

The Influence of Pathogenic Microflora on the Occurrence of Periodontitis in Combination with Cardiovascular Diseases

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ABSTRACT

The purpose of this study is to assess the role of the pathogenicity of odontogenic infection in the development of CVD. The objects of the study were 64 patients, of which 31 patients were included in group 1 at the stages of treatment for CVD, in which the pathogen (*Porphyromonas gingivalis*) was detected by microbiological examination. Group 2 (34) patients were patients with CVD without periodontal pathology. Microbiological, immunological and enzyme immunoassay methods were used. Revealed a high prevalence of gingivitis, respectively, in patients with CVD. A decrease in the synthesis of a chemotactic factor in patients with CVD is accompanied by a significant decrease in the content of IL-8 in mixed saliva, which leads to a reduction in the life span of neutrophils.

KEYWORDS: periodontitis, CVD, pathogenic microflora, chemotaxis, IL-8

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INTRODUCTION

Most dentists believe that the condition of the periodontium is determined by the power (quantitative and qualitative) of microbial attack. But there is more and more evidence that the state of periodontal tissues is due to the structural and functional features of the periodontal complex and the organism as a whole. And inflammatory disease only provides a more favorable environment for the existence of bacteria, so it can be assumed that bacterial invasion of periodontal tissues, apparently, is one of the features of advanced periodontitis. Thus, the presence of bacteria in periodontal tissues is an important pathogenetic factor in periodontitis and undoubtedly supports inflammation in it (6,9,12). An analysis of the literature on this problem should indicate the relevance of studying the relationship between diseases of the oral cavity and general somatic pathology. As you know, the oral cavity is a specific, special, complex and independent microbiocenosis, with stable conditions for the existence and maintenance of bacteria. As a result, the species and quantitative composition of

microorganisms in the oral cavity is very diverse. In the oral cavity, populations of microorganisms are constantly changing both in morphological and physiological terms, self-regulating "living" systems, which under certain conditions, such as a decrease in the level of resistance, stressful effects, can change, and lead to a predisposition to the occurrence of various acute and chronic inflammatory diseases of the oral cavity. The most common cause of inflammatory processes in the oral cavity is due to the pathological effects of obligate anaerobes and facultative anaerobic cocci. (1,3,4,7,8,9,11,12,15). According to numerous researchers, in the etiopathogenesis of inflammatory periodontal diseases, the main place is occupied by periodontopathogenic microflora - *Treponemadenticola*, *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis*, *Streptococcus mutans*, *Peptostreptococcus anaerobius*, *Neisseria sp.*, *Corinebacterium sp.*, *S. sanguis*, *S. sobrinus*, *S. oralis*, *S. salivarius* and *S. macacae* and others. At the same

time, the severity and severity of inflammation depend on the type and pathogenicity of these microorganisms. (2,5,10,13,16,18,20,22,24). В свою очередь, взаимосвязь микрофлоры полости рта с заболеваниями различных органов и систем организма, характеризует полиморбидность патологий. При этом, дисбактериоз ротовой полости усугубляет тяжесть и прогноз сопутствующего заболевания, а эффективная борьба с дисбиотическими нарушениями в полости рта дает лучшие результаты при лечении основного сопутствующей патологии. Коморбидность сердечно-сосудистых заболеваний и патологии пародонта отмечено многими авторами (14,17,19,21,23,25), however, the microbiological and immunological components of this state of the oral cavity have not been fully studied, which, in our opinion, is one of the main links in the development of cardiovascular pathology. In this regard, the necessity and timeliness of this study is beyond doubt. The aim of this study was to study the role of the pathogenicity of odontogenic chronic infection in the course of diseases of the cardiovascular system of the body.

Material and research methods

To achieve the goal and implement the set tasks, we conducted a comparative study of the characteristics of microbiocenosis and the severity of general and local immunity of the oral cavity in patients with gingivitis combined with cardiovascular disease (CVD). This study was conducted at the clinical bases of the Department of Dentistry BukhMI. The objects of the study were 64 patients who were divided into the main study group and the control group.

