FcγRIIIb Genotypes and Smoking in Periodontal Disease Progression Among Community-Dwelling Older Adults in Japan

Akihiro Yoshihara,* Noriko Sugita,† Kouji Yamamoto,† Tetsuo Kobayashi,†‡ Toshinobu Hirotomi,* Hiroshi Ogawa,* Hideo Miyazaki,* and Hiromasa Yoshie†

Background: FcγRIIIb genotypes and smoking are risk factors for periodontal disease. However, the interaction of FcγRIIIb-NA1-NA2 polymorphism with smoking remains unclear. The purpose of this study was to determine if FcγRIIIb-NA1-NA2 polymorphism and smoking are associated with periodontal disease progression among elderly people.

Methods: Among 70-year-old subjects, 164 with neither diabetes mellitus nor blood sugar ≥140 mg/dl, who had more than 20 teeth and who could participate in both the baseline and the follow-up examinations were included in the study. The NA1 group comprised subjects with FcγRIIIb-NA1NA1 genotype (N = 53), while the NA2 group included subjects with FcγRIIIb-NA1NA2 or NA2NA2 genotype (N = 111). We examined the progression of periodontitis by measuring attachment loss during 3 years.

Results: The frequency of subjects who showed ≥4 mm additional attachment loss at one or more sites was 55.6% for smokers and 37.2% for non-smokers. The odds ratio (OR) was 2.13 (confidence interval [CI]: 0.92 to 4.76). We found a better association between periodontal progression and smoking in the NA2 group. The OR for smokers was 3.03 (CI:1.12 to 8.33, $P = 0.028$). Additionally, the mean number of sites with ≥4 mm additional attachment loss per person between smokers and non-smokers in the NA2 group or between smokers and non-smokers in the NA1 group was 2.90 ± 3.42 and 0.74 ± 1.53 or 0.57 ± 0.79 and 0.68 ± 1.03, respectively ($P < 0.001$; analysis of variance [ANOVA]).

Conclusion: Our results may suggest an association between smoking and periodontal disease progression in elderly people with FcγRIIIb-NA2 polymorphism. *J Periodontol 2005;76:250-255.*

KEY WORDS

Elderly; genotypes; periodontal diseases/etiology; polymorphism, genetic; risk factors; smoking/adverse effects.

* Division of Preventive Dentistry, Department of Oral Health Science, Graduate School of Medical and Dental Sciences, Niigata University, Niigata, Japan.
† Division of Periodontology, Department of Oral Biological Science, Graduate School of Medical and Dental Sciences, Niigata University.
‡ General Dentistry and Clinical Education Unit, Niigata University Dental Hospital.

Periodontal destruction is common among elderly people[1,2] and contributes to as much as 40% of tooth loss in this population.[3] Nevertheless, there are some elderly people who show minimum periodontal tissue loss.[4]

Neutrophils have an important role in the control of periodontitis and increased disease susceptibility is observed in patients with defective neutrophil production and/or function.[5-7] Therefore, genetic polymorphism that affects neutrophil effector function may be relevant to disease resistance. FcγRIIIb is a neutrophil-specific receptor and bears the functional NA1-NA2 polymorphism which determines IgG1- and IgG3-mediated neutrophil effector function.

We previously reported a role of FcγRIIIb allele as a risk factor for periodontal disease.[8-14] For an elderly population, we found that FcγRIIIb-NA1 allotype was over-expressed in the periodontitis-resistant group, compared with the periodontitis-susceptible group according to a cross-sectional study.[13] Further, we have found that elderly subjects with >20 teeth showed resistance to periodontal disease progression.[15] In the same group, we found a significant association between additional attachment loss over 2 years and smoking,[16] which is known to be a major contributing factor in periodontitis. In another study, interleukin (IL)-1 polymorphisms appeared to interact with smoking as a significant risk factor for periodontal disease.[17,18] However, the interaction of FcγRIIIb-NA1-NA2 polymorphism with smoking remains unclear. In view of this

background, it is important to evaluate the relationship between genetic polymorphism of FcγRIIIb-NA1-NA2 and progression of periodontitis in longitudinal studies. Accordingly, the purpose of this study was to determine longitudinally how FcγRIIIb-NA1-NA2 polymorphism and smoking are associated with periodontal disease progression among elderly individuals.

MATERIALS AND METHODS

Study Population and Clinical Assessments

In 1998, we sent questionnaires to all 4,542 residents aged 70 years in Niigata City, Japan. The questionnaires were to compile information on the subjects' medical and dental health. The response rate was 81.4% (N = 3,695). Among them, we randomly selected 600 individuals (screened population) to have approximately the same number of males and females all of whom provided informed consent to the study protocol. The study protocol was reviewed and approved by the Ethics Committee of the Faculty of Dentistry, Niigata University. The examinations were performed at local community centers in Niigata City. Four dentists performed the following clinical evaluations: 1) number of teeth present; 2) probing depth (PD); 3) clinical attachment level (CAL). Mouth mirrors incorporating a light and pressure-sensitive plastic periodontal probe, set to give a constant probing force of 20 g and graduated at 1 mm intervals,§ were used. All functioning teeth including third molars were assessed except for partially erupted teeth. PD and CAL were measured at six sites per tooth (mesio-buccal, mid-buccal, disto-buccal, mesio-lingual, mid-lingual, and disto-lingual) and rounded to the nearest whole millimeter. In cases where a restorative margin was apical to the cemento-enamel junction (CEJ), CAL was measured taking the anatomical features of teeth into account and, if present, the CEJ of adjacent tooth/teeth. A personal interview was done to obtain information on smoking.

Subjects (N = 83) with diabetes mellitus and blood sugar ≥140 mg/dL were excluded. We selected the 165 subjects with >20 teeth, one of whom could not participate in the follow-up examination, so that 164 subjects (99.4%) were included in the study. The FcγRIIIb-NA1/NA2 genotype of this population was determined. The NA1 group (N = 53) was defined as subjects with FcγRIIIb-NA1NA1 genotype, while the NA2 group (N = 111) was defined as subjects with FcγRIIIb-NA1NA2 or -NA2NA2 genotype. Follow-up clinical surveys were done by measuring CAL at 3 years.

Prior to data collection, the four examiners were calibrated with each examiner paired with all other examiners on 17 volunteer patients in the Faculty Hospital of Dentistry, Niigata University. The percentage of agreement ranged from 70.0% to 100% for CAL (kappa from 0.62 to 1.00).

Determination of FcγRIIIb-NA1/NA2 Genotype

Genomic DNA was isolated from peripheral blood and genotyped‖ for FcγRIIIb-NA1-NA2 by allele-specific polymerase chain reaction (PCR) as previously described.[8,19] For the FcγRIIIb-NA1 genotyping, 100 ng of genomic DNA was added to 50 μl reaction mixes containing 15 mM Tris-HCl (pH 8.0), 50 mM KCl, 25 mM $MgCl_2$, 25 μM of each dNTP; 400 nM of the sense primer (5′-CAG TGG TTT CAC AAT GTG AA-3′; nt 208-227) or the antisense primer (5′-CAT GCA CTT CTA GCT GCA CCG-3′; nt 329-349), and 1 U Ampli Taq gold DNA polymerase. PCR conditions were as follows: one cycle at 95°C for 9 minutes; 35 cycles at 95°C for 30 seconds, 63°C for 30 seconds, and 72°C for 30 seconds, ending at 72°C for 10 minutes. For the FcγRIIIb-NA2 genotyping, PCR assay was performed with the NA2 sense primer (5′-CTC AAT GGT ACA GCG TGC TT-3′; nt 128-147) and the NA2 antisense primer (5′-CTG TAC TCT CCA CTG TCG TT-3′; nt 277-296). The amplification protocol was as follows: one cycle at 95°C for 9 minutes; 35 cycles at 95°C for 30 seconds, 64°C for 15 seconds, and 72°C for 30 seconds, ending at 72°C for 10 minutes. The end products of the specific NA1 and NA2 reactions were 141 bp and 169 bp, respectively. All PCR products were analyzed by electrophoresis on 2% agarose gels stained with ethidium bromide.

Statistical Analysis

Student *t* test or chi square test was used to compare the clinical parameters between the NA1 and NA2 groups at baseline when applicable. The strength of an association between periodontal disease progression and the genotype of FcγRIIIb or smoking was investigated by the risk for occurrence as the odds ratio with a 95% confidence interval (CI). Periodontal disease progression was defined having one or more sites with additional attachment loss of ≥4 mm during 3 years. If we selected another cutoff point, such as one or more sites with an additional attachment loss of 2 or 3 mm during 3 years, we could not calculate the odds ratio because all the smokers in the NA1 group had one or more sites with a periodontal progressive additional attachment loss.

