Gerodontology

Original article

Association between serum calcium and periodontal disease progression in non-institutionalized elderly

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Objective: To assess the effect of baseline serum calcium on the progression of periodontal disease in non-institutionalized elderly.

Background: Although a few studies have found some evidence of the role played by dietary calcium in periodontal disease process, there is a paucity of information pertinent to longitudinal assessment of serum calcium-periodontal relationships.

Material and methods: Clinical attachment levels of 266 Japanese subjects aged 70 years were recorded at baseline and annually for six consecutive years. Progression of periodontal disease (PPD) was defined as the number of teeth that showed additional attachment loss of ≥3 mm during the 6 years. The number of PPD was calculated for each subject and categorised into four levels, namely, PPD_0, PPD_1, PPD_2 and PPD_3 where the number of teeth with additional attachment loss ranged from 0, 1–10, 11–20 and >20 respectively. The levels of serum calcium, albumin, random blood sugar, immunoglobulin (IgG, IgA and IgM), gender, smoking habits, education, gingival bleeding and the number of teeth present were obtained at baseline.

Results: Serum calcium, IgA, smoking, gingival bleeding and teeth present were associated with PPD at $p \leq 0.10$ and were included in a multinomial logistic regression analysis. Serum calcium was the only variable that was significantly associated with PPD with relative risks of 100 at PPD_1 and PPD_2, respectively, and 1000 at PPD_3.

Conclusion: Serum calcium may be considered a risk factor for periodontal disease progression in non-institutionalized elderly.

Keywords: clinical attachment loss, elderly, periodontal progression, serum calcium.

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Introduction

Calcium is undoubtedly the most abundant mineral in humans. Virtually 99% of the total calcium in the human body exists in the bones and teeth and provides a structural function, while the remaining 1% found in tissues and fluids is crucial for the maintenance of cell metabolism, nerve transmission and muscle contraction[1]. Researchers have been exploring the role played by calcium in the aetiology and/or progression of periodontal diseases (PPD) for well over four decades. Several animal and human studies point to an association between dietary calcium and periodontitis. For instance, Oliver[2], Abe *et al.*[3] and Amano[4] have observed a relationship between calcium-deficient diet and the progression of periodontitis in rats whereas Osborn et al[5], Vogel and Wechsler[6], Nishida *et al.*[7] and Krall *et al.*[8] have obtained similar findings in human studies. Accordingly, it has been hypothesized that low dietary intake of calcium may contribute to progression of periodontitis[1,7]. However, there is a lack of information pertaining to the longitudinal assessment of the relation between serum calcium and periodontitis. Thus, it was envisaged that the current study would make a contribution to the scientific understanding of this subject. It was, therefore, hypothesised that the

levels of serum calcium in elderly affect the PPD. Accordingly, the main aim of this study was to ascertain the effect of baseline serum calcium on the PPD in non-institutionalised elderly by means of a longitudinal approach.

Material and methods

This study was a component of the ongoing oral and general health survey that has been conducted in Niigata city, Japan since 1998 and the methodology, including selection of the subjects, has been described in detail elsewhere[9,10]. In brief, 4542 (males = 2099 and females = 2443) 70-year-old registered citizens of Niigata city, Japan were originally invited to participate in the survey. Having obtained the ethical clearance from the School of Dentistry, Niigata University, Japan, a final sample of 600 subjects (males = 306 and females = 294) was randomly included into the study. All participants agreed to take part in the investigation by signing informed consent forms prior to the study. They were neither institutionalised nor hospitalised, and competent enough to carry out their routine activities independently. The current study was confined to 266 (males = 147; females = 119) dentate subjects for whom the data of total serum calcium levels were available at baseline and follow-up for six consecutive years.

The basic socio-demographic characteristics including gender, education, and smoking habits were obtained by conducting an interview prior to the intra-oral clinical examination. Biochemical parameters such as total serum calcium, albumin, random blood sugar (RBS) and immunoglobulin (IgG, IgA and IgM) were assessed at baseline. The total serum calcium concentration was measured by the *o*-cresolphthalein complexion method with the Calcium C-test (Wako Pure Chemical Industries, Osaka, Japan).

The periodontal status was ascertained by measuring clinical attachment loss (CAL) at six sites for all teeth present using pressure-controlled Vivacare, TPS Probe® (Vivacare, Schann, Liechtenstein under artificial illumination at baseline and at each annual examination. Four well-trained and calibrated investigators recorded the CAL and rounded-up the measurements to the nearest millimetre. In addition to the CAL, the number of teeth present, probing pocket depth (PD) and the percentage of sites with bleeding on probing (BOP) were also determined. The investigators were calibrated before and during the survey at baseline as well as at each follow-up visit, and the same 4 investigators were employed throughout the study. The calculated *kappa* values for the examiner consistency ranged from 0.66 to 1.00.

STATA® was the standard statistical software package employed in statistical analysis[11]. Throughout the analysis, the PPD was the dependent variable, which was defined as the number of teeth showing additional attachment loss (AAL) of ≥3 mm in a given subject during the period of 6 years and was treated as a categorical variable. The definition of AAL was based on the criteria employed by Brown *et al.*[12] to estimate the incidence of attachment loss in community dwelling elderly. From the independent variables studied, gender (0: male, 1: female) smoking (0: no, 1: yes) education (0: <10 years, 1: ≥10 years) and RBS (0: <140 mg/dl, 1: ≥140 mg/dl) were considered as dichotomous categorical variables whereas the number of teeth present, PD, BOP, the levels of serum calcium, albumin, and immunoglobulin (IgG, IgA and IgM) were continuous variables. Statistical analysis was based on a stepwise procedure where a predetermined p-value (0.1), which was higher than the conventional level (0.05), was employed to select independent variables at bivariate level to augment the number of independent variables considered for further analysis at the multivariate level[11]. Accordingly, the association between two variables was determined using Pearson's or Spearman correlation techniques wherever applicable and the independent variables that reached $p \leq 0.10$ in the bivariate analysis were included in a multinomial logistic regression analysis where PPD was the multi-categorical dependent variable. The level of significance was fixed at $p \leq 0.05$ in multinomial logistic regression analysis.

Results

The serum calcium concentration in this group of elderly was 3.6–5.1 mEq/l with a mean of 4.48 mEq/l (SD = 0.21). From the 266 subjects at baseline, seven (2.63%) became edentulous during the 6-year period and consequently, the current analysis was confined to 259 dentate subjects who were present at each annual examination throughout the study period and for whom the data of total serum calcium levels were available. The baseline periodontal characteristics including CAL, PD and BOP of these elderly are presented in Table 1. The number of teeth present in the subjects at baseline (n = 266) ranged from 1 to 32 with a mean of 19.27 (SD = 8.21) at baseline, while the mean number of teeth present at the end of the 6-year period (n = 259) was 18.11(SD = 8.28). There was no significant difference between the

Table 1 Summary of periodontal parameters at baseline.

