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The importance of the presence of aggregatibacter actinomycetemcomitans in sulcus gingivalis of patients with cardiovascular diseases

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

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Summary

Background: Over-replication of periodontal pathogens in the periodontium induces production of proinflammatory cytokines and C-reactive protein that can stimulate systemic inflammatory status and can initiate atherosclerosis and its consequences. In our pilot study we examined whether periodontal status and serum levels of interleukin-6 and C-reactive protein are associated with the presence of *Aggregatibacter actinomycetemcomitans* in the periodontium of patients with cardiovascular diseases (CVD).

Material/Methods: We randomly selected 38 of 166 outpatients with CVD, of which 21 patients had chronic ischemic heart disease (IHD) only and 17 had both IHD and essential hypertension (HT). The presence of *Aggregatibacter actinomycetemcomitans* (*A.a.*) in the periodontium evaluated by PCR was compared with the values of periodontal indices, namely probe depth (PD) and Community Periodontal Index of Treatment Need (CPITN), as well as with interleukin-6 (IL-6) and CRP serum levels.

Results: When comparing *A.a.*-positive and *A.a.*-negative groups of patients, no statistically significant differences were noticed as to the age and values of PD and CPITN, respectively. However, the proportion of CRP and IL-6 positive values was significantly higher ($p \leq 0.001$) among *A.a.*-positive than in *A.a.*-negative patients.

Conclusions: The presence of A.a. in patients with CVD may be associated with significantly higher serum levels of some proinflammatory markers.

key words: **cytokines • CRP • periodontal indexes • periodontal diseases**

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Background

The different forms of periodontitis are mostly chronic inflammations caused by specific anaerobic infections of the subgingival environment [1]. However, destruction of periodontal tissue contributes to proteolytic and osteolytic components of immunological and inflammatory reactions of the host, induced by persisting bacteria in the periodontium [2]. Apart from classic periodontal Gram-negative bacteria such as *A.a.*, *Porphyromonas gingivalis, Tanerella forsythia, Prevotella intermedia*, and *Eikenella corodens* [3], mammalian herpetic viruses can be also of importance [4]. In addition, human cytomegalovirus (CMV) is indicated as a potential etiological factor in atherosclerosis (5).

A.a. is one of the dominant periodontal pathogens with virulence factors that cause oral and non-oral infections [6]. It occurs in 30–100% of cases of different forms of periodontitis [7]. A.a. was also detected in aneurysms and atheromas of patients with cardiovascular diseases [8–10]; however, in some studies the presence of *A.a.* in atheromas was not confirmed [11]. Systemic infection with A.a. can initiate and accelerate inflammation in atherosclerotic plaque of the aortic sinus in hyperlipidemic mice [12,13]. Endothelial dysfunction can be induced by chronic bacterial infection of the periodontal tissues and is considered not only an initiative subclinical stage of the atherosclerotic process, but also a measurable clinical predictive factor of cardiovascular risk [14]. Moreover, *A.a.* and especially its lipopolysaccharide endotoxin significantly participates in the stimulation of production of different components of non-specific immune response (eg, increased aggregation of platelets, activation of B-lymphocytes, monocytes and macrophages, and stimulation of production of proinflammatory cytokines (IL-1, IL-6, IL-8) and CRP, whose increased serum levels were found to be associated with periodontitis and cardiovascular diseases [14–19].

Material and Methods

We randomly selected 38 of 166 outpatients with CVD, of which 21 patients had chronic ischemic heart disease (IHD) and only 17 had both IHD and essential hypertension (HT). We thought it would be useful to differentiate the group of patients with IHD +HT from those with IHD only, because other studies showed only a weak association between periodontitis and hypertension. Other clinical characteristics for the whole group of patients and in the control group, including age, sex, smoking history, hypercholesterolemia, diabetes mellitus and hypertension, were described in our previous study [24].

A standard calibrated probe was used to measure the depth of periodontal sulci of individual sextants for each tooth, thus evaluating a mean depth of periodontal sulcus of each tooth. From the values obtained, a mean value for each patient and then for the whole group of patients was calculated. Determination of Community Periodontal Index of Treatment Needs **(CPITN)** and Peridontal Depth (PD) were determined by measurement of CPITN probes and Williams periodontological probes.

Samples of *A.a.* detection were collected with the use of sterile paper points from the periodontal pocket of the teeth 11, 16, 26, 36 and 46 of each individual patient. In parallel, blood of patients was collected and buffy coats were examined for the presence of *A.a.*, CMV and *Chlamydia pneumoniae*. After DNA extraction from each sample, PCR analysis was performed using the primers specific for given microorganism.

In parallel, sera of patients were examined for the concentration of IL-6 and CRP. Detection of IL-6 was performed by IL-6 ELISA kit (Immunotech, France) with a positive value ⩾3 ng/L. CRP was detected by C-reactive protein ELISA kit (Immunodiagnostik, Germany) with a positive value ⩾3 mg/L. Both ELISA analyses were performed and calculated according to the manufacturers' instructions.

Cholesterol levels were evaluated by a standard method on biochemical analyser Vitros-250 with a positive value ⩾4.5 mmol/L.