In addition, the number of sites with an additional attachment loss of ≥4 mm or more per person in the NA1 and NA2 groups between smokers and non-smokers was evaluated using analysis of variance. The differences between the groups were tested by Bonferroni correction as a post-hoc comparison.

We carried out Poisson regression analysis to determine the characteristics of the combination of smoking habit and FcγRIIIb genotype in subjects who experi-

§ Vivacare TPS Probe, Schaan, Liechtenstein.
‖ Easy DNA Kit, Invitrogen, San Diego, CA.

enced attachment loss. In this model, the number of sites with an additional attachment loss of ≥4 mm was used as the dependent variable and the following six variables as the independent variables: number of teeth lost over the 3 years, a combination of smoking habit and FcγRIIIb genotype, number of sites with CAL ≤4 mm at baseline, and gender.

RESULTS

Table 1 shows the baseline variables in the NA1 and NA2 groups. The percentages of males in the groups were 54.7% and 55.9%, respectively. Percentages of smokers were 13.2% and 18.2%, respectively. The differences in percentage of males or smokers between the NA1 and NA2 groups were not statistically significant. There were no significant differences in clinical parameters such as number of teeth present, PD, or CAL between the NA1 and NA2 groups. Among the study population, 98 (59.8%) had additional attachment loss of ≥4 mm at one or more sites over the 3 years. Mean number of sites with additional attachment loss of ≥4 mm per person was 1.49 ± 4.79.

Table 1.

Biological, Medical, and Periodontal Characteristics at Baseline

Variables	Screened Population (N = 600)	Study Population (N = 164) NA1NA1* (N = 53)	Study Population (N = 164) NA1NA2 or NA2NA2† (N = 111)	P Value (NA1* versus NA2†)
Male/female (N)	306/294	29/24	62/49	0.910
Smokers (%)	18.7	13.2	18.2	0.437
Subjects under medical treatment for diabetes (%)	5.0	0	0	–
Subjects with blood sugar ≥140 mg/dl (%)	7.0	0	0	–
Edentulous subjects (%)	7.5	0	0	–
N teeth (mean ± SD)	17.4 ± 0.4	25.94 ± 2.82	25.11 ± 2.91	0.089
Probing depth (mean ± SD)	2.03 ± 0.03	1.88 ± 0.47	1.91 ± 0.51	0.677
Clinical attachment level (mean ± SD)	3.10 ± 0.05	2.58 ± 0.85	2.71 ± 0.78	0.348
N sites PD or CAL (mean ± SD)				
PD 4-5 mm	8.48 ± 10.30	9.66 ± 11.69	8.70 ± 11.53	0.621
PD ≥6 mm	1.21 ± 2.97	0.85 ± 2.07	1.08 ± 3.49	0.655
CAL 4-5 mm	23.69 ± 20.39	25.47 ± 26.07	28.57 ± 25.41	0.469
CAL ≥6 mm	5.71 ± 9.21	5.51 ± 11.75	4.61 ± 8.25	0.612

* With FcγRIIIb-NA1NA1 genotype (NA1 group).
† With FcγRIIIb-NA1NA2 or NA2NA2 genotype (NA2 group).

Table 2 shows the occurrences and OR between smokers and non-smokers or between the NA1 and NA2 groups. The frequency of subjects who presented with an additional attachment loss of ≥4 mm at one or more sites was 55.6% (N = 15) for smokers compared with 37.2% (N = 51) for the non-smokers. OR was 2.13 (CI: 0.92 to 4.76, P = 0.080). There was no significant difference in the frequency of subjects who presented with an additional attachment loss of ≥4 mm at one or more sites between the NA1 and NA2 groups.

Table 2.

Odds Ratios for Smoker Versus Non-Smoker and NA1 Group Versus NA2 Group

Variables	≥4 mm Additional CAL* (N)	<4 mm Additional CAL (N)	OR†	SE	P	95% CI
Smoker (N = 27)	15	12	2.13	0.20	0.080	0.92-4.76
Non-smoker (N = 137)	51	86				
NA2 group (N = 111)	42	69	1.35	0.25	0.364	0.70-2.63
NA1 group (N = 53)	24	29				

* N subjects exhibiting ≥1 sites with an additional loss of ≥4 mm over 3 years.
† OR for exhibiting ≥1 sites with an additional loss of ≥4 mm over 3 years.

As shown by the data in Table 3, we found a better association between periodontal progression and smoking habit in the NA2 group. The frequency of subjects who presented with an additional attachment loss of ≥4 mm at one or more sites was 60.0% (N = 12) for smokers compared with 33.0% (N = 30) for non-smokers. The OR increased to 3.03 (CI:1.12 to 8.33, P = 0.028) in the NA2 group.

The mean number of sites with an additional attachment loss of ≥4 mm per person between smokers and

Table 3.

Odds Ratios for Smoker Versus Non-Smoker by Genotype Group

Genotype	Smoking	≥4 mm Additional CAL (N)*	<4 mm Additional CAL (N)	OR†	SE	*P*	95% CI
NA2 group	Smoker	12	8	3.03	0.17	0.028	1.12-8.33
	Non-smoker	30	61				
NA1 group	Smoker	3	4	0.89	0.92	0.890	0.18-4.55
	Non-smoker	21	25				

* N subjects exhibiting ≥1 sites with an additional loss of ≥4 mm over 3 years.
† OR for exhibiting ≥1 sites with an additional loss of ≥4 mm over 3 years.

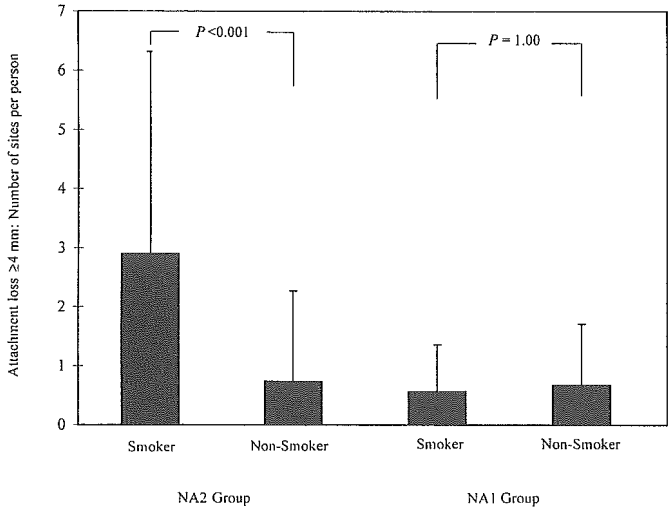

Figure 1.
The number of sites with an additional attachment loss ≥4 mm over 3 years between smokers and non-smokers in the NA1 and NA2 group.

Table 4.

Poisson Regression and Associated *P* Values

Independent Variables	Dependent Variable (N sites with an additional attachment loss of ≥4 mm) IRR*	SE	*P* Value	95% CI
N teeth lost over 3 years	0.95	0.05	0.337	0.86-1.05
Combination type (dummy)				
Smoker in the NA2 group	4.94	2.56	0.002	1.79-13.64
Non-smoker in the NA2 group	1.76	0.92	0.274	0.64-4.88
Smoker in the NA1 group	2.66	1.39	0.062	0.95-7.41
Non-smoker in the NA1 group (reference)	1.00			
N sites with an attachment level of ≥4 mm at baseline	0.99	<0.01	0.180	0.99-1.00
Gender	2.22	0.09	<0.001	1.52-3.23

* Incidence rate ratios.
$P<0.001$, pseudo $R^2 = 0.121$.

non-smokers in the NA2 group or between smokers and non-smokers in the NA1 group was 2.90 ± 3.42 and 0.74 ± 1.53 or 0.57 ± 0.79 and 0.68 ± 1.03, respectively (Fig. 1). There was a significant ($P<0.001$) difference in the mean number of sites among four groups (ANOVA). Further, we found a significant relationship between smokers and non-smokers in the NA2 group by Bonferroni correction as a post hoc comparison ($P<0.001$). Again, there was no significant relationship between smokers and non-smokers in the NA1 group.

Table 4 shows the result of the Poisson regression analysis. In the model, the combination type smokers in the NA2 group was significantly estimated to be 4.94 times larger than that for any other combination type. In addition, it was significant that males were estimated to be 2.22 times more susceptible to periodontitis than females.

DISCUSSION

In this study, we were interested in understanding the influence of FcγRIIIb genotype and smoking on periodontal disease progression. We found that the smokers in the NA2 group showed a higher number of sites with an additional attachment loss of ≥4 mm compared to other groups. The results suggested that smoking contributes to periodontal disease progression in subjects with FcγRIIIb-NA1NA2 or -NA2NA2 genotype. It might mean that FcγRIIIb influences periodontal disease progression in smokers.

