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ASSOCIATION OF THE SERUM IGG LEVEL AGAINST PERIODONTAL BACTERIA WITH PERIODONTAL STATUS AND SERUM LIPID LEVELS

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ABSTRACT

Background and Objective

Several studies have reported that serum antibodies against periodontal pathogens are related not only to periodontal status but also to systemic conditions, such as glycemic control and serum lipids. Therefore, this study examined the associations of serum IgG antibodies against periodontal pathogens with periodontal status and serum lipid levels in community dwellers.

Materials and Methods

A community-based cross-sectional study of 409 subjects (140 men, 269 women) aged ≥ 40 years who had at least 5 teeth was conducted in Goto, Japan in 2009 and 2010.

Results

The serum IgG levels against *Porphyromonas gingivalis* were significantly associated with periodontal parameters and serum high-density lipoprotein cholesterol (HDL-C) in men. According to a multiple linear regression analysis adjusted for covariates, the serum IgG levels against *P. gingivalis* were inversely related to serum HDL-C ($B = -0.1$; $P = 0.004$).

Conclusion
This study suggests that an elevated immune response to the periodontal pathogen is independently correlated with lower serum HDL-C level in community-dwelling men.

Key words*: Periodontitis; Periodontal bacteria; Serum IgG antibody, HDL-cholesterol*

Periodontal disease is a chronic inflammatory condition characterized by the destruction of the supportive connective tissues surrounding the roots of teeth in response to subgingival infection by various periodontal pathogens, mainly Gram-negative anaerobes.[1] Serum antibody levels against infectious agents are frequently used as diagnostic tools in clinical laboratories and epidemiological studies. Serum immunoglobulin G (IgG) antibody levels to periodontal pathogens may be a useful index of whether a patient has experienced infection by periodontal pathogens.[2,3] Multiple periodontal pathogens, rather than a single periodontopathic bacterial species, are associated with the occurrence of periodontitis. Generally, infection with periodontal bacteria leads to elevated IgG antibody levels to multiple periodontopathic bacterial species.[4]

Several studies have suggested an association between periodontitis and the components of metabolic syndrome, such as obesity, diabetes, dyslipidemia, and hypertension that are responsible for atherosclerosis.[5–8] Atherosclerosis is a leading cause of vascular disease and is considered to be an inflammatory disorder of the arteries. Elevated levels of low-density lipoprotein cholesterol (LDL-C) and triglyceride (TG), and reduced high-density lipoprotein cholesterol (HDL-C) in the blood are widely accepted as risk factors for atherosclerosis because they progress to the deposition of plaque on artery walls and artery wall thickening. The uptake of LDL-cholesterol by macrophages via endocytosis is a key step in the development of plaque, leading to the formation of foam cells in the arterial walls.[9] In comparison, HDL-C is thought to reverse cholesterol transport from the arterial walls. Many studies have reported that deterioration in periodontal conditions is related to atherosclerosis, suggesting that cytokines involved in the inflammatory process of periodontitis and molecules from periodontal pathogens, such as lipopolysaccharides (LPSs), contribute to atherosclerotic modifications.[10,11] Therefore, this study examined the associations of serum IgG antibody against periodontal pathogens with periodontal status and serum lipid levels in community dwellers.

METHODS

Study Population

This study enrolled 409 individuals (140 men and 269 women) aged ≥ 40 years who attended the "Specific Health Check-up and Guidance in Japan" program in 2009 and 2010 in Goto, Nagasaki Prefecture, Japan and underwent an oral assessment. All subjects provided written informed consent to participate in this study. The basic inclusion criteria consisted of the availability of all values and laboratory data, at least 5 remaining teeth, and having had a periodontal examination.

This study was approved by the Ethics Committee of Nagasaki University Graduate School of Biomedical Sciences (project registration number 090528160) and was performed in accordance with the Declaration of Helsinki.

