Antibodies to Periodontal Pathogens Are Associated With Coronary Heart Disease

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Objective—We analyzed the association of coronary heart disease (CHD) and serology of periodontitis in a random sample (n=1163) of men (aged 45 to 74 years) by determining serum IgG-antibodies to Actinobacillus actinomycetemcomitans and Porphyromonas gingivalis.

Methods and Results—CHD (n=159) was more prevalent among edentulous than dentate subjects (19.8% and 12.1%, P=0.003). In the dentate population, CHD was more common among subjects seropositive for P. gingivalis compared with those seronegative (14.0% and 9.7%, P=0.029). Accordingly, CHD was more prevalent in subjects with a high combined antibody response than those with a low response (17.4% and 11.1%, P=0.026). When adjusted for age and several CHD risk factors, the subjects with a high combined antibody response had an odds ratio of 1.5 (95% CI, 0.95 to 2.50, P=0.077) for prevalent CHD. In a linear regression model, the combined antibody response was directly associated with prevalent CHD (P=0.046) and inversely with serum HDL cholesterol concentration (P=0.050).

Conclusions—In conclusion, edentulousness and serum antibodies to major periodontal pathogens were associated with CHD. This suggests that periodontal infection or response of the host against the infection may play a role in the pathogenesis of CHD. (Arterioscler Thromb Vasc Biol. 2003;23:1250-1254.)

Key Words: atherosclerosis ■ cardiovascular diseases ■ antibodies ■ infection ■ inflammation

Recent evidence suggests that chronic infectious diseases increase atherogenesis and risk for CHD. Periodontitis is a persistent bacterial infection causing chronic inflammation in periodontal tissues. This common disease is characterized by the formation of deep periodontal pockets and destruction of connective tissue attachment and alveolar bone and may eventually lead to tooth loss. The systemic immunological response to periodontitis can be measured as elevated serum antibody levels against certain periodontopathogenic bacteria.

The most important pathogens responsible for periodontitis are gram-negative bacteria, particularly Actinobacillus actinomycetemcomitans and Porphyromonas gingivalis. They exhibit interspecies differences in virulence characteristics, such as leukotoxin production, endotoxin activity, and capability to adhere and invade host cells. Both pathogens have been found in atherosclerotic plaques, and certain clones of A. actinomycetemcomitans may also exert a particular potency to cause nonoral infections. The aim of this cross-sectional study was to investigate in a random population-based sample whether serum antibodies to periodontal pathogens are associated with CHD. The associations between antibodies to periodontal pathogens and established risk factors of CHD were also studied.

Methods

The participants of the Finnish Platelet Aggregation and Inflammation Study (PAIS) are a subsample of a large population-based risk factor study, which was conducted in Finland in 1997. The PAIS sample originally comprised 2000 men aged 45 to 74 years from eastern and southern Finland. From the 1163 men included in the present analyses, 159 (14%) were classified as CHD patients on the basis of the register of the National Social Insurance Institution on persons entitled to special reimbursement for CHD drugs. To receive the special reimbursement, the diagnosis of CHD is assigned by an internist or a cardiologist. The findings, which lead to the diagnosis, must be documented in terms of symptoms, ischemic changes in ECG, findings in coronary angiography, or a history of a previous CHD event. An expert physician of the Social Security Institute reviews the certificate written by the patient’s own physician, and if the evidence for CHD is found to be adequate, the right to special reimbursements is granted. Data on smoking, alcohol consumption, and education were collected by a questionnaire. Trained nurses measured blood pressure, height, and weight, as well as counted teeth and fillings. Serum lipid, fibrinogen, and sensitive C-reactive protein (CRP) concentrations were available from our previous study.

