Periodontitis as a risk factor of coronary heart diseases?

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Abstract

Background: Unstable atherosclerotic plaque is a dangerous clinical state, possibly leading to acute coronary deficiency resulting in cardiac infarction. Inflammatory factor's role in creating pathological lesions in the endothelium of coronary vessels is frequently raised. This state may be caused by bacteria able to initiate clot formation in blood vessel and destabilizing atherosclerotic plaque already present. Source of these pathogens are chronic inflammatory processes occurring in organism, among them periodontal disease as one of more frequent. Aim of the work was to evaluate incidence of selected anaerobic bacteria in subgingival plaque and in atherosclerotic plaque in patients treated surgically because of coronary vessels' obliteration.

Methods: Study was performed on 20 individuals with chronic periodontitis. Subgingival plaque was collected from periodontal pockets deeper than 5 mm DNA test was used for marking eight pathogens responsible for periodontal tissues destruction. In the same patients, as well as in 10 edentulous individuals material from atherosclerotic plaque was collected during by-pass implantation procedure, and identical DNA testing occurred.

Results: In 13 of 20 patients pathogens most frequent in severe chronic periodontitis were found in coronary vessels. In 10 cases those bacteria were also present in atherosclerotic plaque. Pathogens linked with periodontal disease were also found in 7 of 10 edentulous individuals. Most frequently marked bacteria were: *Porphyromonas gingivalis* and *Treponema denticola*.

Conclusions: It seems that advancement of periodontal disease does not have influence on bacteria permeability to coronary vessels. Important is the presence of active inflammatory process expressed by significantly higher bleeding index in patients with marked bacteria in atherosclerotic plaque.

Key words: dental plaque, atherosclerosis.

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The incidence of acute cardiac syndromes (ACS) is closely associated with atherosclerotic plaque destabilization as the result of local inflammatory processes occurring its interior. Inflammation in patients at risk for ACS is manifested as elevated serum concentrations of inflammatory mediators such as C-reactive protein, fibrinogen, serum amyloid, or interleukin 6 (IL-6) [1]. For many years, attempts have been made to identify the microorganisms responsible for the progression and maintenance of this inflammatory reaction. In the 1990's several microorganisms were isolated from atherosclerotic plaque structures and identified: *Herpes simplex virus*, *Cytomegalovirus*, *Mycoplasma pneumoniae*, *Helicobacter pylori* and *Chlamydia pneumoniae* [2-5]. The last pathogen triggers macrophage activation resulting in increased secretion of proinflammatory cytokines, such as interleukin 1 beta (IL-1β), tumor necrosis factor alpha (TNF-α), interleukin 6 (IL-6). It also increases thrombogenic activity through activation of the thrombogenic system and disruption of fibrinolysis. All of these factors may be the cause of atherosclerotic plaque destabilization that may result in ACS [6,7].

The role of inflammatory factors in the etiopathogenesis of destructive changes in periodontal structures is unquestionable and has been studied for many years [8]. It is known that Gram-negative bacteria in developing dental plaque tend to overpopulate saprophytic Gram-positive microflora. They are a source of enzymes, toxins and many other metabolites directly or indirectly causing connective tissue and alveolar bone destruction.

Lipopolysaccharides (LPS), endotoxins released as the result of bacterial cell membrane fragmentation, are factors triggering the cascade of processes leading to further destruction. LPS cause macrophage activation, which results in the release of IL-1β, TNF-α, prostaglandin E_2 (PGE_2) and proteolytic enzymes – metalloproteinases (MMP's). Released cytokines acting on connective tissue cells (fibroblasts, neutrophils) stimulate them for further production of MMP's. The increase of the PGE_2 concentration activates osteoclasts, leading to destruction of the alveolar bone, while MMP's directly cause the destruction of extracellular connective tissue [9-12].

It seems reasonable to pose the question: is this immuno-inflammatory activity of bacteria limited to the periodontal environment, or can it influence, directly or indirectly, distant structures of the body? Recent evidence suggests that chronic infectious diseases increase atherogenesis and the risk of acute cardiac syndromes, and periodontitis is a persistent bacterial infection causing chronic inflammation in periodontal tissues.

