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ASSESSMENT OF THE MICROBIAL CONTENT OF PERIODONTAL POCKETS IN PATIENTS WITH CHRONIC GENERALIZED PERIODONTITIS AND CORONARY ARTERY DISEASE

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ABSTRACT

The aim: To study the rate of detection of specific periodontopathogenic microbiota in patients with chronic generalized periodontitis (CGP) and coronary artery disease (CAD) and assessment of the risk of periodontal pathogens in the development of CAD.

Materials and methods: A microbiological study of the content of periodontal pockets was carried out in 64 patients with CGP and CAD of the study group (mean age – 56.9±7.9 years) and 20 patients of the comparison group (mean age – 45.2±11,8 years) who were not burdened with CAD.

Results: It was established that in patients with CGP and CAD the following periodontal pathogens were found more frequently than in the comparison group: *Aggregatibacter actinomycetemcomitans* (56.3±6.20% vs 25.0±9.68%; p=0.01), *Prevotella intermedia* (54.7±6.22% vs. 20.0±8.94%; p=0.01), and *Fusobacterium spp.* (34.4±5.94 vs. 10.0±6.71%; p=0.04). The increase in the percentage of the association of the periodontal pathogens was revealed in patients with CAD, which increased with the severity of the pathological process in periodontal tissues. The results of the study indicate the association of *A. actinomycetemcomitans, P. intermedia, Fusobacterium spp.* with CAD: *A. actinomycetemcomitans*: OR=3.86 (95% CI: 1.25-11.90), p=0.015; *P. intermedia*: OR=4.83 (95% CI: 1.45-16.05), p=0.007; *Fusobacterium spp.:* OR=4.71 (95% CI: 1.00-22.20), p=0.035.

Conclusions: Analysis of the microbiological study indicates a high rate of detection of specific periodontal pathogens in patients with CGP and CAD. It can be assumed that the presence of such periodontal pathogens as *A. actinomycetemcomitans*, *P. intermedia, Fusobacterium spp.*, significantly increases the risk of CAD.

KEY WORDS: periodontitis, periodontal pathogens, coronary artery disease

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INTRODUCTION

Periodontal diseases invariably remain among the most common dental diseases. According to the epidemiological data from international studies, the prevalence of periodontal diseases is 80-100% of the world population [1]. The leading place in the structure of clinical forms of generalized lesions of periodontal tissue belongs to chronic generalized periodontitis (CGP) as the main disease that leads to premature tooth loss.

Currently, there is a great number of scientific papers devoted to the in-depth study of the relationship of CGP with general somatic pathology, in particular with cardiovascular diseases (CVD) [2-4], which remains the most common cause of death worldwide [5].

Coronary artery disease (CAD), which results from the atherosclerotic vascular lesion, is diagnosed in 15-20% of the adult population of different countries [5]. An active discussion of the etiopathogenetic links and the impact of CGP on the development and course of CVD, including CAD, received a response from cardiologists. The European guidelines on cardiovascular disease prevention in clinical practice (ESC, 2016), which aim to identify and control the main risk factors for diseases of the circulatory system, indicate the important role of CGP in the development of CAD [6]. CGP is considered an independent risk factor for cardiac diseases associated with atherosclerotic vascular lesions; in particular, special emphasis is placed on the presence of periodontal pathogenic microflora. The existence of a close relationship between disorders of lipid metabolism and hypercholesterolemia with CGP is evidenced by the INVEST study (Oral Infections and Vascular Disease Epidemiology Study), which found that improving the clinical and microbiological parameters of periodontal tissues reduces the rate of progression of thickening of carotid intima-media complex, i.e. the progression of atherosclerosis [7].

In recent years, the increase in the number of scientific studies has been observed to raise the questions of direct or indirect pathogenetic effects of specific microbiota of periodontal pockets on the process of atherogenesis [4,7]. The main factor in the virulence of periodontal gram-negative microorganisms is endotoxins, which are similar to lipopolysaccharides by their chemical nature that are a part of cell membranes. The very presence of lipopolysaccharides leads to the activation of the immune system, increase in plasma concentrations of proinflammatory cytokines (IL-6, TNF-α), biochemical parameters such as C-reactive protein, fibrinogen, total cholesterol, triglycerides, etc., which in turn cause systemic inflammation and then atherosclerosis [8].

