Original Article

Interrelation of Cardiovascular Diseases with Anaerobic Bacteria of Subgingival Biofilm

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Abstract

Aims: The aim of this study is to study the colonization of subgingival biofilm (SGB) with periodontopathogenic bacteria species and endothelium-dependent vasodilation in patients with coronary heart disease and concomitant periodontitis. **Subjects and Methods:** Forty-five patients with cardiovascular diseases (CVDs) were examined – 28 women (62%) and 17 men (38%) aged 53–76 years, including 15 patients with acute myocardial infarction (AMI), 15 patients with exertional angina (pectoris), and 15 patients with chronic periodontitis (CP) without CVD. Dental and cardiological health conditions were determined, a biochemical blood test was conducted, endothelium-dependent vasodilation in the brachial artery was measured, and DNA of periodontopathogenic bacteria in SGB was detected. **Results:** A reliable interrelation between the colonization of SGB with periodontopathogenic bacteria and development of AMI was established. In AMI patients, the frequency of *Porphyromonas gingivalis, Tannerella forsythia,* and *Aggregatibacter actinomycetemcomitans* detection was significantly higher than in the group of participants without cardiovascular disease. The presence of *P. gingivalis* and *A. actinomycetemcomitans* in patients with CP directly correlated with severity of periodontal tissue destruction. Endothelium-dependent vasodilation in the brachial artery moderately correlated with patient's cardiological condition (r = 0.3284), biochemical markers of atherosclerosis development (r = 0.6465), and frequency of *P. intermedia* detection in periodontal pockets (r = 0.3828). **Conclusions:** Periodontal status in patients with AMI is characterized by unsatisfactory and poor hygiene, increased indices of bleeding on probing, and periodontal pocket depth in comparison to groups of patients with angina pectoris and CP without cardiovascular pathology.

Keywords: *Bacteria, biofilms, DNA, endothelium-dependent relaxing factors, inferior wall myocardial infarction, periodontitis*

Introduction

In recent years, many authors studied the role of intravascular infection as a risk factor for atherosclerosis, as well as its complications associated with thrombosis and embolism. It is believed that the cause of these processes is the bacterial invasion of the endothelium, which leads to the disruption of endothelial function and blood coagulation, and consequently, to the progression of atherogenesis.[1,2] It is confirmed that the polymicrobial infectious component is linked to atheromatous damage, which opens a new direction for the research in vascular infectology and study of atherosclerosis development.[3,4]

The question of whether specific etiological agents contribute to microbial invasion in vascular pathology remains open. It has been established that bacterial antigens or genetic material of periodontopathogenic microbial species, such as *Aggregatibacter actinomycetemcomitans*, *Fusobacterium nucleatum*-periodonticum, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Prevotella nigrescens,* and *Tannerella forsythia* can be detected in the areas of atherosclerotic damage of the coronary arteries. The most commonly identified bacteria include *P. gingivalis*, *A. actinomycetemcomitans,* and *P. intermedia.*[3,5,6]

Some studies established the links between the composition of subgingival microflora and microbes that determined in the areas of atherosclerotic vascular damage.[7-9] There is evidence showing that the bacteria in atherosclerotic plaques are viable. A significant amount of periodontopathogenic bacterial species

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DNA in periodontitis patients are detected in atherosclerotic plaque samples. This fact makes possible that the presence of these bacterial species in sites of vascular injury is not accidental, and periodontopathogens can indeed contribute to the development of cardiovascular diseases (CVD).[10,11]

However, at the moment, available data on the health condition of the oral cavity in patients with acute myocardial infarction (AMI) are insufficient. Several studies[12-14] show that patients suffering from myocardial infarction tend to have worse health conditions of the oral cavity than those without CVDs.