Of these, group 1 included 31 patients (22 men and 9 women) aged 18 to 65 years (mean age 47.6 years) who were at the stages of treatment for CVD in whom microbiological examination revealed the pathogen "Porphyromonas gingivalis". Group 2 - (34) patients were patients with CVD without periodontal pathology. The control group consisted of 14 people, aged 18 to 65 years (9 men and 5 women), who applied for dental care at the Department of Dentistry BukhGMI. The diagnosis is "healthy" was put on the basis of the clinical and radiological picture and the conclusions of the therapist. Comprehensive dental examination and laboratory examination of patients of 3 groups was carried out at the clinical bases of BukhSMI. To assess the hygienic state of the oral cavity, the following indices were used: the index of caries intensity of permanent teeth KPU (h), the simplified index of oral hygiene IGR-U, the papillary-marginal-alveolar index, the assessment of bleeding of the gingival sulcus using the MRMuhlemann

method, the periodontal index (PI) according to A. Russel, gingivitis index GI. In addition, the presence of non-carious lesions of the teeth and diseases of the oral mucosa (OM) was noted in the dental chart. The study of the composition of the microflora of the oral cavity for the presence of pathogenic and opportunistic microflora in the examined persons was carried out in the microbiological laboratory of the BukhSMI. To conduct a microbiological study, all patients were sampled with a sterile cotton swab, which was then placed in a tightly closed sterile tube. The material was taken on an empty stomach, before brushing the teeth. With the help of the cultural method of bacteriological research, identification and counting of pure cultures of certain microorganisms is carried out, which makes it possible to most accurately judge the etiological affiliation of the obtained microorganisms. The number of colonies of microorganisms was expressed using units of CFU/ml. Identification of the obtained cultures of microorganisms was carried out in accordance with the determinant of bacteria Bergey (1997) and guidelines for microbiology. The immunological study was carried out on the basis of the clinical laboratory of BukhGMI. To assess local and general immunity, blood was taken in the morning, on an empty stomach, as well as oral fluid. The evaluation of indicators of both cellular and humoral immunity was carried out using the enzyme immunoassay method. The concentration of interleukin 8 was determined by enzyme immunoassay on a Mindray analyzer using reagents from Human. Determination of the phagocytic function of free oral neutrophils liquids were evaluated by the ability to absorb latex particles according to the method Freidlin. To study the ability of neutrophils to free oral liquid to phagocytosis 0.2 ml of oral secretion suspension was mixed with 0.02 ml of latex suspension with a diameter of 1.7 μm (10 particles/ml), obtained from

All-Union Research Institute of Synthetic Rubber. Academician S. V. Lebeden

(St. Petersburg). After a half-hour incubation at 37°C, smears were prepared, dried, fixed with 96% ethanol, and stained

Romanovsky-Giemsa. When analyzing phagocytosis, the activity, intensity of phagocytosis, and phagocytic number were calculated. Phagocytosis activity was calculated as the number of neutrophils containing latex particles per 100 counted cells. The intensity of phagocytosis was determined by the number of latex particles per 100 cells in terms of 1 cell. The results are presented as the arithmetic mean with the value of the standard deviation. Significance of differences was assessed using the STATISTICA software

package using Student's t-test.

Research results and discussion

An analysis of the dental status of patients with CVD showed that periodontal diseases in this group of patients result from the accumulation of plaque, with or without the development of an inflammatory process, destruction of the periodontium, including the gum itself, periodontal ligament and alveolar bone. Clinically, the gingival sulcus deepens, forming a periodontal pocket, the attachment of the gingiva to the root surface is disrupted, while the biofilm on the surface of the teeth migrates apically, attachment of connective tissue occurs and alveolar bone loss, gingival loss. When studying the papillary-marginal-alveolar index PMA in the modification of C. Parma (1960) in the main group of patients, on average, it was $62.3 \pm 4.6\%$, which corresponds to an average degree of inflammation. A mild degree of gingival inflammation according to this index is observed in 31.7%, a severe degree - 6.0%. In the control group, this indicator was significantly lower and equal to $19.8 \pm 2.1\%$, which is typical for mild inflammation.

