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BIOCHEMICAL PARAMETERS OF ORAL FLUID OF CHILDREN WITH HYPERTROPHIC GINGIVITIS DURING DENTAL TREATMENT

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The study is dedicated to assessing the impact of the developed treatment-and-prophylactic complex on the biochemical parameters of the oral fluid of children with hypertrophic gingivitis. The study involved 30 children aged 13–16 years with a diagnosis of hypertrophic gingivitis. The conducted studies testify to the rather high efficiency of the proposed treatment-and-prophylactic therapy for children with hypertrophic gingivitis, which included a universal natural biological complex of drugs with a broad therapeutic and prophylactic effect. The developed therapeutic and prophylactic complex helped to normalize the biochemical parameters of oral fluid in children, improving antioxidant protection, reducing the intensity of inflammation, the degree of lipid peroxidation, the level of contamination of the oral cavity and increasing non-specific antimicrobial protection.

Key words: children, periodontal tissue diseases, dental disease, biochemical markers, oral fluid.

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БІОХІМІЧНІ ПОКАЗНИКИ РОТОВОЇ РІДИНИ ДІТЕЙ З ГІПЕРТРОФІЧНИМ ГІНГІВІТОМ У ПРОЦЕСІ СТОМАТОЛОГІЧНОГО ЛІКУВАННЯ

Дослідження присвячено проведенню оцінки впливу розробленого лікувально-профілактичного комплексу на біохімічні показники ротової рідини дітей з гіпертрофічним гінгівітом. В дослідженнях приймали участь 30 дітей віком 13–16 років з діагнозом гіпертрофічний гінгівіт. Проведені дослідження свідчать про достатньо високу ефективність запропонованої лікувально-профілактичної терапії для дітей з гіпертрофічним гінгівітом, яка включала універсальний природний біологічний комплекс препаратів з широкою лікувально-профілактичною дією. Розроблений лікувально-профілактичний комплекс сприяв у дітей нормалізації біохімічних показників ротової рідини, покращуючи антиоксидантний захист, зменшуючи інтенсивність запалення, ступінь перекисного оксислення ліпідів, рівня обсіменіння порожнини рота та підвищуючи неспецифічний антимікробний захист в ній.

Ключові слова: діти, захворювання тканини пародонту, стоматологічне захворювання, біохімічні маркери, ротова рідина.

The work is a fragment of the research project "Correction of pathogenetic mechanisms of disorders of carbohydrate and lipid metabolism in the body and tissues of the oral cavity in patients depending on environmental and nutritional factors affecting carbohydrate and lipid metabolism", state registration No. 0118U006966.

Periodontal diseases are the most common inflammatory processes in the human population [8, 11, 12]. In one form or another, they occur in more than half, and, according to some estimates, in more than 80 % of the adult population of the planet [13]. General risk factors for periodontal disease can be divided into hereditary and acquired (including socio-economic factors, poor oral hygiene, cigarette smoking and diabetes) [7]. Gingivitis, the initial, most common and reversible form of the disease, is characterized by

inflammation of the gums, manifested by edema, redness of the mucous membrane and bleeding at the slightest mechanical impact (eating, brushing teeth), without compromising the integrity of the gingival junction. The anatomy of the teeth and roots, the density of the teeth in the mouth, previous dental therapy, mouth breathing, dental caries and other dental factors may affect the intensity and nature of clinical symptoms [15]. At the same time, gingivitis is widespread among adults, children and adolescents. According to the etiology of the disease, in addition to gingivitis caused by dental plaque, there are infectious, food, hormonal and medicinal gingivitis [15].

Hypertrophic gingivitis (HG) is accompanied by hyperplastic processes in the form of reactive growth of fibrous elements of the connective tissue base and basal cells of the gum epithelium. The immediate causes of hypertrophic gingivitis can be local factors such as occlusal and individual tooth abnormalities, unsatisfactory orthopedic structures, improperly installed fillings, dental plaque, poor hygiene when wearing orthodontic appliances, and common factors: diseases of the nervous system, endocrine system leukemic reticulosis), medication, vitamin C deficiency, hormonal changes in the body [1, 2, 5, 10]. Therefore, the development of a special complex for the treatment of children with such pathology is an urgent task of modern dentistry.

The purpose of the study was to assess the impact of the developed treatment and prevention complex on the biochemical parameters of oral fluid in children with hypertrophic gingivitis.