In some works, it was shown that in patients suffering from myocardial infarction, the condition of the oral cavity is worse than in patients without cardiovascular disease. Clinical evidence also suggests that periodontitis is associated with a host systemic response and a mild inflammatory state, as measured by the elevated serum C-reactive protein and endothelial dysfunction.[12,13,15] The relationship between the altered lipid profile and periodontitis has been investigated in several studies.[16,17] However, since both myocardial infarction and periodontal disease are multifactorial in nature, some authors suggest that no relationship between periodontal disease and myocardial infarction could have been exactly replicated or confirmed.[18,19]

Therefore, in this study, we conducted an investigation of oral periodontopathogenic bacterial species and their possible connection to the development of cardiovascular pathology. The aim of our work is to study the bacterial colonization of subgingival biofilm (SGB) in patients with coronary heart disease and concomitant periodontitis.

Subjects and Methods

We examined 45 patients with cardiovascular pathology: 28 women (62%) and 17 men (38%) aged 53–76 years. Group I included 15 patients with AMI and Group II consisted of 15 persons with angina pectoris I-II functional classes (AP I-II FC). These patients were receiving treatment at the Cardiological Center of the Russian Academy of Medical Sciences (Moscow). Group III included 15 patients with moderate chronic periodontitis (CP) without CVD who were under the supervision at the Research Institute of Medicine and Dentistry by A.I. Evdokimov Moscow State University of Medicine and Dentistry (MSUMD).

Clinical and laboratory examination of dental patients was carried out taking into account the requirements of International Statistical Classification of Diseases and Related Health Problems (ICD 10), the State Standard (Russian) and guidelines for the examination of dental patients with periodontal diseases, based on the identification of oral hygiene, the severity of the inflammatory reaction, gum recession, periodontal destruction, and bone resorption of the dental alveoli.

Clinical examination of the patients included the analysis of complaints, anamnesis, parameters of patients' health condition, and local changes in periodontal tissues. Patients underwent a standard dental examination.

The DNA determination of *P. intermedia*, *T. forsythia*, *Treponema denticola*, *A. actinomycetemcomitans* (Actinobacillus actinomycetemcomitans), and *P. gingivalis* was carried out through PCR using a set of «Multident 5» reagents produced by «Genlab» and A. I. Evdokimov MSUMD laboratory of molecular biology investigations. The sensitivity of the kit is not more than 10^4 copies/ml for each pathogen, which makes it possible to detect bacteria in amounts representative of areas with a risk of development or progression of periodontitis.[8]

Sample of SGB was placed in a test tube with 0.9% sodium chloride solution immediately after removal from the periodontal pocket. Periodontopathogen DNA was isolated by accelerated sample preparation method using the «Realex» reagent. The amplification of the isolated genetic material was carried out in the thermocycler "Terzik MS-2" ("DNA-Technology," Moscow, Russia). Cloned DNA samples were analyzed by electrophoresis in a 1.6% agarose gel after coloring with ethidium bromide. The gels were studied and photographed in TCP-25M transilluminator (Vilber Lourmat, France) with an ultraviolet wavelength of 312 nm.[8]

The statistical analysis was performed using the STATISTICA 9.0 program (DELL, Round Rock, Texas, USA). All obtained data had no signs of normal distribution based on the Shapiro–Wilk test, and therefore, were represented as median and 25% and 75% quantiles for continuous data and the assessment of percentage distributions for categorical data. The differences in continuous and categorical data were determined using the Chi-square tests (χ^2) and Mann–Whitney U tests, respectively. The Spearman correlation coefficient was also used. The differences between the groups were considered statistically significant at the level of $P < 0.05$.

Results and Discussion

Forty-five people with moderate CP were examined. Group I consisted of 15 patients with angina pectoris I-II FC, 7 men (47%) and 8 women (53%); Group II included 15 people with AMI, 6 men (40%) and 9 women (60%); and Group III consisted of 15 patients with CP without CVD, 4 men (27%) and 11 women (73%).