Data collection and Laboratory Measurements

Each subject's height and weight were measured, and body mass index (BMI: kg/m^2) was calculated as an index of obesity. Systolic (SBP) and diastolic (DBP) blood pressure were recorded at rest. Blood samples were collected from each participant after an overnight fast. Serum was separated and stored at −20ºC for less than 3 days until assayed. The TG levels were measured by enzymatic methods,[12,13] and HDL-C was measured by a direct method[14]; LDL-C levels were calculated using the Friedewald equation.[15] Hemoglobin A_{1c} (HbA_{1c}) levels were measured by hexokinase by the latex agglutination reaction.[16,17]

Serum IgG antibody levels against periodontal pathogens were determined with an enzyme-linked immunosorbent assay (ELISA). Sonicated preparations of *Aggregatibacter actinomycetemcomitans* ATCC29523, *Eikenella corrodens* FDC1073, *P. gingivalis* FDC381, and *Prevotella intermedia* ATCC25611 were used as

antigens. Serum from 10 healthy participants without periodontitis was pooled and used to calibrate the analyses. Standard titration curves were prepared with serial dilutions of the pooled control serum. The assays and determinations of antibody titers (EU) were performed by Leisure (Tokyo, Japan), as described elsewhere.[3]

Oral Examination

The periodontal examination was performed by one of 4 trained dentists using the method modified from the Third National Health and Nutrition Examination Survey, as described previously.[18,19] Probing pocket depth (PPD), clinical attachment loss (distance from the cementoenamel junction to the bottom of the pocket, CAL) and bleeding on probing (BOP) were measured using a periodontal probe at the mesiobuccal and mid-buccal sites for all present teeth, excluding the third molars. Before starting this study, examiners were trained and calibrated using a chart, periodontal models, and volunteers at the Nagasaki University Hospital.

Statistical Analysis

The results of analyses involving continuous variables are expressed in terms of means (± standard deviations) or medians (25th–75th percentiles). The results of analyses involving categorical variables are expressed as percentages. Continuous variables were compared using the Student's t test or the Mann-Whitney *U*-test, as appropriate. Differences in prevalence were assessed using the chi-square test. Pearson's correlation coefficient was calculated to compare 2 continuous variables. In men, we evaluated the association between serum IgG antibody against *P. gingivalis* and HDL-C using simple and multivariate linear regression analyses. IBM SPSS statistics ver. 22.0J (IBM Japan, Tokyo, Japan) was used for the statistical analyses. Values of $P < 0.05$ were considered significant.

RESULTS

Table 1 shows the characteristics of the study participants. The mean PPD, mean clinical attachment loss, DBP, current prevalence of smoking, and alcohol consumption of men were significantly higher than those of women, whereas HDL-C and LDL-C were lower in men than in women. There were no differences between men and women in the serum IgG titers against periodontal pathogens. The serum anti-*P. gingivalis* IgG titers were widely distributed among the subjects, whereas the serum IgG titers against *A. actinomycetemcomitans*, *E. corrodens*, and *P. intermedia* were in the same range as those of pooled serum from healthy volunteers.

Table 2 shows the correlation analyses for serum anti-*P. gingivalis* IgG and other variables. In all subjects, there were significant positive correlations between serum anti-*P. gingivalis* IgG and the mean PPD, mean clinical attachment loss, and sites of BOP. In men, there were significant positive correlations between serum anti-*P. gingivalis* IgG and the mean PPD, but a negative correlation was found with HDL-C. No relationship between serum anti-*P. gingivalis* IgG and TG or LDL-C was found.

Table 3 shows the linear regression models for serum anti-*P. gingivalis* IgG as an independent variable and HDL-C as a dependent variable. The multiple linear regression analysis revealed that serum *anti-P. gingivalis* IgG was negatively correlated with HDL-C after adjusting for age, BMI, TG, HbA1c, SBP, current alcohol consumption, and current smoking.

DISCUSSION

In comparison to periodontal conditions in the NHANES III,[18] the mean PPD (1.6 mm) in this subjects was comparable to that (1.6 mm) in the NHANES III, however, the mean CAL (2.7 mm) and the mean BOP (17.5 %) were higher than those (around 2.0 mm and around 12 %, respectively) in the NHANES. Therefore, periodontal status was considered to be more severe in this population than in the NHANES population.

Cases of persistent chronic periodontitis involve the recruitment of host cells and the mediators of adaptive immunity, the activation of macrophage populations, and an increased antibody response.[20] Many studies have reported that serum IgG antibodies against periodontal pathogens were elevated in patients with periodontitis.[3,21] In this study, serum IgG against *P. gingivalis* was positively related to periodontal status, including periodontal probing depth and clinical attachment loss. This result is consistent