Serum IgG-class antibodies against A. actinomycetemcomitans and P. gingivalis were determined by a multisertype ELISA, where mixtures of 6 strains representing 5 serotypes and a nonserotyptable A. actinomycetemcomitans and 3 serotypes of P. gingivalis were used as antigens in the form of formalin-killed whole cells. Two dilutions (1:1500 and 1:3000 for A. actinomycetemcomitans and 1:100 and 1:200 for P. gingivalis) of each serum (stored at −70°C) were used as antigens in the form of formalin-killed whole cells.
in duplicate were used for the measurements, and the results (ELISA units [EU]) consisting of mean absorbances were calculated as continuous variables. The subjects were considered seropositive for \textit{A. actinomycetemcomitans} or \textit{P. gingivalis} when the corresponding antibody value was \(5.0\) EU, which represents the mean antibody level plus \(1.5\ SD\) of the periodontally healthy subjects in our earlier study.\textsuperscript{3} The threshold value of \(14.0\) EU for the high level of the combined antibody response (antibodies to \textit{A. actinomycetemcomitans} plus antibodies to \textit{P. gingivalis}) represents the corresponding mean value plus \(3\ SD\) of the periodontally healthy subjects.\textsuperscript{3}

Differences in continuous and categorical variables were examined with \(t\) tests and \(\chi^2\) tests, respectively. Variables with skewed distribution, such as triglyceride and fibrinogen concentrations, were log transformed before testing. Subjects with a systolic blood pressure \(\geq 140\) mm Hg, with a diastolic blood pressure \(\geq 90\) mm Hg, or using antihypertensive medication were categorized as hypertensive. The associations of combined antibody response as a continuous variable with CHD and its risk factors were examined for edentulous, dentate, and all subjects with linear regression analyses. In the basic model (model I), the independent variables included age, CHD status, serum total and HDL cholesterol concentration, hypertension status, and smoking. In a more extensive model (model II), the associations of antibody levels with education, body mass index (BMI), fibrinogen (or CRP), and triglyceride concentration, as well as the number of fillings and natural teeth, were also examined. The odds ratio of CHD for the combined antibody response was calculated using logistic regression, adjusting for established CHD risk factors. The statistical analyses were carried out using SAS program version 6.0 for VAX computers.

### Results

In this population-based random sample of middle-aged men, the subjects with CHD had significantly fewer teeth than the CHD-free subjects (12.2 versus 17.7, \(P<0.001\)) (Table 1). In the dentate subjects, the mean serum antibodies to \textit{A. actinomycetemcomitans}
mycetemcomitans did not differ significantly between those with CHD and those without CHD (4.86 ± 2.80 versus 4.72 ± 3.03 EU, P = 0.60), but the mean serum antibodies to P. gingivalis tended to be higher among subjects with CHD than those without CHD (7.05 ± 3.61 versus 6.42 ± 3.60 EU, P = 0.08). The prevalence of CHD was slightly but nonsignificantly higher among A. actinomycetemcomitans-seropositive than among seronegative subjects (13.6% versus 11.2%, P = 0.173), whereas among P. gingivalis-seropositive subjects, the prevalence of CHD was significantly higher than among seronegative ones (14.0% versus 9.7%, P = 0.029) (Table 1). In men with CHD, the number of teeth increased linearly (P for trend < 0.001) in the quartiles of the combined antibody response (antibodies to A. actinomycetemcomitans and P. gingivalis) (Figure). Throughout the antibody quartiles, the subjects with CHD had clearly fewer teeth than the CHD-free subjects; the difference was statistically significant in the first, second, and fourth quartile.

CHD was significantly (P = 0.026) more prevalent among dentate subjects with a high (≥ 14.0 EU) combined antibody response (n = 270) than among subjects with lower (< 14.0 EU) antibody response (Table 1). Among the subjects with a high combined antibody response, the prevalence of CHD increased in the categories of 1 to 10, 11 to 20, and > 20 remaining teeth (4.8%, 5.2%, and 7.4%, respectively). In these 3 groups, the proportion of subjects with a high antibody response was 1.3- to 1.6-fold higher in men with CHD than in those without CHD. Of the edentulous subjects, 13.8% (n = 30) had a high combined antibody response. In a logistic regression model after adjustment for age, the subjects with a high combined antibody response had an odds ratio of 1.4 (95% CI, 0.91 to 2.18; P = 0.121) for CHD. After additional adjustment for the CHD risk factors age, serum total and HDL cholesterol concentration, smoking, education, BMI, and hypertension status, the odds ratio of a high combined antibody response for CHD was 1.5 (0.95 to 2.50, P = 0.077). For the edentulous subjects with a high combined antibody response, the corresponding odds ratios for CHD were 2.1 (0.65 to 6.80, P = 0.213) and 3.2 (0.78 to 13.06, P = 0.107), respectively.

