Serum Antibody Levels to *Actinobacillus actinomyctetemcomitans* Predict the Risk for Coronary Heart Disease

Pirkko J. Pussinen, Kristiina Nyysönen, Georg Alfthan, Riitta Salonen, Jari A. Laukkanen, Jukka T. Salonen

**Objective**—The association between serum antibody levels to major periodontal pathogens and coronary heart disease (CHD) was analyzed in a prospective population-based study.

**Methods and Results**—The population comprised 1023 men (aged 46 to 64 years) in the Kuopio Ischemic Heart Disease Study. The subjects with CHD at baseline (n=113) were more often seropositive for *Porphyromonas gingivalis* IgA (38.9% versus 28.5%, *P*=0.021) and IgG (60.2% versus 46.7%, *P*=0.007) than those without CHD. During the 10-year follow-up, 109 men free from CHD at baseline experienced an acute myocardial infarction or CHD death. The men with an end point were more often seropositive for *Actinobacillus actinomycetemcomitans* IgA (15.5% versus 10.2%, *P*=0.019) than those who remained healthy. In the highest tertile of *A. actinomycetemcomitans* IgA-antibodies compared with the lowest one, the relative risk (RR) for an end point adjusted for CHD risk factors was 2.0 (95% confidence interval [CI], 1.2 to 3.3). In the *Porphyromonas gingivalis* IgA-antibody tertiles, the highest RR of 2.1 (1.3 to 3.4) was observed in the second tertile. All antibody levels correlated positively with the carotid artery intima-media thickness.

**Conclusions**—High-serum antibody levels to major periodontal pathogens are associated with subclinical, prevalent, and future incidence of CHD. Periodontal pathogens or host response against them may contribute to the pathogenesis of CHD. (*Arterioscler Thromb Vasc Biol. 2005;25:833-838.*)

**Key Words:** atherosclerosis ■ cardiovascular diseases ■ infection ■ inflammation ■ periodontitis

Periodontitis is a common bacterial infection of the tooth-supporting tissues. The prevalence of severe periodontitis is 10% to 15% in most populations and it increases with age.\(^1\) The host response to the persistent bacterial insult in periodontitis leads to chronic inflammation, which causes the symptoms of the disease: gingival bleeding, formation of deepened periodontal pockets, destruction of tooth attachment, and eventually loss of tooth. Bacteria, their components, and local inflammation may act as triggers leading to systemic inflammation. This can be observed as elevated concentrations of acute-phase reactants and inflammatory mediators, especially C-reactive protein, which has been shown to be directly associated with atherosclerosis.\(^2\)

A selection of epidemiological studies from the past decade suggests that periodontitis is an important risk factor for coronary heart disease (CHD).\(^3\) Most of the literature is based on clinical periodontal examinations or self-reported periodontitis. Therefore, interpretation of the results may experience the absence of standard measures for periodontal disease. However, data on the role of periodontal pathogens in atherogenesis is limited, although some bacterial species have been identified as etiologic agents in periodontitis.\(^4\) In 3 recent serological studies, however, high antibody levels to a periodontal pathogen, *Porphyromonas gingivalis*, have been found to associate with prevalent CHD,\(^5\) high degree of stenosis in plaque segments,\(^6\) and incidence of acute myocardial infarction (AMI) in a small study population.\(^7\)

The aim of the study was to analyze if antibody levels to the major periodontal pathogens, *Actinobacillus actinomyctemcomitans* and *P. gingivalis*, are associated with the risk of CHD and carotid intima-media thickness (IMT) in a prospective population-based study.

**Subjects and Methods**

A total of 2682 men were enrolled in the Kuopio Ischemic Heart Disease Risk Factor (KIHD) study conducted in Finland between 1984 and 1989.\(^8\) Data on socioeconomic status were collected during this examination. The Research Ethics Committee of the University of Kuopio approved the study, and all study subjects gave their written informed consent.
Study Design, Population, and Outcome

The present study population comprises 1023 men aged 46 to 64 years, who were enrolled in 1987 to 1989 and re-examined after 4 years in 1991 to 1993 (baseline). The subjects visited the study site of re-examination (1991 to 1993) twice with a 7-day interval. Blood pressure and body mass index were measured at the first visit. The IMT of the common carotid artery was scanned ultrasonographically and blood samples were drawn at the second visit. A self-administered questionnaire provided information on sociodemographic background, diseases, medication, and smoking habits.

