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CHARACTERISTICS OF CYTOKINE PROFILE OF ORAL FLUID IN PATIENTS WITH CHRONIC SIMPLE MARGINAL GINGIVITIS

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ABSTRACT — Biomarkers of inflammation can be used as indicators for monitoring the treatment of periodontal diseases, as well as for finding the safest and most effective drugs with different mechanisms of action. The concentration of biomarkers in saliva was determined by enzyme immunoassay and chemiluminescent analysis. The obtained results reflect the degree of activity of inflammatory processes in periodontal tissues that occur in gingivitis and reveal an imbalance in the processes of free radical oxidation and antioxidant protection of the oral cavity.

KEYWORDS — biomarkers of inflammation, periodontal diseases, gingivitis.

INTRODUCTION

Nowadays, periodontal diseases contribute to the highest dental morbidity among the population of the Russian Federation, causing early tooth loss and negatively affecting the quality of human life [1,6].

The nonspecific mechanism of anti-bacterial protection in oral cavity is represented by several components. These include mechanical factors (barrier function of mucous membranes), microbiological component (role of normal microflora), chemical (humoral) and cellular immunological factors of the oral fluid [2]. In inflammatory periodontal diseases (IPD), the number of phagocytes is increased as a result of decreased apoptosis intensity. Activated neutrophils secrete a number of cytokines, which, in turn, prolong cellular response to the pathogen. In IPD, the imbalance between pro- and anti-inflammatory cytokines develops. Pro-inflammatory cytokines represent the central mediators in the pathogenesis of IPD. [3].

Diagnostic approaches based on the determination of molecular biomarkers of inflammation, alteration, immune system, antioxidant status, etc. have become widespread, allowing to predict the outcome of disease and effectiveness of treatment [5, 12, 17, 30]. This are primarily cytokines, defensins, cathelicidins, components of the complement system, immunoglobulins, growth factors, markers of oxidative stress [8, 9, 11, 15, 23].

Recent fundamental studies convincingly demonstrate that these biologically active compounds have a wide spectrum of action and play a decisive role in the immunopathogenesis of many diseases, including periodontal disease [4, 10]. It is known that depending on the set of secreted cytokines, transcription factors and signaling pathways, effector CD4 + T-helper lymphocytes are subdivided into Th1, Th2, Th3, and Th17 subpopulations.

Cytokines produced by Th1, in particular IFN-γ, IL-2, TNF-α, control cellular defense mechanisms through macrophages, providing a delayed-type hypersensitivity reaction and activation of cytotoxic T-lymphocytes (CD8+). The result of the action of mediators synthesized by Th2 (IL-4, -5, -6, -10) is the activation of B-lymphocytes, followed by their differentiation into plasma cells and the formation of antibody synthesis [22, 24].

Defensins act as one of the main triggers for the induction of cytokine synthesis and are involved in the gene expression regulation of most cytokine and chemokine receptors [21]. Stimulation of CD4+ T-cells with defensins increases the production of IFN-γ, IL-2, IL-6, IL-10, IL-8. HNPs stimulate monocyte production of TNF-γ and IL-1β. Defensins are also involved in the initiation of humoral response against microbial antigens, initiate the production of ovalbumin-specific IgG [26, 29].

Another important protective peptide, LL-37, is also a product of neutrophils and, to a lesser extent, epithelial gingival cells. LL-37 belongs to a group of major mammalian antimicrobial proteins called cathelicidins. The origin of the name is associated with the presence of 37 amino acids and two leucine residues at the N-terminus of the molecule [13]. The study of antimicrobial activity of LL-37 against oral bacteria showed specificity for P. gingivalis, A. actinomycetemcomitans, Streptococcus gordonii, Prevotella intermedia, Fusobacterium nucleatum and Streptococcus sanguinis, with the greatest activity noted against A. actinomycetemcomitans and Capnocytop. [19, 20].

The level of cathelicidins is subject to significant fluctuations in various pathological conditions. Thus, the concentration of LL-37 in the gingival fluid is significantly increased in patients with chronic periodontitis, but this was uncommon for gingivitis [18].

The complement system also plays a significant role in the pathogenesis of periodontal diseases [14, 15]. It was found that inhibition of complement cascade at the level of its main component C3 by local administration of the oligopeptide Cp40/AMY-101 provides a therapeutic effect, protecting primates from induced or natural periodontitis [15].