The basic multivariate linear regression model (model I) for all subjects revealed a positive association between the combined antibody response as a continuous variable and prevalent CHD (P = 0.046) and a negative association with HDL cholesterol concentration (P = 0.050) (Table 2). In a more extensive model (model II) for all participants, significant predictors of the combined antibody response included age, smoking (inversely), number of teeth, number of amalgam fillings (inversely), and dental status (edentulous or dentate), whereas prevalent CHD (P = 0.060) and HDL cholesterol concentration (P = 0.170) did not reach statistical significance. In edentulous subjects, the combined antibody response was positively associated with prevalent CHD (P = 0.002), smoking (inversely), and fibrinogen (P = 0.027) or sensitive CRP (β ± SE = 1.928 ± 0.651, P = 0.003).

**Discussion**

Diagnosis of periodontitis is usually based on clinical or radiographic examination. A more practical diagnostic tool in large epidemiological studies would be to use serology. Finding a suitable antigen for determining serum antibodies, which indicate clinical periodontitis, has been problematic, because pathogens in this infection comprise several genetically and serologically heterogeneous bacterial species.

However, our recently developed and validated multiseroype ELISA has sensitivity and specificity of 71% and 90% for finding clinically and radiographically diagnosed periodontitis. To our knowledge, the present study is the first to explore the association between CHD and serology of periodontitis. In the present dentate male population, the proportion of subjects with a high combined antibody response against periodontal pathogens was 29.3%, which is of the same magnitude as the earlier radiographically determined prevalence of severe periodontitis in the Mini-Finland Oral Health Study (32%).

In a linear regression model, we found a significant positive association between the combined antibody response and prevalent CHD. In addition to age, the only established CHD risk factor that the combined antibody response was associated with was serum HDL cholesterol concentration. The result suggests that periodontitis may be associated with a major CHD risk factor and low HDL cholesterol concentration and may impair reverse cholesterol transport. The actual association between smoking and antibodies to periodontal pathogens was not settled by this study. The combined antibody response was negatively associated with smoking (expressed as a 3-level variable: 1, never smokers; 2, ex-smokers; 3, current smokers), which was most probably attributable to the fact that the smokers had significantly
fewer teeth (14.6 ± 12.3) than the nonsmokers (19.7 ± 11.2) or subjects who had quit smoking (15.3 ± 12.5).

As in several earlier reports, also in this study CHD was associated with missing teeth and edentulousness. The number of remaining teeth, especially edentulousness, was an important confounder for the antibody values. The fact that the CHD patients were older and more frequently toothless caused a bias toward high and low antibody levels. In the lowest antibody quartile, the men with CHD had markedly fewer teeth than the CHD-free subjects. This suggests that among subjects with CHD, the tooth loss was a result of periodontitis followed by a notable decrease of corresponding serum antibodies. Actually, IgG-class antibodies to periodontal pathogens remain elevated after periodontal treatment but decrease soon after tooth extraction. In the present study, we did not have information on when the teeth were lost; however, the fact that 13.8% of the edentulous subjects had high antibody levels suggests that they had lost the teeth recently. It is well known that, besides periodontitis, untreated caries can eventually lead to the loss of teeth. However, the only data concerning caries in the present study was the number of fillings, which did not differ between the subjects with and without CHD when calculated per number of teeth.

Our investigation shows that studying the serology of a multipathogen infection is not without problems but that our multiserotype ELISA holds promises as a diagnostic aid for future epidemiological studies regarding associations between periodontal infection, response of the host to the infection, and systemic health. In conclusion, our results from this population-based study indicate that edentulousness, seropositivity for P. gingivalis, and high antibody response against the major periodontal pathogens are associated with prevalent CHD. The present study suggests that periodontal infection or response of the host against the infection may play a role in the pathogenesis of CHD.

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### References

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