Many previous reports indicated a significant association between smoking and periodontal disease progression.[20-23] Nicotine is thought to be a major harmful substance found in tobacco. Nicotine derivatives are known to be vasoconstrictive, not only on peripheral vessels, but also on coronary, placental, and gingival blood vessels as well.[20] Tobacco use may reduce the functional activities of polymorphonuclear leukocytes including chemotaxis and phagocytosis.[21,22] Smoking has a significant systemic effect on plasma IgG levels as well.[23] According to these findings, our results might suggest that there is an association between smoking and the FcγRIIIb-NA1NA2 or FcγRIIIb-NA2NA2 genotype.

In earlier studies, a significant association between smoking and periodontal disease progression in subjects with A2/A2 allele of composite genotype IL-1α/IL-1β or IL-1α/IL-1RN was found.[17,18] However, the mechanism which relates smoking to genetic factors such as FcγRIIIb or IL-1 has not been fully understood.

FcγRIIIb-NA2 is less efficient in neutrophil phagocytosis. In addition, effects of smoking on neutrophils were examined in the previous reports.[17,18] Nicotine inhibited production of superoxide anion and hydrogen peroxide.[24] Our results suggest that there might be the synergism of smoking and the FcγRIIIb genotype for neutrophil function.

On the other hand, our subjects in the NA2 group did not have a higher progressive rate than the NA1 group. According to previous reports, the FcγRIIIb-NA2 carriers were over-expressed in periodontitis patients even if some smokers were included as study subjects.[10-14] As our study was aimed at elderly subjects aged 70 years who had more than 20 teeth present, the subjects which we examined might have been periodontitis resistant. Therefore, it was difficult for FcγRIIIb polymorphism to contribute to the inter-individual difference in resistance to periodontitis. This might be a reason for the insignificant difference in periodontal disease condition at baseline and progression during 3 years between the NA1 and NA2 groups.

In this study, we chose to restrict the age of subjects to 70 years. Hence, we eliminated the influence of age on periodontal disease progression. In addition, subjects with fewer than 20 teeth were excluded because the cause and the time of tooth loss were not clear. Similarly, diabetes patients, who are known to be at a high risk for periodontitis, were also not included. Therefore, it was possible to evaluate how FcγRIIIb-NA1-NA2 polymorphism and smoking are associated with periodontal disease progression accurately by excluding confounding factors. However, this study has some limitations. We used restriction to control for confounding on a number of variables such as age, diabetes mellitus, blood sugar, and the number of teeth present. The restriction may not have detected potential weak associations. In addition, the number of smokers included in the analysis was 27, which might not be adequate to confirm the reliability of the findings. Another survey with a larger number of subjects should provide more solid data.

In conclusion, our results might suggest a better association between smoking and periodontal progression in elderly with the FcγRIIIb-NA2 polymorphism. Additional studies with larger cohorts of patients are needed to fully understand the interaction between genetic polymorphisms and smoking.

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Correspondence: Dr. Akihiro Yoshihara, Department of Preventive Dentistry, Department of Oral Health Science, Graduate School of Medical and Dental Sciences, Niigata University 2-5374 Gakkocho-Dori, Niigata, Japan 951-8514. Fax: 81-25-227-0807; e-mail: akihiro@dent.niigata-u.ac.jp.

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Periodontal Conditions in an Elderly Japanese Population Influenced by Smoking Status and Serum Immunoglobulin G2 Levels

Keisuke Nakashima,* Takao Kobayashi,* Akihiro Yoshihara,† Jun Fujiwara,* Hideo Miyazaki,† and Yusuke Kowashi*

Background: A Japanese population residing in the same area may be suitable for evaluation of the association among IgG subclass levels, smoking status, and periodontal disease due to similar racial and environmental factors, as these factors can interact to influence serum IgG subclass levels. The present cross-sectional investigation attempted to examine the influence of serum IgG subclass levels and smoking status on periodontal condition in a population of elderly Japanese subjects.

Methods: Elderly individuals (N = 451, age, 71 years) residing in Niigata City, Japan participated in the present study. Clinical evaluations, which consisted of probing depth (PD), clinical attachment level (CAL), bleeding on probing (BOP), and calculus, and serological determinations including serum IgG subclass levels and anti-*Porphyromonas gingivalis* fimbriae IgG subclass titers were conducted. All participants were asked to complete questionnaires regarding smoking status and were then divided into one of three groups: never smoker, former smoker, or current smoker.

Results: Distributions of the number of teeth did not differ significantly across the groups. The proportion of sites with CAL ≥4 mm (%CAL4) in current smokers was significantly higher in comparison with never smokers. Multiple linear regression analysis revealed that %CAL4 was influenced by number of teeth, serum IgG2 levels, gender, and smoking status ($R^2 = 0.253$, $P < 0.001$). The effect of IgG2 was greater than that of smoking status. Distribution of IgG2 was not significantly different across the three groups.

Conclusion: These data indicate that serum IgG2 levels influences periodontal conditions in an elderly Japanese population independent of smoking status. *J Periodontol 2005;76:582-589.*

KEY WORDS

Cross-sectional studies; elderly; IgG; Japanese; periodontal diseases/etiology; smoking/adverse effects.

* Department of Periodontology and Endodontology, School of Dentistry, Health Sciences University of Hokkaido, Hokkaido Japan.
† Division of Preventive Dentistry, Department of Oral Health, Graduate School of Medical and Dental Sciences, Niigata University, Niigata Japan.

Experimental evidence accumulated over the last two decades indicates that tobacco smoking is probably a true risk factor for periodontitis.[1] Smokers display an increased prevalence and a more severe extent of periodontal disease, as well as higher prevalence of tooth loss and edentulism compared to non-smokers. Salvi et al. reviewed the influence of tobacco smoking on the pathogenesis of periodontitis.[2] Smoking appears to exert both local and systemic effects. Systemic alternations of the host response in smokers, which have been evaluated by several investigators, include impaired chemotaxis and phagocytosis of oral and peripheral neutrophils. Smoking also reduces immunoglobulin G (IgG) levels in human serum. This finding is consistent with that of a number of studies involving human subjects from such diverse locations as the United States, the United Kingdom, Sweden, and Australia.[3] In addition, IgG levels in serum tend to increase when subjects cease smoking.[4] Four IgG subclasses are known, which possess distinct biological properties, such as complement activation and Fc receptor binding on phagocytes.[5] However, whether all four subclass antibodies are equally affected by smoking is unclear.

Wilton et al. demonstrated that serum IgG2 levels of patients with adult periodontitis were elevated significantly relative to corresponding levels observed in controls.[6] Tew et al. focused on IgG subclass

response in patients exhibiting early-onset periodontitis in a series of studies.[7-9] Serum IgG2 levels of patients with localized juvenile periodontitis (LJP) were elevated in comparison with age- and race-matched controls; this observation indicated that the high levels of serum IgG2 in LJP patients are beneficial in terms of localization of periodontal destruction. Gunsolley et al. reported that genetic and environmental factors can interact to influence levels of individual IgG subclasses.[10] A Japanese population residing in the same area is characterized by similar racial and environmental backgrounds; consequently, these individuals may be suitable for evaluation of serum IgG subclass levels. Documentation regarding the association among IgG subclass levels, smoking status, and periodontal condition in the Japanese population is strictly limited. The purpose of the present cross-sectional investigation was to examine the influence of serum IgG and IgG subclass levels, especially IgG2, and smoking status, on periodontal condition in a population of elderly Japanese subjects of identical age.

MATERIALS AND METHODS

Study Population and Clinical Evaluations

An oral health survey of the elderly population was conducted in 1998 by the Ministry of Health and Welfare of Japan. Questionnaires were sent to all 6,629 residents 70 or 80 years old in Niigata City, Japan. Six hundred persons 70 years of age (as of 1998) agreed to undergo the medical and dental examinations. Signed informed consent for the protocol, which was reviewed and approved by the Ethics Committee of the Faculty of Dentistry, Niigata University, was received from all participants. In the present investigation, 451 subjects (239 males, 212 females) of the 600 respondents then aged 71 years, who participated in the 2-year longitudinal study (1999), were selected (screened population). Sera were obtained from all subjects by venipuncture at the time of the clinical evaluations. These samples were stored at –80°C until the day prior to the assay. Four dentists clinically evaluated the following: 1) number of teeth; 2) probing depth (PD); 3) clinical attachment level (CAL); 4) bleeding on probing (BOP); and 5) calculus. PD, CAL, BOP, and calculus were assessed with a Williams probe at six sites per tooth.

