0,05 p_2>0,05	8,7±0,8 p>0,8 p_1<0,01 p_2<0,01

Note: p is an index of reliability, calculated relative to the control group;
p1 – in relation to the index "before the treatment";
p2 – in relation to the comparison group.

is more than 1, and the more pronounced the degree of dysbiosis is, the higher is this index. Namely: from 1.5 to 3.0 – subclinical compensated form of oral dysbiosis, from 3.0 to 8.0 – clinical subcompensated form and from 8.0 to 20.0 – clinical decompensated form of dysbiosis stage.

The statistical processing of the results of the study was carried out using methods of variation statistics using the EXCEL program (the standard package of Microsoft Office). The average values of the variables, standard deviations, confidence intervals of reliability according to the parametric criteria using the Student's t-test were determined.

RESULTS AND DISCUSSION

The dynamics of changes in biochemical parameters of oral fluid in patients of study groups with crowded teeth during the orthodontic treatment is presented in Table I.

In the main group that received the application of the developed gel, the level of markers of inflammation in the oral fluid decreased in 2 weeks. Thus, the MDA level was 1.27 times less than the index before the fixation (p> 0.05), and 1.5 times less than the data obtained in the comparison group (p <0.05). After 2 months, the MDA level in the main group significantly decreased (p <0.05), both in comparison with the baseline data and with the main group.

Activity of elastase in the patients of the main group decreased throughout the study period: by 25% after 2 weeks, by 31.8% after 2 months (p <0.05), and at the end of the observation it was equal to the indices of the control group. In the comparison group, the activity of elastase after 2 weeks increased by 16.7% (p <0.05), and after 2 months it decreased to the indices that were observed before the treatment.

Before the treatment, the activity of urease was increased by 2.7 times (p <0.01) and lysozyme activity was reduced by 1.2 times in patients of both groups. In patients of the main group, urease activity significantly decreased after 2 weeks (1.7 times) (p <0.05), and after 2 months it did not differ from the norm. The activity of urease in the oral fluid of patients in the comparison group is also reducing, but still remains above the norm (2.3 times). In the process of orthodontic treatment, the activity of lysozyme increases, however it is only significant in patients of the main group (1.5 times) (p <0.05), which used the developed gel.

The results of the test of catalase activity and the API index showed that the use of the developed gel significantly increases antioxidant indices, and in 2 months it practically normalizes them. Thus, the catalase level in the main group increased by 1.5 times in 2 weeks, and by 1.9 times in 2 months in relation to the baseline values. As for the comparison group it increased slightly within 2 months.

Figure 1 presents data on the comparative characteristics of the level of oral dysbiosis among the study groups at the stages of orthodontic treatment with a bracket system. The dysbiosis stage is statistically reduced in the comparison group only after 2 months, and in the main group – after