The high risk of periodontal pathology is also evidenced by the presence of signs of oxidative stress, and this phenomenon is considered as a criterion for the severity of the disease course, which can be used for timely prescription and/or adjustment of treatment [31].

The most commonly measured markers of oxidative stress are malondialdehyde and thiobarbituric acid-reactive substances (TBARS), indicating oxidative damage to lipids, especially lipid membranes. These analytes are widely used due to their low labor intensity and speed of execution, for rapid assessment of oxidative status and activity of inflammatory process in clinical medicine, including dentistry [7].

Thus, the imbalance of the pro- and antioxidant systems of plasma, saliva, and gingival fluid is an integral diagnostic sign of various clinical forms of gingivitis/periodontitis, which predetermines the need to analyze the redox state of these biological media in inflammatory processes of oral cavity. Current data do not support the use of any one universal indicator of oxidative stress. Obviously, a minimum set of markers is required, including the assessment of both oxidative damage and antioxidant status.

Particular attention is focused on the central role of oxidative stress in the pathogenesis and progression of chronic periodontal inflammation and the need to correct the redox imbalance in this area using antioxidants of various nature [27, 28].

Molecular markers of damage and adaptation have great potential for new diagnostic, preventive and therapeutic strategies in dentistry, and a deeper understanding of the mechanisms of their regulation as a component of the body's compensatory-adaptive systems is of great scientific and practical importance [16, 25]. The aim of the research is to analyze the level of biomarkers of inflammation and degree of oxidative stress in patients with chronic simple marginal gingivitis.

MATERIALS AND METHODS

All patients were divided into 2 groups — control group (n = 35) and a group of patients with chronic simple marginal gingivitis (CSMG) (n = 45). Oral fluid intake was carried out in the morning within the period from 8:00 to 10:00 a.m., on an empty stomach according to the standard method. Before taking the oral fluid, three times rinsing the mouth with saline sodium chloride solution (0.9% NaCl) was carried out. Samples of the oral fluid were collected in disposable sterile 1.5-ml Eppendorf. Delivery of biomaterial to the laboratory was carried out within an hour at a temperature of 20° to 37° C. In the summer, due to the impossibility of rapid delivery, the material was frozen, and the samples were stored in low-temperature refrigerator at –20° C for no more than 6 months.

The content of the analytes was determined by biochemical and immunological methods using standard test systems in accordance with the manufacturer's instructions. The concentration of interleukins was determined with test systems "Bender MedSystems" (Austria) on an enzyme immunoassay analyzer "Anthos 2020". The assessment of the state of free radical processes and antioxidant activity in the oral cavity was also carried out using chemiluminescent analysis with a portable domestic chemiluminometer HL-003.

RESULTS AND DISCUSSION

We studied the indicators of cytokine status, oxidative stress, activity of the complement system and the level of some antimicrobial peptides in oral fluid in patients with CSMG and patients of the control group.

Table 1 shows the results of a study of the level of some components of complement system in the oral fluid of patients with CSMG and patients of the control group.

The obtained data shows that there is a significant increase by almost 68% in concentration of C3a in oral fluid of patients with chronic simple marginal gingivitis as compared to parameters of healthy individuals — an increase in the level of C3a as indicator of hyperstimulation of complement system indicates the activation of hydrolysis of its precursor C3 component. The increase in C3a has an important independent significance in the development of gingivitis, since it is able to initiate chemotaxis of leukocytes to the focus of inflammation and release of histamine from mast cells and platelets.

The level of various antimicrobial peptides, in particular, defensins and cathelicidins, is considered as a reliable indicator of the activity of inflammatory phenomena.

As it is shown in the Table 2, the concentration of α-defensins in patients with CSMG exceeded those in the control group ($p \leq 0.05$).

Table 1. *Content of components of the complement system in the oral fluid (M±m)*

	Control group		Patients with CSMG	
	C1 inhibitor, μg/ml	C3a, ng/ml	C1 inhibitor, μg/ml	C3a, ng/ml
Indicator	34,72±3,13	36,4±7,52	13,23±3,05*	61,16±8,33*

Note: ** statistical significance of differences with the control group*

Table 2. *The content of some antimicrobial peptides in the oral fluid in patients of the control group and with CSMG (M±m)*

	Control group		Patients with CSMG	
Indicator	α-defensin, pg/ml	LL-37, ng/ml	α-defensin, pg/ml	LL-37, ng/ml
	0,82±0,09	28,4±3,51	3,15±0,41*	55,6±7,8*

Note: ** statistical significance of differences with the control group*

The analysis of the concentration of some cytokines in our patients is of particular interest (Table 3). This table also shows the analysis of matrix metalloproteinase-8 (MMP-8) activity since there is data on the high sensitivity and specificity of the combination of the determination of this enzyme together with IL-6 in the diagnosis of CSMG.