Determination of Serum IgG and IgG Subclass Levels

IgG levels were determined by immunoprecipitation assay. IgG subclass levels were assessed by enzyme-linked immunosorbent assay (ELISA). First, 96-well microtiter plates‡ were coated with one of the following anti-human IgG subclass monoclonal antibodies:§ HP6069A (anti-G1), HP6002 (anti-G2), HP6047A (anti-G3), or HP6023 (anti-G4) overnight at 4°C. Unoccupied sites were blocked with phosphate-buffered saline (PBS) containing 5% non-fat dry milk; serial 2-fold dilutions of a human standard serum (1:500 to 1:512,000)‖ or diluted serum samples (IgG1, 1:4,000; IgG2 and 4, 1:16,000; IgG3, 1:64,000) were then added to the wells and incubated overnight at 4°C. A 1:1,000 dilution of alkaline phosphatase-conjugated goat anti-human IgG antibody¶ was introduced; subsequently, plates were incubated for 4 hours at room temperature. Plates were washed three times with PBS containing 0.05% Tween 20 between each step. The color was developed with *p*-nitrophenylphosphate as the substrate; optical density (OD) at 405 nm was measured. The data were fitted to a graph of the common logarithm of serum dilutions versus OD by 4-parameter logistic regression analysis. IgG subclass levels in serum samples were calculated from the standard curve.

Twenty randomly selected serum samples were evaluated in terms of IgG subclass levels via single radial immunodiffusion (SRID) assay.# IgG subclass levels measured by ELISA displayed significant correlation with those levels determined by SRID (correlation coefficients were 0.799 [IgG1], 0.853 [IgG2], 0.649 [IgG3], and 0.625 [IgG4]).

Determination of Anti-Fimbriae IgG and IgG Subclass Titers

Porphyromonas gingivalis 381 was anaerobically cultured in GAM broth** at 37°C. Fimbriae was prepared from culture supernatant and purified by the method of Yoshimura et al.[11] to verify purity on SDS-PAGE gels. Microtiter plates‡ were coated with purified fimbriae (0.2 μg/ml) overnight at 4°C. Unoccupied sites were blocked; subsequently, serial 2-fold dilutions of pooled high-titer human sera (1:64 to 1:8,192) or serum samples (1:500 dilution) were introduced to the wells and incubated overnight at 4°C. A 1:4,000 dilution of one of the anti-human IgG subclass monoclonal antibodies was added, followed by the introduction of a 1:1,000 dilution of alkaline phosphatase-conjugated goat anti-mouse IgG antibody;¶ subsequently, plates were incubated for 4 hours at room temperature. Plates were washed three times between each step. Color was developed as described above. The data were fitted to a graph of the common logarithm of serum dilutions versus OD by 4-parameter logistic regression analysis. Anti-fimbriae IgG subclass titers were calculated from the standard curve of pooled high-titer human sera, and expressed as ELISA units (EU).

‡ Nunc-Immuno Plate II, Nalge Nunc International Co., Rochester, NY.
§ Calbiochem-Novabiochem Co., San Diego, CA.
‖ NOR-01, batch 4674, Nordic Immunology Laboratories, Tilberg, The Netherlands.
¶ Sigma Chemical Co., St. Louis, MO.
# Human IgG subclass SRID kit; The Binding Site Ltd., Birmingham, U.K.
** Nissui Pharmaceutical, Co., Ltd, Tokyo, Japan.

Determination of Smoking Status

All participants were asked to complete questionnaires regarding smoking status. Subjects were then partitioned into one of three groups: never smoker, former smoker, or current smoker. Exposure to tobacco products was also assessed via measurement of serum cotinine, a stable metabolite of nicotine, by liquid-phase radioimmunoassay.†† Initially, 25 µl of serum sample or standard, 100 µl of ^{125}I-labeled cotinine and 100 µl of nicotine metabolite antiserum were introduced to polypropylene tubes; subsequently, tubes were incubated for 30 minutes at room temperature. Following the addition of cold precipitating solution, tubes were centrifuged for 15 minutes at 3,000 × g. Following removal of supernatant, the precipitate was measured with a Gamma counter. Serum cotinine levels were calculated from the standard curve and used to exclude active smokers from never smokers or former smokers.

Diagnosis of Diabetes

Plasma glucose and HbA1c levels were determined. Subjects displaying plasma glucose levels ≥200 mg/dl and HbA1c values ≥6.5% were diagnosed as diabetic according to the classification and diagnostic criteria of diabetes mellitus by the Japan Diabetes Society.[12]

Statistical Analysis

Statistical analyses were conducted with a software program.‡‡ Difference in the ratio of males to females among the groups (never smoker, former smoker, and current smoker) was evaluated via the likelihood ratio chi square test. Proportion of sites with PD ≥4 mm, CAL ≥4 mm, BOP (+) and calculus (+) to total examined sites were calculated for each subject, and expressed as %PD4, %CAL4, %BOP+ and %calculus+, respectively. Results were expressed as mean ± SD. One-way analysis of variance was employed to analyze the manner in which the distribution of each continuous variable differed across the groups. Tukey-Kramer HSD (honestly significant difference) test was utilized to test differences with respect to group means. Multiple linear regression analysis was performed for evaluation of the influence of smoking status and IgG subclass levels on periodontal condition. Four clinical variables, %PD4, %CAL4, %BOP+ and %calculus+, served as dependent variables. Smoking status and IgG2 were included in the final models as independent variables, the remaining variables characterized by *P* value <0.25 in stepwise regression analysis were also included. Two categorical variables, gender and smoking status, were coded with dummy variables in a hierarchical fashion as the followings: male (gender [A] = −1), female (gender [A] = 1), never smoker (smoking status [A] = 1, smoking status [B] = 0), former smoker (smoking status [A] = −1, smoking status [B] = 1), and current smoker (smoking status [A] = −1, smoking status [B] = −1).

Table 1.

Gender and the Number of Teeth Among Never Smokers, Former Smokers, and Current Smokers

	Smoking Status			
	Never (N = 197)	Former (N = 133)	Current (N = 63)	*P* Value
Gender (male:female)	26:171	121:12	58:5	<0.01
Number teeth	18.99 ± 7.97	19.34 ± 8.41	17.98 ± 8.16	0.55

RESULTS

Diabetes is one of the most important risk factors for periodontal disease. Moreover, data pertaining to clinical variables were not available regarding edentulous subjects. Therefore, 58 participants were excluded from the screened population based on the following criteria: 1) diabetes because it is an important risk factor for periodontitis; 2) edentulism, since clinical measurements could not be taken; or 3) serum cotinine levels ≥100 ng/ml in never or former smokers. Comparison of gender and the number of teeth among the groups in 393 non-diabetic, dentulous subjects is presented in Table 1. The ratios of males to females differed markedly among never smokers, former smokers, and current smokers (P <0.01). However, distributions of the number of teeth were not significantly different across the groups.

Comparison of %PD4, %CAL4, %BOP+, and %calculus+ among the groups is presented in Figure 1. Distributions of %PD4, %CAL4, and %calculus+ were significantly distinct across the groups. Means of these three variables for current smokers were substantially higher than corresponding variables for never smokers. Means of %PD4 and %CAL4 for former smokers were higher than corresponding variables for never smokers. Distribution of %BOP+ was not significantly different across the groups. Comparison of total IgG, IgG1, IgG2, IgG3, and IgG4 among the groups is presented in Figure 2. Distributions of total IgG and IgG1 displayed significant differences across the groups, although those of IgG2, IgG3, and IgG4 were not significantly different across the groups. Moreover, mean of total IgG for current smokers was substantially lower compared with the mean for never smokers. Mean of

†† Double antibody nicotine metabolite kit; Diagnostic Products Co., Los Angeles, CA.
‡‡ JMP4, SAS Institute Inc., Cary, NC.

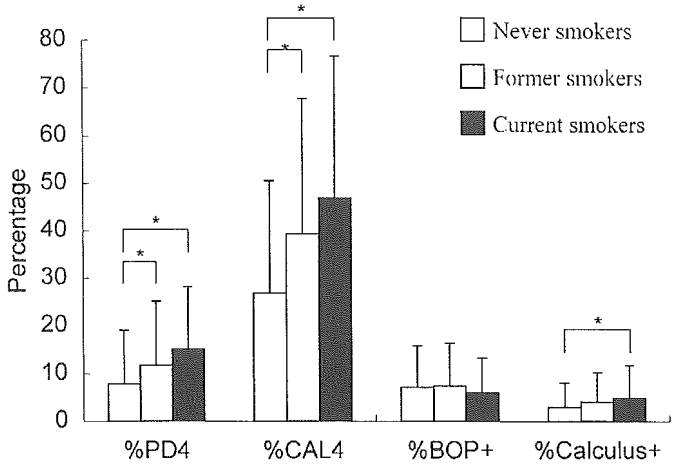

Figure 1.
*Comparison of %PD4, %CAL4, %BOP+, and %calculus+ among never smokers, former smokers and current smokers. *Significant differences with respect to group means by Turkey-Kramer HSD.*

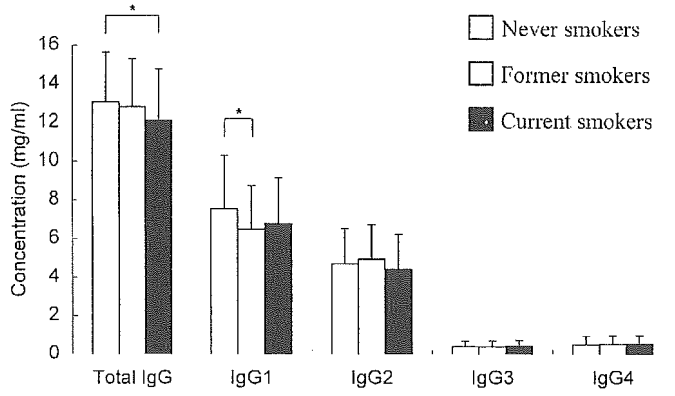

Figure 2.
*Comparison of total IgG, IgG1, IgG2, IgG3, and IgG4 among never smokers, former smokers and current smokers. *Significant differences with respect to group means by Turkey-Kramer HSD.*

IgG1 for former smokers was lower than the mean for never smokers. Comparison of anti-fimbriae total IgG, IgG1, IgG2, and IgG4 among the groups is presented in Figure 3. Distributions of those variables did not display significant differences across the groups.