Aim

The aim of the study was to evaluate the incidence of selected anaerobic bacteria in subgingival and atherosclerotic plaques of patients treated surgically because of coronary vessel obliteration.

Material and methods

The study group consisted of 30 individuals with a mean age of 57 years, hospitalized for coronary vessel obliteration at the Clinical Department of Cardiac Surgery, Medical University of Warsaw. These patients were qualified and prepared for by-pass procedures. In the study group, 10 individuals were edentulous, in 20 individuals severe generalized chronic periodontitis was diagnosed (more than 30% of dental pockets affected with clinical attachment loss – CAL), at least two pockets with a depth exceeding 5 mm).

Anamnesis and clinical examination was performed in all subjects, including detailed periodontal examination in patients with preserved teeth. The number of teeth, simplified plaque and bleeding indices, pocket depth (PD), clinical attachment loss (CAL), were recorded.

Bacteriological examination was performed with the use of the DMDx® DNA Test (MicroDenteX). This test is based on DNA hybridization on a nitrocellulose membrane ("slot blot" procedure) and analyzes the incidence of eight selected pathogens in the collected sample: *A.a. – Actinobacillus actinomycetemcomitans*, *P.i. – Prevotella intermedia*, *P.g. – Porphyromonas gingivalis*, *E.c. – Eikenella corrodens*, *C.r. – Campylobacter rectus*, *T.f. – Tanerella forsythensis*, *T.d. – Treponema denticola*, *F.n. – Fusobacterium nucleatum*. In the laboratory, the samples were exposed to a factor causing lysis of bacterial cells, resulting in the release and hybridization of DNA. Material so prepared was placed on a nitrocellulose membrane having the ability to bind denatured DNA. Following this the membrane was exposed to radiologically labeled DNA samples specific for each of the pathogens. If a given pathogen was present in the studied sample, hybridization with radioactive DNA occurred. Autoradiography was then performed with irradiation being proportional to the number of pathogens. An autoradiography film was placed on the membrane and left in the dark for several hours. The developed film was then scanned by video densitometry. The paraquantitative results of the test are presented as follows: negative result (below 10^3 pathogens), low level of bacteria (10^3-10^4 of pathogens in the sample), moderate level (10^4-10^5), and high level of bacteria (above 10^5).

To ensure reliability, the manufacturer suggests verification of patient selection. According to those instructions material was collected from pockets deeper than 5 millimeters, with no suppuration. None of the patients had undergone scaling or taken antibiotics within 6 months prior to the study. No antiseptic mouthwash was used by the patients for 12 hours before sampling.

Selected teeth were cleaned from bacteria and into two pockets deeper than 5 mm sterile paper points were inserted and left for 10 seconds. Points with collected material were inserted into a test-tube, labeled with a bar code exactly the same as the code on the patient's card. Material prepared in this manner was sent to the manufacturer.

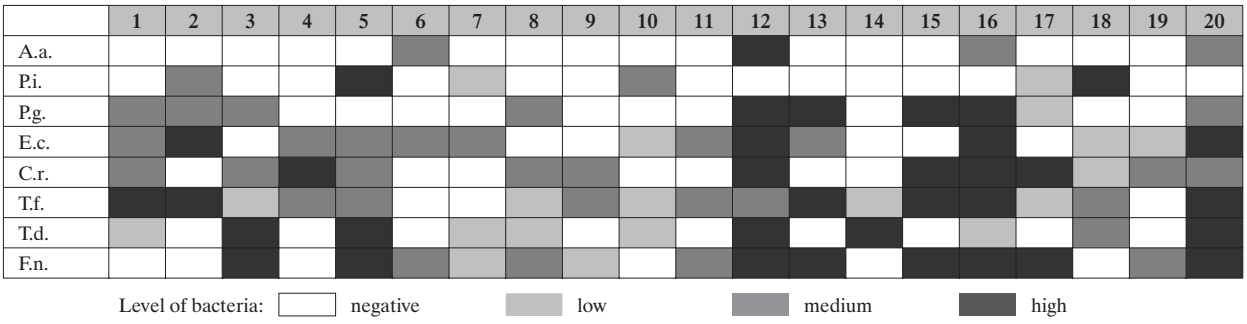
Subgingival plaque was examined in 20 patients with preserved teeth. Atherosclerotic plaque was sampled in all 30 patients during by-pass implantation surgical procedure. Sterile paper points were inserted into atherosclerotic plaque of open coronary vessels and after 10 seconds packed and sent to the laboratory.