Materials and methods. The study involved 30 children aged 13–16 years with a diagnosis of hypertrophic gingivitis (15 people in the main group and 15 in the comparison group). Children of the main group, in addition to basic therapy, received twice a year developed treatment and prevention complex (TPC), which included drugs “Phenigidine” (FC Zdorovya, Ukraine), “Microcirculin with Lecithin”, TM (Biola, Ukraine) and “Phytor” (PJSC Phytoria, Ukraine). The comparison group received only basic therapy – oral sanitation and professional hygiene. In the oral fluid of the studied children, the activity of lysozyme, catalase, elastase, urease and the content of malondialdehyde were studied [3, 4].

The principle of the method of determining the activity of lysozyme in oral fluid was performed by bacteriolytic method based on the ability of lysozyme to lyse bacterial membranes. When lysozyme interacts with the substrate of Micrococcus lysodeikticus, the substrate is clarified, which is recorded spectrophotometrically. The degree of clarification is proportional to the activity of lysozyme, which was expressed in units/kg of homogenate.

The principle of the method for determining catalase activity is based on the ability of residual hydrogen peroxide to form a stable colored complex with molybdenum salts [3]. Color intensity is inversely proportional to catalase activity expressed in millicatals/kg.

Elastase activity was assessed by the degree of hydrolysis of the synthetic substrate N-t-BOC-L-alanine-p-nitrophenyl ester (BOC) (Sigma, USA) by the Visser method. Under the action of elastase in the oral fluid or tissue, yellow p-nitrophenol is cleaved from the substrate, the intensity of which is proportional to the activity of elastase [4].

The method for assessing the activity of urease in oral fluid is based on the ability of urease to break down urea to ammonia, which, under the action of Nesler's reagent, gives a yellow color. The color intensity of the sample is directly proportional to the activity of urease in the homogenate [3].

The method for determining the content of malondialdehyde is that at high temperature in an acidic environment, malondialdehyde reacts with 2-thiobarbituric acid, forming a colored trimethyl complex, with an absorption maximum at 532 nm [3].

The results were processed by variational statistical methods of analysis using the Microsoft Office Excel 2016 software. Statistical processing of the experimental study results was carried out by the methods of variation analysis using the Student's test. The difference was considered statistically significant at $p<0.01$.

Results of the study and their discussion. Lysozyme is a major factor in nonspecific antimicrobial protection in the oral cavity and is an enzyme that destroys bacteria and viruses, as well as activates phagocytic leukocytes and immunoglobulins. It is known that the activity of this antimicrobial enzyme in oral fluid correlates with the level of nonspecific and specific antimicrobial factors. Due to the decrease in the level or activity of lysozyme in the oral cavity there is increased growth and reproduction of opportunistic and pathogenic microflora. Table 1 shows that in our study at the initial stage there was a significant decrease in the activity of lysozyme in the oral fluid of the studied children with HG compared to normal, which indicates an insufficient level of nonspecific antimicrobial protection in their oral cavity.

Table 1

Activity of lysozyme in the oral fluid of children with hypertrophic gingivitis in the course of complex treatment, units/ml

Groups / Terms	Comparison group n = 15	Main group n = 15
Norm	0.114±0.010	
Initial state	0.039±0.005 p<0.001	0.047±0.004 p<0.001
In 6 months	0.071±0.009 p<0.01 p_1>0.05	0.082±0.009 p<0.01 p_1<0.01
In 1 year	0.042±0.006 p<0.001 p_1>0.05	0.132±0.015 p>0.05 p_1<0.001
In 2 years	0.051±0.007 p<0.001 p_1>0.05	0.106±0.008 p>0.05 p_1<0.001

Note. p – the index of the reliability of differences relative to the norm; p_1 – the index of the reliability of differences relative to initial state.

After six months of TPC in children of the main group, lysozyme activity increased by 1.74 times, and after 2 years – by 2.25 times, which almost corresponded to normal values. In the comparison group for 2 years of observations, this indicator has not changed significantly.

The enzyme catalase is responsible for the degree of antioxidant protection in the body. Table 2 shows that in the initial state in both the main group and in the comparison group, this value was below normal – by 2.1 times and 2.7, respectively.