On the other hand, the relationship between periodontal microbiota and cardiovascular pathology, including CAD, endocarditis, etc., is associated with transient bacteremia that occurs after such dental manipulations as professional hygiene, tooth extraction, and other surgical interventions in the oral cavity [8-10]. According to Mazur I.P. (2018) and Kharchenko N.L. (2012), the presence of pathogenic microorganisms – *Staphylococcus aureus* (16.3%), *Staphylococcus pyogenes* (11.6%), and *Streptococcus viridans* (14.0%) was revealed in the bloodstream at the 15th minute in 44.1% of patients who underwent dental interventions. These microorganisms also remained in the blood at the 30th minute after manipulation and were detected in 16.2% of patients [10]. The increased risk of transient bacteremia is more typical for people with periodontal diseases because periodontal pockets contain a large number of pathogenic microflora [9,10]. Short-term or long-term episodes of bacteremia lead to the spread of microorganisms in the bloodstream and their attachment to the walls of blood vessels, endocardium, heart valves, and atherosclerotic plaques [9]. The results of polymerase chain reaction studies indicate the presence of rDNA of such periodontal pathogens as *Porphyromonas gingivalis*, *Aggregatibacter Actinomycetemcomitans*, *Bacteroides forsythus*, *Treponema denticola* in atherosclerotic plaques of human carotid arteries [8, 12, 13]. Despite the significant amount of data that indicate the presence of periodontal pathogens in atherosclerotic lesions, there are also studies that contain conflicting results of similar studies [14, 15].

Thus, determining the types of periodontal pathogens that increase the risk of CAD, as well as determining the impact of their quantitative composition and the role of virulence in this process remain incompletely studied and require further research.

THE AIM

To analyze the rate of detection of specific periodontopathogenic microbiota in patients with CGP and CAD, in particular, to study the risk of the association of these periodontal pathogens with the development of CAD.

MATERIALS AND METHODS

A microbiological study of the contents of periodontal pockets of 64 patients with CGP and CAD of the study group (mean age – 56.9±7.9 years), who were admitted to the Cardiology department and 20 persons with CGP not burdened with CAD or other systemic diseases, who were in the comparison group (mean age – 45.2±11,8 years). Among 64 patients of the study group, CGP initial-I degree was diagnosed in 24 patients (37.5%), CGP II degree – in 26 patients (40.6%), and CGP III degree – in 14 patients (21.9%). In the comparison group, CGP initial-I degree was diagnosed in 15 patients (75.0%), CGP II degree – in 4 subjects (20.0%) and CGP III degree was observed in one examined patient (5.0%).

Diagnosis of periodontal status of patients was performed according to the classification by Danilevsky M.F. (1994) on the basis of history taking, filling the periodontal examination card, which included assessment of periodontal tissue, in particular determining the degree of inflammation of gum tissue using PMA periodontal index (papillary-marginal-alveolar index by M. Massler modified by C. Parma, 1960).

The diagnosis of CAD and the results of laboratory tests were obtained by analyzing the medical records of the inpatients. The exclusion criteria were patients with a history of other systemic diseases and patients with CAD and complete secondary adentia.

Material for microbiological examination (the content of periodontal pockets) was collected with sterile paper points and immediately introduced into the semi-liquid Thioglycolate broth medium to detect the anaerobic bacteria. Culture from Thioglycolate medium was plated in basic agar for anaerobes, Schaedler agar with 5% Sheep Blood and Schaedler neomycin vancomycin agar. The system GENbox anaer (BioMerieux, France) was used to cultivate anaerobes. Studies of anaerobic microflora were continued after 3-4 days. Further identification of the group and the type of microorganisms was performed by morphotinctorial, cultural, and biochemical properties. Biochemical characteristics of the microorganisms were determined using test kits API 20E (BioMerieux, France). The identification of microorganisms was performed according to Bergey's classification.

According to the requirements of the basic bioethical provisions of the European Convention on Human Rights and Biomedicine from 04.04.1997 and the Helsinki Declaration of the World Medical Association on ethical principles for medical research involving human subjects (1964-2008), patients signed consent for examination and research (protocol No.3 from 25.03.2019, discussed and approved by the Committee on ethics of scientific research, experimental development and scientific works of Danylo Halytsky Lviv National Medical University).

Statistical analysis of the results was performed using Excel and IBM SPSS Statistics 20. The results of the study are presented as mean±standard deviations (M±SD) (PMA index) and relative values: fractions with error ($P \pm m_p$). Pearson's chi-squared test ($\chi 2$) was used to estimate the probable difference in the results in the compared groups. The constructed tables 2×2 and the criterion of conditional independence (Cochran) were used to compare the odds ratio (OR) with 95% confidence intervals (95% CI). The difference between the groups was considered significant at $p<0.05$.