Parameter	*Mean*	*SD*	*Range*
CAL (mm)	3.04	1.05	1.31–8.00
PD (mm)	1.98	0.56	1.00–5.42
BOP (%)	6.38	7.30	0.00–50.00

CAL, clinical attachment loss; PD, probing pocket depth; BOP, bleeding on probing.

Table 2 Distribution of progression of periodontal disease (PPD).

*PPD**	*n (%)*
0 (PPD_0)	7 (2.7)
1–10 (PPD_1)	141 (54.4)
11–20 (PPD_2)	96 (37.1)
>20 (PPD_3)	15 (5.8)
Total	259 (100.0)

*PPD was defined as the number of teeth that develops additional attachment loss of ≥3 mm in a given person during the 6-year period.

former and latter means ($p = 0.11$). The PPD was in the range of 0–31 (mean = 10.12, SD = 6.47) during the 6-year follow-up period. Based on the mean value, the PPD was converted into a multi-categorical variable where PPD_0 = no teeth with AAL, PPD_1 = 1–10 teeth with AAL, PPD_2 = 11–20 teeth with AAL and PPD_3 = >20 teeth with AAL. The consequent distribution of the PPD is shown in Table 2.

Table 3 shows the relationships between PPD and the baseline independent variables under study. Accordingly, the variables with $p \leq 0.10$, namely, BOP, teeth present, smoking, IgA and total serum calcium were included in multinomial logistic regression analysis where PPD was the multi-categorical dependent variable and PPD^0 was the reference group. Table 4 shows the multinomial logistic regression model for the associations between PPD and independent variables at three different levels of PPD. None of the variables, excluding the total serum calcium, was significant at any level of PPD. Serum calcium was the only variable that remained significant at all three levels of PPD, with relative risk ratios of 0.01, 0.01 and 0.001 at PPD_1, PPD_2 and PPD_3, respectively.

Discussion

The findings of this study indicate that there was an inverse relationship between the total serum

Table 3 Relationship between the independent variables and PPD.

Variable	*Correlation coefficient*	*p-value*
Albumin	−0.01	0.88
Calcium*	−0.10	0.04
IgG	0.04	0.50
IgA*	0.10	0.08
IgM	0.04	0.58
Random blood sugar (0:<140, 1: ≥140)	−0.06	0.42
Smoking* (0: no, 1: yes)	0.15	0.02
Education (0: <10 years, 1: ≥10 years)	0.03	0.65
Gender (0: M, 1: F)	−0.02	0.80
Number of teeth present*	0.10	0.03
BOP* (%)	−0.10	0.09
PD	−0.05	0.45

PPD, progression of periodontal disease; PD, probing pocket depth; BOP, bleeding on probing.
*Variables with *p*-values ≤0.10 were included in multinomial logistic regression analysis.

Table 4 Multinomial logistic regression model for PPD.

	RR	*SE*	*p-value*	*95% CI for RR*
PPD_1				
Calcium	0.01	0.02	0.02	2×10^{-4}–0.48
IgA	1.003	0.004	0.37	0.99–1.01
Smoking	1.30	1.43	0.81	0.15–11.27
BOP	0.53	2.00	0.87	3×10^{-4}–877.71
Number of teeth present	1.03	0.04	0.43	0.96–1.11
PPD_2				
Calcium	0.01	0.02	0.03	2×10^{-4}–0.69
IgA	1.01	0.004	0.20	0.99–1.01
Smoking	3.02	3.36	0.31	0.35–26.43
BOP	0.04	0.15	0.43	1×10^{-5}–119.74
Number of teeth present	1.06	0.04	0.19	0.98–1.14
PPD_3				
Calcium	0.001	0.001	0.004	3.0×10^{-6}–0.09
IgA	1.004	0.004	0.32	1.0–1.01
Smoking	1.59	2.11	0.73	0.12–21.47
BOP	2.31	11.77	0.87	1×10^{-4}–50248.9
Number of teeth present	1.05	0.05	0.36	0.95–1.16

PPD, progression of periodontal disease; BOP, bleeding on probing.
PPD_0 is the comparison group; Pseudo $R^2 = 0.07$; $\chi^2 = 25.90$ ($p = 0.04$).

calcium concentration and periodontal disease progression, as denoted by PPD at all three levels. This was statistically significant, notwithstanding smoking, BOP, the number of teeth intact and the levels of IgA. In other words, elderly subjects with low serum calcium levels and AAL in ≤20 teeth and >20 teeth, respectively, would have 100 and 1000 times greater chance of developing further periodontal disease progression compared to those who had high serum calcium levels and no teeth with AAL. This, in turn, suggests that the elderly with more extensive periodontal progression were at a greater risk of being affected by low total serum calcium levels than those with less extensive periodontal breakdown. In this connection, it is also noteworthy that this is the first longitudinal study, which has been reported to date and which demonstrates a significant association between the total serum calcium levels and periodontal disease progression in elderly patients.

It should be highlighted that recent cross-sectional studies, which investigated the relationship between dietary calcium/total serum calcium and periodontal disease[7] and serum Mg/Ca ratio and periodontal disease[13] have obtained findings similar to the present study despite the fact that this sample was confined to the elderly. Interestingly, Nishida *et al.*[7] have demonstrated that females aged 20–39 years with low total serum calcium levels, had 6.11 odds of developing periodontal disease, notwithstanding tobacco use, gingival bleeding and dietary calcium intake even though such an association was not observed in males or older females. They also reported a dose–response effect of dietary calcium intake on periodontal disease in females. In contrast, in this study, gender was not significantly associated with either the total serum calcium level or PPD. Meisel *et al.*[13], on the other hand, reported a significant relationship between serum Mg/Ca ratio and CAL even after controlling for age, sex, gender, education, smoking and HbA_{1C}. Moreover, a randomised placebo-controlled clinical trial in subjects aged ≥65 years has shown that tooth loss was 2.5 times greater in controls compared with the experimental group who received dietary calcium and vitamin D supplementation during a 3-year period, whilst tooth loss was almost halved in subjects whose dietary calcium intake was ≥1000 mg/day in comparison to those who consumed less during the 2-year follow-up period[8]. Although not directly comparable with the findings of this study, it points to the importance of dietary and/or serum calcium-periodontal relationships, especially, in elderly patients.