Results of multiple linear regression analysis are presented in Tables 2 through 5. There was no confounding or collinearity among independent variables in the fitting models. %PD4 functioned as a dependent variable in Table 2. smoking status [A] (estimate = –2.814, P <0.001), smoking status [B] (estimate = –1.926, P = 0.037), number of teeth (estimate = –0.223, P = 0.003), IgG2 (estimate = 0.789, P = 0.024), and anti-fimbriae IgG4 (estimate = –0.004, P = 0.008) exhibited significant correlation with %PD4 (R^2 = 0.129, P <0.001). % CAL4 served as a dependent variable in Table 3. Gender [A] (estimate = –5.059, P = 0.010), smoking status

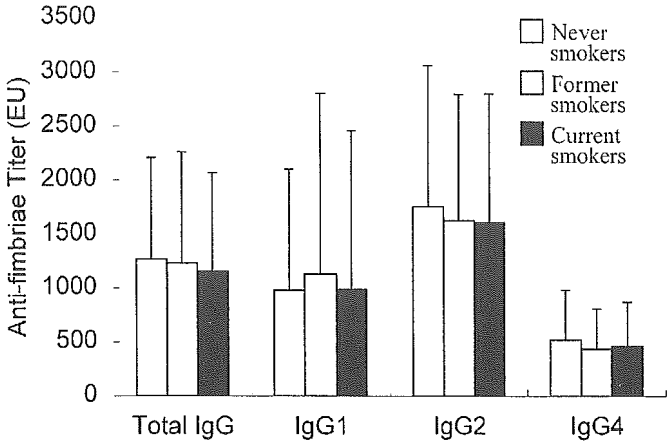

Figure 3.
Comparison of anti-fimbriae total IgG, IgG1, IgG2, and IgG4 among never smokers, former smokers and current smokers.

Table 2.

Multiple Linear Regression Analysis (%PD4)

	Dependent Variable: %PD4				
Independent Variables	Estimate	Standard Error	*P* Value	95% CI	
Intercept	5.491	3.600	0.128	–1.586	12.569
Smoking status [A]	–2.814	0.631	<0.001	–4.054	–1.574
Smoking status [B]	–1.926	0.921	0.037	–3.737	–0.116
Number teeth	–0.223	0.075	0.003	–0.370	–0.077
Total IgG	0.582	0.249	0.020	0.093	1.071
IgG2	0.789	0.347	0.024	0.107	1.470
IgG3	2.894	2.096	0.168	–1.228	7.016
IgG4	–1.998	1.437	0.165	–4.824	0.828
Anti-fimbriae IgG4	–0.004	0.001	0.008	–0.007	–0.001

CI: confidence interval.

[A] (estimate = –4.319, P = 0.030), number of teeth (estimate = –1.246, P <0.001), and IgG2 (estimate = 1.378, P = 0.048) displayed significant correlation with %CAL4 (R^2 = 0.253, P <0.001). %BOP+ functioned as a dependent variable in Table 4. Gender [A] (estimate = 1.728, P = 0.012), total IgG (estimate = 0.348, P <0.044), and IgG3 (estimate = 3.638, P = 0.014) were significantly associated with %BOP+ (R^2 = 0.064, P = 0.001). %CAL+ served as a dependent variable in Table 5. Smoking

Table 3.

Multiple Linear Regression Analysis (%CAL4)

	Dependent Variable: %CAL4				
Independent Variables	Estimate	Standard Error	*P* Value	95% CI	
Intercept	43.083	7.053	<0.001	29.215	56.951
Gender [A]	−5.059	1.953	0.010	−8.900	−1.219
Smoking status [A]	−4.319	1.981	0.030	−8.214	−0.424
Smoking status [B]	−3.461	1.850	0.062	−7.098	0.176
Number teeth	−1.246	0.150	<0.001	−1.541	−0.950
Total IgG	0.889	0.500	0.076	−0.093	1.872
IgG2	1.378	0.695	0.048	0.011	2.744
IgG4	−4.884	2.889	0.092	−10.565	0.797

CI: confidence interval.

Table 4.

Multiple Linear Regression Analysis (%BOP+)

	Dependent Variable: %BOP+				
Independent Variables	Estimate	Standard Error	*P* Value	95% CI	
Intecept	3.120	2.528	0.218	−1.850	8.091
Gender [A]	1.728	0.682	0.012	0.388	3.068
Smoking status [A]	−1.128	0.695	0.105	−2.494	0.238
Smoking status [B]	0.624	0.647	0.336	−0.648	1.895
Number teeth	−0.093	0.052	0.076	−0.196	0.010
Total IgG	0.348	0.173	0.044	0.009	0.687
IgG2	0.116	0.243	0.634	−0.362	0.595
IgG3	3.638	1.475	0.014	0.737	6.539
Anti-fimbriae IgG4	−0.002	0.001	0.091	−0.004	0.000

CI: confidence interval.

status [A] (estimate = −0.972, $P = 0.002$) and IgG1 (estimate = 0.251, $P = 0.046$) demonstrated meaningful correlation with %CAL+ ($R^2 = 0.038$, $P = 0.010$).

Table 5.

Multiple Linear Regression Analysis (%Calculus+)

	Dependent Variable: %Calculus+				
Independent Variables	Estimate	Standard Error	*P* Value	95% CI	
Intercept	−0.691	1.797	0.701	−4.225	2.843
Smoking status [A]	−0.972	0.307	0.002	−1.576	−0.369
Smoking status [B]	−0.442	0.445	0.321	−1.318	0.433
Number of teeth	0.047	0.036	0.191	−0.024	0.118
IgG1	0.251	0.125	0.046	0.005	0.497
IgG2	0.118	0.172	0.494	−0.221	0.457

CI: confidence interval.

DISCUSSION

Questionnaires have been utilized exclusively to examine smoking status in most epidemiological studies. As a result, some true current smokers were misclassified as former smokers or never smokers and such misclassification may bias estimates of morbidity associated with smoking.[13] Detection of smoking by measurement of nicotine metabolites; i.e., cotinine, is preferred over other methods, such as carboxyhemoglobin or thiocyanate determination. Suadicani et al.[14] employed serum cotinine as an objective marker of tobacco use to examine characteristics of potentially misclassified smokers with respect to mortality, morbidity, and risk factors. A serum concentration of 100 ng/ml was regarded as a relevant threshold for active smoking.[14] In the present investigation, identical threshold levels of serum cotinine were applied, which led to the exclusion of three active smokers from the never smoker and former smoker groups.