RESULTS

The analysis of the obtained results of the microbiological study of the contents of periodontal pockets of the study group and the comparison group did not reveal significant differences in the species composition of periodontopathogenic microbiota, but the proportion of detected individual anaerobic microorganisms differed in the comparison group. According to the prevalence of periodontal pathogens, the following species were more common in

Table I. Rate of detection of periodontal pathogens in periodontal pockets (P±m_p, %)

Periodontal pathogens	Study group (n=64)		Comparison group (n=20)		χ2	p
	n	P±m_p, %	n	P±m_p, %		
Porphyromonas gingivalis	57	89.1±3.90	18	90.0±6.71	0.01	0.91
Aggregatibacter actinomycetemcomitans	36	56.3±6.20	5	25.0±9.68	5.96	0.01
Prevotella intermedia	35	54.7±6.22	4	20.0±8.94	7.37	0.01
Fusobacterium spp.	22	34.4±5.94	2	10.0±6.71	4.44	0.04
Peptostreptococcus anaerobius	33	51.6±6.25	11	55.0±11.12	0.07	0.79

Table II. Rate of detection of periodontal pathogens in periodontal pockets of patients with CAD depending on the severity of CGP (P±mp, %)

Periodontal pathogens	CGP Initial-I degree (n=24)		CGP II degree (n=26)		CGP III degree (n=14)		χ2	p
	N	P±m_p, %	n	P±m_p, %	n	P±m_p, %		
Porphyromonas gingivalis	21	87.5±6.75	22	84.6±7.08	14	100±0	1χ2=0.09	0.77
							2χ2=1.90	0.17
							3χ2=2.39	0.12
Aggregatibacter actinomycetemcomitans	10	41.7±10.06	13	50.0±9.81	13	92.9±6.88	1χ2=0.35	0.55
							2χ2=9.70	0.002
							3χ2=7.35	0.01
Prevotella intermedia	15	62.5±9.88	15	57.7±9.69	5	35.7±12.81	1χ2=0.12	0.73
							2χ2=2.54	0.11
							3χ2=1.76	0.18
Fusobacterium spp.	5	20.8±8.29	8	30.8±9.05	9	64.3±12.81	1χ2=0.64	0.42
							2χ2=7.17	0.01
							3χ2=4.18	0.04
Peptostreptococcus anaerobius	8	33.3±9.62	17	65.4±9.33	8	57.1±13.23	1χ2=5.13	0.02
							2χ2=2.06	0.15
							3χ2=0.26	0.61

Notes:
1χ2 – the value of Pearson's criterion of the probability of difference between the indicators of CGP initial-I degree and CGP II degree;
2χ2 – the value of Pearson's criterion of the probability of difference between the indicators of CGP initial-I degree and CGP III degree;
3χ2 – the value of Pearson's criterion of the probability of difference between the indicators of CGP II degree and CGP III degree;

patients with CGP and CAD: *A. actinomycetemcomitans*, *P. intermedia*, and *Fusobacterium spp*. In particular, *A. actinomycetemcomitans* were detected in 56.3±6.20% of the patients in the study group against 25.0±9.68% of those in the comparison group (p=0.01). *P. intermedia* and *Fusobacterium spp.* detected in 54.7±6.22% and 34.4±5.94% of patients in the study group and 20.0±8.94% (p=0.01) and 10.0±6.71% (p=0.04) in the comparison group, respectively (Table I). The study did not find a statistically significant difference in the rate of detection of *P. gingivalis* and *P. anaerobius* in both groups (p>0.05).

Analyzing the data on the rate of detection of periodontal pathogens in patients with CAD, depending on the degree of CGP (Table II), it was found that *P. gingivalis* (87.5±6.75%), *P. intermedia* (62.5±9.88%), and *A. actinomycetemcomitans* (41.7±10.06%) were detected the most frequently in patients with CGP initial-I degree in the settings of the inflammatory process of moderate severity (PMA index was 50.3±12.6%).

There was a slight percentage increase in the rate of detection of gram-negative anaerobic microflora: *A. actinomycetemcomitans* (50.0±9.81%) and *Fusobacterium spp.* (30.8±9.05) in patients with CAD with CGP II degree and severe inflammatory process (PMA – 76.8±12.5%). The percentage of detection of *P. anaerobius* gram-positive anaerobic microorganism also increased (65.4±9.33%; p=0.02).