To evaluate the degree of advancement of periodontal disease, point classification of periodontal disease it was scored according to Czerniuk; the results are, presented in *Tab. 1* was used according to Czerniuk [13]. Each of 4 examined parameters was evaluated on a numerical scale ranging as the number in the range from 1 to 4, where 1 means low, and 4 – a high value of the measured parameter (a patient with a total of 4 points

Tabele 1. **Numerical scale of advancement of periodontal disease**

Measured parameter	Value of selected parameter	Aquired points
Plaque index	0-25	1
	26-50	2
	51-75	3
	76-100	4
Bleeding index	0-25	1
	26-50	2
	51-75	3
	76-100	4
Pocket depth	0-1	1
	2-3	2
	4-5	3
	6 and more	4
Clinical attachment loss	0-2	1
	3-4	2
	5-6	3
	7 and more	4

Figure 1. **Incidence of bacteria in periodontal pockets**



In columns – patients; in verses – *A.a. – Actinobacillus actinomycetemcomitans*, *P.i. – Prevotella intermedia*, *P.g. – Porphyromonas gingivalis*, *E.c. – Eikenella corrodens*, *C.r. – Campylobacter rectus*, *T.f. – Tanerella forsythensis*, *T.d. – Treponema denticola*, *F.n. – Fusobacterium nucleatum*

Figure 2. **Incidence of bacteria in atherosclerotic plaque**

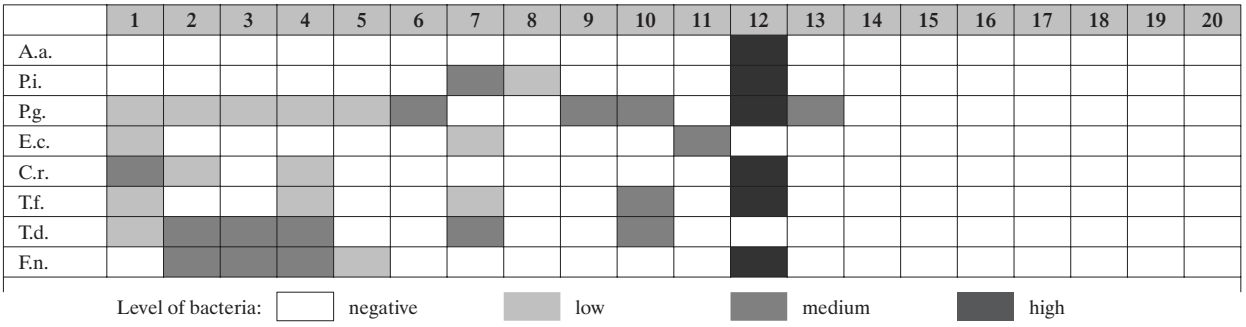


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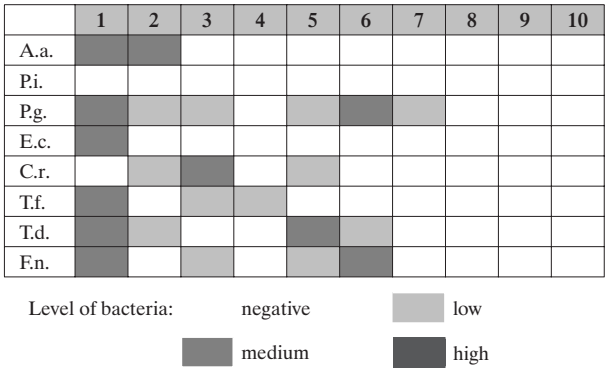
Figure 3. **Incidence of bacteria in atherosclerotic plaque (edentulous patients)**



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generally has a healthy periodontium, an individual with very advanced periodontal disease has got 16 points).