Inadequate dietary intake of calcium in adults may be associated with osteopenia and osteoporosis, which are systemic skeletal disorders characterised by reduced bone mineral density[14]. As systemic bone mineral density has been shown to be linked with oral bone mineral density[15–17] as well as periodontal destruction[14,18] and that there are indications for osteoporosis-periodontal[19,20] as well as calcium-alveolar bone loss-periodontal interrelationships[21,22], it may be plausible that the effect/s of calcium on periodontal disease progression could be mediated through the changes that occur in alveolar bone mass. In this connection, it is worth mentioning that Devlin *et al.*[23] have recently described a novel approach to diagnose osteoporosis based on routine dental radiographs. It has also been suggested that the imbalance of calcium/phosphorous concentrations in the blood would stimulate the secretion of parathyroid hormone, which in turn, would result in a loss of calcium from the skeleton, including alveolar bone[7]. In spite of the fact that the exact role played by calcium on the aetiology and progression of periodontal diseases, as well as the biological phenomena underlying such a role, have not been precisely defined, the significant association between total serum calcium level and PPD observed in the present sample of non-institutionalized elderly, even after controlling for potential confounders, could be explained by the biological mechanisms mentioned hitherto.

Interestingly, only 14 (5.4%) of the elderly showed total serum calcium levels below the normal range of that for Japanese elderly (4.1–5.0 mEq/l)[24]. Apparently, the levels of serum calcium in these 14 subjects were approaching the lower limit of the normal range (3.6–4.0 mEq/l; results not shown). Consequently, neither the categorisation of the individuals into low/high total serum calcium levels nor the demonstration of serum calcium-PPD dose–response relationships was possible in the current analysis owing to the presence of relatively few subjects with low total serum calcium levels. Conversely, even though the concentration of total serum calcium was within the normal range in almost 95% of the study group, the serum ionised calcium that is considered as the metabolically active portion of calcium could not be analysed. It has also been reported that the reduced levels of ionised calcium might be linked with the normal ageing process in otherwise healthy elderly subjects[25]. Moreover, the information pertinent to the serum magnesium levels that have been said to be associated with not only periodontitis, but also the biological activity of serum

calcium[13] was not available for the participants in this study. The dynamic, competent and otherwise healthy status of the non-institutionalized elderly population concerned would also render them less susceptible to severe periodontal break down compared to their institutionalised and/or hospitalised counterparts who are less active and dependent[9,10].

Although smoking is a well-established risk factor for periodontal disease, it was not reflected in the multinomial logistic regression model of the current analysis: the relatively small sample of smokers (n = 45) in the present study, who could also be considered as light smokers given that 89% of them smoked less than 10 cigarettes per day: results not shown. Accordingly, the impact of smoking on PPD might have been diluted in the multivariate analysis where the influence of several factors was simultaneously assessed. It might also be possible that a proportion of the subjects could have developed diabetes mellitus (type II) during the course of the study, which in turn could have influenced the current findings whereas gingival recession could have contributed to PPD in this elderly population – neither the information relevant to any of these nor radiographic analysis of bone height/density was available for the present sample.

Given such limitations mentioned, one should exercise caution in interpreting the present findings and consequently further studies, especially longitudinal assessments and clinical trials, involving populations of institutionalised and hospitalised individuals who are at a greater risk for periodontitis should be carried out. Also, it may be relevant to assess serum ionized calcium levels in such investigations involving elderly populations.

In conclusion, the findings of our 6-year longitudinal study in this non-institutionalised elderly population clearly demonstrate that the total serum calcium levels were significantly associated with PPD regardless of other potential confounding factors including smoking, RBS levels and gingival bleeding as well as the levels of immunoglobulin and the number of teeth present at baseline. Accordingly, the total serum calcium could be considered as a risk factor for progression of periodontal disease in this population of non-institutionalised elderly.

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Salivary spinability and periodontal disease progression in an elderly population

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ABSTRACT

Objective: To explore the relationship between the spinability of stimulated whole saliva and periodontal disease progression over 12 months in an elderly population.

Methods: Three hundred and thirty-two subjects aged 76 years at baseline were studied. Attachment loss was calculated on a site-by-site basis, and periodontal disease progression was defined as an attachment loss of ≥3 mm. Stimulated whole saliva was collected and salivary spinability (SS) was measured. A multiple linear regression analysis was performed to assess the relationship between periodontal disease progression and SS after controlling for other covariates. The independent variables were selected from those which had significant relationships with disease progression in the bivariate analyses.

Results: Mean SS was 1.94 ± 0.42 mm in males and 1.88 ± 0.32 mm in females; this difference was not significant. Simple linear regression analysis showed a significant positive relationship between periodontal disease progression and SS ($P = 0.026$), whereas there was no significant relationship between periodontal disease progression and salivary flow rate. Multiple regression analysis revealed a significant positive relationship between periodontal disease progression and SS ($P = 0.024$) after controlling for the number of remaining teeth and baseline periodontal conditions. The model explained 15.5% of the variance in the percentage of sites where the disease had progressed.

Conclusions: These findings suggest that elderly subjects with viscous saliva are prone to periodontal disease progression.

1. Introduction

As saliva plays an important role in the maintenance of oral function, decreased salivary flow has an adverse effect on oral conditions. In patients whose salivary glands had been irradiated for head and neck cancer, it was reported that damage to the glands led to diminished salivary flow[1] and the development of dental root caries.[2] However, few epidemiological studies have elucidated the relationship between salivary flow and periodontal health in healthy individuals.[3]

Saliva has the rheological properties of viscosity, solubility, elasticity and adhesiveness as a result of the unique chemical and structural characteristics of its mucins.[4] The lubricating action of saliva is essential for oral health. It facilitates movement of the tongue and lips in swallowing and eating. The efficacy of saliva as a lubricant depends on its viscosity.[5] The viscosity of a fluid composed of small molecules depends

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on the forces of intermolecular attraction present, and on the degree of friction between different molecular layers moving in parallel within the fluid.[6] It has been shown that increased salivary viscosity is associated with an increase in dental caries in rats.[7] Additionally, the viscosity of stimulated whole saliva is greater in vomiting bulimics with severe dental erosion.[8] However, no studies, except one,[9] have investigated the relationship between the physical properties of saliva and periodontal conditions.

Saliva forms a thin mobile layer over the oral mucosa. This layer is thought to be the first line of defence against chemical, physical and biological insult, and a change in this barrier will compromise oral health. It has been shown that the surface films contain very high concentrations of calcium; almost 40% of total salivary calcium was concentrated in the surface layer.[10] Moreover, it has been suggested that surface rheology depends on protein interactions mediated by calcium, and that the surface layer enhances the function of saliva as a protective layer.[10] Salivary viscosity has been shown to increase with the addition of calcium to saliva, while removal of calcium by EGTA caused a decrease in viscosity.[11] In addition, several studies have indicated that salivary calcium concentration is an important factor in periodontal health.[12–14] Thus, it is possible that there is an association between salivary viscosity and periodontal conditions.