The ratios of males to females differed significantly among the groups (Table 1). Most males (179/205) had smoked; additionally, two out of three males (121/179) were former smokers. Most females (171/188) had never smoked. Smoking status in both genders in the present investigation was similar to that of the Japanese elderly population at the age of 70. Means of three clinical variables, %PD4, %CAL4, and %calculus+ for current smokers were substantially higher in comparison with never smokers (Fig. 1). Means of %PD4 and %CAL4 for former smokers were markedly higher than those for never smokers. Mean number of teeth was not significantly different among the groups; it appeared that subjects who had experienced smoking (former smokers and current smokers) exhibited

more severe periodontal destruction compared to never smokers. These findings were consistent with those of previous studies.[1] Interestingly, distribution of %BOP+ was not significantly different across the groups. Smoking might exert a strong and chronic suppressive effect on BOP, as reported by Dietrich et al.[15]

In humans, mean levels of serum IgG1, G2, G3, and G4 are 9, 3, 1, and 0.5 mg/ml, respectively. Mean levels of IgG3 were slightly lower than those of IgG4 in the present study. Wilton et al. documented similar results following measurement of levels of serum IgG subclasses by ELISA.[6] We compared four IgG subclass levels of randomly selected sera measured by ELISA with those determined by SRID. IgG3 and IgG4 values obtained with SRID decreased relative to those obtained with ELISA; moreover, reduction was greatest in terms of IgG3 levels. The contradiction in IgG3 and IgG4 levels may be due to the differences between ELISA and SRID. Qvarfordt et al. examined whether susceptibility to recurrent exacerbations in smokers with chronic bronchitis was associated with altered IgG subclass levels or IgG subclass deficiency; they found lower serum levels of IgG and IgG2 in smokers in comparison with never smokers.[16] Quinn et al. reported the association between smoking-related decrease in serum IgG2 and increased periodontal destruction in white subjects.[17] In the present investigation, current smokers displayed markedly lower total IgG levels than did never smokers; however, differences in serum IgG2 levels among the groups were not meaningful (Fig. 2). Rather, serum IgG1 levels in former smokers were significantly lower than those in never smokers. Correlation coefficient between smoking status [A] and IgG1 was 0.190 ($P<0.001$), while correlation coefficient between smoking status [A] and total IgG was 0.095 ($P=0.059$) (data not shown). Smoking status [B] was not significantly correlated with IgG subclasses. Quinn et al.[17] also noted reduced serum IgG1 and IgG4 levels in black subjects with adult periodontitis who smoked relative to their age-matched controls. Our results suggested that smoking decreases serum total IgG levels and that the decrease is primarily attributable to the IgG1 subclass in an elderly Japanese population. Smoking may influence serum levels of different IgG subclasses in a race-dependent manner.

P. gingivalis possesses many virulence factors including gingipain and fimbriae. Fimbriae may be involved in adult periodontitis; consequently, it was selected as a specific antigen to measure specific IgG response to *P. gingivalis*. In the present investigation, the assay for anti-fimbriae titers failed only in the case of the IgG3 subclass. Antigen-antibody bindings were checked carefully at each step of the assay in order to verify that anti-fimbriae IgG3 antibody was not detectable in sera from the subjects. Ogawa et al. reported that the major response to fimbriae of *P. gingivalis* 381 was IgG3, followed by IgG1, IgG2, and IgG4, in patients with periodontal disease and control subjects.[18] On the other hand, a recent study revealed that the major response to whole cells of *P. gingivalis* 381 was IgG1, followed by IgG2 and IgG4; furthermore, minimal levels of IgG3 were detected.[19] No significant difference was observed in the means of anti-fimbriae total IgG or IgG1, IgG2, and IgG4 titers among never smokers, former smokers, and current smokers in this study (Fig. 3). Neither smoking status [A] nor smoking status [B] was significantly correlated with anti-fimbriae total IgG or IgG subclasses (data not shown). Amano et al. examined the relationship between the prevalence of *fimA* genotypes of *P. gingivalis* and periodontal health status in adults. They found that the most prevalent *fimA* types were type I in healthy adults (76.8%) and type II in periodontitis patients (66.1%).[20] *P. gingivalis* 381, which provided the fimbriae employed in the present investigation, was classified as type I *fimA* genotype. DeNardin et al. reported that antibody reactions to *P. gingivalis* W50 correlated with the presence of periodontal disease in the elderly, while the responses to 381 did not differentiate elderly normal from the elderly diseased group.[21] Meaningful differences may be evident in the mean IgG and IgG subclass titers to fimbriae prepared from type II *fimA* genotype of *P. gingivalis*.

Increased periodontal destruction appeared to be related to smoking in the present investigation (Fig. 1). However, gender and serum IgG, subclass levels, rather than smoking, might be correlated with periodontal destruction. Therefore, multiple linear regression analysis was performed in order to evaluate the influence of smoking status and serum IgG subclass levels, especially IgG2, on periodontal condition (Tables 2 through 5).

%PD4 was significantly influenced by six variables (Table 2). Scaled estimates, which afford coefficients corresponding to factors scaled to a mean of zero and a range of two, were also calculated in order to ascertain the effect size (data not shown). The rank order of effect size was anti-fimbriae IgG4 > total IgG > IgG2 > number of teeth > smoking status [A] > smoking status [B]. Smoking, total IgG, and IgG2 were associated with a increase in the proportion of sites with PD ≥4 mm, while the number of teeth and anti-fimbriae IgG4 were associated with the decrease in the proportion. These findings indicate that smoking, total IgG, and IgG2 are involved in developing deep periodontal pockets although anti-fimbriae IgG4 inhibit the developing.

%CAL4 was significantly influenced by four variables (Table 3). The rank order of effect size was number of teeth > IgG2 > gender [A] > smoking status [A]. Smoking and IgG2 were associated with an increase in the proportion of sites with CAL ≥4 mm, while number of teeth and gender (female = 1) were associated with the decrease in the proportion. The strong influence consequent to number of teeth might be attributable

to significant correlation between number of teeth and %CAL4 (correlation coefficient; –0.38, $P<0.01$, data not shown). Interestingly, the effect size of IgG2 was greater compared with smoking status [A]. IgG2 distribution did not differ significantly across the groups (Fig. 2), moreover, there was no significant correlation between IgG2 and smoking status [A]. In conjunction, IgG2 may influence %CAL4 independently of smoking status. %CAL4 was influenced by both smoking status and gender; however, the effect size of gender was greater. A significant difference in the ratio of males to females among the groups may present difficulty with respect to proper analysis; consequently, future investigations require larger numbers of subjects.

%BOP+ was significantly influenced by three variables (Table 4). The rank order of effect size was IgG3 > total IgG > gender [A]. Total IgG, IgG3, and gender (female) were associated with a increase in the proportion of sites with BOP+. Neither smoking status [A] nor smoking status [B] were associated with %BOP. Vanderas et al. investigated the relationship between gingivitis and emotionally stressful states, showing that dental plaque and proximal decayed surfaces significantly affected gingivitis (gingival index).[22] The fitting model for %BOP+ may explain more than 6% of total variation, if we could include any variables for dental plaque. %calculus+ was significantly influenced by two variables (Table 5). The rank order of effect size was IgG1 > smoking status [A]. Smoking and increased total IgG1 were associated with an increase in the proportion of sites with calculus+. Three of four clinical variables, %PD4, %CAL4, and %calculus, were significantly influenced by smoking status [A], while only %PD4 was slightly influenced by smoking status [B]. Smoking experience rather than smoking at present may affect these clinical variables.

Susceptibility to periodontitis has been defined in numerous ways. Simply, subjects with gingivitis and subjects presenting with chronic adult or rapidly progressive periodontitis of similar ages were defined as periodontitis-resistant and periodontitis-susceptible, respectively.[23-25] Interleukin-1 genotype was also utilized to determine susceptibility to periodontitis.[26] Sugita et al. investigated a Japanese population of 70-year-olds to determine whether FcγRIIIb polymorphism was associated with resistance to periodontitis.[27] In the present study, we examined the influence of smoking status, total IgG subclasses, and fimbriae-specific IgG subclasses on periodontal conditions to find IgG variables to determine susceptibility to periodontitis. The fitting model for %CAL4 had the highest correlation coefficient among the four fitting models. Clinical attachment levels usually indicate periodontal destruction around a tooth. We considered the proportion of sites with CAL ≥4 mm as an index for periodontal destruction at subject levels. Smoking and serum IgG2 levels were significantly associated with %CAL4. The reason why high levels of serum IgG2 are associated with periodontal destruction is not clear; however, this finding is consistent with the result of a previous study.[6] Smoking and serum IgG2 levels may be useful for determination of susceptibility to periodontitis in Japanese populations, although the fitted model with these factors can explain only 25% of total variation.

In summary, the current results suggested that proportions of sites with PD ≥4 mm (%PD4) and proportion of sites with CAL ≥4 mm (%CAL4) are significantly influenced by the number of teeth, serum IgG2 levels, and smoking status in a Japanese elderly population. Moreover, serum IgG2 levels were not affected by smoking status. Numerous other local and systemic factors could affect the periodontal condition. Longitudinal studies are necessary to clarify whether smoking status and serum IgG2 levels influence periodontal condition in Japanese populations.

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Correspondence: Dr. Keisuke Nakashima, Department of Periodontology and Endodontology, School of Dentistry, Health Sciences University of Hokkaido, Ishikari-Tobetsu, Hokkaido 061-0293, Japan. Fax: 81-1332-3-1414; e-mail: ben-chan@hoku-iryo-u.ac.jp.