Table 1 summarizes the general parameters of the patients examined. The average age of AMI patients was 72.7 ± 6.0 years, AP patients – 64.6 ± 3.4 years, and CP – 58.1 ± 4.8 years. The age of patients with CP without cardiovascular pathology was statistically significantly lower than in AMI patients, which is understandable since patient's age is a risk factor for the development of both periodontitis and cardiovascular disease. 9 of

Table 1: General parameters of examined patients

Parameter	Groups		
	AMI	AP I-II FC	CP without CVD
Age, M±Δ (minimum-maximum)	72.7±6.0* (66.7-78.7)	64.6±3.4 (61.2-68.0)	58.1±4.8 (53.3-62.9)
Sex, *n* (%)			
Male	6 (40)	7 (47)	4 (27)
Female	9 (60)	8 (53)	11 (73)
Smokers, *n* (%)	9 (60)	12 (80)	7 (47)
Gastrointestinal disease, *n* (%)	9 (60)	9 (60)	7 (47)
Arterial hypertension, *n* (%)	6 (40)	2 (14)	0
Hyperlipidemia, *n* (%)	12 (80)	10 (67)	4 (27)
Triglycerides (mmol/l), M±Δ (minimum-maximum)	2.15±0.20* (1.95-2.35)	2.14±0.40* (1.75-2.54)	1.02±0.30 (0.73-1.32)
Cholesterol (mmol/l), M±Δ (minimum-maximum)	5.33±0.19* (5.14-5.51)	5.16±0.35* (4.81-5.51)	3.69±0.61 (3.08-4.29)
HDL (mmol/l), M±Δ (minimum-maximum)	0.46±0.04* (0.42-0.50)	0.57±0.11* (0.46-0.67)	1.30±0.10 (1.20-1.40)
LDL (mmol/l), M±Δ (minimum-maximum)	4.59±0.20* (4.39-4.79)	2.88±0.17 (2.71-3.05)	3.12±0.20 (2.91-3.33)
EDVT BA, M±Δ (minimum-maximum)	4.51±0.93* (3.58-5.44)	9.69±0.32* (9.38-10.00)	10.70±0.27 (10.50-11.00)
CPITN, M±Δ (minimum-maximum)	2.73±0.25* (2.48-2.98)	2.77±0.23* (2.54-2.99)	1.53±0.18 (1.36-1.71)
OHIS (plaque), M±Δ (minimum-maximum)	1.63±0.14* (1.50-1.77)	1.41±0.15 (1.26-1.55)	1.19±0.24 (0.96-1.43)
OHIS (tartar), M±Δ (minimum-maximum)	1.17±0.18 (0.99-1.35)	1.24±0.23 (1.01-1.47)	1.12±0.20 (0.93-1.32)
PBI, M±Δ (minimum-maximum)	1.56±0.03* (1.53-1.58)	1.45±0.04* (1.41-1.49)	1.33±0.05 (1.28-1.38)
PDI, M±Δ (minimum-maximum)	7.45±0.08* (7.37-7.53)	7.37±0.06* (7.31-7.42)	7.20±0.03 (7.18-7.23)

*Statistically significant difference compared to CP group (P<0.05). M: Arithmetic mean; Δ: Half confidence interval; *n*: Number of patients surveyed; %: Relative frequency; AP I–II FC: Angina pectoris I–II functional classes; CVD: Cardiovascular disease; CP: Chronic periodontitis; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; PBI: Papilla bleeding index; PDI: Periodontal disease index; CPTIN: Community periodontal index of treatment need; OHIS: Green-vermillion index; FMD: Flow-mediated endothelium vasodilatation of the brachial artery

15 patients (60%) in Group I, 12 patients in Group II (80%), and 7 ones in Group III (47%) were smokers. Six AMI patients (40%) and 2 patients with AP (14%) were suffered from arterial hypertension. Nine patients with AMI (60%), 9 ones with angina (60%), and seven patients with CP without CVD (47%) had gastrointestinal disease. Significant differences in the levels of cholesterol, triglycerides, and some other biochemical indices were also revealed.