Table 3. *The concentration of some cytokines and MMP-8 in the oral fluid in patients of the control group and with CSMG (M±m)*

Group	IL-1β, pg/ml	IL-6, pg/ml	IL-33, pg/ml	ФНОα pg/ml	MMP-8, ng/ml
Control group	14,6±2,6	3,7±0,6	455,4±37,6	16,5±1,9	91,7±14,2
Group of patients with CSMG	97,3±11,4*	20,8±3,7*	692,9±48,2*	28,5±3,3*	334,1±21,6*

Note: ** statistical significance of differences with the control group*

The concentrations of all studied parameters in patients of the main group were significantly higher ($p \leq 0.05$) compared with the group of healthy patients, however, the degree of increase in their levels differed markedly.

Thus, in our work, we confirmed the results of other authors showing high diagnostic value of the combination of biomarkers IL-1β, IL-6, and MMP-8 for verifying periodontal pathology.

There is a promising approach based on the determination of combination of IL-6 and other pro-inflammatory proteins of macrophages in the oral fluid, which makes it possible to perform differentiative diagnosis of gingivitis with a sensitivity of more than 80% and a specificity of more than 70%.

The next stage of our work was devoted to the study of redox status parameters of the oral fluid in patients with CSMG and the control group. The imbalance between generation of reactive oxygen species (ROS) and antioxidant activity of the oral cavity is the main prerequisite for the development of oxidative stress, which is the leading pathogenetic factor of periodontal diseases.

The degree of oxidative stress activity was analyzed according to the level of TBA-reactive products (TBA-RP), and the antioxidant status of the oral fluid was assessed by determining total antioxidant activity (TBA). The parameters of the redox status of the oral fluid in examined patients are shown in Table 4.

The obtained data indicates that in chronic marginal gingivitis, intermediate products of lipid oxidative modification such as TBA-RP accumulate in the oral fluid with increase up to 220% as compared to the control level in patients of the main group.

The total antioxidant activity of the oral fluid in patients with gingivitis was reduced by almost one third and accounted to 69% ($p < 0.02$) from the parameters in the control group.

The obtained results reflect the degree of activity of inflammatory processes in periodontal tissues that occur during gingivitis and are accompanied with induction of lipid peroxidation.

At the next stage of our research, the state of *lipid peroxidation — antioxidant protection* in the oral fluid of patients with CSMG was studied using chemiluminescence analysis in vitro model systems generating ROS (Table 5).

CONCLUSION

Taken together, obtained results indicate the imbalance between processes of free radical oxidation and antioxidant defense in the oral cavity accompanied with the intensification of oxidative stress during CSMG.

Accordingly, such biomarkers as components of compliment system, α-defensin, LL-37, MMP-8, interleukins and TNF-α could be used as informative indicators for controlling the treatment of periodontal diseases, as well as for searching for the safest and most effective drugs with different mechanisms of action.

To conclude, a large group of biologically active compounds of protein origin is directly or indirectly involved in the realization of

Table 4. *Indicators of free radical homeostasis of the oral fluid and patients of the control group and with CSMG (M±m)*

Group	TBA-RP, nM/mg protein	Total antioxidant activity (AAA), nM/ml
Control group	4,19±0,58	2,14±0,19
Group of patients with CSMG	9,23±0,87*	1,45±0,12*

Note: ** statistical significance of differences with the control group*

Table 5. *The level of chemiluminescence (in conv. units) of the oral fluid in patients of the control group and with CSMG (M±m)*

Group	Emission of light	Spontaneous luminosity
Control group	3,83±0,41	1,49±0,15
Group of patients with CSMG	7,15±0,69*	4,43±0,39*

Note: ** statistical significance of differences with the control group*

pathogenic mechanisms in CSMG, including substances with different diagnostic and prognostic potential.

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