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Features of Microbiocenosis and Production of Hydrogen Peroxide by Gum Symbionts in Adolescents with Catarrhal Gingivitis and Chronic Gastroduodenitis

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SUMMARY

Introduction. The key links in the etiology and pathogenesis of periodontal tissue diseases are the quantitative and qualitative changes in the composition of the microflora of the oral cavity, with the simultaneous deterioration of oral hygiene, and reduction of local and general immunity, which occurs more often in the presence of somatic diseases.

Aims. The aim of the paper was to study the features of the microbiocenosis of periodontal tissues and the production of hydrogen peroxide by gum symbionts in adolescents with catarrhal gingivitis and chronic gastroduodenitis.

Methods. The condition of the microbiocenosis of the gums of 83 adolescents from 12 to 18 years, which was divided into groups depending on the diagnosed catarrhal gingivitis and chronic gastroduodenitis, was studied. Bacteriological examination was performed to isolate pure cultures of microorganisms and to identify them according to generally accepted microbiological methods. The ability of the selected cultures to produce hydrogen peroxide was studied on an indicator medium with potassium-iodine-starch system, by the iodometric method.

Results. The results of microbiological studies showed significant changes in qualitative and quantitative indicators of the microbiocenosis of the gingival mucosa in the affected area of patients with gingivitis, compared with dentally and somatically healthy individuals in the control group. In the group of clinically healthy adolescents, hydrogen peroxide producers were found on the mucous membrane of the gums only in 5.0 ± 1.15% of the examinees. In catarrhal gingivitis, hydrogen peroxide producers were found in 52.4 ± 2.4 % of the examined main group subjects ($p < 0.01$) and in the 50.0 ± 2.5 % of the examined comparison group subjects ($p < 0.01$).

Conclusion. Among adolescents with catarrhal gingivitis, which occurs on the background of chronic gastroduodenitis, there were more pronounced quantitative and qualitative changes in the microbiocenosis of the gums. The hydrogen peroxide produced by them can act as an additional damaging factor in the pathogenesis of the inflammatory process of the gingival area.

Keywords: **gingivitis, gastroduodenitis, adolescents, microbiocenosis, hydrogen peroxide**

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INTRODUCTION

Periodontal disease in both children and adults remains an urgent problem of modern dentistry. The prevalence of this pathology among children and adolescents varies widely - from 60% to 99%, which indicates a high level of the disease among these age groups. According to the WHO, 80% of 12-year-old children and almost 100% of children at the age of 14 - 15 years have chronic gingivitis, which often in the early stages of the disease has hidden symptoms. This complicates the timely diagnosis of the disease and without opportune treatment and prevention measures, a risk group for the development of destructive processes in the future is thus formed (1 - 4).

According to the modern concept, the development of periodontal diseases is closely related to the microflora of the oral cavity. The key links in the etiology and pathogenesis of the disease such as a decrease in the amount of normal flora, an increase in opportunistic pathogens, excessive contamination and infection with periodontal pathogens with a simultaneous deterioration of oral hygiene, reduction of local and general immunity, somatic diseases are the main links in the etiology and pathogenesis of the disease (5 - 10). Nowadays, the normal flora of the human body is considered as a set of microbiocenoses which are part of a single system which performs the most important functions in the body; the main among them is the creation of the front line of non-specific protection of the microorganism (11, 12). Disturbance of the balance between the microbiocenosis of the oral cavity leads to a decrease in the number of obligate microorganisms, on the one hand, and an increase in opportunistic and pathogenic bacteria, on the other, which becomes a leading factor in the development of inflammation in periodontal tissues (8, 13).

Pathological processes in the periodontium are especially common among children and adolescents with general somatic diseases, particularly among people with diseases of the gastrointestinal tract (14 - 20). Diseases of the gastrointestinal tract are one of the most common causes and tend to increase with age. In the structure of pathology of the gastroduodenal zone in childhood, the first place is occupied by chronic gastroduodenitis (60 - 70%). According to pediatric gastroenterologists, every third child is diagnosed with this disease. Taking into consideration the fact that chronic gastroduodenitis is most often diagnosed during the adolescence, the study of the intensity and prevalence of periodontal disease and the peculiarities of the microbiocenosis of periodontal tissues in adolescents with this pathology is relevant (21, 22).