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The relationship between bone mineral density and the number of remaining teeth in community-dwelling older adults

A. YOSHIHARA*, Y. SEIDA*, N. HANADA†, K. NAKASHIMA‡ & H. MIYAZAKI*

**Division of Preventive Dentistry, Department of Oral Health Science, Graduate School of Medical and Dental Sciences, Niigata University, Niigata, Japan, †Department of Oral Science, National Institute of Public Health, and ‡Department of Periodontology and Endodontology, School of Dentistry, Health Sciences University of Hokkaido, Hokkaido, Japan*

SUMMARY **Tooth loss and osteoporosis may share common aetiologic agents which influence the process of both diseases. The purpose of this study was to evaluate the relationship between bone mineral density (BMD) of the os calcis and the number of remaining teeth, controlling the known confounding factors. Among all 70-year-old inhabitants in Niigata City, Japan, 600 people were selected randomly in order to have approximately the same number of each gender. We analysed 460 subjects. We evaluated the BMD of the os calcis using an ultrasound bone densitometer, then we counted the number of remaining teeth excluding the third molars. Stiffness is a clinical index which is calculated by the spread speed of supersonic waves. To monitor the general health condition of the subjects, serum levels of disease markers and smoking habits were also investigated. The mean number of remaining teeth for the osteopenia group (OG) and the no-osteopenia group (NOG) was 15·97 ± 9·98 and 18·31 ± 8·06, respectively in females, and 16·32 ± 9·93 and 18·12 ± 9·33, respectively in males (OG versus NOG, $P = 0{\cdot}047$ by two-way ANOVA). In addition, stiffness was significantly associated with the number of remaining teeth (CV = −0·157, $P = 0{\cdot}005$) using linear multiple regression analysis adjusted by four other variables. In this study, there was a significant relationship between the number of remaining teeth and BMD of the os calcis.**

KEYWORDS: **aetiology, general bone loss, tooth loss**

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Introduction

Osteoporosis is the most common metabolic bone disease among the elderly (65 years and older), and the incidence of osteoporotic fractures obviously increases with aging (1). Tooth loss also occurs in the elderly population, and osteoporosis has long been suspected as a risk factor for alveolar bone loss (2). Tooth loss and osteoporosis may share common aetiologic agents which influence the process of both diseases (3).

Numerous studies have been conducted to investigate the association between the number of remaining teeth and skeletal bone mineral density (BMD); alveolar bone loss and BMD; periodontal status and BMD (4–20). Almost all of these studies have focused on elderly women. The methods used to measure alveolar bone loss, skeletal BMD or controlling factors, such as serum levels, have varied among investigators. Therefore, the outcomes have been different. Some studies have shown that post-menopausal women with very low skeletal BMD have fewer teeth remaining than those who have normal skeletal BMD (4, 5). Krall *et al.* (6, 7) suggested that a greater rate of BMD loss at multiple skeletal sites is associated with the loss of one or more teeth in healthy post-menopausal women. According to other reports, mandibular bone mass is significantly correlated with skeletal BMD (8, 9). On the contrary, some previous studies have indicated a relationship between periodontal status, especially

periodontal attachment loss, and osteoporosis or skeletal BMD (5, 10–13). In spite of these findings, several studies have failed to find a significant association between the number of remaining teeth or periodontal status and skeletal BMD (14–19). Another study of adults aged 65 years and over failed to find any significant association between the number of remaining teeth and skeletal BMD in older women, although a weak but significant correlation was seen in older men (20). According to these findings, the relationship between the number of remaining teeth or alveolar bone loss and skeletal BMD remains unclear.

There are some reasons why the results of the aforementioned studies are conflicting. The majority of the studies conducted to date have had relatively small sample sizes and have included groups of subjects with a wide range of age (more than 20 years). This means that the amount of time required to develop moderate to severe alveolar bone loss or skeletal bone loss varied considerably among the subjects. A significant relationship has been difficult to establish, as the results have been easily confounded by other factors such as gender, smoking, race, age, etc.

The purpose of this study was to evaluate the relationship between BMD of the os calcis and the number of remaining teeth in a large-scale study, controlling the known confounding factors.

Materials and methods

Subjects and clinical assessment

Questionnaires were sent to all 4542 70-year-old inhabitants according to a registry of residents in Niigata City, Japan, and they were informed of the purpose of this survey. The response rate was 81·4% (n = 3695). After dividing into groups of males and females, 600 people (the screened population) were randomly selected in order to have approximately the same number of each gender for the study. The subjects for the study agreed to undergo medical and dental examinations, and signed informed consent forms regarding the protocol, which had been reviewed and approved by the Ethics Committee of the Faculty of Dentistry, Niigata University.

From the screened population, 466 subjects participated in this study. The subjects were examined at local community centres in Niigata City. We utilized the data on BMD of the heel, which we measured using an ultrasound bone densitometer (Lunar Achilles^{TM}*). The ultrasound signal is sent to the os calcis. Ultrasound densitometry measures the physical properties of bone, specifically BMD. The ultrasound measurement contains two criteria: the velocity [speed of sound (s); SOS] and frequency attenuation [broadband ultrasound attenuation (dB MHz^{-1}); BUA] of a sound wave as it travels through a bone (21, 22). Stiffness is a clinical index combining SOS and BUA, which is calculated by the spread speed of supersonic waves. The formula is (BUA-50) × 0·67+(SOS-1380) × 0·28. This charts the SOS and BUA into biological relevant ranges. The percentage of stiffness is indicated by the monitor of the bone densitometer. This figure is based on the percentage for the value for 20 year olds. Osteopenia is defined as stiffness ≤85 for 70-year-old males, and ≤69 for females (23). To monitor the general health condition, serum levels of disease markers, and smoking habits were also investigated. These disease markers were immunoglobulin (serum IgG concentration) and nutritional factors [serum albumin, total cholesterol, calcium, vitamin C, vitamin E(α) concentrations]. We counted the number of remaining teeth and checked the grip power/weight to measure physical strength. In addition, the percentage of body fat was measured by bioelectrical impedance analysis (Body composition analyzer/scales^{TM})†. Personal interviews were conducted to obtain information regarding medication taken for osteoporosis, and smoking habits.

Statistical analysis

After excluding six people who were taking oestrogen or medication known to influence calcium metabolism, 460 subjects were included in the analysis. Mean and SD were used for characterizing the continuous variables. We compared the percentage of stiffness, the number of remaining teeth, serum values, smoking habits, percentage of body fat and grip power/weight between males and females. The statistical differences were tested by chi-square test or Mann–Whitney U-test when applicable. The comparison of serum values, percentage of body fat, grip power/weight, and the number of remaining teeth between the osteopenia group (OG) and the no-osteopenia groups (NOG), in both females and males, were investigated using two-way ANOVA.

*Lunar Achilles^{TM}, GE Medical Systems, Madison, WI, USA.
†Body composition analyzer/scales^{TM}, Tanita, Tokyo, Japan.

Furthermore, multiple linear regression analysis was performed to evaluate the relationship between the number of remaining teeth and skeletal BMD. As a dependent variable, the percentage of stiffness based on the value for 20 year olds was used. As independent variables, we selected the variables which had *P*-values which were <0·1 according to the analysis for each variable. In addition, we also selected the number of remaining teeth as an independent variable.

Results

Table 1 shows the comparison by mean values of the percentage of stiffness, the number of remaining teeth, serum albumin concentration, serum IgG concentration, serum vitamin C concentration, serum vitamin E(α) concentration, the percentage of smokers, serum calcium concentration, serum total cholesterol concentration, percentage of body fat and grip power/weight between males and females. All variables except stiffness, the number of remaining teeth, the percentage of smokers, and grip power/weight, were higher in females than in males. There were significant differences in all variables except for the number of remaining teeth.

After dividing the subjects into the OG and the NOG, we compared all the variables again. There were significant differences in the concentration of serum vitamin E(α), and the percentage of body fat between the OG and NOG adjusted by gender (Table 2). Both values were greater in the NOG. Then, we evaluated the number of remaining teeth per person between the OG and NOG. The mean number of remaining teeth for the OG and NOG was 15·97 ± 9·98 and 18·31 ± 8·06, respectively in females, 16·32 ± 9·93 and 18·12 ± 9·33, respectively in males (Fig. 1). The number of remaining

Table 1. Comparison of bone mineral density, remaining teeth, serum values, percentage of body fat, grip power/weight between males and females

Variables	Subjects		*P*-value
	Males (n = 248)	Females (n = 212)	
Stiffness (%)	76·57 ± 12·85	62·53 ± 10·10	<0·001
No. of remaining teeth	16·77 ± 9·79	16·57 ± 9·56	0·625
Albumin (g dL^{-1})	4·07 ± 0·24	4·14 ± 0·23	0·002
IgG (mg dL^{-1})	1269·52 ± 267·62	1322·92 ± 274·88	0·027
Vitamin C (mcg dL^{-1})	6·21 ± 2·92	8·32 ± 3·24	<0·001
Vitamin E(α) (mcg dL^{-1})	11·12 ± 3·41	13·08 ± 4·76	<0·001
Smokers (%)	85·81 ± 168·18*	11·17 ± 36·40†	<0·001
Calcium (mg dL^{-1})	4·42 ± 0·19	4·49 ± 0·35	<0·001
Total cholesterol (mg dL^{-1})	184·11 ± 26·34	210·34 ± 30·42	<0·001
Percentage of body fat (%)	19·87 ± 5·00	28·58 ± 6·43	<0·001
Grip power/weight (kg kg^{-1})	0·68 ± 0·11	0·49 ± 0·09	<0·001

Values given in this table are mean ± s.d.
*n = 246 in total.
†n = 211 in total.