Results

The frequency of selected bacterial pathogens characteristic of severe periodontitis (in 40 pockets of 20 individuals) is presented in *Fig. 1*. In *Fig. 2* the results of bacteriological analysis of material collected from coronary vessels is shown (from the same 20 individuals). In each of 20 patients bacteria regarded as pathogenic for chronic periodontitis were isolated. In 13 of those persons such bacteria were present in coronary vessels; in 10 the same pathogens were present both in periodontium and in coronary vessels.

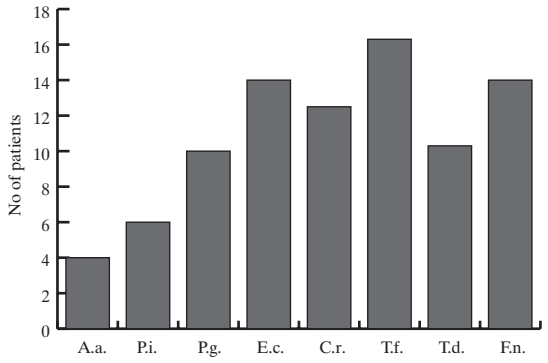
Also in 7 of 10 edentulous patients bacteria responsible for periodontitis were present in atherosclerotic plaque (*Fig. 3*).

To better show the presence of selected bacteria both in the periodontium and coronary vessels, their frequency is presented on *Fig. 4* and *5*. As *Fig. 4* shows, in pockets with a depth exceeding 5 mm, *T. forsythensis* was the most frequent (in 17 individuals), followed by *E. corrodens* and *F. nucleatum* (in 14 individuals), *C. rectus*, *T. denticola* and *P. gingivalis* (in 13, 11 and 10 individuals, respectively).

Fig. 5 presents the frequency of selected bacteria in atherosclerotic plaque of individuals with periodontitis. The most frequent were *P. gingivalis* (in 10 individuals) and *T. forsythensis* (in 6 individuals).

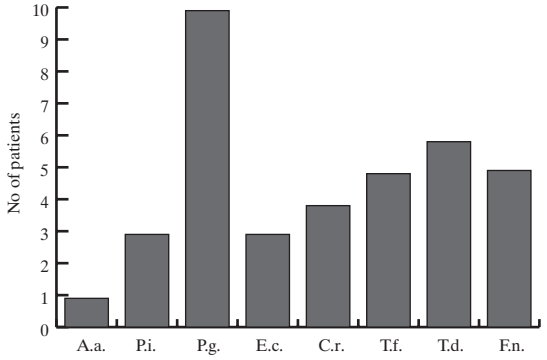
Fig. 6 displays the scores of periodontitis advancement in two subgroups of patients. 13 patients had bacterial pathogens in atherosclerotic plaque, 7 had bacteria present only in the periodontium. Comparison of these two subgroups shows a similar degree of periodontitis advancement (the group with bacteria in the atherosclerotic plaque – 11.77; the group without the those bacteria – 11.57), the slightly difference between the two groups was not statistically significant.

***Figure 4*. Frequency of bacteria in pockets of patients with periodontitis**



A.a. – Actinobacillus actinomycetemcomitans, *P.i. – Prevotella intermedia*, *P.g. – Porphyromonas gingivalis*, *E.c. – Eikenella corrodens*, *C.r. – Campylobacter rectus*, *T.f. – Tanerella forsythensis*, *T.d. – Treponema denticola*, *F.n. – Fusobacterium nucleatum*

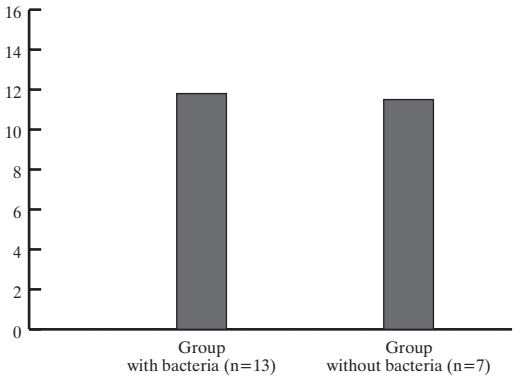
***Figure 5*. Frequency of bacteria in arterial plaque of patients with coronary disease**