Table 2

Catalase activity in the oral fluid of children with hypertrophic gingivitis in the process of complex treatment, mcat/l

Groups / Terms	Comparison group n = 15	Main group n = 15
Norm	0.24±0,02	
Initial state	0.09±0.01 p<0.001	0.11±0.01 p<0.001
In 6 months	0.13 ± 0.02 p<0.001 p_1>0.05	0.19±0.02 p>0.05 p_1<0.05
In 1 year	0.07±0.01 p<0.001 p_1>0.05	0.26±0.03 p>0.05 p_1<0.001
In 2 years	0.12±0.02 p<0.001 p_1>0.05	0.20±0.02 p>0.05 p_1<0.05

Note. p – the index of the reliability of differences relative to the norm; p_1 – the index of the reliability of differences relative to initial state.

The use of the developed TPC allowed in the main group of children to increase this indicator by 2.36 times over 1 year of observation, which corresponded to the normal value. After 2 years of observations, the catalase index remained at the same level. At the same time, in the comparison group, it has not changed compared to the initial state.

Any pathological processes in the body are accompanied by an increase in endogenous lipid peroxides, which act as a damaging factor and disrupt the structural and functional organization of cell membranes (fig. 1).

During the process of lipid peroxidation (LPO) dialdehydes of the malonic type (MDH) are formed, which are mutagens and have pronounced cytotoxicity. The level of MDH in biological objects, in particular in the oral fluid, can be used to judge the degree of LPO in the oral cavity. In our study, MDH levels were significantly higher than normal in both groups at the initial stage. 6 months after the start of TPC, the content of MDH in the oral fluid of children in the main group decreased by 3.29, and after 1 year – by 7.8 times, which almost corresponded to the norm. In the comparison group, there was also a decrease in this indicator, but it still significantly exceeded the norm (4.3 times after 1 year of observation).

The degree of activity of the proteolytic enzyme elastase reflects the intensity of inflammatory processes in the oral cavity (fig. 2).

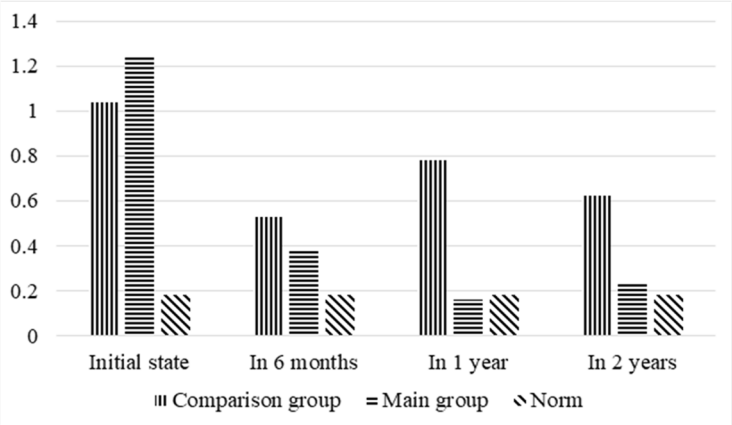

Fig.1. The content of malonic dialdehyde in the oral fluid of children with hypertrophic gingivitis in the process of complex treatment, mmol/l.

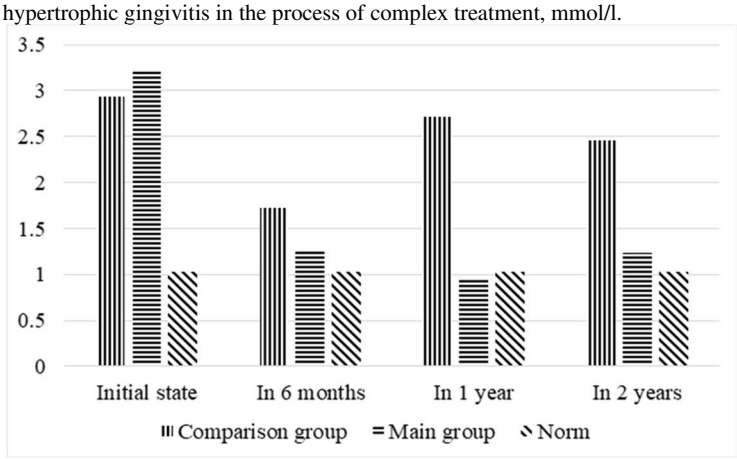

Fig.2. The activity of elastase in the oral fluid of children with hypertrophic gingivitis in the process of complex treatment, μ-cat/l