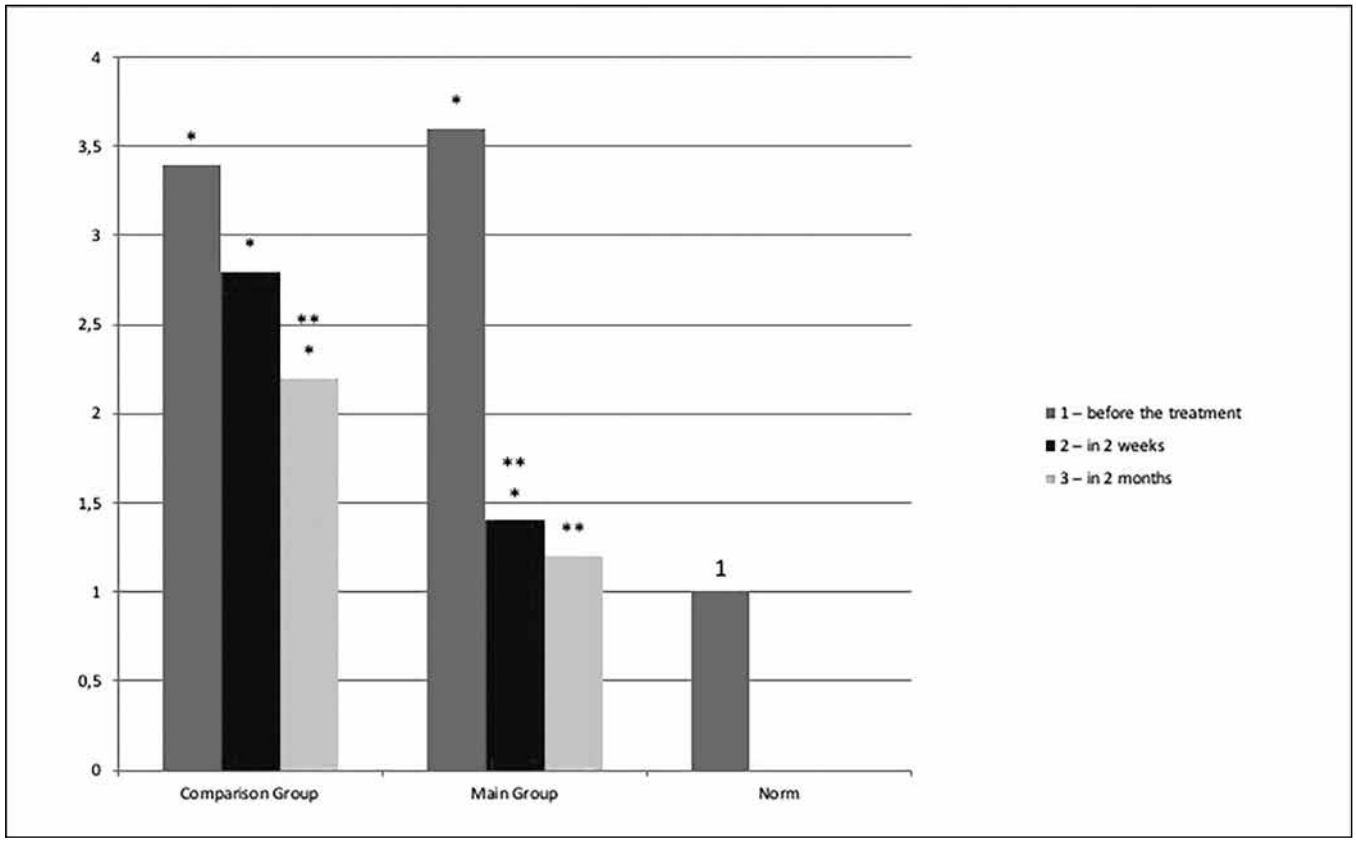

Fig. 1. The effect of the developed gel on dysbiosis stage in the oral fluid of patients with crowded teeth in the process of orthodontic treatment (1 – before the treatment, 2 – in 2 weeks, 3 – in 2 months): * – p <0,05 in comparison with the norm; ** – p <0,05 in comparison with the index before the treatment.

2 weeks. In the main group, the biotic processes are completely stabilized to the normal values after 2 months.

The results of the biochemical study of the oral fluid in patients with crowded teeth position revealed the presence of inflammatory markers in the oral cavity, as indicated by the data in Table 1 in patients before fixation of bracket system in comparison with the control group. Although the observation group did not include patients with severe pathology of the periodontal and mucous membrane tissue. Thus, the activity of elastase in patients with dentofacial anomalies increased to 0.43 ± 0.04 μkat / l (1.6 times, p <0.05), there was a significant increase in the MDA content (1.8 times) and the tendency to a decrease in the activity of catalase (by 33.3%) in individuals with crowded teeth position, which in turn led to a significant decrease in the API index by 2.8 times, indicating a decrease in the antioxidant potential of the oral cavity and the intensification of peroxide lipid oxidation in patients with crowded teeth position. Urease activity, which indirectly reflects the microbial contamination of the oral cavity, was significantly increased in the patients of the main group (2.7 times), indicating imbalance of the microflora towards dysbiosis. The activity of lysozyme, on the contrary, was significantly reduced (by 18%), which indicates a decrease in nonspecific resistance of the oral cavity in people with crowded teeth position. The degree of dysbiosis testifies to the increase in the microbial contamination of the oral cavity of the patients of the main group, which is confirmed by its reliable growth (3.4 times) in patients with crowded teeth position.

The obtained data on the presence of inflammatory markers and dysbiotic disorder in patients with crowded teeth necessitates the influence of the pathogenesis of periodontal inflammatory diseases on this link and substantiates the use of probiotic agents for correcting the oral microbiocenosis.

Subsequent observations of the dynamic pattern in biochemical parameters after fixation of bracket system confirmed studies by a number of scientists that orthodontic treatment additionally leads to activation of the inflammatory process in periodontal tissues. First of all, these processes occur on the basis of reducing the level of oral hygiene and the presence of a stressful situation in the oral cavity, especially during the early stages of treatment.

Studies have shown that the developed mucosal gel has a therapeutic and prophylactic effect in relation to the gums within the conditions of dysbiosis, normalizing the effect on the biochemical parameters of the pathological condition of the oral cavity. Gel application significantly reduces the activity of urease and it significantly reduces (almost 6 times) the dysbiosis stage. It is important to emphasize that the protective effect of the gel is carried out during its local application on the oral mucosa in significantly lower doses compared with oral administration of the drug. Considering the properties of a normal indigenous microflora (antimicrobial, biosynthetic, trophic, regulatory, detoxification), the use of probiotic flora

for the prevention of dental diseases will be appropriate, since probiotic microorganisms are able to optimize the physico-chemical and biological characteristics of the ecosystem.

CONCLUSIONS

The data suggest that the use of mucosal gel with probiotic as a medical support for orthodontic treatment improves oral hygiene, reduces inflammatory process in the periodontium, increases antioxidant defence, improves nonspecific resistance and normalizes microbiocenosis in the oral cavity.

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