Data on deaths were obtained from the National Death Certificate Register, and underlying cause of death was taken as that assigned at the Central Statistical Office of Finland. Data on nonfatal AMI were derived from the National Hospital Discharge Data Register. At baseline, 113 men reported to have CHD, angina pectoris, coronary bypass, or other coronary disease. Of the 910 men free from CHD at baseline, a total of 109 subjects experienced a cardiac end point, AMI, or CHD death during a mean follow-up of 10 years until year 2002.

Serum and Plasma Determinations

Venous blood samples were taken after fasting for 12 hours at baseline (1991 to 1993). Lipoproteins were separated from fresh serum samples by combination of ultracentrifugation and precipitation. Serum total, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, and triglyceride concentrations were determined by enzymatic methods, and apolipoprotein B (apoB) and apoA-I by immunoturbidimetry. The concentrations of serum glutamyltransferase were measured by the kinetic method following the recommendation of the European Committee for Clinical Laboratory Standards. Concentrations of plasma fibrinogen were determined based on clotting of diluted plasma with excess thrombin by a coagulometer.

Serum IgG and IgA class antibodies to A. actinomycetemcomitans and P. gingivalis were determined by multiserotype enzyme-linked immunosorbent assay (ELISA) from serum samples stored at −20°C. Two dilutions of each serum in duplicate were used and the results (ELISA units [EU]) consisting of mean absorbances were calculated as continuous variables. We included on each plate a high material was analyzed. The subjects were considered seropositive for A. actinomycetemcomitans and P. gingivalis when the corresponding IgG value was ≥50 EU or the IgA value was ≥20 EU, which represent the mean antibody levels plus 1.5×SD of periodontally healthy subjects. Combined antibody response in dentate subjects was computed by summing IgG levels to A. actinomycetemcomitans and P. gingivalis, and the value was considered “high” when it exceeded 14.0 EU, which represents the corresponding mean antibody level of periodontally healthy subjects plus 3×SD.

Statistical Analyses

The significance of differences between cases and controls were tested using t test or χ2 test. The significance of differences between IMT values in the tertiles of antibody levels was tested by t test. Cumulative survival as well as the relative risks and their 95% confidence intervals of cardiac end points in tertiles of A. actinomycetemcomitans and P. gingivalis antibody levels were estimated using Cox regression models. Confounding factors included in the model were age, smoking, plasma fibrinogen, diabetes, body mass index, medication for hypertension, socioeconomic status, and serum LDL and HDL cholesterol concentrations. The statistical analyses were performed using SPSS program version 10.0 for Windows.

Results

Both IgA and IgG antibody levels correlated positively with mean IMT, especially among dentate subjects (n=459): the correlation coefficients (r) for mean IMT and A. actinomycetemcomitans IgA and IgG levels, and for P. gingivalis IgA and IgG levels were 0.116 (P=0.013), 0.097 (P=0.038), 0.166 (P=0.001), and 0.146 (P=0.002), respectively. In all subjects free from CHD at baseline (n=907), mean IMT increased in the tertiles of A. actinomycetemcomitans IgA (P for trend 0.021), and P. gingivalis IgA (P<0.001) and IgG antibody levels (P=0.023) (Figure 1). The same trend was observed in the tertiles of combined antibody response in dentate subjects free from CHD (n=418) (P=0.005) (Figure 1). The edentulous subjects (n=481), who were free from CHD at baseline, had higher mean IMT than the subjects with natural teeth (Figure 1). The combined antibody response correlated negatively with serum HDL cholesterol (r=−0.073, P=0.019) and positively with HDL triglyceride concentration in the dentate population (r=0.133, P=0.004).

At baseline, a total of 113 men had already signs of CHD. They were older (60.6±5.1 versus 55.7±6.6 years), had higher serum cholesterol (5.61±1.08 versus 5.52±0.92 mmol/L), apoB (1.04±0.28 versus 0.96±0.25 g/L), triglyceride (1.96±1.18 versus 1.57±1.01 mmol/L), and plasma fibrinogen (3.41±0.66 versus 3.13±0.56 g/L) concentrations, and lower serum HDL cholesterol (1.00±0.29 versus 1.11±0.29 mmol/L) and apoA-I (1.15±0.18 versus 1.21±0.18 g/L) concentrations than the men free from CHD (n=910), respectively (for all differences P<0.001). They also more frequently had diabetes (13.3% versus 5.8%, P=0.003) and hypertension (81.4% versus 22.0%, P<0.001), and were reported to more often have claudication (22.1% versus 2.5%, P<0.001) and history of any CVD in the family (96.5% versus 84.5%, P=0.001), respectively. The men with CHD less often had their own teeth (34.8% versus 46.6%, P=0.019), and they were more often seropositive for P. gingivalis IgA (38.9% versus 28.5%, P=0.021) and IgG (60.2% versus 46.7%, P=0.007) than the men free from CHD. Furthermore, the dentate subjects with CHD (n=39) had more fre-
quently a high combined antibody response compared with the dentate subjects without CHD (n = 420) (35.9% versus 18.8%, \( P = 0.011 \)).