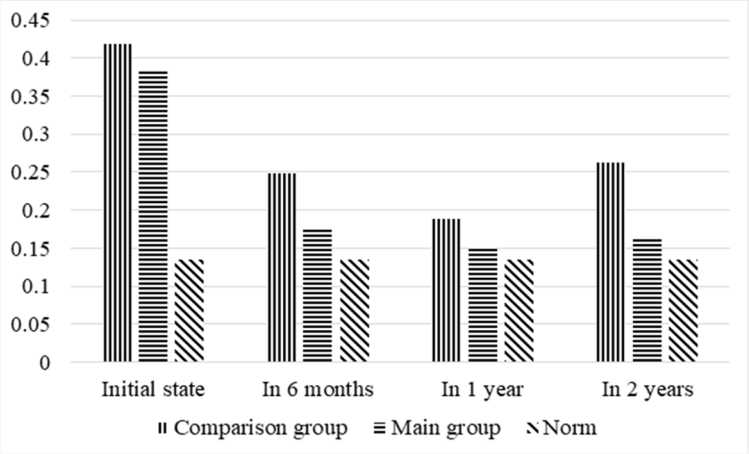

Fig.3. Urease activity in the oral fluid of children with hypertrophic gingivitis in the process of complex treatment, μ-cat/l

Fig. 2 shows that in the initial state, this indicator in the main group and in the comparison group was higher than the norm by 3.12 times and 2.85 times, respectively. The use of the developed therapeutic therapy allowed in the main group of children with HG for 1 year of observation to reduce the activity of elastase by 3.37 times, while in the comparison group it decreased only by 1.6 times. After 2 years in the main group of children, elastase activity almost corresponded to the norm, and in the comparison group, there were no significant changes in this indicator relative to the initial state.

The level of activity of the enzyme urease in the oral fluid indirectly judges the degree of contamination of the oral cavity, as this enzyme is not produced by somatic cells and probiotic bacteria, and is released only by opportunistic and pathogenic microflora (fig. 3).

At the initial stage, both in the main group and in the comparison group, this indicator significantly exceeded the norm – by 2.84 times and by 3.10 times, respectively. After 6 months of treatment and prophylactic therapy in children of the main group, this indicator decreased by 2.16 times, and after 1 year – by 2.56 times, while in children of the comparison group under the influence of basic therapy, urease activity decreased by 1.68 times and 2.21 times. After 2 years of observation in the main group, urease activity remained practically unchanged and almost corresponded to the normal values. In the comparison group, this indicator exceeded the norm by almost 2 times.

Diseases of periodontal tissues in children have a powerful driving factor – a violation of the interaction of antimicrobial systems of the oral cavity. In the process of human development, nature has selected biosystems that can protect against the harmful effects of many factors, including microbes [6, 9]. A special biosystem of antimicrobial protection is the secretory enzyme lysozyme. Lysozyme, as a hydrolytic enzyme that breaks down specific polysaccharides of bacterial cell membranes, has a wide range of physiological effects: bacteriological, bacteriostatic, immunomodulatory, regulatory, etc. [14]. It is important to determine the content of urease, which is a product of the vital activity of microorganisms. A decrease in the activity of lysozyme in the oral fluid indicates a weakening of the antimicrobial action. An increase in the enzymatic activity of urease indicates the inoculation of the oral cavity with microorganisms. The imbalance of these indicators causes dysbiosis of the oral cavity and leads to pathological changes in the organs of the oral cavity and, in particular, periodontal tissues [7]. The conducted studies indicate a fairly high efficiency of the proposed treatment and prophylactic therapy. The obtained results, in our opinion, should be taken

into account in order to assess the effectiveness of preventive measures, it is recommended to evaluate biochemical markers of antioxidant protection, the degree of lipid peroxidation and the intensity of inflammation, nonspecific antimicrobial protection and the level of contamination of the oral cavity in children with hypertrophic gingivitis.

Conclusions

1. The conducted studies indicate a rather high efficiency of the proposed therapeutic and prophylactic therapy, which contributed to the normalization of antioxidant protection, the degree of lipid peroxidation and the intensity of inflammation, nonspecific antimicrobial protection and the level of contamination of the oral cavity in children with hypertrophic gingivitis.

2. The use of complex dental treatment in children with hypertrophic gingivitis led to an increase in lysozyme activity in the oral fluid by 2.8 times, catalase activity by 1.82 times and a decrease in the content of malondialdehyde by 7.8 times, elastase activity by 3.38 times, ureases – 1.63 times.

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