It is known that in the development of dysbiotic disorders of the body, preference is given to opportunistic pathogens, among which are clones with drug resistance and genetic determinants that stipulate the virulence and pathogenicity of bacteria (23, 24), but it is known that at the stage of primary inflammation streptococci (representatives of the normal flora play an important role in the development of the pathological process, for example, fixation of *P. gingivalis* and *P. intermedia* on the surface of the gums occurs after the appearance of *Streptococcus mitis* and *Streptococcus sanguis* in these areas, which promote the attachment of periodontopathogenic microflora, forming an intermediate layer between them and the outer membrane of epithelial cells (25 - 27).

The negative impact of microorganisms is especially noticeable in case of reduced protective capacity of the organism; namely, the imbalance between the protective properties of the organism and the growth of persistent potential of opportunistic and pathogenic microflora leads to pathological changes in the periodontium. It is proved that the presence of persistence factors in microorganisms can contribute to the long-term development of the disease. It was found that one of the markers of persistence are the activated forms of oxygen, primarily hydrogen peroxide, which affect the ecological balance in the oral cavity and the development of inflammation of the gums (28, 29).

AIMS

The aim of the study was to study the features of the microbiocenosis of periodontal tissues and the production of hydrogen peroxide by gum symbionts in adolescents with catarrhal gingivitis and chronic gastroduodenitis.

PATIENTS AND METHODS

To achieve this goal, the state of the microbiocenosis of the gums of 38 adolescents with catarrhal gingivitis and chronic gastroduodenitis from 12 to 18 years, who formed the main group, was studied. Verification of the diagnosis of chronic gastroduodenitis was carried out by doctors of the gastroenterology department of the Ivano-Frankivsk Regional Children's Clinical Hospital on the basis of current national and international agreements and recommendations (based on clinical and instrumental examination in dynamics, international classification of diseases). The comparison group included 25 adolescents of the same age, diagnosed with catarrhal gingivitis, who at the time of the examination did not complain of somatic health disorders and were not registered at the dispensary with related specialists. The control group included 20 somatically and dentally healthy adolescents. Patient groups were homogeneous in significant indicators and representative. Patients were divided into groups by randomization.

All manipulations with adolescents were carried out only after the parents had read and signed an informed consent for clinical trials, and with children older than 14 years in compliance with the basic provisions of GCP (1996), The Convention on Human Rights and Biomedicine) (from 04.04.1997), World Medical Association Declaration of Helsinki) on the ethical principles for medical research involving human subjects (1964 - 2013), orders of the Ministry of Health of Ukraine № 690 from 23. 09. 2009, № 616 from 03.08.2012. In accordance with the requirements of bioethics" On conducting laboratory tests of biological material", a written consent was obtained from the parents (guardians) of each child and adolescent for the study of biomaterial. The protocol of clinical and laboratory research was approved by the Ethics Commission of Ivano-Frankivsk National Medical University (Protocol № 83/15 dated 03. 06. 2015 and protocol № 106/19 dated 07. 02. 2019). The authors declared no conflict of interest. The study was conducted without the participation of pharmaceutical companies.

Material was collected for bacteriological examination to detect aerobic and facultative anaerobic microflora from the gingival sulcus on an empty stomach, before brushing the teeth, after their preliminary drying, using a calibrated bacteriological loop № 1. Microbiological culture test was performed immediately after the collection of material on blood agar base, salt egg yolk agar base, Endo and Saburo media, as well as on indicator medium with potassium-iodine-starch system (to detect producers of hydrogen peroxide). Tests were performed by Gold's method, which allows to quantify the level of microbial contamination (28, 30, 31). The plates were incubated for one day at 37°C under aerobic and anaerobic conditions (in a hermetically sealed desiccator in a CO_2-enriched atmosphere). Pure streptococcal cultures were isolated on 5% blood agar.