Hyperlipidemia was detected in 12 patients in Group I (80%), in 10 patients in Group II (67%), and in 4 patients in Group III (27%). Instances of hyperlipidemia in Groups II and III were 3 and 2.5 times less frequent than in Group I, respectively (χ^2 = 9.47, P = 0.09, η = 2). The cholesteric index of atherogenicity was determined according to the formula: AI = [ ] total cholesterol – [ ] high-density lipoprotein HDL/[ ] HDL. In Group III (AI = 1.8 ± 0.4), in Group II (AI = 8.1 ± 3.2), and in Group III (AI = 10.6 ± 3.3); that is, the index of atherogenicity in patients with AMI and AP corresponded to a high risk of coronary heart disease [Table 1].

The prevalence and intensity of dental caries in patients of all examined groups corresponded to the average level (index ICE). However, indices of oral hygiene (Oral Hygiene Index [OHI's] plaque and OHI's tartar), papillary marginal attached, Papillary Bleeding Index (PBI), and periodontal pockets depth differed significantly between the groups. The most significant difference was observed in Group I in comparison to Group III.

According to earlier studies, inflammatory periodontal diseases could negatively affect endothelial function in direct and indirect ways.[1,11,13] In patients with periodontitis, severe endothelial dysfunction was observed through endothelium-dependent vasodilation in the brachial artery. Among the examined patients, the highest values of this test were detected in patients of Group III (10.70% ± 0.27%). In Group II, they were 1.1 times lower (9.69% ± 0.32%), and in Group I, 2.4 times lower (4.51% ± 0.93%) with $P < 0.05$.

Using molecular genetics methods, we conducted the detection of periodontopathogenic species: *A. actinomycetemcomitans*, *T. forsythia*, *P. gingivalis*, *P. intermedia,* and *T. denticola* in SGB in all the examined patients [Table 2].

We discovered that *T. forsythia* DNA was most frequently found in patients of all the examined groups: In 12 patients with AMI (80%), in 11 with AP (73%), and in 13 with CP without CVD (87%), χ^2 = 2.13, η = 2, P = 0.345 (Tab. 2). T. denticola markers were frequently detected: In 10 patients with AMI (67%), in 7 ones with CP without CVD (47%), and only in 5 patients with AP (33%). However, the difference was not statistically significant between the compared groups (χ^2 = 3.38, η = 2, P = 0.185). Similar results were obtained in *A. actinomycetemcomitans* detection: It was found in 3 patients with AMI (20%), in 1 patient with AP (7%), and in 4 patients with CP without CVD (27%) (χ^2 = 2.13, η = 2, P = 0.345). *P. gingivalis* was discovered in 10 patients with AMI (67%), in 9 patients

Table 2: Frequency of periodontopathogenic bacterial species detection in examined patients' subgingival biofilm, *n* (%)

Bacterial species	Groups (%)			χ^2	η	*P*
	AMI	AP	CP without CVD			
Prevotella intermedia	2 (13.0)	1 (6.7)	8 (53.0)	10.3	2	0.006
Tannerella forsythia	12 (80.0)	11 (73.0)	13 (87.0)	2.13	2	0.345
Treponema denticola	10 (67.0)	5 (33.0)	7 (47.0)	3.38	2	0.185
Aggregatibacter actinomycetemcomitans	3 (20.0)	1 (6.7)	4 (27.0)	2.13	2	0.345
Porphyromonas gingivalis	10 (67.0)	9 (60.0)	5 (33.0)	7.2	2	0.027

AMI: Acute myocardial infarction; η: Number of degrees of freedom; *P*: Significance level; CVD: Cardiovascular disease; CP: Chronic periodontitis; AP: Angina pectoris

with AP (60%), and in 5 patients with CP (33%). That is, in patients with CVDs, it is detected statistically significantly two times more frequently than in persons without them (χ^2 = 7.2, η = 2, P = 0.027). *P. intermedia* was found in eight patients with CP without CVD (53%) and only in 2 patients with AMI (13%) and in 1 (7%) with angina (7%) (χ^2 = 10.3, η = 2, 0.006).