During the mean follow-up of 10 years, 46 (40.7%) men with a history of CHD at baseline experienced a secondary coronary event, an AMI, or CHD death. Antibody levels to periodontal pathogens were not associated with this coronary event. However, the men who had a secondary event more frequently wore dentures than the men who remained free from a coronary event (76.1% versus 57.6%, \( P = 0.046 \); RR for an end point 2.3, 95% CI, 1.02 to 5.40).

A total of 109 men free from CHD (n = 910) at baseline experienced an AMI or CHD death during the follow-up time of 10 years. The characteristics of these subjects with and without an end point are summarized in Table 1. The subjects with an end point were more frequently seropositive for \textit{A. actinomycetemcomitans} IgA than subjects without an end point (15.5% versus 10.2%, \( P = 0.019 \)). During the follow-up, 46 (5.1%) men with a high IgA class antibody level to \textit{A. actinomycetemcomitans} (third tertile) and 23 (2.5%) men with a low antibody level (first tertile) experienced an end point (\( P = 0.004 \) for difference) (Figure 2). The corresponding rates for the second and first tertiles of \textit{P. gingivalis} IgA class antibody levels were 48 (5.3%) and 24 (2.6%), respectively (\( P = 0.002 \) for difference) (Figure 2). The risk for CHD end point increased with increasing tertiles of \textit{A. actinomycetemcomitans} IgA antibody levels (Table 2). The multivariate RR for the second tertile of \textit{P. gingivalis} IgA antibody levels was also significant (\( P = 0.004 \)) compared with the first tertile, but the association was not completely linear (\( P \) for trend 0.015).

**Discussion**

We found that elevated serum IgA class antibody levels to periodontal pathogens, \textit{P. gingivalis} and especially to \textit{A. actinomycetemcomitans}, were associated with CHD incidence in a population-based prospective study among men free from CHD at baseline. The risk ratio for CHD was 2.0 to 2.1, which is of the same range as reported earlier for

<table>
<thead>
<tr>
<th>Parameter</th>
<th>With an End Point Mean (SD)</th>
<th>Without an End Point Mean (SD)</th>
<th>( P^* )</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age, y</strong></td>
<td>57.4 (6.5)</td>
<td>55.4 (6.6)</td>
<td>0.003</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>28.1 (3.7)</td>
<td>27.4 (3.5)</td>
<td>NS</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>1.00 (0.04)</td>
<td>0.98 (0.04)</td>
<td>0.005</td>
</tr>
<tr>
<td>Alcohol/wk, g</td>
<td>89.2 (116.6)</td>
<td>79.4 (115.1)</td>
<td>NS</td>
</tr>
<tr>
<td>GGT, U/L</td>
<td>32.7 (26.8)</td>
<td>31.3 (39.2)</td>
<td>NS</td>
</tr>
<tr>
<td>S-chol, mmol/L</td>
<td>5.77 (0.86)</td>
<td>5.48 (0.92)</td>
<td>NS</td>
</tr>
<tr>
<td>S-apoB, g/L</td>
<td>1.05 (0.29)</td>
<td>0.95 (0.24)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>S-HDL, mmol/L</td>
<td>1.07 (0.31)</td>
<td>1.11 (0.28)</td>
<td>NS</td>
</tr>
<tr>
<td>S-apoA-I, g/L</td>
<td>1.21 (0.19)</td>
<td>1.21 (0.18)</td>
<td>NS</td>
</tr>
<tr>
<td>S-TG, mmol/L</td>
<td>1.83 (1.30)</td>
<td>1.54 (0.96)</td>
<td>0.004</td>
</tr>
<tr>
<td>Mean IMT, mm</td>
<td>3.29 (0.53)</td>
<td>3.11 (0.57)</td>
<td>0.001</td>
</tr>
<tr>
<td>Mean maximal IMT, mm</td>
<td>0.94 (0.20)</td>
<td>0.86 (0.19)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>N, %</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>109 (12.0)</td>
<td>801 (88.0)</td>
<td></td>
</tr>
<tr>
<td>Diabetic</td>
<td>11 (10.1)</td>
<td>42 (5.2)</td>
<td>0.043</td>
</tr>
<tr>
<td>Hypertensive</td>
<td>39 (35.8)</td>
<td>161 (20.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Smokers</td>
<td>38 (34.9)</td>
<td>208 (26.0)</td>
<td>0.050</td>
</tr>
<tr>
<td>Any CVD in family</td>
<td>98 (89.9)</td>
<td>671 (83.8)</td>
<td>NS</td>
</tr>
<tr>
<td>Claudication</td>
<td>5 (4.6)</td>
<td>18 (2.2)</td>
<td>NS</td>
</tr>
<tr>
<td>Own teeth</td>
<td>50 (45.9)</td>
<td>370 (46.2)</td>
<td>NS</td>
</tr>
</tbody>
</table>

