ORIGINAL ARTICLE

Level of serum antibody against a periodontal pathogen is associated with atherosclerosis and hypertension

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Inflammation has a role in the pathogenesis of atherosclerosis, which causes hypertension. Results from some studies have suggested links between periodontal disease and atherosclerosis, but links between periodontal disease and hypertension have been seldom studied. We investigated whether periodontal disease and serum antibody level were associated with hypertension. We studied 127 patients (93 men and 34 women, mean age 68 ± 9 years) who were admitted with ischemic heart disease to our institution. A composite periodontal risk score was calculated from five periodontal vector scores. The levels of serum antibody against *Porphyromonas gingivalis* (Pg) were measured. Pulse pressure, mean blood pressure (BP) and pulse wave velocity were used as indices of atherosclerosis. We divided patients into two groups according to the levels of serum antibody against Pg: higher or equal to the median (high Pg antibody group) and lower than the median (low Pg antibody group). There was no difference in the use of antihypertensive agents between the two groups. The composite periodontal risk score ($P=0.0003$), systolic BP ($P=0.030$), diastolic BP ($P=0.038$), pulse pressure ($P=0.050$) and mean BP ($P=0.055$) were higher in the high Pg antibody group than in the low Pg antibody group. The composite periodontal risk score ($r=0.320$, $P=0.0003$), systolic BP ($r=0.212$, $P=0.017$), diastolic BP ($r=0.188$, $P=0.035$) and mean BP ($r=0.225$, $P=0.011$) correlated with the level of serum antibody against Pg, even after adjustment for age. An elevated antibody level against Pg indicates advanced periodontal disease and suggests advancement of atherosclerosis and hypertension.

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INTRODUCTION

Atherosclerosis is the major cause of ischemic heart disease (IHD). Although the traditional risk factors, hypertension, dyslipidemia, diabetes and smoking, are established, they do not fully account for the risk of IHD, suggesting that other mechanisms contribute to the pathogenic process.^{1,2} Emerging evidence suggests that inflammation in response to specific pathogens is an additional risk factor for atherosclerosis.³ The nature and source of the inflammation, however, remain unclear. Periodontal disease, a very common chronic infection of the tissue surrounding teeth and a significant predictive factor for IHD,⁴ is caused by infection with predominantly Gram-negative bacteria.

Infectious agents might also provide inflammatory stimuli that accentuate atherogenesis.^{5,6} Chronic extravascular infections (for example, gingivitis, prostatitis and bronchitis) can augment extravascular production of inflammatory cytokines, which may

accelerate the evolution of remote atherosclerotic lesions. *Porphyromonas gingivalis* (Pg) is the primary pathogenic agent of adult periodontal disease⁷ among the well-known periodontal pathogens, for example, *Actinobacillus actinomycetemcomitans*, *Prevotella intermedia*, *Treponema denticola*, *Tannerella forsythensis* and Pg. Antibodies against Pg, a prominent periodontal pathogen, can be harvested from atheromas during human autopsies,^{8,9} as can viable bacteria.¹⁰ Animal models have demonstrated that bacterial seeding with strains of Pg accelerates the formation of aortic and coronary atherosclerotic plaques in pigs¹¹ and aortic lesions in mice.¹²

Despite the high prevalence of hypertension in the general population and its prognostic importance, limited data are available on the relationship between elevated blood pressure (BP), hypertensive organ damage and periodontal disease.¹³ Therefore, we investigated whether periodontal disease was associated with hypertension in patients with IHD.

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METHODS

Study population

We studied 127 patients who were admitted with IHD. There were 93 men and 34 women, with a mean age of 68 ± 9 years. All patients with IHD had angiographic documentation of $>50\%$ organic stenosis in one or more major coronary arteries, or had a history of percutaneous coronary intervention or coronary artery bypass grafting.

Patients with heart failure, valvular heart disease, cardiomyopathy, severe arrhythmia and infection were excluded from this study. Written informed consent was obtained from each patient. The study protocol was in agreement with the guidelines of the ethics committees at our institutions.

Blood sampling

Samples for serum antibody levels against Pg were obtained from an antecubital vein in all patients on admission. All blood samples were immediately centrifuged at 3000 r.p.m. for 10 min at 4°C , and aliquots of samples were stored at -80°C until analysis.