There was a significant increase of gram-negative anaerobic microorganisms in patients with CAD and CGP III degree with the PMA indicator of 86.9±8.3%, which corresponds to the severe degree of inflammation in gums, compared with groups of patients with CAD and

Table III. Association between periodontal pathogens and the risk of CAD

Periodontal pathogens	Study group		Comparison group		Odds ratio		Criterion of conditional independence (Cochran)	
	R*	95% CI	R	95% CI	OR**	95% CI***	χ2	P
Porphyromonas gingivalis	0.99	0.84-1.17	1.09	0.25-4.85	0.91	0.17-4.75	0.014	0.91
Aggregatibacter actinomycetemcomitans	2.25	1.02-4.95	0.58	0.40-0.85	3.86	1.25-11.90	5.956	0.015
Prevotella intermedia	2.73	1.11-7.76	0.57	0.40-0.80	4.83	1.45-16.05	7.372	0.007
Fusobacterium spp.	3.44	0.88-13.36	0.73	0.58-0.92	4.71	1.00-22.20	4.436	0.035
Peptostreptococcus anaerobius	0.94	0.59-1.49	1.08	0.62-1.86	0.87	0.32-2.39	0.07	0.79

Note. * – ratio; ** – odds ratio; *** – confidence interval.

CGP initial-I and II degree. Thus, the detection rate of *A. actinomycetemcomitans* increased to 92.9±6.88% (p=0.01), and *Fusobacterium spp.* – 64.3±12.81% (p=0.04). It should be noted that *P. gingivalis* was detected in 100% of the examined patients with CAD and CGP III degree.

Analysis of the results of the odds ratio indicates that the following periodontal pathogens were significantly associated with CAD: *A. actinomycetemcomitans*: OR=3.86 (95% CI: 1.25-11.90), p=0.015; *P. intermedia*: OR=4.83 (95% CI: 1.45-16.05), p=0.007; *Fusobacterium spp .:* OR=4.71 (95% CI: 1.00-22.20), p=0.035 (Table III).

Thus, the probability of detection of *A. actinomycetemcomitans* in patients with CAD is 3.86 times higher than in patients with CGP without CAD. A similar situation is also characteristic of *P. intermedia* and *Fusobacterium spp*. – the probability of detection of these microorganisms in patients with CGP and CAD is 4.83 and 4.71 times higher than in the comparison group, respectively.

DISCUSSION

Reviewing the researches that studied the relationship between periodontal pathogens and CVD, including CAD, it may be assumed that most authors confirm the hypothesis of the association of periodontal pathogens with CAD [4,7,8,16,17]. Therefore, in the study of the contents of periodontal pockets of patients with CAD, the most common periodontal pathogens were: *P. gingivalis*, *P. intermedia*, *A. actinomycetemcomitans, Fusobacterium nucleatum, T. forsythensis (Tannerella forsythensis)*, *T. denticola (Treponema denticola)*, and others [16,17,18,19]. However, the results of studies of the species composition namely, the association of individual periodontal pathogens with CAD, are ambiguous. For example, a study by Spahr A. et al. CORODONT (The Coronary Event and Periodontal Disease) did not find a significant statistical difference in the prevalence of periodontal pathogens in patients with CAD and in subjects who have not been diagnosed with CAD [19]. At the same time, the total periodontal pathogenic infection in periodontal pockets was significantly higher in patients with CAD, with the main portion of *A. actinomycetemcomitans* and, to a lesser extent, *P. intermedia*.

The high rate of detection and association of *A. actinomycetemcomitans* and *P. intermedia* with CAD are indicated by the results of studies by Mantyla P. et al. and Nonnemacher C. et al., respectively [17,18]. It should be noted that the periodontal pathogen *P. intermedia*, which is associated with CAD, has also been frequently detected in patients who suffered from myocardial infarction, as confirmed by a study by Andriankaja O. et al. [16].

According to the analyzed literature data, which is also confirmed by the results of our study, there is no reliable association between CAD and *P. gingivalis*, which is considered one of the most common pathogens of periodontal disease, in particular, CGP. There was no significant difference in the prevalence and total amount of *P. gingivalis* in the content of periodontal pockets of patients with CAD compared with those who were not burdened with CAD.

Moreover, our study found an association between CAD and *Fusobacterium spp*. However, the available literature has insufficiently covered information about the role of this periodontal pathogen.

Thus, the results of the study complemented and confirmed the scientific data about the importance of the relationship between periodontal pathogens and the risk of CAD. Further study of the quantitative and qualitative properties of periodontal pathogens and their probable impact on the development of CAD will improve approaches to the comprehensive treatment of patients with mutually aggravating diseases – CGP and CAD.

CONCLUSIONS

Analysis of the microbiological studies of the contents of periodontal pockets of patients with CGP and CAD indicates a high rate of detection of specific periodontal pathogens, the percentage of which increases with the severity of the pathological process in the periodontal tissues. It can be assumed that the presence of such periodontal pathogens as *A. actinomycetemcomitans*, *P. intermedia*, *Fusobacterium spp.* significantly increases the risk of CAD by 3.86-4.71 times.

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The Authors declare no conflict of interest.

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