Table 3 shows the frequency of simultaneous detection of virulent bacterial species in periodontal pockets. DNA of periodontopathogenic bacterial species was detected in all patients of Group I. Among them, only one species of bacteria was identified in 3 patients (20%), two species simultaneously in 6 patients (40%), three species in 2 patients (13%), and four species in 4 patients (27%). In Group II, periodontopathogens were not detected in only 1 person (7%). One virulent bacterial species were identified in 5 patients (33%), two species simultaneously in 5 patients, and three types of microbes in four patients (27%). In Group III, bacteria were not detected in 1 patient (7%), one species in 1 person (7%), two species in 4 (27%), three species in 8 (53%), and four types of periodontopathogens in 1 (7%) patient.

Therefore, in the AMI group, three types of periodontopathogenic bacteria were detected with statistically significant frequency, which was four times less than in AP and CP groups – in 2 (13%) and 8 (53%) cases, respectively (χ^2 = 5.4, η = 1, P = 0.02). Although the associations of four bacterial species were more frequent in AMI patients than in those with angina and CP, the difference was not statistically reliable.

The bacterial load in the contents of periodontal pockets in patients of all the groups was approximately the same. At the same time, in AMI patients, the most virulent type of periodontopathogens, *P. gingivalis*, was detected more often than *P. intermedia*.

Our data confirmed the higher frequency of *P. gingivalis* presence in periodontal pockets in AMI and AP groups, in comparison with CP group, which is consistent with the results obtained by other authors. Thus, Stein *et al*. consider that *P. gingivalis* is a predictor of AMI since the relative risk of the disease is 13.6 (95% DI 3.1–59.8, P = 0.0005), even after adjusting of other AMI risk factors (age, sex, smoking, body mass index, and HDL level).[19] In patients with AMI, the incidence of *P. gingivalis* and *A. actinomycetemcomitans* or *A. actinomycetemcomitans*, *P. gingivalis,* and *T. forsythia* in periodontal pockets is significantly higher than in those without cardiovascular disease. The presence of *P. gingivalis* and *A. actinomycetemcomitans* in patients with periodontitis is associated with severe periodontal damage. These associations can influence the course of CVDs and can be a link to their development.[11,20] Earlier we had shown the low frequency of *P. intermedia* detection in the gingival sulcus of patients with periodontitis and CVDs.[8]

Adaptive immunity is assumed to enhance the inflammatory response in atheromatous plaques, which can lead to their rupture. Antibodies produced against plaque bacteria have a pro-inflammatory effect because they crossreact with endothelial cells and modified LDL and increase the incorporation of lipids into the inflammatory cells in blood-vessel wall. Some of these antibodies, as well as pro-inflammatory cytokines, can induce a Th1-type response in atheromas, activating macrophages, and increasing inflammation. The levels of immunoglobulin G against *P. gingivalis* are related to both myocardial infarction and periodontal disease, and hence, *P. gingivalis* could be a link between cardiovascular pathology and oral health condition.[20,21]

Khosravi Samani *et al*. reported that the risk of AMI in patients with 3 mm or more epithelial attachment loss is 4.0 (P = 0.0001), and Prosthodontic Diagnostic Index (PDI) in patients with AMI is significantly higher than in those with CP without CVD.[22] Nevertheless, the strength of correlation increases if more indicators of periodontal conditions are used.[23] Alman *et al*.(2011) reported a nonlinear positive correlation between bone loss and CVD, even if bone loss is mild (10%–20%).[24]

We analyzed all the data we received through Spearman correlation method. Significantly high correlation ($P < 0.05$) was found between dental diagnosis (moderate CP) and PDI (r = 0.9624), PBI (r = 0.9526), and periodontal pocket depth (r = 0.9241). We discovered direct moderate correlation between dental diagnosis and Community Periodontal Index of treatment Needs (CPITN) (r = 0.7336), tooth mobility (r = 0.6358),

Table 3: Frequency of detection periodontopathogenic bacterial compositions in the subgingival biofilm of examined patients, *n* (%)