\*t test.
†χ² test.
CVD indicates cardiovascular disease; EU, ELISA units; GGT, glutamyltransferase; TG, triglycerides.
clinically diagnosed periodontitis. In addition, edentulousness and seropositivity for *P. gingivalis* were associated with prevalent CHD at baseline. Both of these pathogens are connected to aggressive forms of periodontal disease. *A. actinomycetemcomitans* occurs particularly in periodontal diseases manifesting at young age (younger than 35 years), but periodontitis caused by *P. gingivalis* develops usually among middle-aged or elderly people. Both *A. actinomycetemcomitans* and *P. gingivalis* may also be found in low proportions among periodontally healthy subjects and can therefore be considered part of the endogenous oral flora. DNA of these pathogens has been found in atherosclerotic plaques, and certain clones of *A. actinomycetemcomitans* may exert potency to cause nonoral infections.

Periodontitis is accompanied by a strong immune response to periodontal pathogens, which can be measured from saliva and serum. Individual IgG class antibody levels are quite stable over time and do not necessarily display activity or severity of the disease; for example, periodontal treatment decreases IgG class antibody levels only temporarily. The cutoff level to seropositivity in our assay is relatively low (mean antibody level plus 1.5×SD of periodontally healthy subjects) and validated using results from polymerase chain reaction detection. Therefore, seropositivity indicates that the individual is exposed to the pathogens, but that individual may be in a state of “carrier” rather than with an active disease. Another cutoff limit, which we have validated for our assay, specifies individuals with “a high combined antibody response.” Combining the IgG antibody levels of *A. actinomycetemcomitans* and *P. gingivalis* results in an assay with sensitivity of 71% and specificity of 90% for finding periodontitis in dentate subjects. In the present population, the percentage of subjects with “serologically diagnosed” periodontitis was 36% among men with CHD versus 19% among men free from CHD. The proportions are in the same range as in our earlier cross-sectional study conducted in 1997, in which percentages were 38% and 27%, respectively. These percentages are of the same magnitude as the earlier radiographically determined prevalence of severe periodontitis in the Mini-Finland Oral Health Study (32%), suggesting that the multisertype ELISA may be a suitable method to identify subjects with periodontitis in large-scale studies, in which only serum samples are available.

![Figure 2. Cumulative survival rates for coronary events. The men free from CHD at baseline were divided into tertiles according to serum *A. actinomycetemcomitans* and *P. gingivalis* IgA class antibody levels. The cumulative survival of AMI or cardiac death was analyzed by Cox regression model adjusted for age, smoking, plasma fibrinogen, diabetes, hypertension, socioeconomic status, and serum LDL and HDL cholesterol concentration.](image)

<table>
<thead>
<tr>
<th>Antibody to</th>
<th>Tertile</th>
<th>1st</th>
<th>2nd</th>
<th>3rd</th>
<th>P for Trend</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. actinomycetemcomitans</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgG Univariate</td>
<td>1.0</td>
<td>1.2 (0.74–1.90)</td>
<td>1.3 (0.79–2.00)</td>
<td>0.616</td>
<td></td>
</tr>
<tr>
<td>Multivariate*</td>
<td>1.0</td>
<td>1.2 (0.72–1.86)</td>
<td>1.2 (0.76–1.94)</td>
<td>0.695</td>
<td></td>
</tr>
<tr>
<td>IgA Univariate</td>
<td>1.0</td>
<td>1.8 (1.09–3.04)</td>
<td>2.2 (1.32–3.60)</td>
<td>0.009</td>
<td></td>
</tr>
<tr>
<td>Multivariate*</td>
<td>1.0</td>
<td>1.5 (0.91–2.58)</td>
<td>2.0 (1.21–3.33)</td>
<td>0.025</td>
<td></td>
</tr>
<tr>
<td><strong>P. gingivalis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgG Univariate</td>
<td>1.0</td>
<td>1.6 (1.01–2.60)</td>
<td>1.5 (0.91–2.39)</td>
<td>0.120</td>
<td></td>
</tr>
<tr>
<td>Multivariate*</td>
<td>1.0</td>
<td>1.3 (0.79–2.08)</td>
<td>1.2 (0.70–1.88)</td>
<td>0.606</td>
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<tr>
<td>IgA Univariate</td>
<td>1.0</td>
<td>2.2 (1.36–3.62)</td>
<td>1.8 (1.10–3.06)</td>
<td>0.006</td>
<td></td>
</tr>
<tr>
<td>Multivariate*</td>
<td>1.0</td>
<td>2.1 (1.26–3.37)</td>
<td>1.5 (0.87–2.47)</td>
<td>0.015</td>
<td></td>
</tr>
</tbody>
</table>