Since saliva is a non-Newtonian fluid, measurement of its viscosity requires the use of a special device called a viscometer which is expensive and complicated to use.[15–17] Spinability is the thread-forming capacity of mucus under the influence of a large-amplitude elastic deformation, and provides information about the internal cohesive forces of mucus.[18] Factors that control mucus spinability are the concentration of mucous glycoproteins, the degree of intermolecular and intramolecular cross-linkings, and the hydration of mucus.[19–21] Recently, salivary spinability (SS) has been shown to correlate positively with salivary viscosity and to be quick and easy to measure.[22]

Previously, the authors reported that a low salivary flow rate (SFR) and high SS had a deleterious effect on periodontal disease.[9] However, since that study had a cross-sectional design, the longitudinal relationship between salivary parameters and periodontal disease progression was not clear. Thus, the purpose of the present study was to explore the relationship between the spinability of stimulated whole saliva and the progression of periodontal disease over 12 months in an elderly population.

2. Methods

The baseline and follow-up studies were performed in June 2004 and 2005, respectively. Subjects included in the baseline study were recruited from people born in 1927, residing in the city of Niigata, Japan. The study population consisted of community-dwelling, independently living elderly people aged 76 years at baseline. Six hundred subjects were selected at random from the target population, and 355 dentate subjects participated in the baseline study.[9] Of these 355 subjects, this longitudinal study analysed 332 (93.5%) subjects who participated in both the baseline and follow-up examinations. In this epidemiological study, all subjects gave informed consent and the examination protocol was reviewed and approved by the Ethics Committee of the Faculty of Dentistry, Niigata University.

At baseline and follow-up, periodontal examinations were undertaken by four trained dentists. All subjects were examined at local community centres in Niigata City. Mouth mirrors incorporating a light and pressure-sensitive plastic periodontal probes, set to give a constant probing force of 20 g and graduated at 1-mm intervals (Vivacare TPS Probe), were used. All functioning teeth including the third molars were assessed, except for partially erupted teeth. The pocket depth (PD) and attachment level (AL) 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 millimetre. In cases where a restorative margin was apical to the cemento-enamel junction (CEJ), AL was measured taking into account the anatomical features of the teeth and, if present, the CEJ of the adjacent tooth/teeth. Also, bleeding on probing (BOP) was measured at six sites per tooth. To evaluate the progression of periodontal disease during the 12-month period, attachment loss was calculated by subtracting baseline AL from follow-up AL on a site-by-site basis. In order to distinguish between genuine change and measurement error, periodontal disease progression was defined as an attachment loss of $\geq$3 mm at each site assessed, in common with other epidemiological studies.[23–26] Before and during the survey, calibrations were conducted in an institution for the aged and the Faculty Hospital of Dentistry, Niigata University. Inter-examiner agreements ranged from 86.6% to 95.9% and from 65.8% to 94.4% for PD and AL, respectively. Kappa values ranged from 0.79 to 0.93 and from 0.56 to 0.92 for PD and AL, respectively.

Saliva samples were collected in the morning between 9:00 and 11:00 h or in the afternoon between 13:00 and 15:00 h. Oral intake and smoking were forbidden for at least 1 h prior to saliva collection. Stimulated whole saliva was collected from 332 subjects at baseline. Subjects chewed a 1-g piece of paraffin wax for 1 min, and after swallowing once, they expectorated secreted saliva into a test tube. The collection time was 3 min and SFR was calculated in ml/min. After stimulated whole saliva was collected, SS was measured immediately using the Neva Meter (IMI-001 Ishikawa Ironworks Co. Ltd., Japan). The Neva Meter can measure SS objectively with acceptable reproducibility,[22] and is based on the principle that electrical resistance approaches infinity at the cutting position. After a saliva sample is introduced to the bottom reservoir of the device, it is automatically stretched at a constant rate of 5 mm/s. Next, application of an electrical current (5 V) to the liquid induces a microcurrent, which stops when the thread breaks. The device detects the point at which the current stops and then measures the maximum length (in millimetres) of the thread, i.e. the spinability. Five consecutive measurements were taken, and SS was calculated by averaging three of the five values, excluding the highest and lowest readings. As room humidity may also affect SS,[20,21] temperature and humidity were maintained throughout the measurements at 22–26 °C and 55–60%, respectively.

Baseline information about the subjects' smoking status was obtained using a questionnaire. Subjects were asked

about their use of cigarettes and categorised as current smokers, former smokers or never smokers. The questionnaire also obtained information about oral hygiene habits, i.e. frequency of interdental cleaning (daily/frequently versus rarely/never) and the last dental visit (within 1 year versus >1 year). In addition, data in relation to periodontal treatment during the follow-up period were collected.

Since all subjects had participated in a medical examination prior to the oral examination, the number of prescription medications that they were taking and the number of diseases for which they were currently being treated were also confirmed from physicians' examination records at baseline. The number of prescription medications was dichotomised: no medication, between one and four medications, and five medications or more. Similarly, subjects were categorised according to the number of systemic diseases: no disease, between one and two diseases, and between three and five diseases.

For salivary and periodontal parameters, means and standard deviations were calculated; Student's t-test and one-way analysis of variance (ANOVA) combined with Scheffe's post hoc test were used to analyse differences between the groups. As there are no universally accepted standards for SFR and SS, the associations between salivary parameters and periodontal disease progression were explored by simple linear regressions. Simple linear regressions were also used to analyse the effect of the baseline periodontal conditions on disease progression over 12 months. Finally, multiple linear regression analysis was performed to assess the relationship between periodontal disease progression and SS after controlling for other covariates. The dependent variable was the percentage of sites with periodontal disease progression over 12 months, and the independent variables were selected from those which had significant relationships with disease progression in the bivariate analyses. The chosen level of statistical significance was 5%. Data analysis was performed using STATA software (Stata 9 for Windows, Stata Corporation, College Station, TX, USA).

3. Results

Although baseline periodontal parameters, including the percentage of sites with PD ≥6 mm and AL ≥6 mm, were compared between subjects who were followed-up ($n = 332$) and subjects who dropped out of the study ($n = 23$), there were no significant differences. Similarly, smoking status, mean number of medications and mean number of systemic diseases did not differ between the two groups. However, a significantly higher mean number of remaining teeth was found in subjects who were followed-up (18.4 teeth) compared with subjects who dropped out (14.7 teeth) ($P = 0.04$). The mean number of teeth lost was 0.34 in the 332 subjects who were followed-up.

Table 1 shows the mean values for salivary parameters at baseline by gender, smoking status, number of prescription medications and number of systemic diseases. The mean SFR was significantly higher in males (1.62 ± 0.91 ml/min) than females (1.24 ± 0.66 ml/min). Subjects who took more medications or who had more systemic diseases exhibited a trend towards decreased SFRs, although these differences did not reach statistical significance. Mean SS was 1.94 ± 0.42 mm in males and 1.88 ± 0.32 mm in females; this difference was not significant. Mean SS values in current, former and never smokers were 1.99 ± 0.39, 1.97 ± 0.47 and 1.87 ± 0.30 mm, respectively ($P = 0.04$, ANOVA; not significant within the three possible pair-wise comparisons, Scheffe's test). There was no significant relationship between SS and the number of medications or diseases.