Bacteriological examination was performed to isolate pure cultures of microorganisms and to identify them, according to generally accepted microbiological methods based on morphological, tinctorial, cultural and biochemical properties of bacteria according to Bergey ("Bergey's manual of systematic bacteriology") (32). Identification of isolated pure cultures was performed by a set of morphological, cultural and biochemical properties (set STREPTO test 16, STAPHY test 16, Erba Lachem 9a, Czech Republic).

The bacteriological study took into account the presence of the following microorganisms in the culture tests: 1) α-hemolytic *Streptococcus* sp., 2) β-hemolytic *Streptococcus pyogenes*, 3) *Staphylococcus aureus*, 4) *Staphylococcus epidermidis*, 5) *Stomatocose mucilaginosus*, 6) *Neisseria* sp., 7) *Micrococcus* sp., 8) *Corynebacterium* sp., 9) enterobacteria (*Escherichia coli*, etc.), 10) yeast-like fungi *Candida* sp.

Quantitative evaluation of colonies was carried out taking into consideration their species (or genus) affiliation. The results of quantitative study of the microflora were expressed in colony-forming units in terms of 1,0 ml - CFU/ml, taking into account only those microorganisms the concentration of which in the pathological material was not less than 1 × 103 CFU/ml. Based on the analysis of seeding results for microorganisms of each group, the population level (PL, which was expressed in lg CFU/ml) and the constancy index (CI) were determined (31).

To obtain isolated colonies of bacteria under aerobic conditions, 5% blood agar, 10% bile salt agar and Endo medium were used. Pure cultures of anaerobic microorganisms were obtained using 5% blood agar with hemin.

The ability of isolated cultures to produce hydrogen peroxide was studied on an indicator medium with potassium-iodine-starch system (28, 30).

The concentration of produced hydrogen peroxide was determined by iodometric method. The intensity of production was estimated by a semi-quantitative method based on the appearance of purple color on the indicator medium, which was expressed in conventional units:

1. Pale purple color of the colonies and no color of the surrounding agar;

2. Violet color of the colonies with colored agar zone around colonies up to 1 mm;

3. Dark purple color of the colonies and surrounding agar with a diameter > 1 mm.

In order to assess the hygienic condition of the oral cavity, all subjects were evaluated by the Oral Hygiene Index of Green-Vermillion (Oral Hygiene Index-Simlified, Green-Vermillion, 1964) (OHI-S), which allowed us to detect not only plaque but also tartar.

Computer programs based on Microsoft Excel were used for statistical processing of the material at all stages of the study, where the materials were grouped according to the study contingent (calculation of relative and average values, their level of accuracy, t-test). Some of the data development tasks were performed using licensed packages for statistical analysis in Microsoft Excel and Statistica 12.0, for example, programs based on descriptive statistics, pair and multiple correlation-regression analysis and graphical representation. The reliability of the obtained indicators was confirmed by calculating the error (± m) for relative values according to the well-known formula. The probability of data difference in the compared groups, taking into consideration the large number of observations and the proximity to the normal distribution, was proved on the basis of calculating the Student's t-distribution and was determined according to the table of accuracy of an error-free forecast (p); the results were considered reliable at $p < 0,05$ (33 - 36).

RESULTS

The results of microbiological studies showed significant changes in the qualitative and quantitative indicators of the microbiocenosis of the gingival mucosa in the affected area among patients with gingivitis, compared with dentally and somatically healthy individuals from the control group (Table 1).

Representatives of the resident microflora of the oral cavity - α-hemolytic streptococci were found in all patients of the main group and the comparison group without exceptions, as well as in all healthy adolescents of the control group (CI 100,0 %). However, adolescents in the main group had a significantly higher level of colonization of the gingival mucosa by α-hemolytic streptococci than adolescents of the comparison group ($p < 0,01$) and control group ($p < 0,05$).