Enzyme-linked immunosorbent assay

Serum antibody levels against Pg were measured by two technicians blinded to the patients' characteristics. An enzyme-linked immunosorbent assay was used to measure the serum levels of IgG antibody against Pg (Pg-IgG) and an antibody titer was grown in Modified Gifu anaerobic medium (Nissui Pharmaceutical, Tokyo, Japan) broth under anaerobic conditions at 37°C for 2 days. Bacteria were washed in phosphate-buffered saline (PBS) and suspended at an optical density of 0.6 (at 600 nm). After a 1:100 dilution in PBS, 100 μl aliquots of the suspension were incubated at 4°C overnight in 96-well microtiter plates Breakable EB (Thermo Electron, Vantaa, Finland) with a cover. Plates were washed three times with PBS, and nonspecific binding was blocked with 350 μl of 2% bovine serum albumin, 5% sucrose and 0.1% sodium azide in PBS and incubated covered at 37°C for 4 h. The plates were then sealed up and stored in aluminum bags at 4°C until the assays.

All serum samples and reference serum were diluted in pH 7.0 1 mol l^{-1} phosphoric acid buffer solution (PABS) containing 1% bovine serum albumin, 0.1% sodium azide, 5 mmol l^{-1} EDTA- Na_2 and 5 mmol l^{-1} MgCl_2 . Reference IgG was purified with a Protein G column (Amersham Biosciences, Uppsala, Sweden) from high IgG titer serum against Pg, and was used to create a standard curve to calculate arbitrary enzyme-linked immunosorbent assay units. Reference IgG was diluted from 10- to 390-fold in PABS. Each patient's serum and the reference IgG were diluted 420-fold in PABS. Aliquots (100 μl) of each dilution of patient or reference IgG were added in duplicate to the wells, and plates were incubated covered at room temperature for 1 h. After washing six times with PBS containing 0.05% tween-20, the wells were incubated with 100 μl of antihuman IgG labeled with peroxidase (DakoCytomation, Glostrup, Denmark) at room temperature for 1 h covered. Plates were washed eight times in PBS containing 0.05% tween-20 and incubated with peroxidase substrate (3,3',5,5'-tetramethylbenzidine; DakoCytomation) at room temperature for 30 min. The reaction was stopped by the addition of 100 μl 2 N H_2SO_4 and the optical density was read at 450 nm and a subwavelength of 650 nm using a Dynatech MR 5000 plate reader (Dynatech, Yokohama, Japan). A standard curve of the optical density of reference IgG was plotted against the serum dilution. Individual serum IgG antibody levels were calculated from the IgG reference curves and expressed as relative arbitrary enzyme-linked immunosorbent assay units. We then divided study patients into two groups according to the serum antibody values. Patients with serum antibody values greater than or equal to the median were assigned to high Pg-IgG group, and those with values lower than the median were assigned to low Pg-IgG group.

Periodontal examination

All patients received a thorough periodontal examination comprising a full-mouth series of dental radiographs and a comprehensive oral examination with assessments of the presence of plaque, bleeding on probing and pocket probing depths (at four surfaces per tooth: mesiobuccal, mid-buccal, distobuccal and mid-lingual). The proportional distributions of the presence of plaque, bleeding on probing and pocket probing depth (<4.0 , 4.0 – 5.0 and ≥ 6 mm)

were calculated and used as subject-based data for the analysis. The number of teeth with visible signs of gingival recession and the number of remaining teeth were also accounted for. A dentist who was blinded to patients' characteristics evaluated the periodontal status based on the assessments and radiographs.

Periodontal risk scores based on vector scores

We calculated periodontal scores for all patients based on the data sheets written by the dentist, while referring to the periodontal pentagon risk diagram reported by Renvert *et al.*¹⁴ Briefly, the five vectors were the following: (1) the proportion of sites with bleeding on probing, (2) the number of sites with a pocket probing depth ≥ 6.0 mm, (3) the number of teeth lost in the past deducted from a total of 28 teeth, (4) the proportion of mesial/distal sites with evidence of a distance from the cement–enamel junction to bone level (bone loss) greater than or equal to half the tooth length and (5) smoking status with regard to packs per year. The five vector scores were calculated, and the total was used as the composite periodontal risk score.