In statistical analysis, Student`s test, Mann-Whitney's test, and chi-square test were employed. All tests were performed at the significance level α=0.05, with the use of statistical software SPSS 15.02022 for Windows.

Results

A.a. detection in the gingival sulcus by PCR, along with the age, sex, depth of periodontal pockets, CRP and IL-6 positivity in relation to the IHD only and IHD+HT is shown in Table 1. Of 38 examined patients, the presence of *A.a.* was confirmed in 17 (44.7%) – 6 with IHD only and 11 with IHD+HT. The mean age was similar in both groups of patients, irrespective of whether they were *A.a*-positive or *A.a.*-negative. Sex of patients was also evenly distributed, except for higher proportion of females in the group of *A.a.*-positive IHD patients. The numbers of CRP- and IL-6-positive patients among males and females was very similar.

No significant difference in the indices of the probe depth in relation to the presence of *A.a.* in the periodontal pockets or CVD presentation was observed (ie, the mean values of PD and CPITN in *A.a.*-negative patients were 2.80 and 2.32 mm, respectively, as compared to 2.77 and 2.48 mm in *A.a.*-positive patients [p=0.729 for PD and p=0.445 for CPITN]).

There was also no significant difference in the cholesterol levels and smoking habits in the followed CVD patients (data not presented). Both CRP and IL-6 occurred in significantly higher proportions of *A.a.*-positive than *A.a*-negative CVD patients ($p<0.001$ for both CRP and IL-6), regardless of whether they were recruited from the group of those with IHD only or those with both IHD+HT.

Using the same DNA extraction procedure and PCR analysis, we detected CMV in 6 patients; 4 were *A.a.*-positive (3 with IHD+HT and 1 with IHD only) and 2 were *A.a.*-negative (both with IHD only). *C. pneumoniae* in the followed patients was not detected.

Discussion

The presence of bacterial and viral pathogens in the periodontium induces a whole scale of inflammatory and immunological reactions. Several products of Gram-negative

Table 1. Some atherosclerosis risk factors in *A.a.*-positive and *A.a.*-negative cardiovascular patients.

Patients No.	*A.a.*	Age	Gender	PD	CPITN	CMV	CRP	
IHD only 6	Posit.	66±5.9	5 F, 1 M	3.00±0.60	2.42±0.62	1 83.3%	5 66.6%	4
IHD+HT 11	Posit.	57±10.4	5 F, 6 M	2.65±0.45	2.51±0.47	3 81.8%	9 81.8%	9
Total 17		60±9.9	10 F, 7 M	2.77±0.52	2.48±0.51	4 82.3%	14 76.5%	13
IHD only 14	Negat.	57±7.5	7 F, 7 M	2.84±0.59	2.41±0.74	2 21.4%	3 14.3%	2
IHD+HT 6	Negat.	60±4.4	2 F, 4 M	2.68±0.67	2.12±0.57	0 16.6%	1 –	0
Total 20		58±6.8	9 F, 11 M	2.80±0.60	2.32±0.69	2 20.0%	4 10.0%	2

P-values for PD, CPITN, IL-6, CRP and presence/absence of A.a. are given in section Results.

periodontal pathogens, particularly endotoxin, can lead to an increased inflammatory response of the host, thus representing an important risk factor for atherogenesis [20]. Pussinen et al. [21] found an association between an increased level of antibodies directed to *A.a.* and *P.g.* and increased endotoxemia with elevated serum concentrations of CRP and IL-6. They concluded that the relationship of the host to periodontal pathogens is an independent predictive factor of cardiovascular disease and that the simultaneous presence of *A.a.* and *P.g.* in the periodontal pockets in association with elevated concentrations of CRP and IL-6 significantly increase the risk of atherosclerosis.

In our study, we similarly found significant correlation between the presence of *A.a.* in the gingival sulcus and increased concentrations of IL-6 and CRP. Similar results were confirmed in another study, in which the presence of 1 bacterial pathogen (*A.a.*) was considered to be a possible risk factor of atherosclerosis. In that study, statistically significant correlation between CPITN values and cardiovascular diseases were detected [22]. In our previous studies on cardiovascular patients, we found a correlation between increased proportion of sera containing antibodies to *C. Pneumoniae* [23], CMV [24], CRP and IL-6. One can conclude that the presence of *A.a.* in the gingival sulcus of patients with CVD can stimulate atherosclerosis due to the increased serum concentrations of IL-6 and CRP.

Even though we detected *A.a.* in the periodontal pockets by PCR, the same technique did not reveal this agent in the buffy coat of patients who were studied. A similar discrepancy between detection of the agent in periodontal pocket and atheromatous plaques was also observed by Aimetti et al [11].

Conclusions

The presence of *A.a.* in patients with CVD was associated with significantly higher serum levels of some proinflammatory markers. These findings support the hypothesis that

the presence of *A.a.* added to other periodontal pathogens of the gingival sulcus (pocket) of patients with CVD can initiate and stimulate inflammatory process in atherosclerosis. Results of this study support the importance of individual and professional oral hygiene procedures in cardiac patients. However, the results should be interpreted with caution because this was only a pilot study with small sample sizes. Larger scale studies of patients are warranted to examine the role of *A.a.* and other periodontopathic bacteria in etiopathogenesis of atherosclerosis.

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