The vast majority of cultures of α-hemolytic streptococci from dentally healthy individuals have been identified as *Streptococcus salivarius*, *Streptococcus mitis* and *Streptococcus oralis*. With catarrhal gingivitis on the surface of the affected gingival mucosa in 68,4 ± 3,32% of patients in the main and 64,0 ±

Table 1. *Characteristics of oral microbiocenosis in the area of inflammation of the gingival mucosa (n = 83)*

Groups of microorganisms	Main group (n = 38)		Comparison group (n = 25)		Control group (n = 20)	
	PR	CI	PR	CI	PR	CI
α-hemolytic *Streptococcus* sp.	6.35 ± 0.11*/†	100.0	5.02 ± 0.21	100.0	4.74 ± 0.30	10.00
β-hemolytic *Streptococcus pyogenes*	5.38 ± 0.20*/†	26.3 ± 3.15*/†	4.18 ± 0.07*	16.0 ± 2.62*	3.00 ± 0.03	5.0 ± 1.56
Staphylococcus aureus	3.67 ± 0.11*	18.4 ± 2.77*	3.94 ± 0.18*	20.0 ± 2.86*	0	0
Staphylococcus epidermidis	4.46 ± 0.20*/†	52.6 ± 3.57*	3.64 ± 0.15	48.0 ± 3.57*	3.78 ± 0.20	30.0 ± 3.27
Stomatococcus mucilaginosus	4.81 ± 0.23*/†	50.0 ± 3.57	4.31 ± 0.15*	52.0 ± 3.56	3.39 ± 0.08	45.0 ± 2.44
Neisseria sp.	3.82 ± 0.15	18.4 ± 2.76	4.02 ± 0.19*	20.0 ± 2.86*	3.57 ± 0.22	15.0 ± 2.55
Micrococcus luteus	3.85 ± 0.03*	5.2 ± 1.59	4.00 ± 0.03*	4.0 ± 1.40	0	0
Corynebacterium sp.	4.07 ± 0.12*	15.8 ± 2.60	3.76 ± 0.13*	20.0 ± 2.86*	3.00 ± 0.03	10.0 ± 2.14
Candida sp.	4.23 ± 0.16*/†	23.7 ± 3.03*/†	3.50 ± 0.14*	8.0 ± 1.94*	0	0

CI- constancy index (%), PL - population level (lg CFU/ml).

*- $p < 0,05$ when compared with the control; † - when compared with the comparison group

3,43 % of patients in the comparison group, the dominant species were *Streptococcus gordonii*, *Streptococcus sanguinis*, *Streptococcus constellatus*, *Streptococcus anginos*.

The frequency of seeding and the overall population level of the main representatives of the pathogenic coccal microflora - *Staphylococcus aureus* and β-hemolytic streptococci (*Streptococcus pyogenes* and Streptococcus group G) among patients of the main group were significantly higher than of the comparison group ($p < 0.05$). In somatically healthy adolescents, β-hemolytic Streptococcus on the gingival mucosa was found in a single case (with minimal colonization), and *Staphylococcus aureus* was completely absent. In adolescents with catarrhal gingivitis, especially with chronic gastroduodenitis, there was also an increase in the levels of colonization of the gingival mucosa in the affected area by such representatives of the transient oral microflora as *Epidermal staphylococcus*, *Stomatococcus* and *Corynebacteria* (*diphtheroids*). In addition, on the mucous membrane of the gums in 23.7 ± 3.03% of patients in the main group and in 8.0 ± 1.94% of patients in the comparison group the presence of yeast-like fungi of the genus *Candida* was found; the volume of colonization was 4.23 ± 0.16 CFU/ml and 3.50 ± 0.14 CFU/ml, respectively. In none of the adolescents from the control group, yeast-like fungi in the amount of ≥ 3.0 CFU/ml (which is the limit of sensitivity of the applied research method) were found.