Measurement of pulse wave velocity

We used pulse wave velocity (PWV) as the index for atherosclerosis. A medical technologist measured the PWV and BP of each subject twice using a PWV/ABI device (Nippon Colin, Komaki, Japan) while the subject was at rest in a supine position. This device, approved by the US Food and Drug Administration as VP-2000/1000, can monitor bilateral brachial and ankle pressure wave forms.

Statistical analysis

Data are given as mean $\pm$ s.d. or median. The comparisons of continuous data between the two groups were performed with an unpaired *t*-test for patient characteristics or Mann–Whitney *U*-test for periodontal scores and antibody levels. The frequency data between the two groups were compared by the χ^2 -test. Linear regression analysis was used to determine the correlation between two measurements. Probability levels <0.05 were considered statistically significant.

RESULTS

Patient characteristics

All study patients had at least one coronary risk factor. Of the study patients, 78 (61.4%) were diagnosed with hypertension, 58 (45.7%)

Table 1 Comparison of clinical characteristics between high Pg-IgG group and low Pg-IgG group

Characteristics	High Pg-IgG group	Low Pg-IgG group	P
Age (years)	70.0 $\pm$ 9.3	66.3 $\pm$ 9.0	0.024
Men/women	46/18	47/16	0.729
Body mass index (kg m^{-2})	24.1 $\pm$ 4.1	24.3 $\pm$ 3.3	0.774
Hypertension	41	38	0.666
Diabetes mellitus/impaired glucose tolerance	28/17	30/12	0.688
HbA1c (%)	6.5 $\pm$ 1.4	6.4 $\pm$ 1.1	0.783
Dyslipidemia	45	43	0.803
LDL cholesterol (mg dl^{-1})	108 $\pm$ 28	105 $\pm$ 27	0.571
HDL cholesterol (mg dl^{-1})	52 $\pm$ 14	53 $\pm$ 14	0.557
Triglyceride (mg dl^{-1})	118 $\pm$ 45	118 $\pm$ 55	0.966
Smoking (current/past)	8/25	13/22	0.465
Number of antihypertensive agent	1.8 $\pm$ 0.9	1.8 $\pm$ 1.0	0.955
Angiotensin-converting enzyme inhibitor	21 (31.3%)	19 (28.6%)	0.8950
Angiotensin II receptor blocker	25 (39.1%)	27 (42.9%)	0.6667
Alpha blocker	1 (1.6%)	1 (1.6%)	0.9911
Beta blocker	18 (26.6%)	24 (38.1%)	0.1672
Calcium channel blocker	49 (76.6%)	39 (61.9%)	0.0744
Diuretic	1 (1.6%)	2 (3.2%)	0.5534

Abbreviations: HbA1c, hemoglobin A1c; HDL, high-density lipoprotein; LDL, low-density lipoprotein; Pg-IgG, IgG antibody against *Porphyromonas gingivalis*.

with diabetes mellitus, 29 (22.8%) with impaired glucose tolerance and 87 (68.5%) with dyslipidemia. There were 21 (16.5%) current smokers and 47 (37.0%) past smokers. There were 117 (92.1%) patients using antihypertensive agents, and the average numbers of antihypertensive agents used were 1.8 ± 0.9 and 1.8 ± 1.0 in the high Pg-IgG and low Pg-IgG groups, respectively. There was no significant difference in the frequency of each hypertensive agent between the groups. A comparison of clinical characteristics according to serum antibody levels is shown in Table 1. There were no significant differences in the clinical characteristics, except for age, between the high Pg-IgG group and the low Pg-IgG group.

Table 2 Comparison of clinical vectors between high Pg-IgG group and low Pg-IgG group

Characteristics	High Pg-IgG group	Low Pg-IgG group	P
Systolic blood pressure (mmHg)	132 ± 18	125 ± 22	0.030
Diastolic blood pressure (mmHg)	76 ± 12	72 ± 12	0.038
Pulse pressure (mmHg)	57 ± 15	51 ± 16	0.050
Mean blood pressure (mmHg)	94.7 ± 12.3	90.1 ± 14.6	0.055
Pulse (per min)	73 ± 11	68 ± 13	0.023
Mean pulse wave velocity (cm s^{-1})	1760 ± 359	1659 ± 281	0.096
Composite periodontal risk score	12.2 ± 5.7	8.5 ± 5.4	0.0003
Bleeding on probing	3.08 ± 1.99	1.89 ± 1.99	0.001
Pocket probing depth ≥ 6.0 mm (%)	1.21 ± 1.65	0.33 ± 0.62	0.0001
Number of teeth lost	3.25 ± 1.89	2.86 ± 1.86	0.239
Bone loss greater than or equal to half the tooth length	2.25 ± 2.07	1.27 ± 1.82	0.005
Smoking status (pack per year)	2.41 ± 2.48	2.11 ± 2.40	0.490

Abbreviation: Pg-IgG, IgG antibody against *Porphyromonas gingivalis*.