A.a. – Actinobacillus actinomycetemcomitans, *P.i. – Prevotella intermedia*, *P.g. – Porphyromonas gingivalis*, *E.c. – Eikenella corrodens*, *C.r. – Campylobacter rectus*, *T.f. – Tanerella forsythensis*, *T.d. – Treponema denticola*, *F.n. – Fusobacterium nucleatum*

Periodontitis advancement expressed as the mean of each clinical parameter measured in both subgroups is displayed in *Tab. 2*. No difference was found in the number of preserved teeth between both subgroups, i.e. between – groups with and without bacteria atherosclerotic plaque (10.69 and 12.71, respectively). The mean percentage plaque index was high in both subgroups, higher in the subgroup without bacteria in atherosclerotic plaque (77.92% vs 93.00% in the other subgroup). There was no statistically significant difference in pocket depth and or clinical attachment loss between subgroups with and without bacteria in atherosclerotic plaque (3.19 mm vs 3.79 mm and 5.53 mm vs 5.69 mm, respectively). The only statistically significant difference was found in the mean percentage bleeding index ($p<0.005$), where a higher mean value was in patients with bacteria in atherosclerotic plaque (55.54%) compared with patients without bacteria in coronary vessels (31.00%).

***Figure 6*. Advancement of periodontal disease in correspondence to bacteria incidence in atherosclerotic plaque**



Discussion

Recent years have brought much research related to the potential connection between periodontitis and coronary disease. Many authors have demonstrated such a relationship; among others in the USA, De Stefano et al., basing on 14 years of research on 9 760 individuals aged 25-74 years, reported an increased risk of coronary disease in the group with periodontitis (25% higher) [14]. Also Beck et al. showed a possible connection between periodontitis and coronary disease. There may also be an association with increased mortality. The authors recorded the periodontal status in 1 147 individuals subjected to 20-year-long observation. In the group with advanced periodontal disease there was an increased risk of severe cardiac episodes and cerebral stroke (1.9 and 2.8 times, respectively) compared with the group without periodontitis [15]. Finnish authors have attempted to evaluate the relationship between chronic tooth-related infections, expressed as the number of lost teeth, and coronary disease. They showed that the incidence of coronary disease in individuals who lost less than a half of their teeth is 10% higher, rising to 2-fold higher in the group that lost more than a half of their dentition. The number of teeth lost proved to be a significant risk factor of coronary disease incidence, but only in smokers [16].

It has been hypothesized that Gram-negative bacteria can migrate from pathogenic dental plaque to the bloodstream, passing through the inflammatory, damaged epithelial attachment. The epithelial lining of the periodontal pocket, which frequently becomes thin and ulcerated in the course of periodontitis, may then provide an entry point for bacteria of subgingival plaque (regardless of their importance to periodontal disease) to gain an access to the underlying tissues and – eventually – to the vasculature. Spreading with the blood, they can affect inflammatory cells present in atherosclerotic plaque and stimulate a cascade of processes leading to atherosclerotic plaque instability [17-19].

The presented study does not unequivocally prove this theory, because bacteria were less frequent in atherosclerotic than in dental plaque, and there was no regular correlation between amount of bacteria in the subgingival pocket and in coronary vessel.

Tabele 2. **Mean values of parameters in relation to bacterial incidence in atherosclerotic plaque**

	Group with bacteria n=13			Group without bacteria n=7			p-level Mann--Whitney
	Mean	Median	SD	Mean	Median	SD	
Age	59.3077	57.0000	9.22302	55.0000	55.0000	6.87992	0.392879
No of teeth	10.6923	9.0000	7.33013	12.7143	12.0000	5.18698	0.274923
Plaque index	77.9231	79.0000	17.19720	89.1429	93.0000	6.81734	0.114628
Bleeding index	**55.5385**	**56.0000**	**21.60099**	**31.0000**	**34.0000**	**12.08305**	**0.006347**
Pocket depth (PD)	3.1923	3.1000	0.68369	3.7857	3.4000	1.09458	0.274923
Clinical attachment loss (CAL)	5.5308	5.4000	1.99348	5.6857	5.2000	1.53886	1.000000