Depending on the severity of catarrhal gingivitis, the nature of the gum microflora of patients of both groups was also analyzed. Patients in the main group with catarrhal gingivitis of moderate severity have significantly higher levels of colonization of the gingival mucosa with β-hemolytic streptococci, *Staphylococcus aureus* and *Stomatococcus* ($p < 0.05$) than those with mild catarrhal gingivitis. At the same time, there is an inverse relationship between the severity of catarrhal gingivitis and the massiveness of colonization of the mucous membrane by *Neisseria* and *Corynebacteria*. Adolescents in the comparison group with catarrhal gingivitis of moderate severity had significantly higher values of PL of *Staphylococcus aureus*, *Stomatococcus* and yeast-like fungi of the genus *Candida*, compared with adolescents with catarrhal gingivitis of mild severity ($p < 0.05$). In both the main group and the comparison group, and in patients with mild and with moderate catarrhal gingivitis, the massiveness of colonization of the gingival mucosa by β-hemolytic streptococci, *Staphylococcus aureus* and fungi of the genus *Candida* was significantly higher than in adolescents of the control group.

In a complex set of relationships between different types of bacteria that colonize the mucous membrane of the oral cavity and gums, as well as in the interaction of individual microflora with the macroorganism, an important role belongs to the activated forms of oxygen, especially hydrogen peroxide. Therefore, one of the tasks was to study hydrogen peroxide production activity of microorganisms isolated from the mucous membrane of the gums among adolescents with catarrhal gingivitis and chronic gastroduodenitis.

It was discovered that in the group of somatically healthy adolescents without periodontal disease, hydrogen peroxide producers were found on the mucous membrane of the gums quite rarely - only in 5,0 ± 1,15% of subjects. In catarrhal gingivitis, hydrogen peroxide producers were found in 52.4 ± 2.4% of the examined main group subjects ($p < 0.01$) and in 50.0 ± 2.5% of the examined comparison group subjects ($p < 0.01$).

The analysis of the obtained data among all subjects, depending on the severity of the disease showed that the intensity of hydrogen peroxide production by streptococci isolated from the gingival area of adolescents with catarrhal gingivitis of moderate severity was higher – 1.90 ± 0.09 relative units than those isolated from adolescents with catarrhal gingivitis of mild severity – 0.61 ± 0.17 relative units ($p < 0.05$).

Taking into account both the severity and the presence of somatic disease, it was found that in adolescents of the main group there was a sharp increase in the frequency of excretion from the gingival area of hydrogen peroxide producers as well as the increase in the average intensity of hydrogen peroxide production by oral streptococci contrasted to the adolescents of the comparison group (Table 2).

Thus, the frequency of excretion of hydrogen peroxide producing bacteria in adolescents of the main group with the catarrhal gingivitis of mild severity was 46.1 ± 2.62%, which was 1.2 times higher than in adolescents of the comparison group – 38.5 ± 2.56%, ($p < 0.05$). With the catarrhal gingivitis of moderate severity, the frequency of excretion of hydrogen peroxide producing bacteria in adolescents of the main group was 85.0 ± 1.88%, which was 1.1

Table 2. *Production of hydrogen peroxide (H_2O_2) by microorganisms of the gums of patients with catarrhal gingivitis (CG) of varying severity (n = 83)*

H_2O_2 production by gum microorganisms	Main group		Comparison group		Control group
	CG easy degree	CG medium degree	CG easy degree	CG medium degree	
Frequency of H_2O_2 producers %	46.1 ± 2.62*	85.0 ± 1.88*/†	38.5 ± 2.56*	78.1 ± 2.18*/†	5.0 ± 1.15
Intensity of H_2O_2 production by streptococci, um. from	0.50 ± 0.15*	2.40 ± 0.09*	0.83 ± 0.20*	1.15 ± 0.24*	0.15 ± 0.10

*- $p < 0.05$ in comparison with the control; † - in comparison with patients with easy and medium degree of CG in the respective groups

times higher than in adolescents of the comparison group - (78.1 ± 2.18) % ($p < 0.05$).