Number of periodontopathogenic bacterial species detected simultaneously	AMI	AP	CP without CVD
0	0 (0.0)	1 (6.7)	1 (6.7)
1	3 (20.0)	5 (33.3)	1 (6.7)
2	6 (40.0)	5 (33.3)	4 (26.6)
3	2 (13.3)	4 (26.6)	8 (53.3)
4	4 (26.6)	0 (0.0)	1 (6.7)
5	0 (0.0)	0 (0.0)	0 (0.0)

CVD: Cardiovascular disease; CP: Chronic periodontitis; AP: Angina pectoris; AMI: Acute myocardial infarction

OHIs (r = 0.5507), patients' age (r = 0.3541), frequency of *P. intermedia* detection in periodontal pockets, serum levels of cholesterol (r = 0.3007), triglycerides (r = 0.2563), and LDL (r = 0.2783). The relation between dental diagnosis and endothelial dysfunction (r = −0.5653) and dental diagnosis and HDL serum level (r = −0. 3182) was moderate inverse.

Direct moderate correlations were defined between cardiologic diagnosis and serum HDL level (r = 0.4178), endothelium-dependent vasodilation in the brachial artery (r = 0.3284), and detection of three periodontopathogenic bacterial species in the periodontal pocket. Cardiologic condition weakly correlates with age (r = 0.1510), ICE (r = 0.2179), PDI (r = −0.1405), and PBI (r = −0.1708). Moderate inverse relations are found between the cardiological diagnosis and OHIs (r =-0.2148), hyperlipidemia (r = −0.2605), serum levels of LDL (r = −0.4656), triglycerides (r = −0.3438), and cholesterol (r = −0.3626).

The values of endothelium-dependent vasodilation in the brachial artery moderately correlated with cardiological diagnosis (r = 0.3284), HDL level (r = 0.6465), frequency of *P. intermedia* detection in periodontal pockets (r = 0.3828) and weakly correlated with gender (r = 0.1489), ICE index (r = 0.1599), and the presence of three periodontopathogenic bacteria species (r = 0.2702).

In this way, moderate inverse correlations were found between endothelial function and oral condition (r = −0.5653), PDI (r = −0.6595), PBI (r = −0.6618), CPITN (r = −0.6362), periodontal pockets' depth (r = −0.3825), OHIs (r = −0.33273), tooth mobility (r = −0.3897), hyperlipidemia (r = −0.44524), levels of triglycerides (r = −0.4675), cholesterol (r = −0.5078), and LDL (r = −0.4795). It should be noted that dysbiosis is accompanied not only by deterioration in oral health but also by the levels of peripheral blood lipids and endothelial dysfunction, especially in smokers despite the prehospital use of statins and other drugs. The distribution of age, smoking status, and the number of missing teeth did not differ depending on the group.

Conclusions

The data presented in this article emphasize the concept that the condition of periodontal tissues in patients with moderate chronic generalized periodontitis and AMI is characterized by unsatisfactory and poor hygiene, increased bleeding on probing, and other periodontal indices compared with groups of patients with AP and moderate generalized CP without cardiovascular pathology.

In the examined cohort of patients with AMI and AP in the SGB, *P. gingivalis* DNA was detected statistically significantly more often, but *P. intermedia* less often than in the control group. The levels of serum total cholesterol, LDL, and triglycerides were statistically significantly higher, and HDL lower in patients with AMI and AP than in those with CP without CVD.

In addition, endothelial dysfunction was observed in all the examined groups of patients. However, endothelium-dependent vasodilation in the brachial artery was statistically significantly lower in patients with AMI and AP than in participants with CP without CVD. These results indicate a scope for the prevention of CVD through the already well-established advice for the optimal oral health.

Periodontal status in patients with AMI is characterized by unsatisfactory and poor hygiene, increased indices of bleeding on probing, and periodontal pocket depth in comparison to groups of patients with angina pectoris and CP without cardiovascular pathology.

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Conflicts of interest

There are no conflicts of interest.

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