Table 1 – Mean values for salivary parameters by gender, smoking status, number of prescription medications and number of systemic diseases

Subject characteristics	n	Salivary flow rate (ml/min)	Salivary spinability (mm)
Gender			
Male	174	1.62 ± 0.91***	1.94 ± 0.42 NS
Female	158	1.24 ± 0.66	1.88 ± 0.32
Smoking status[a]			
Current	43	1.57 ± 0.82 NS	1.99 ± 0.39*
Former	109	1.51 ± 0.91	1.97 ± 0.47
Never	178	1.36 ± 0.76	1.87 ± 0.30
Number of medications			
0	141	1.51 ± 0.84 NS	1.92 ± 0.35 NS
1–4	124	1.40 ± 0.81	1.91 ± 0.38
5≤	67	1.36 ± 0.80	1.91 ± 0.43
Number of diseases[b]			
0	74	1.57 ± 0.84 NS	1.94 ± 0.39 NS
1–2	229	1.40 ± 0.82	1.91 ± 0.39
3–5	28	1.43 ± 0.79	1.87 ± 0.27

Mean values ± standard deviation are given.
***$P < 0.001$; *$P < 0.05$; NS, not significant. P-values between genders were obtained with Student's t-test, and for other variables, with one-way analysis of variance.
[a] Data missing for two subjects.
[b] Data missing for one subject.

The mean percentage of sites with periodontal disease progression over 12 months by various subject characteristics is shown in Table 2. Although males had a higher percentage of sites with disease progression, there was no significant difference between genders. An inverse relationship was found between disease progression and the number of remaining teeth ($P < 0.001$, ANOVA; $P < 0.001$ between subjects with 1–9 teeth and 10–19 teeth and between subjects with 1–9 teeth and 20–32 teeth; not significant between subjects with 10–19 teeth and 20–32 teeth, Scheffe's test). In subjects with 1–9 teeth, 9.7 ± 15.1% of sites showed periodontal disease progression, compared with 2.4 ± 4.2% in subjects with 20–32 teeth. Although current and former smokers experienced more disease progression than never smokers, the difference did not reach statistical significance. Similarly, the percentage of sites with disease progression did not differ with the frequency of interdental cleaning or the last dental visit. Although subjects who had not received periodontal treatment during the study period experienced more periodontal disease progression, there was no significant difference between the two groups.

The results of the simple linear regression analysis are shown in Table 3. A significant positive relationship was found

Table 2 – Mean percentage of sites with periodontal disease progression over 12 months by subject characteristics

Subject characteristics	n	% of sites with progression
Gender		
Male	174	5.0 ± 9.2 NS
Female	158	3.4 ± 7.6
Number of teeth present		
1–9	64	9.7 ± 15.1***
10–19	91	4.0 ± 6.9
20–32	177	2.4 ± 4.2
Smoking status[a]		
Current	43	5.3 ± 7.2 NS
Former	109	5.2 ± 10.7
Never	178	3.5 ± 7.3
Interdental cleaning[b]		
Daily/frequently	179	4.2 ± 9.0 NS
Rarely/never	150	4.5 ± 8.0
Last dental visit[b]		
Within 1 year	233	4.1 ± 8.5 NS
>1 year	96	4.6 ± 8.6
Periodontal treatment during follow-up period[b]		
Received	160	3.4 ± 5.6 NS
Not received	169	5.1 ± 10.6

Mean values ± standard deviation are given.
***$P < 0.001$; *$P < 0.05$; NS, not significant. P-values for variables with two alternatives were obtained with Student's t-test, and for variables with three alternatives, with one-way analysis of variance.
[a] Data missing for two subjects.
[b] Data missing for three subjects.

between periodontal disease progression and SS ($P = 0.026$), whereas there was no significant relationship between periodontal disease progression and SFR. Baseline periodontal parameters significantly associated with disease progression were percentage of sites with PD ≥6 mm and percentage of sites with AL ≥6 mm. BOP had no impact on disease progression.

Table 4 shows the result of the multiple regression analysis. A significant positive relationship was found between periodontal disease progression and SS ($P = 0.024$) after controlling for the number of remaining teeth, percentage of sites with PD ≥6 mm and percentage of sites with AL ≥6 mm. The model explained 15.5% of the variance in the percentage of sites where the disease had progressed.

Table 3 – Simple linear regressions for assessing the effect of baseline salivary and periodontal parameters on periodontal disease progression over 12 months

Independent variables	Coefficient	P	95% CI
Baseline salivary parameters			
Salivary spinability (mm)	2.76	0.026	0.33–5.18
Salivary flow rate (ml/min)	0.14	0.809	−0.99 to 1.27
Baseline periodontal parameter			
% of sites with PD ≥6 mm	0.69	0.000	0.43–0.96
% of sites with AL ≥6 mm	0.09	0.008	0.02–0.17
% of sites with BOP	−0.01	0.814	−0.08 to 0.06

CI, confidence intervals; PD, pocket depth; AL, attachment level; BOP, bleeding on probing. Dependent variable was the percentage of sites with periodontal disease progression over 12 months.

4. Discussion

This is the first longitudinal study to assess the relationship between the spinability of stimulated whole saliva and the progression of periodontal disease over 12 months. In this elderly population, a significantly higher rate of disease progression was found in subjects with a higher SS, and this relationship persisted after controlling for other covariates with the use of a multiple regression analysis. One possible explanation for this finding is that higher salivary viscosity results in impairment of the salivary substrate clearance mechanism, due to an increase in molecular cohesion which inhibits the ability of saliva to flow.[7] Another explanation may be that increased salivary viscosity contributes to a change in salivary lubricant function. In addition, high-molecular-weight mucin and structurally similar molecules have been identified in the pellicle formed on root cementum after exposure to saliva.[27–29] It is possible that cleansing, one of the most important roles of saliva, would be less effective and that the accumulation of plaque would be encouraged due to altered properties of saliva. Thus, these findings suggest that subjects with viscous saliva would be prone to periodontal disease progression.