The main producers of hydrogen peroxide among the representatives of the aerobic and facultative-anaerobic microflora of the gingival sulcus of adolescents with catarrhal gingivitis were α- and β-hemolytic streptococci – 96.4 ± 0.45% of all positive cultures. The ability to produce hydrogen peroxide was detected in 50.9 ± 0.52 % of the total number of tested cultures of α-hemolytic and in all cultures of β-hemolytic streptococci without exception. β –hemolytic streptococci are characterized by high intensity of hydrogen peroxide production - on average 2.0 ± 0.32 relative units.

Various types of α-hemolytic streptococci from gingival microbiocenoses of patients with catarrhal gingivitis differ significantly in their ability to produce hydrogen peroxide. Among the strains of *S. salivarius*, *S. mitis* and *S. oralis*, which are more inherent in normal microbiocenoses of the oral mucosa, producers of hydrogen peroxide were found infrequently - in 16.7 ± 1.96%, 25.0 ± 2.27% and 40.0 ± 2.58% of cases, respectively. The intensity of hydrogen peroxide production by these cultures was minimal - the average level was in the range of relative units (0.25 – 0.6).

Strains of *S. gordonii*, *S. sanguinis*, *S. constellatus* and *S. anginosus*, which were present on the surface of the affected gingival mucosa in 64.0% – 68.4% of patients with catarrhal gingivitis and only in 5.0% ± 1.15% of healthy individuals had the ability to produce hydrogen peroxide much more often. This feature is a special property of cultures of *S. gordonii*, *S. sanguinis, S. constellatus* - with the frequency of 78.6% ± 2.15%, 66.7% ± 2.48% and 66.7% ± 2.48%, respectively. *S. gordonii* (1.93 ± 0.38 relative units), *S. sanguinis* (1.53 ± 0.36 relative units) and *S. constellatus* (1.33 ± 0.32 relative units) were characterized by the highest intensity of hydrogen peroxide production. In this context, the fact that these types of α-hemolytic streptococci are characterized by increased pathogenic potential deserves attention.

Analysis of oral hygiene in the surveyed adolescents showed that the structure of the OHI-S index in the study groups showed the worst performance in adolescents of the main group and was as follows: in adolescents of the main group, unsatisfactory oral hygiene was found according to the OHI-S index (1.74 ± 0.01) points. The state of oral hygiene in the examined comparison groups was satisfactory, which was confirmed by the value of the OHI-S index - (1.32 ± 0.03) points. In adolescents of the control group, the state of oral hygiene according to the OHI-S index was good and was equal to 0.35 ± 0.02 points ($p < 0.001$).

Analysis of the indicators of the hygienic index depending on the severity of catarrhal gingivitis in adolescents with chronic gastroduodenitis and without somatic pathology showed an increase in values relative to the presence of somatic pathology. On average, in adolescents of the main group with mild catarrhal gingivitis, the OHI-S index with the value 1.53 ± 0.01 was 1.2 times higher than the corresponding values in the comparison group (1,26 ± 0.03) score ($p < 0.001$). In catarrhal gingivitis of moderate severity, in the examined main group, the OHI-S index was 1.3 times higher, relative to the data obtained in the comparison group, 1.94 ± 0.02 points, against 1.45 ± 0.01 score ($p < 0.001$). The analysis of the obtained results of the level of oral hygiene in the

examined adolescents did not reveal persons with poor and very poor condition. Unsatisfactory oral hygiene prevailed in the adolescents of the main group, and satisfactory oral hygiene prevailed in the adolescents of the comparison group. However, in adolescents with a healthy periodontium and without somatic pathology, mostly, good oral hygiene was registered.

DISCUSSION

Numerous studies confirm that the leading role in the occurrence of periodontal tissue diseases belongs to plaque bacteria (gram-positive and gram-negative cocci, gram-positive and gram-negative rods, spirochetes, *Bacteroides melaninogenicus, Actinomyces viscosus, Actinomyces naesludii*) and their toxins, which destroy the periodontal ligament and cause inflammation of the gums. Some researchers suggest identifying the microflora based on the main role in the development of periodontal disease, as follows: pathogenic microorganisms that have been strongly associated with the development of periodontal disease (*Actinobacillus actinomycetemcomitans*, etc.) and pathogenic microorganisms that have been moderately associated with the development of periodontal disease (*Prevotella intermedia*, *Campilobacter rectus*) (8, 9, 11, 12, 25).