When studying the condition of the periodontium using the GI gingivitis index, it was found that the average value in the main group is 1.98 ± 0.14 , which corresponds to moderate inflammation in the gums. Mild inflammation (an index in the range (from 0.1 to 1.0) was determined in 24.5% of patients; moderate inflammation (within 1.1 - 2.0) - in 72.1% and severe inflammation (interval of 2.1-3.0) - in 3.4%. In the control group, the average GI value was 0.37 ± 0.04 , this figure is significantly lower than in the study group. A mild degree of gingival inflammation was established in 76% patients, and moderate - in 24%. The highest prevalence among periodontal diseases in the main study group, as shown in the table, was catarrhal marginal gingivitis and hypertrophic gingivitis, respectively, in 46 and 12 patients with CVD. At the same time, CHPST in patients with CVD was observed in 6 patients of the main group and averaged 9.3%. It should be noted that the group of gingivitis included catarrhal and hypertrophic gingivitis and amounted to 71.9% and 18.8%, respectively.

Table №1 Index assessment of the state of periodontal tissues in patients with CVD (M±m)

Periodontaldisease	1 group (n=31)	2 group (n=33)	Control group (n=14)
PMA, %	$62,3 \pm 4,6\%$	$27,3 \pm 2,1\%$	$19,8 \pm 2,1\%$
Bleeding score by M.R.Muhlemann method	$1,5 \pm 0,67$	$0,7 \pm 0,62$	$0,4 \pm 0,12$
PII by A. Russel	$3,6 \pm 0,51$	$1,8 \pm 0,24$	$1,4 \pm 0,37$
CPITN	$3,1 \pm 0,44$	$1,9 \pm 0,13$	$1,3 \pm 0,95$
GingivitisindexGI	$1,9 \pm 0,14$	$0,9 \pm 0,08$	$0,3 \pm 0,04$

Various microorganisms, including *Porphyromonasgingivalis*, colonize the glycoprotein-containing layer (plaque) above and below the gingival margin to form supragingival and subgingival layers of plaque. Further, bacterial-endothelial cellular interactions occur in periodontal pockets, creating and exchanging signals between microorganisms and neighboring cells of the immune system. *Porphyromonasgingivalis* are attracted to and metabolized through receptor-mediated endocytosis in the cells of the epithelium lining the gingival sulcus. Thus, endotoxin (eg, lipopolysaccharide)-producing *Porphyromonasgingivalis* are protected from the immune system and can multiply within cells and possibly spread systemically through the circulation, causing a generalized immune response.

Table №2 Analysis of the structure of periodontal diseases in the examined patients with CVD is presented in the table.(%)

Diseasesperiodontal	1 group (n=31)		2 group (n=33)		Control group (n=14)	
	abs.number	%	abs.number	%	abs.number	%
Healthyperiodontium	-	-	4	11,8	9	64,3
Catarrhalmarginalggingivitis	22	71,9	-	-	-	-
Hypertrophicgingivitis	6	18,8	-	-	-	-
Acuteperiodontitis	-	-	-	-	-	-
Chronicgeneralizedmoderateperiodontitis	3	9,3	30	88,2	5	35,7
periodontaldisease	-	-	-	-	-	-

The anti-infective protection of mucous membranes, along with the epithelial barrier, includes the cellular link of anti-infective protection, humoral immunity factors and normal microflora of the oral cavity, which exists in the form of biofilms on the surface of the epithelium or in planktonic form in the free oral fluid. To assess the functional activity of neutrophils in free oral fluid, spontaneous and latex-induced reduction of NBT to diformazan was studied, and the ability of neutrophils to phagocytosis of latex particles was determined.

As can be seen from the presented research results, the parameters of the NBT-test of oral fluid neutrophils in patients with CVD had a peculiar dynamics when compared with those of healthy individuals. Thus, during a spontaneous test, the activity of neutrophils in patients with CVD decreased on average by 1.75 times, while in the case of an induced decrease in NST, the activity of neutrophils was more pronounced and, on average, it decreased by 3.5 times when compared with the indicators of healthy individuals, thereby indicating very low phagocytic activity of neutrophils. Indicators of the intensity of phagocytosis in the examined individuals had similar dynamics. As you know, porphyromonas (*Porphyromonasgingivalis*) - bacteria that actively contribute to inflammation. As a rule, these bacteria are located on the areas of the gums that are directly adjacent to the tooth. They are obligately anaerobic and often form protective biofilms based on resistant tooth tissues.