No significant relationship was found between SFR and the progression of periodontal disease in this elderly population. It was also confirmed in the baseline study that none of the periodontal parameters were related to SFR.[9] An epidemiological study targeting healthy individuals reported that major salivary gland flow did not impact on gingival and periodontal conditions.[3] In addition, a case–control study showed that in

Table 4 – Multiple linear regression analysis to explore factors for periodontal disease progression

Independent variables	Coefficient	P	95% CI
Salivary spinability (mm)	2.61	0.024	0.35–4.87
Number of teeth present at baseline	−0.30	0.000	−0.42 to −0.19
% of sites with PD ≥6 mm at baseline	0.73	0.000	0.43–1.04
% of sites with AL ≥6 mm at baseline	−0.09	0.039	−0.17 to 0.00
Constant	4.43	0.090	−0.69 to 9.56

CI, confidence intervals; PD, pocket depth; AL, attachment level. $n = 332$. Explained variance, adjusted for degrees of freedom (R^2 adj.) = 15.5%. Dependent variable was the percentage of sites with periodontal disease progression over 12 months.

spite of the significantly lower salivary flow in patients with Sjögren's syndrome, periodontal conditions did not differ between these patients and controls.[30] The results of the present longitudinal survey are consistent with these other studies. Thus, it is suggested that the low SFR alone could not increase the risk of developing periodontal disease.

In this elderly population, current and former smokers experienced more disease progression than never smokers. However, the difference did not reach statistical significance. It is reasonable to assume that this may be caused by a relatively short period of observation, and that this relationship may become significant over a longer period.

This study found that mean SS in current, former and never smokers decreased gradually, while SS did not differ between genders. Thus, the significant relationship between SS and smoking status was not biased by gender difference. On the other hand, it has been found that the ultrasound value of the heel representing bone mineral density was lower in heavy smokers compared with never smokers, and that the salivary calcium concentration of smokers was higher than that of never smokers.[31] Thus, it could be suggested that the decrease in skeletal bone density may increase the amount of calcium in saliva. Briefly, general calcium turnover would be reflected in saliva. In addition, it has been shown that elevated levels of salivary calcium made saliva more viscous.[11] The involvement of calcium in the structure of mucus may enhance intermolecular cross-links between mucins. Thus, these findings suggest that calcium would be a key component of saliva, which links smoking status and saliva viscosity.

Again, this longitudinal study showed a positive association between SS and periodontal disease progression. As cited above, elevated levels of salivary calcium were shown to make saliva more viscous.[11] In addition, several studies have indicated that a high level of salivary calcium increases the risk of periodontal disease.[12–14] A high level of salivary calcium has been shown to be closely related to rapidly mineralising plaque,[12] which may lead to further plaque retention and poor oral hygiene. The continuous, apically growing calcifying plaque may be enough to cause periodontal disease progression. Therefore, these findings suggest that salivary calcium would mediate the positive association between SS and periodontal disease progression.

It has been reported that the daily intake of multiple drugs and multiple systemic diseases resulted in less salivary secretion.[32,33] However, it has also been stated that when a person uses several medicines, it is difficult to determine which has the most detrimental effect on SFR.[34] This study could not show an association between stimulated SFRs and the number of medications or systemic diseases. A possible explanation for the lack of an association may be that this elderly population was relatively healthy and that the number of subjects who had numerous systemic diseases was small. It may also be because stimulated SFRs were measured in this study instead of resting SFRs, which have been reported to be more sensitive to the influence of medication.[33]

The present study investigated stimulated saliva. As saliva was collected in the morning or in the afternoon, circadian rhythm may have affected the results. However, the viscosity of stimulated saliva remained stable throughout the repeated measurements within a day, while significant within-subject variation was found in the viscosity of unstimulated saliva.[17] This could be due to the increased proportion of parotid secretion during masticatory stimulation, because the parotid gland is known to be the greatest contributor to stimulated salivary flow and to produce more serous saliva.[35–37]

Unstimulated salivary flow is a measure of the amount of saliva that is constantly secreted into the oral cavity, whereas stimulated salivary flow is a measure of the functional capacity of the gland. The major contributor to unstimulated flow is the submandibular gland, which produces the less serous, mucin-rich saliva.[35] Moreover, secretions of the submandibular gland have been shown to be significantly more viscous than secretions of the parotid gland.[36,37] Thus, it is reasonable to assume that the spinability of unstimulated saliva may be higher than that of stimulated saliva.

In the multiple linear regression analysis, a negative relationship was found between periodontal disease progression and severe (≥6 mm) AL at baseline. This contradictive finding can be partly explained by the effect of tooth loss. Further analysis of the data revealed that 26.2% of the subjects with severe AL at baseline had lost at least one tooth during the 12 months, while 15.0% of the subjects without severe AL had experienced tooth loss ($P < 0.05$, Chi-square test, data not shown). In other words, subjects with severe AL at baseline tended to lose more teeth than subjects without severe AL. Thus, it is possible that periodontal disease progression was underestimated in the subjects with severe AL, because teeth lost during the study period could not be included in the longitudinal data analysis. However, the impact of tooth loss on the results of this study would be small, since the 113 teeth lost represented just 1.9% of the total number of teeth examined at both baseline and follow-up.

In conclusion, these findings suggest that elderly subjects with viscous saliva are prone to periodontal disease progression.

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The relationship between periodontal condition and serum levels of resistin and adiponectin in elderly Japanese

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Background and Objective: Diabetes and periodontitis are associated with each other. Adipokines, specifically adiponectin and resistin, are secreted from adipocytes and are thought to cause insulin resistance in rodents. Additionally, adiponectin and resistin may play a role in inflammation and immune responses. The aim of this study was to clarify the relationship between serum levels of adipokines and periodontal conditions in elderly Japanese people with and without periodontitis.

Material and Methods: A total of 158 Japanese men and women (76 years old) with or without periodontitis were selected for the study. Serum adiponectin, resistin, interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) concentrations were compared between subjects with and without periodontitis.

Results: Serum resistin levels and total leukocyte counts in subjects with periodontitis were higher than in control subjects. No significant differences were observed in adiponectin, IL-6 and TNF-α levels between subjects with and without periodontitis. Logistic regression analysis showed that periodontitis with at least one tooth that displayed a probing pocket depth of ≥6 mm was significantly associated with higher serum resistin levels (odds ratio, 2.0; 95% confidence interval, 1.0–4.0). When excluding periodontitis subjects with ≤ 10% of bleeding on probing and excluding control subjects with >10% bleeding on probing, differences between groups and odds ratio increased. Serum adiponectin tended to decrease in patients with periodontitis, albeit not significantly.

Conclusion: Increased serum resistin levels were significantly associated with periodontal condition, especially when considering bleeding on probing, in elderly Japanese people. There was also a trend, though non-significant, toward decreased levels of adiponectin in subjects with periodontitis.

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The local host response to periodontopathogens and their products includes the proliferation and release of macrophages and cytokines. These immune components are thought to play a crucial role in periodontitis. Various cytokines, including interleukin-1β and tumor necrosis factor-α (TNF-α), are determinants of the progression of periodontitis (1). Additionally, increased circulating interleukin-6 (IL-6) levels appear to be correlated with disease severity (2).