*Cox regression model adjusted for age, smoking, plasma fibrinogen, diabetes, medication for hypertension, socioeconomic status, and serum LDL+HDL cholesterol.*

Relative risk for AMI and CHD death (95% CI).
High IgA class antibody levels to periodontal pathogens in saliva indicate persistent periodontitis with active tissue destruction, whereas the significance of high IgA antibody levels in serum is not fully understood. However, local and systemic antibody levels correlate strongly with each other. Therefore, our results suggest that prevalent CHD and future risk of CHD are associated with active forms of periodontitis. Persistently elevated antibodies belonging to IgA class also appear to be one of the best serological markers of chronic *Chlamydia pneumoniae* infection.

The serum antibody levels to periodontal pathogens had a moderate positive correlation with IMT at baseline, indicating that exposure to periodontal pathogens may be related already to subclinical atherosclerosis. In 3 earlier studies, carotid artery plaque has been more common in subjects with severe or moderate periodontitis, high *P. gingivalis* IgG titers, or ≥10 missing teeth. Edentulousness or missing teeth has been found to be associated with CHD in several studies, and they can be considered as markers of past periodontal disease. Also in the present study, the use of dentures was associated with CHD, but it did not predispose to future coronary events in CHD-free subjects. However, the mean IMT was higher among the CHD-free men wearing dentures than those without dentures. These observations imply that in the final stage of periodontitis, when the teeth are already lost, “the damage is done” regarding atherosclerosis. However, denture-related mucosal lesions are also important determinants of CVD risk in edentulous subjects and associated with elevated C-reactive protein concentrations. Furthermore, edentulosity is strongly associated with low socioeconomic status and thereby also with other chronic infections, which have been recognized as risk factors for CHD separately and as contributors to the “infectious burden.” Because chronic infections and CHD usually share common etiopathogenic factors, eg, smoking and low socioeconomic status, the nature of their association is difficult to explore in humans.

The underlying mechanisms explaining the association between periodontitis and increased risk for CHD are still unclear. In periodontitis, the local inflammatory state and/or the long-term systemic exposure to the pathogens or their virulence factors act as triggers for systemic inflammation, which can be detected as elevated concentrations of inflammation markers. This is supported by a recent study showing that mice administered via oral and anal application with *P. gingivalis* displayed local periodontal infection and exacerbated early atherosclerotic lesions. Furthermore, recent studies on human subjects indicate that periodontitis increases the proatherogenic properties of LDL, thereby directing lipoprotein metabolism in an unfavorable direction. Similarly, as in our earlier study, in the present study the combined antibody response correlated inversely with serum HDL cholesterol concentration. Furthermore, here the antibody response correlated directly with HDL triglyceride concentration, which is the first to be affected by inflammation/infection. These observations suggest that periodontitis may interfere with HDL metabolism and thereby decrease proper removal of excess peripheral cholesterol.

Recent studies on the role of periodontal pathogens in atherosclerosis have concentrated on *P. gingivalis*. In addition to studies on animal models, cell culture studies demonstrate that vesicles shed from the surface of *P. gingivalis* promote binding of LDL to macrophages, LDL modification, and macrophage-derived foam cell formation, a sign of early atherosclerosis. Periodontitis is, however, a multibacterial disease, and studies on the role of other pathogens than *P. gingivalis* in CHD are scarce. To our knowledge, the present study is the first to suggest that an infection by *A. actinomycetemcomitans* may be associated with the incidence of CHD. Consistent with our findings, high serum levels of IgA antibodies to *A. actinomycetemcomitans* have been found to predispose to cerebrovascular stroke in subjects free from CVD at baseline.

Our study suggests that high IgA antibody levels to both *P. gingivalis* and *A. actinomycetemcomitans* are associated with CHD. These results also indicate that serum antibodies to periodontal pathogens might be worth screening when mapping individual risk factors for CVD.

**Acknowledgments**

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**References**


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