TABLE 1 Characteristics of the Subjects

Characteristics	All subjects (n=409)	Men (n=140)	Women (n=269)	*P* value
Serum anti-*Aa* IgG antibody median, (IQR)	0.0 (−0.2, 0.5)	0.1 (−0.2, 0.7)	0.1 (−0.2, 0.4)	0.363
Serum anti-*Ec* IgG antibody	−0.2 (−0.4,0.1)	−0.1 (−0.4, 0.2)	−0.2 (−0.4, 0.0)	0.524
Serum anti-*Pg* IgG antibody	10.9 (2.9, 26.1)	9.9 (2.6, 27.8)	11.1 (3.2, 25.4)	0.517
Serum anti-*Pi* IgG antibody	−0.1 (−0.3, 0.3)	−0.1 (−0.30, 0.1)	−0.1 (−0.3, 0.2)	0.286
Age(years) mean±SD	66.0 ± 9.4	67.0 ± 10.0	65.4 ± 9.1	0.114
Mean PPD (mm)	1.6 ± 0.6	1.7 ± 0.7	1.5 ± 0.6	0.003
Mean CAL (mm)	2.7 ± 1.0	3.0 ± 1.2	2.5 ± 0.9	<0.001
BOP (%)	17.5 ± 18.5	17.2 ± 17.4	17.6 ± 19.0	0.859
BMI (kg/m^2)	23.1 ± 3.1	23.4 ± 2.8	22.9 ± 3.3	0.089
HbA_{1c} (%)	5.3 ± 0.4	5.2 ± 0.4	5.3 ± 0.4	0.29
HDL-C (mg/dL)	60.5 ± 15.8	55.7 ± 15.3	63.1 ± 15.5	<0.001
LDL-C (mg/dL)	120.7 ± 30.4	113.2 ± 28.9	124.6 ± 30.5	<0.001
TG (mg/dL)	106.5 ± 63.7	105.6 ± 61.3	106.9 ± 65.1	0.845
SBP (mmHg)	138.2 ± 19.6	140.0 ± 18.8	137.2 ± 20.0	0.171
DBP (mmHg)	80.8 ± 10.3	82.3 ± 10.0	80.0 ± 10.3	0.027
Dyslipidemia (%)	87 (21.8)	38 (27.1)	49 (18.2)	0.042
Hypertension (%)	257 (62.8)	93 (66.4)	164 (61.0)	0.284
Medication for Diabetes Melitus (%)	15 (3.7)	6 (4.3)	9 (3.3)	0.594
Medication for Dyslipidemia (%)	43 (10.5)	9 (6.4)	34 (12.6)	0.061
Medication for Hypertension (%)	120 (29.3)	34 (24.3)	86 (32.0)	0.111
Current Smoker, Yes (%)	26 (6.4)	21 (15.0)	5 (1.9)	<0.001
Current Alcohol Drinker, Yes (%)	99 (24.2)	73 (52.1)	26 (9.7)	<0.001

Student's t test or Mann-Whitney U test for continuous variables between men and women, as appropriate.
Chi-squared test for categorical variables between men and women.
BMI = body mass index; BOP = bleeding on probing; DPB = diastolic blood pressure; HDL C = high-density lipoprotein cholesterol; LDL C = low-density lipoprotein cholesterol; PPD = probing pocket depth; SBP = systolic blood pressure; TG = triglycerides.

with a report that the levels to *P. gingivalis* antibodies best reflected periodontal status, indicating that the antibody response to *P. gingivalis* is strongly enhanced by its colonization of periodontal lesions.[3]

Epidemiological studies have shown that HDL-C is a strong predictor of cardiovascular risk, with 1-mg/dL increments in the serum HDL-C levels associated with a 2–3% reduction in mortality.[22] In this study, serum IgG against *P. gingivalis* was negatively correlated with HDL-C in men, although it was not correlated with LDL-C or TG. Furthermore, a multiple linear regression model showed that elevated serum IgG against *P. gingivalis*, as well as age, BMI, TG, SBP, current alcohol consumption, and current smoking, were independent risk factors for lowering HDL-C. During the acute and chronic phase responses induced by infection and inflammation, plasma HDL cholesterol and apolipoprotein (Apo) AI levels are reduced in humans.[23,24] The acute phase response induced following the LPS injection of mice causes decreased production of ApoAI in the liver, which is the major protein component of HDL particles.[25] Although

TABLE 2 Correlation of Serum Anti-Pg IgG Antibody to Periodontal/Systemic Conditions