Periodontal risk score and serum antibody values

The composite periodontal risk score was higher in the high Pg-IgG group than in the low Pg-IgG group ($P = 0.0003$; Table 2), and it correlated with Pg-IgG levels ($r = 0.320$, $P = 0.0003$; Figure 1a). In addition, the vector score for the proportion of sites with bleeding on probing was higher in the high Pg-IgG group than in the low Pg-IgG group ($P = 0.001$; Table 2), and it correlated with Pg-IgG ($r = 0.273$, $P = 0.002$; Table 3). The vector score for the number of sites with a pocket probing depth ≥ 6.0 mm was higher in the high Pg-IgG group than in the low Pg-IgG group ($P = 0.0001$), and this correlated with Pg-IgG ($r = 0.314$, $P = 0.0004$; Table 3). The vector score for bone loss greater than or equal to half the tooth length was higher in the high Pg-IgG group than in the low Pg-IgG group ($P = 0.006$; Table 2), and it correlated with Pg-IgG ($r = 0.307$, $P = 0.0005$; Table 3). In addition, the periodontal risk score significantly correlated with Pg-IgG, even after adjustment for age (Table 3).

BP and serum antibody values

Systolic BP was higher in the high Pg-IgG group than in the low Pg-IgG group ($P = 0.030$; Table 2), and it correlated with Pg-IgG levels ($r = 0.212$, $P = 0.017$; Figure 1b). Diastolic BP was also higher in the high Pg-IgG group than in the low Pg-IgG group ($P = 0.038$), and it correlated with Pg-IgG ($r = 0.188$, $P = 0.035$; Figure 1c). In addition, systolic and diastolic BP remained correlated with Pg-IgG after adjustment for age (Table 3).

Atherosclerotic index and serum antibody values

Pulse pressure was higher in the high Pg-IgG group than in the low Pg-IgG group ($P = 0.050$; Table 2). Mean BP tended to be higher in the high Pg-IgG group than in the low Pg-IgG group ($P = 0.055$; Table 2), and it correlated with Pg-IgG ($r = 0.225$, $P = 0.011$; Figure 1d), even after adjustment for age (Table 3). Mean PWV tended to be higher in the high Pg-IgG group than in the low Pg-IgG group ($P = 0.096$; Table 2), and tended to correlate with Pg-IgG

Figure 1 Correlation between the levels of IgG antibody against *Porphyromonas gingivalis* (Pg-IgG) and each factor: (a) composite periodontal risk score, (b) systolic blood pressure, (c) diastolic blood pressure and (d) mean blood pressure.

Table 3 Correlation with serum level of Pg-IgG before and after adjustment for age

	r	P	r*	P*
Systolic blood pressure (mm Hg)	0.212	0.017	0.265	0.012
Diastolic blood pressure (mm Hg)	0.188	0.035	0.275	0.008
Pulse pressure (mm Hg)	0.140	0.118	0.195	0.091
Mean blood pressure (mm Hg)	0.225	0.011	0.300	0.003
Pulse (per min)	0.038	0.669	0.170	0.163
Mean pulse wave velocity (cm s ⁻¹)	0.171	0.067	0.223	0.056
Number of antihypertensive agent	0.025	0.778	0.168	0.176
Composite periodontal risk score	0.320	0.0003	0.351	0.0004
Bleeding on probing	0.273	0.002	0.315	0.002
Pocket probing depth ≥6.0 mm (%)	0.314	0.0004	0.366	0.0002
Number of teeth lost	0.141	0.116	0.185	0.119
Bone loss greater than or equal to half the tooth length	0.307	0.0005	0.342	0.0005
Smoking status (pack per year)	0.048	0.592	0.178	0.139

Abbreviation: Pg-IgG, IgG antibody against *Porphyromonas gingivalis*.
r*, P*: after adjustment for age.