In addition, it is known that *Streptococci* (representatives of the normal flora) at the stage of primary gingivitis play an important role in the development of the pathological process, for example fixation of *P. gingivalis* and *P. intermedia* on the gum surface occurs only after the appearance of *Streptococcus mitis* and *Streptococcus sanguis*, which contribute to the attachment of periodontopathogenic microflora, forming an intermediate layer between them and the outer membrane of epithelial cells (11, 12, 25). It was found that with catarrhal gingivitis on the surface of the affected gingival mucosa in 68,4 ± 3,32% of patients in the main and 64,0 ± 3,43% of patients in the comparison group, the dominant species were *Streptococcus gordonii*, *Streptococcus sanguinis*, *Streptococcus constellatus*, and *Streptococcus anginosus*. These types of α-hemolytic streptococci are characterized by increased pathogenic potential. They have the ability to produce streptolysin-O, inhibit complement activation, bind to fibronectin, actively form biofilms on both enamel and gum epithelial surface, and can act as an initiator of adhesion of periodontal pathogens (8, 11).

Moreover, the normal flora exhibits antagonistic properties against pathogens. Biologically active compounds that synthesize bacteria normal floral take part in this process: organic acids, short-chain fatty acids, microbial lysozyme (muramidase), hydrogen peroxide and antibiotic-like peptide substances – microcins, bacteriocins, which posess a wide range of antibacterial activity.

It is known that the biological action of hydrogen peroxide is diverse. On the one hand, the production of hydrogen peroxide by microorganisms that form biofilms on the surface of the epithelium of the mucosa and early dental plaque, are factors that counteract the colonization of environmental micro-niches of the oral cavity by cariogenic *S. mutans* (29). On the other hand, hydrogen peroxide secreted by microorganisms may be involved in the development of the inflammatory process in the gum epithelium.

The bactericidal activity of hydrogen peroxide is associated with its high oxidizing ability, namely with the action of toxic products formed by lipid peroxidation. The biological activity of bacteria – producers of hydrogen peroxide, is provided by the functioning of a number of enzymes and products of biologically active metabolites (28, 30).

Another aspect of the effect of hydrogen peroxide on oral microorganisms has recently been identified. The hydrogen peroxide produced by *S. gordonii* can promote the release of DNA from streptococcal cells and facilitate the intraspecific transfer of antibiotic resistance genes (erythromycin and kanamycin resistance cassettes). Due to its mutagenic properties and ability to inhibit DNA repair processes, hydrogen peroxide can induce resistance of *S. gordonii* to rifampicin (23).

At the same time, experimental data unequivocally indicate the participation of hydrogen peroxide produced by oral streptococci which exhibits cytotoxic properties in the development of inflammation and inflammatory alteration of tissues. Due to the production of hydrogen peroxide, *S. oralis* and *S. sanguinis* cause the death of macrophages (37, 38), which develops due to destabilization of lysosomal membranes (26, 39), and also inhibit their protective functions by reducing the expression of proinflammatory cytokines TNF-α, IL-6 (24). The cytotoxic activity of hydrogen peroxide-producing strain *S. sanguinis* against neutrophils has also been demonstrated (19). *S. oralis* and *S. sanguinis* cause the death of epithelial cells of various origins: nasopharyngeal

Detroit 562, bronchial Calu-3, cervical HeLa (40). This effect is also mediated by the action of hydrogen peroxide because it does not appear when adding catalase to the experimental system. Closely related to oral streptococci of the mitis and sanguinis groups is *Streptococcus pneumoniae*, which shows with them the identity of the 16S rRNA sequence at the level of 99,5% (11). It also has the ability to produce hydrogen peroxide, thereby inducing apoptosis and death of neutrophils and alveolar epithelial cells of the lungs (19, 39).