On the other hand, our data does not exclude the bacterial theory, since *P. gingivalis*, *T. forsythensis* and *T. denticola* were found in the material collected from cardiac vessels; these bacteria are intimately related to chronic periodontitis. It is possible that a major role is played by *P. gingivalis*. Madianos showed its presence and ability to reproduce in the epithelium of the periodontal pocket [20], whereas Ishihara showed an increased level of *P. gingivalis* in atherosclerotic plaque, which was correlated to its presence in subgingival pockets [21]. Our own research corroborates this data, as *P. gingivalis* in material from atherosclerotic plaque was the most frequently isolated microorganism in our study. The role of this pathogen in the formation of atherosclerotic plaque is confirmed by research conducted on animals. It proved that periodontitis experimentally generated by *P. ginigvalis* causes greater accumulation of fat in the aorta compared with the control group [22].

Pussinen's research confirms the relationship between periodontitis and cardiac diseases. He showed a higher incidence of cardiac disease in seropositive *P. gingivalis* patients than in seronegative ones (14.0% vs 9.7%). He also reported a higher frequency of coronary disease in individuals with a higher level of antibodies (17.4% vs 11.1%). When discussing the results of linear regression models showing an association of combined antibody response (both for *A. actinomycetemcomitans* and *P. gingivalis*) with the incidence of coronary heart disease (direct) and serum HDL concentration (inverse), he suggests that periodontitis may impair reverse cholesterol transport [23].

Given the ability of *P. gingivalis* to multiply in the epithelium, its ability to penetrate into the bloodstream, the incidence of *P. gingivalis*-stimulated antibodies and role of this pathogen in thrombocyte aggregation through the presence of platelet aggregation-associated protein (PAAP) on its surface, it seems that *P. gingivalis* may play a significant role in the initiation of pathological processes in coronary vessels [24]. The lack of a correlation between the presence of *P. gingivalis* in subgingival and atherosclerotic plaque may be the result of immune reactions and elimination of the bacteria, both through the "first line of defense" (granulocyte infiltration) and specific reactions (humoral and cellular responses). It is possible that permeation of bacteria to the heart results from a disordered immunological response.

Our study also showed the presence of bacteria in atherosclerotic plaque regardless of edentulism. This confirms the findings of Pussinen, who reported a higher incidence of cardiac disease in edentulous patients (19.8% vs 12.1%) [23]. The observed results are difficult to interpret. They may indicate that pathogens present in atherosclerotic plaque could have infiltrated coronary vessels while the patients still had teeth (atherosclerotic plaque formation is a long-term process). The role of bacteria present in nasopharyngeal mucosa and removable prostheses can not be excluded, since they also can permeate through damaged epithelial tissue into blood vessels.

We also have found the same bacteria both in subgingival pockets ≥5 mm and in atherosclerotic plaque (10 of 20 individuals). The statistically significant correlation between bleeding index and presence of bacteria in atherosclerotic plaque may point indicate that active periodontal inflammation favors their infiltration into coronary vessels.

Ishihara has shown differences in the presence of bacteria in subgingival and atherosclerotic plaques in patients having 4 or more pockets deeper than 4 mm, compared with individuals having less than 4 such pockets [21]. He found higher levels of *P. gingivalis*, *T. denticola* and *A. actinomycetemcomitans* in the former subgroup. *P. gingivalis* was isolated from atherosclerotic plaques in 11 of 51 patients (in our material, from 10 of 20). In 11 of those patients, this bacteria was also isolated from the subgingival plaque in 10 (in our material, from 5 of 10).

Our results seem to confirm the possibility that bacteria associated with periodontitis can permeate into coronary vessels. It therefore seems important to limit this process. To achieve this goal, systematic dental plaque elimination is crucial, as well as elimination of retention points favoring its accumulation. The reduction of bacteria present in supragingival and subgingival plaque may be important prophylaxis of both of periodontal and coronary disease.

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