($r=0.171$, $P=0.067$; Table 3). Pulse pressure and PWV tended to correlate with Pg-IgG after adjustment for age.

BP and atherosclerotic index

Systolic BP correlated with pulse pressure ($r=0.786$, $P<0.0001$), mean BP ($r=0.878$, $P<0.0001$) and PWV ($r=0.369$, $P<0.0001$). Diastolic BP correlated with mean BP ($r=0.843$, $P<0.0001$) and PWV ($r=0.183$, $P=0.0483$).

DISCUSSION

Our results showed that the composite periodontal risk score was higher in the high Pg-IgG group than in the low Pg-IgG group. The level of serum antibody against Pg significantly correlated with periodontal risk score, which is an index for the severity of periodontal disease.¹⁴ This finding is consistent with previously reported data showing that high antibody levels against Pg are strongly associated with periodontitis.¹⁵ We previously demonstrated that levels of antibody against *P. intermedia* correlated with the composite periodontal risk score in patients with acute coronary syndrome.¹⁶ In the present study, serum levels of antibody against Pg were associated with three vector scores (the proportion of sites with bleeding on probing, the number of sites with a pocket probing depth ≥6.0 mm and bone loss greater than or equal to half the tooth length) and were significantly associated with the composite periodontal risk score. Thus, these data indicate that the serum level of antibody against Pg reflects the severity of periodontal disease.

Our data demonstrated that systolic and diastolic BP were higher in the high Pg-IgG group than in the low Pg-IgG group, and they correlated with Pg-IgG levels. Current epidemiological evidence also supports the association between periodontal disease and BP. A recent large study on data derived from the Third National Health and Nutrition Examination Survey showed a positive linear relationship between systolic BP and increased severity of periodontal disease.¹⁷ Another study reported that study patients with elevated antibodies against Pg and *A. actinomycetemcomitans* had a higher systolic BP.¹⁸ It has also been reported that etiological bacterial burden was positively associated with both systolic and diastolic BP.¹⁹ These findings are consistent with our data showing an association between BP and the level of serum antibody against Pg.

The intima-media wall thickness (IMT) of the carotid artery, pulse pressure, mean BP and PWV are common indices of clinical atherosclerosis. Several studies reported that IMT was associated with the severity of periodontal disease.^{20,21} Pulse pressure serves as an indicator of large-artery stiffness, whereas mean BP denotes peripheral resistance.²² Although PWV is an atherosclerotic index, as well as IMT,²³ the relationship of periodontal disease to subclinical measures of atherosclerosis, such as PWV, has not been examined. In this study, we evaluated the association with pulse pressure, mean BP and PWV. Pulse pressure was higher in the high Pg-IgG group than in the low Pg-IgG group. Similarly, mean BP and PWV tended to be higher in the high Pg-IgG group than in the low Pg-IgG group. These results suggest that atherosclerotic indices, including pulse pressure, mean BP and PWV, are related to the serum level of antibody against Pg.

Several periodontal organisms, including some periodontopathic bacteria, have been detected directly within the atherosclerotic plaque lesion of the vessel wall.^{8,24} Furthermore, periodontal organisms such as Pg have been reported to induce several pathological responses²⁵ and, in an animal model, long-term systemic injection of Pg was reported to accelerate atherogenic plaque progression.²⁶ These findings are consistent with the concept that inflammation has an important role in the development of atherosclerosis.²⁷ Another possibility is that periodontal pathogen accelerates atheroma formation by deteriorating lipid metabolism.²⁸

Dustan²⁹ showed that hypertension and atherosclerosis are intimately associated; hypertension leads to atherosclerosis, and less frequently, atherosclerosis begets hypertension. In our data, systolic BP was actually correlated with PWV, pulse pressure and mean BP. Elevated systolic BP was part of the mechanism by which the periodontal pathogen response might influence atherosclerosis.¹⁸

Limitation of this study is that study subjects are not a general population but IHD patients. In this study, we could show the relationship between periodontal disease and atherosclerosis in a relatively small population. In a general population, we may need to study in a large population to conform the relationship because they have less-advanced atherosclerosis than IHD patients.

In conclusion, our results suggest that an elevated level of antibody against Pg indicates advanced periodontal disease and suggests advancement of atherosclerosis and hypertension.

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