Detected during the study in the group of somatically healthy adolescents without periodontal disease, producers of hydrogen peroxide on the mucous membrane of the gums was quite a rare phenomenon – detected only in 5,0 ± 1,15% of subjects. Among patients with catarrhal gingivitis, hydrogen peroxide producers were found in 52,4 ± 2,4% of the examined main group subjects ($p < 0,01$) and in 50,0 ± 2,5% of the examined comparison group subjects ($p < 0,01$). Therefore, the ability of microflora to intensively produce hydrogen peroxide can be considered as an additional factor of pathogenicity, along with the ability to form biofilms and act as an initiator of adhesion of periodontal pathogens, produce streptolysin-O, inhibit complement activation (11).

CONCLUSION

Among adolescents with catarrhal gingivitis, which occurs on the background of chronic gastroduodenitis, there are more pronounced quantitative and qualitative changes in the microbiocenosis of the gums. The obtained data may indicate high competitiveness of gingivitis pathogens in the formed oral microbiocenoses. In addition, the results of microbiological studies suggest that the most probable cause of inflammation of the gums may be the development of oral dysbiosis on the background of somatic pathology (chronic gastroduodenitis) combined with poor oral hygiene. The hydrogen peroxide produced by them can act as an additional damaging factor in the pathogenesis of the inflammatory process of the gingival area.

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Karakteristike mikrobiocenoze i produkcija hidrogen-peroksida od strane simbiotičkih organizama gingive kod adolescenata sa kataralnim gingivitisom i hroničnim gastroduodenitisom

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SAŽETAK

Cilj. Cilj rada bilo je ispitivanje karakteristika mikrobiocenoze parodontalnih tkiva i produkcije hidrogen- peroksida od strane simbiotičkih organizama gingive kod adolescenata sa kataralnim gingivitisom i hroničnim gastroduodenitisom.

Metode. Ispitivano je stanje mikrobiocenoze gingiva 83 odolescenta uzrasta od 12 do 18 godina, koji su bili podeljeni u dve grupe u zavisnosti od dijagnoze kataralnog gingivitisa i hroničnog gastroduodenitisa. Urađen je bakteriološki pregled, kako bi se izolovale čiste kulture mikroorganizama i uradila identifikacija prema opšteprihvaćenim mikrobiološkim metodama. Mogućnost odabranih kultura da proizvedu hidrogen-peroksid ispitivana je na indikovanom medijumu sa potasijum-jod-skrob sistemom pomoću jodometrijske metode.

Rezultati. Rezultati mikrobioloških studija pokazali su značajne promene u kvalitativnim i kvantitativnim indikatorima mikrobiocenoze mukoze gingive zahvaćene gingivitisom u poređenju sa ovim indikatorima kod dentalno i somatski zdravih osoba u kontrolnoj grupi.

Utvrđeno je to da su u grupi klinički zdravih adolescenata pronađeni mikroorganizmi koji su proizvodili hidrogen-peroksid na sluzokoži gingiva kod samo 5,0% ± 1,15% ispitanika. U slučaju kataralnog gingivitisa, organizmi koji su proizvodili hidrogen-peroksid pronađeni su kod 52,4% ± 2,4% ispitanika glavne grupe ($p < 0,01$) i kod 50,0% ± 2,5% ispitanika kontrolne grupe ($p < 0,01$).

Zaključak. Kod adolescenata sa kataralnim gingivitisom, koji se javlja u prisustvu već postojećeg hroničnog gastroduodentisa, zabeležene su izraženije kvantitativne i kvalitativne promene u mikrobiocenozi gingiva. Proizvedeni hidrogen-peroksid ponaša se kao dodatni faktor oštećenja u patogenezi inflamatornog procesa koji zahvata gingive.

Ključne reči: **gingivitis, gastroduodenitis, adolescenti, mikrobiocenoza, hidrogen peroksid**