	All Subjects		Men		Women	
	r	***P* value**	**r**	***P* value**	**r**	***P* value**
Mean PPD	0.187	<0.001	0.173	0.041	0.210	0.001
Mean CAL	0.168	<0.001	0.141	0.097	0.211	<0.001
BOP	0.182	<0.001	0.060	0.484	0.219	<0.001
BMI	−0.052	0.293	−0.026	0.764	−0.057	0.350
HbA_{1C}	−0.019	0.700	−0.072	0.397	−0.008	0.902
HDL-C	0.004	0.937	−0.176	0.037	0.046	0.448
LDL-C	−0.077	0.121	−0.029	0.734	−0.099	0.104
TG	−0.066	0.183	−0.063	0.458	−0.069	0.258
SBP	0.029	0.557	−0.023	0.784	0.046	0.452
DBP	0.008	0.868	−0.136	0.109	0.052	0.396

r, Pearson's correlation coefficient
BMI = body mass index; BOP = bleeding on probing; DPB = diastolic blood pressure; HDL C = high-density lipoprotein cholesterol; LDL C = low-density lipoprotein cholesterol; PPD = probing pocket depth; SBP = systolic blood pressure; TG = triglycerides.

TABLE 3 Association between Serum Anti-P.g. IgG Antibody and HDL-C in Men using Linear Regression Model

	Variables	**B**	**95% CI**	**R^2**	***P* value**
Model 1					
	Serum anti-Pg IgG Antibody	−0.087	−0.168 , −0.005	0.031	0.037
Model 2					
	Serum anti-Pg IgG Antibody	−0.089	−0.167, −0.011	0.127	0.025
	Age	−0.041	−0.285, 0.202		0.739
	BMI	−1.675	−2.534, −0.815		<0.001
Model 3					
	Serum anti-Pg IgG Antibody	−0.100	−0.167, −0.033	0.393	0.004
	Age	−0.315	−0.551, 0.078		0.009
	BMI	−1.727	−2.525, −0.929		<0.001
	TG	−0.096	−0.134, −0.059		<0.001
	HbA_{1c}	−0.292	−5.712, 5.127		0.915
	SBP	0.144	0.022, 0.266		0.021
	Medication for Dyslipidemia	−0.109	−8.760, 8.542		0.980
	Alcohol Intake, Yes	8.421	4.243, 8.542		<0.001
	Current Smoking, Yes	−6.953	−13.282, −0.624		0.032

B, regression coefficient; 95% CI, 95% confidence interval; R^2, coefficient of determination
BMI = body mass index; BOP = bleeding on probing; DPB = diastolic blood pressure; HDL C = high-density lipoprotein cholesterol; LDL C = low-density lipoprotein cholesterol; PPD = probing pocket depth; SBP = systolic blood pressure; TG = triglycerides.

periodontitis is a chronic infectious disease caused by periodontal pathogens, sustained stimulation by LPS of periodontal pathogens from periodontal lesions carried by the blood stream may reduce the HDL particles in the liver. In addition, myeloperoxidase (MPO) which is released from neutrophils was known to impair HDL production.[26] MPO generated oxidants modify apoA1, blocking its lipidation and leading to decreased nascent HDL biogenesis. A recent study reported that serum myeloperoxidase levels were higher in subjects with periodontitis than periodontally healthy subjects.[27] Considering the above, one plausible explanation for an inverse relationship between serum IgG against *P. gingivalis* and HDL-C in men in this study is that decreased HDL biogenesis is caused by chronic inflammatory response to *P. gingivalis* infection. Therefore, control of periodontal inflammation by treating periodontitis and performing routine health checkups may be important for maintaining normal levels of serum HDL-C as well as for maintaining periodontal health.

This study found a difference between men and women in the association between serum IgG against *P. gingivalis* and HDL-C. However, the female hormone estrogen increases circulating HDL-C in women.[28] At post menopause, decreased estrogen leads to lower HDL-C. Therefore, one possible explanation for the sex difference in the association between serum IgG against *P. gingivalis* is that estrogen has an impact on serum HDL-C rather than does periodontal inflammation in women.

Our study had several limitations. First, no causal relationship between serum IgG antibody against *P. gingivalis* and serum lipids could be determined because of the cross-sectional study design. Second, subjects participated in this study on a voluntary basis and might not be representative of the Japanese population; therefore, the results of this study may not be generalizable to a non-Japanese population. Third, data related to menoposal status, levels of sex hormones, diet and physical activity were not available for analysis in this study.

CONCLUSIONS

A linear association between serum IgG against *P. gingivalis* and HDL-C was revealed in men. Further study is needed to examine how the inflammation of the periodontal disease response to periodontal pathogens is related to serum HDL-C level.

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