Recent evidence indicates that periodontitis may have profound effects on

systemic health. Several studies have evaluated the relationship between diabetes, metabolic syndrome and periodontal disease (3–6). Most epidemiological evidence indicates that individuals with diabetes tend to have a more rapid progression of periodontitis than non-diabetics (7).

Adipose tissue produces and releases a variety of inflammatory factors, including adiponectin, resistin, leptin and visfatin, as well as cytokines such as TNF-α, IL-6 and monocyte chemoattractant protein-1. These factors and cytokines influence insulin resistance and are thought to play a role in inflammation and immune responses (8). Resistin received its name from the original observation that it induced insulin resistance in mice. Additionally, resistin is downregulated in mature murine adipocytes cultured in the presence of insulin-sensitizing drugs, including thiozolidinediones (9). Recent studies in humans suggest that very little resistin is expressed in adipocytes; resistin is largely expressed in monocytes, macrophages (10) and bone marrow (11), which are all linked to the immune response (8).

In contrast, adiponectin levels are decreased in individuals with obesity, type 2 diabetes and cardiovascular disease (12). In addition, adiponectin influences a wide range of inflammatory pathologies, such as rheumatoid arthritis (13). Furthermore, adiponectin inhibits osteoclast formation stimulated by lipopolysaccharide (LPS) from *Actinobacillus actinomycetemcomitans* (14). Regulation of adiponectin is provided by inflammatory cytokines such as IL-6 (15) and TNF-α (16). Inflammatory endotoxins induce resistin in human macrophages via a cascade involving the secretion of inflammatory cytokines such as IL-6 and TNF-α (17). Although altered adipokine levels have been observed in a variety of systemic inflammatory conditions, only a few studies reported an association between periodontitis and adipokines such as leptin and adiponectin. Leptin levels in gingival crevicular fluid decreased as the periodontal disease progressed (18). Although Iwamoto *et al.* demonstrated that periodontal treatment did not influence circulating adiponectin levels (19), a relationship between periodontal conditions and resistin levels has not been examined. Bleeding frequency, which is a direct indicator of gingival inflammation, is considered to be a strong risk factor for the progression of periodontal disease in elderly people (20). Here we investigated the relationship between periodontal conditions and adiponectin, resistin, IL-6 and TNF-α in a population of elderly people.

Material and methods

Subjects

In 1998, a total 4542 people who were at 70 years old and resided in Niigata, Japan, were sent a written request to participate in the survey and were informed of the purpose of this survey. After two requests, 81.4% (3695) responded positively to participate in the survey. After considering the availability of resources, 600 subjects were randomly selected. The participants signed informed consent forms that described the protocol and were approved by the Ethics Committee of Niigata University Graduate School of Medical Dental Sciences. The methods used in this study have been described in detail elsewhere (20,21). Among a total of 418 subjects who attended the 2004 examination, 158 subjects (80 males and 78 females) who were 76 years old and had at least ten teeth with and without periodontitis were selected.

Periodontal examination

The periodontal examination included the assessment of probing pocket depth, attachment level (AL) and bleeding on probing (BOP). Parameters were measured at six sites on every tooth. Four trained dentists used calibrated pressured plastic periodontal probes set to give a probing force of 20 g and measured at 1 mm intervals. All functioning teeth were assessed except those that were partly erupted. A calibration of periodontal examination among all dentists was held; κ (kappa value) ranged from 0.81 to 1.00 for probing pocket depth and from 0.74 to 1.00 for AL.

For the selection of subjects, two criteria for periodontal conditions were defined as follows. Model 1: periodontitis, having at least one tooth with a probing pocket depth ≥6 mm; and control, having teeth without a probing pocket depth ≥6 mm. Model 2: periodontitis with bleeding, excluding subjects with ≤ 10% of sites with BOP from periodontitis in model 1; and control without bleeding, excluding subjects with > 10% BOP from control.

A personal interview was conducted to obtain information regarding smoking habits. Body mass index (BMI) was calculated as an indicator of obesity, and subjects were divided into two groups by BMI: normal BMI (< 25.0) and high BMI (≥25). Fasting glucose levels were defined as either normal (< 110 mg/dL) or high (≥110 mg/dL).

Enzyme-linked immunosorbent assay (ELISA)

Blood samples from the anticubital vein were obtained in the morning for measurement of adiponectin, resistin and other biochemical components. All sera were frozen and stored at −80°C until further measurement. Adiponectin, resistin, TNF-α and IL-6 levels in serum samples were examined using ELISA kits KHP0041, KHP0051, KHC3014 and KHC0064 (Biosource International Inc., CA, USA), respectively, according to the manufacturer's protocol. In addition, each plate was checked before use to ensure that the calibration curve measuring the standard was accurate. All samples were run in duplicate. Absorbance of the substrate color reaction was measured using Microplate manager (Bio-Rad Laboratories, Hercules, CA, USA) at a primary wavelength of 405 nm.

Data analysis

Statistical analyses were conducted using SPSS version 12.0J (SPSS Japan, Tokyo, Japan). Quantitative data are presented as the means ± SD and the median. Statistical significance was

estimated using either a chi-squared test or an independent non-parametric test (Mann–Whitney *U*-test). Correlations were calculated using Spearman's rank correlations. Logistic regression analysis was performed to determine the association between periodontitis and the levels of serum resistin and adiponectin. Odds ratios and 95% confidence intervals (CI) were calculated. Each mean value was used as a cut-off point for high or low levels of serum resistin and adiponectin. Adjusted mean values of serum resistin and adiponectin in the subjects with each periodontal condition were calculated by analyses of covariance (ANCOVA), adjusting for sex, smoking, BMI and fasting glucose levels.

Results

Table 1 outlines characteristics of subjects for each periodontal condition. In model 1, there were significantly higher concentrations of total leukocytes (5.96 ± 1.43 vs. 5.46 ± 1.25 × 10^3/μL; $p = 0.015$) and neutrophils (57.78 ± 8.78 vs. 52.88 ± 9.96%; $p = 0.001$) in subjects with periodontitis compared with control subjects. There was a tendency of increased resistin levels and decreased adiponectin levels in periodontitis; however, this difference was not significant. Median values of TNF-α (0.71 vs. 0.60 pg/mL) and IL-6 (0.37 vs. 0.29 pg/mL) were higher in periodontitis patients than in control subjects, albeit not significantly.

Model 2 showed a similar tendency, with significantly higher concentrations of leukocytes (6.25 ± 1.64 vs. 5.44 ± 1.23 × 10^3/μL; $p = 0.006$) and neutrophils (59.09 ± 9.30 vs. 53.44 ± 9.97%; $p = 0.004$) in subjects with periodontitis with bleeding than in control subjects without bleeding. Additionally, subjects with periodontitis showed significantly higher concentrations of resistin (6.10 ± 3.54 vs. 4.78 ± 2.95 ng/mL; $p = 0.024$) and higher BMI (not significant).