Table 2. Comparison of serum values, percentage of body fat, grip power between the osteopenia group and the no-osteopenia group

Variables	Subjects		*P*-value*
	Osteopenia (n = 342)	No-osteopenia (n = 118)	
Albumin (g dL^{-1})	4·10 ± 0·24	4·10 ± 0·22	0·895
IgG (mg dL^{-1})	1280·58 ± 254·67	1333·60 ± 314·94	0·068
Vitamin C (mcg dL^{-1})	7·13 ± 3·15	7·38 ± 3·50	0·470
Vitamin E(α) (mcg dL^{-1})	11·70 ± 3·82	13·05 ± 5·07	0·002
Smokers (%)	51·3	56·0	0·211
Calcium (mg dL^{-1})	4·45 ± 0·29	4·47 ± 0·21	0·416
Total cholesterol (mg dL^{-1})	195·49 ± 32·26	198·32 ± 27·66	0·325
Percentage of body fat (%)	23·30 ± 7·11	25·60 ± 7·09	<0·001
Grip power/weight (kg kg^{-1})	0·60 ± 0·14	0·58 ± 0·14	0·682

Values given in this table are mean ± s.d.
**P*-value by an analysis of two-way variance adjusted by gender.

teeth was significantly lower in the OG. Two-way ANOVA was performed to discriminate the effects of stiffness, gender, and stiffness-gender interaction from the number of remaining teeth. As shown by the data in the Fig. 1, the F statistic on stiffness was 3·950 and significant effects of stiffness (osteopenia/no-osteopenia, $P = 0·047$) were observed. However, we could find no significant effects in gender or in stiffness–gender interaction.

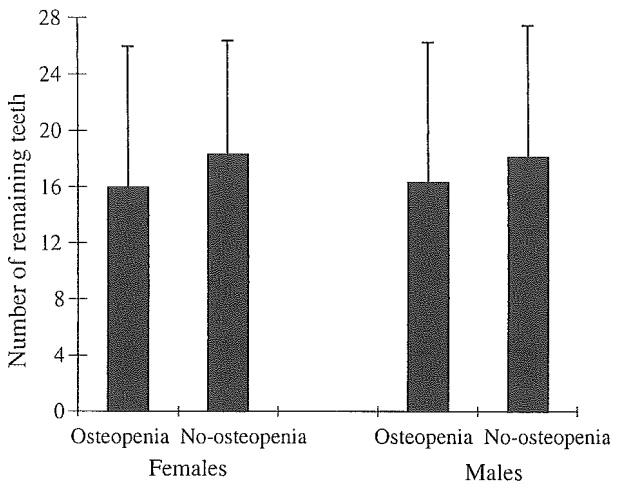

Figure 1. Relationship between the number of remaining teeth and bone mineral density. [a]Osteopenia group (OG; $n = 158$) and no-osteopenia group (NOG; $n = 54$) for females, OG ($n = 184$) and NOG ($n = 64$) for males. Data in plot represent mean ± s.d. The results of analysis of variance for the evaluation between the number of remaining teeth and stiffness (osteopenia/no-osteopenia) or gender.

Variables	Sum of squares	d.f.	Mean square	*F*-value	*P*-value
Stiffness (osteopenia/ no-osteopenia)	368·36	1	368·36	3·950	0·047
Gender (male/female)	4·84	1	4·84	0·050	0·820
Stiffness × gender	373·62	2	186·81	2·000	0·130
Residual	42 320·26	454	93·22		
Total	42 693·88	456	93·63		

To evaluate the relationship between BMD (stiffness) and the number of remaining teeth, four other variables [the percentage of body fat, serum IgG concentration, gender, and serum vitamin E(α) concentration] were selected as the independent variables in the final model. The results of linear multiple regression analysis in the final model are presented in Table 3. The number of remaining teeth (CV = 0·157, $P = 0·005$), percentage of body fat (CV = 0·396, $P < 0·001$), gender (CV = −18·169, $P < 0·001$) and serum vitamin E(α) concentration (CV = 0·262, $P = 0·048$) were significantly associated with stiffness ($R^2 = 0·318$, $P < 0·001$).

Discussion

Many studies have been conducted on the association between the number of remaining teeth and skeletal BMD, and the association between alveolar bone loss and BMD. As the results of these studies have been conflicting, a relationship controlling the confounding factors should be established in a large-scale study. Therefore, variables such as serum levels of disease markers and smoking habits were selected to monitor the general health condition of the subjects in this study. Furthermore, we selected a large number of subjects, and restricted the age to 70 years to exclude the influence of age variation in the results.

In addition, the difference in methodology to assess skeletal BMD may limit the comparability of various studies. Ultrasonic bone density measurements were used to evaluate BMD of the os calcis in this study. Dual energy X-ray absorptiometry (DEXA) can accurately measure skeletal BMD and is considered to be the gold standard. However, it was impossible to use DEXA in this study, because the BMD examinations were

Table 3. Multiple linear regression and associated *P*-values

Independent variables	Dependent variable: Stiffness (%) CV	s.e.	*P*-value	95% CF
No. of remaining teeth	0·157	0·055	0·005	0·264 to 0·049
Percentage of body fat (%)	0·396	0·097	<0·001	0·205 to 0·587
IgG (mg dL^{-1})	0·002	0·002	0·272	−0·002 to 0·006
Gender (1, male; 2, female)	−18·169	1·379	<0·001	−20·880 to −15·458
Vitamin E(α) (mcg dL^{-1})	0·262	0·132	0·048	0·002 to 0·522
Constant	83·004	3·406	<0·001	76·308 to 89·699

$P < 0·001$, $R^2 = 0·318$.

conducted at local community centres. According to previous reports, ultrasound densitometry is highly reproducible and has a high correlation with BMD measured by DEXA in different parts of the skeleton (24–26). Especially in field studies, Ultrasound bone densitometry of the os calcis is useful to assess skeletal BMD.

The results of this study showed that the subjects in the OG had a lower number of remaining teeth than the subjects in the NOG. This study clearly demonstrated that there is a significant relationship between BMD of the os calcis and the number of remaining teeth in both males and females.

Some systemic factors which contribute to the loss of bone mass have been identified. Smoking, nutritional deficiencies, age, use of medication and immune dysfunction are shown to be common factors between skeletal BMD and alveolar bone loss (1, 27, 28). It is important that this study showed a significant relationship between BMD of the os calcis and the number of remaining teeth after controlling the known confounding factors.

However, the R^2 value of the multiple regression analysis was only 0·32. This means that the amount of variance explained by the multiple regression analysis was not so high. Although the four independent variables had significant probabilities, there might be other explanatory factors. While the skeleton is heterogenic, bone density, bone turnover rate and bone remodelling ability differ in each part of the skeleton, suggesting that each type of bone has some degree of independence. Furthermore, there could be some influence from local factors such as masticatory muscles, or earlier periodontitis, on alveolar bone loss.

Furthermore, we found a significant relationship between stiffness and serum vitamin E(α) concentration, and between stiffness and the percentage of body fat in the multiple regression analysis (Table 3). A vitamin E(α) deficiency has been linked with calcium loss in bones, and according to previous reports, this could be because of increased free radical activity or decreased calcium availability for bone deposition (29, 30). An association between low amounts of fat tissue and decreased skeletal BMD has been described in some reports (31, 32). In particular, women with a higher percentage of body fat have better oestrogen metabolism and thus have a smaller risk of osteoporosis than women with a lower percentage of body fat (33). Body composition is considered to be one of the strongest predictors of bone density at multiple skeletal sites.

Finally, limitation of the present study should also be taken into consideration. In spite of finding a significant relationship between the BMD of the os calcis and the number of remaining teeth, our study is cross-sectional. Therefore, the ability to address the issue of whether osteopenia is causally related to tooth loss or not is limited. Further longitudinal studies should be undertaken to confirm a causal relationship.

In conclusion, this well-designed, large-scale study suggested that there is a significant relationship between the number of remaining teeth and BMD of the os calcis.

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Correspondence: A. Yoshihara, Division of Preventive Dentistry, Department of Oral Health Science, Graduate School of Medical and Dental Sciences, Niigata University, 1-757, Asahimachi-Dori, Niigata, 951-8510, Japan.
E-mail: akihiro@dent.niigata-u.ac.jp