Table№3 Parameters of the NST-test of oral fluid neutrophils in patients with CVD

Indicator		1 group (n=31)	2 group (n=33)	Control group (n=14)
NST spontaneous	Activity, %	12,01±0,76	15,01±1,04	21,04±0,98
	Intensity r.u.	0,13±0,01	0,18±0,01	0,28±0,01
NST induced	Activity, %	7,46±0,57	16,23±1,12	26,27±1,23
	Intensity r.u.	0,068±0,01	0,14±0,01	0,22±0,01

Note: * - differences relative to group data comparisons are significant (P<0,05)

This is how dental plaque is formed. Therefore, as the disease progresses, acute inflammation becomes chronic. The reason for this is *P. gingivalis*, which disrupts the synthesis of the chemotactic factor IL-8 and the adhesion molecule E-selectin in the gum cells in order to normally feed on activated neutrophils. As can be seen from the presented research results (table), a decrease in the synthesis of the chemotactic factor in patients with CVD is accompanied by a significant decrease in the content of IL-8 in mixed saliva, which leads to a reduction in the life span of neutrophils. As a result, neutrophils become the main mediators involved in damage to the gums and alveolar bone during the development of periodontitis.

One of the important indicators of the course of the inflammatory process is the intensity of phagocyte mobility (chemotaxis). It is the mobility of macrophages that is one of the mechanisms that determine the number of cells - phagocytes in the focus of inflammation and ultimately determine the effectiveness of the phagocytic process. Analysis of the presented research results indicates a decrease in the intensity of macrophage chemotaxis. One of the reasons for the decrease in the intensity of neutrophil chemotaxis and the decrease in the level of IL-8 is the high demand for neutrophils by pathogens. This is probably why the blocking of chemotaxis has a short duration in time, which leads to an excessive accumulation of neutrophils in the focus of inflammation.

Table№4 the intensity of phagocytosis, macrophage chemotaxis in the dynamics of periodontal inflammation caused by *Porphyromonasgingivalis*

Indicator	1 group (n=31)	2 group (n=33)	Control group (n=14)
Interleukin-8 (IL-8) pg/ml in mixed saliva	167,31±12,04	90,54±8,16*	124,32±9,73
Intensity of macrophage chemotaxis (mg protein/ml)	0.08±0.001	0.05±0.001*	0.07±0.001*

Note: * - differences relative to data control group are significant (- P<0,05)

Thus, in patients with periodontitis, a pronounced absolute leukocytosis was found in the periodontal tissues and in the oral fluid, which indicates the preservation of signs of local inflammation. Thus, the primary damage to periodontal tissues caused by (*Porphyromonasgingivalis*) begins to gradually increase over time. In this case, the subsequent inflammatory process spreads from the gum to the alveolar bone along the periodontium, neurovascular bundles. In the places where the vessels exit the alveolar bone, the inflammatory process of bone begins fabrics.

Conclusions

1. The highest prevalence among periodontal diseases in the main study group, as shown in the table, was marked by catarrhal marginal gingivitis and hypertrophic gingivitis, respectively, in 46 and 12 patients with CVD disease.
2. Endotoxin (eg, lipopolysaccharide) - producing *Porphyromonasgingivalis*, are protected from the immune system and can multiply within cells and

possibly spread systemically through the circulation, causing a generalized immune response.

3. The decrease in the synthesis of the chemotactic factor in patients with CVD is accompanied by a significant decrease in the content of IL-8 in mixed saliva, which leads to a reduction in the life span of neutrophils. As a result, neutrophils become the main mediators involved in damage

to the gums and alveolar bone during the development of periodontitis.

4. Primary damage to periodontal tissues caused by Porphyromonas gingivalis over time, the damage zone begins to gradually increase. In this case, the subsequent inflammatory process spreads from the gum to the alveolar bone along the periodontium, neurovascular bundles.

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