Furthermore, we conducted simple correlation analyses for all subjects between resistin and adiponectin and mean probing pocket depth, mean AL, percentage of BOP, or leukocyte

Table 1. Characteristics of subjects with and without periodontitis

Model 1[a]

	Periodontitis (n = 84)		Control (n = 74)		
	Mean ± SD	Median	Mean ± SD	Median	p^b
Periodontal condition					
Probing pocket depth (mm)	2.52 ± 0.44	2.44	1.77 ± 0.21	1.76	<0.001
AL (mm)	3.45 ± 0.88	3.26	2.77 ± 0.63	2.78	<0.001
BOP (%)	15.60 ± 12.65	10.97	5.58 ± 7.32	3.25	<0.001
Adipokine and cytokine level					
Resistin (ng/mL)	5.58 ± 3.23	4.62	4.86 ± 2.90	4.01	0.131
Adiponectin (μg/mL)	10.92 ± 4.96	10.42	12.09 ± 6.27	10.66	0.199
TNF-α (pg/mL)	0.82 ± 0.74	0.71	1.19 ± 2.20	0.60	0.954
IL-6 (pg/mL)	0.53 ± 0.61	0.37	0.86 ± 2.15	0.29	0.273
Blood components					
Leukocyte count (× 10^3/μL)	5.96 ± 1.43	5.70	5.46 ± 1.25	5.30	0.015
Platelet count (× 10^4/μL)	20.27 ± 4.15	19.55	20.14 ± 4.78	19.35	0.686
Monocyte (%)	6.21 ± 1.99	5.80	6.25 ± 1.72	6.10	0.650
Neutrophil (%)	57.78 ± 8.78	57.50	52.88 ± 9.96	52.10	0.001
General condition					
Male (%)	51.2		50.0		0.882
Smoking (%)[c]	46.4		43.2		0.690
BMI (kg/m^2)	22.77 ± 2.69	22.66	22.39 ± 2.56	22.20	0.384
Fasting glucose (mg/dL)	122.96 ± 39.73	114.0	118.37 ± 29.58	112.0	0.409

Model 2[a]

	Periodontitis with bleeding (n = 47)		Control without bleeding (n = 60)		
	Mean ± SD	Median	Mean ± SD	Median	p^b
Periodontal condition					
Probing pocket depth (mm)	2.65 ± 0.46	2.58	1.74 ± 0.20	1.75	<0.001
AL (mm)	3.64 ± 0.89	3.50	2.70 ± 0.60	2.75	<0.001
BOP (%)	23.62 ± 11.50	20.83	2.88 ± 2.67	1.93	<0.001
Adipokine and cytokine level					
Resistin (ng/mL)	6.10 ± 3.54	5.07	4.78 ± 2.95	3.94	0.024
Adiponectin (μg/mL)	10.85 ± 5.62	9.24	11.90 ± 6.55	10.66	0.283
TNF-α (pg/mL)	0.86 ± 0.80	0.84	1.22 ± 2.27	0.60	0.995
IL-6 (pg/mL)	0.59 ± 0.66	0.45	0.94 ± 2.31	0.32	0.272
Blood components					
Leukocyte count (× 10^3/μL)	6.25 ± 1.64	6.10	5.44 ± 1.23	5.30	0.006
Platelet count (× 10^4/μL)	20.92 ± 4.55	20.50	20.35 ± 4.91	19.45	0.444
Monocyte (%)	6.34 ± 2.36	5.80	6.29 ± 1.74	6.10	0.751
Neutrophil (%)	59.09 ± 9.30	57. 80	53.44 ± 9.97	52.35	0.004
General condition					
Male (%)	46.8		51.7		0.622
Smoking (%)[c]	42.6		43.3		0.936
BMI (kg/m^2)	23.01 ± 2.44	22.71	22.38 ± 2.46	22.20	0.145
Fasting glucose (mg/dL)	123.53 ± 43.00	113.0	117.23 ± 29.94	110.0	0.398

[a]In model 1, the selection criterion of periodontal condition was only with or without ≥6 mm of probing pocket depth, whereas in model 2, 10% of BOP was considered a selection criterion in addition to probing pocket depth.
[b]The p-values were calculated by Mann–Whitney *U*-test except for percentages of male and smoking by chi-squared test.
[c]Past or current smoking habit.

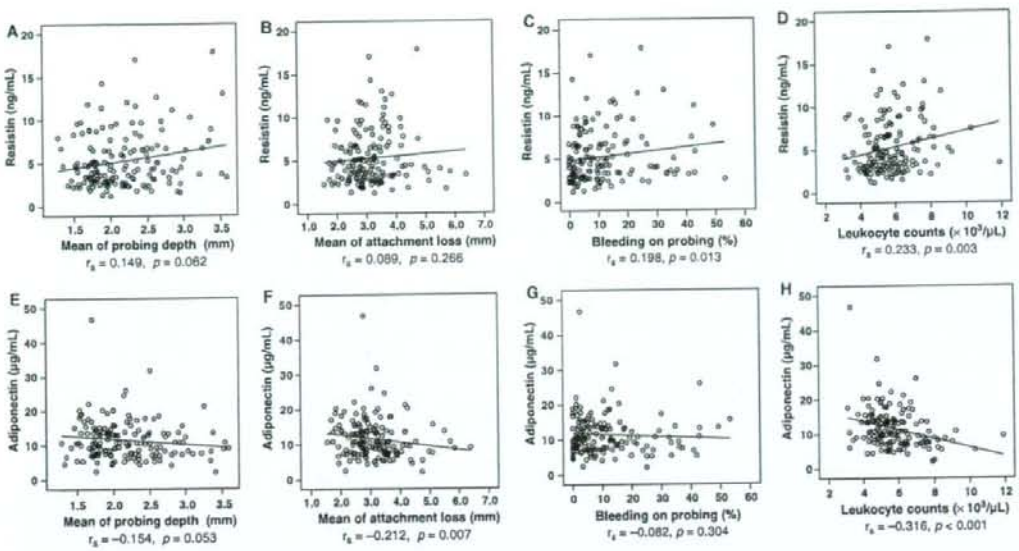

Fig. 1. Correlations between serum levels of resistin or adiponectin and mean probing depth, mean attachment loss, percentage of bleeding on probing and leukocyte counts. Serum levels of resistin and adiponectin were determined by ELISA. Mean values of probing depth, mean values of attachment loss and percentage of bleeding on probing for each subject were determined by periodontal examination. The figure shows Spearman's rank correlation analyses between mean probing depth (A), mean attachment loss (B), percentage of bleeding on probing (C) and leukocyte counts (D) with serum levels of resistin; also Spearman's rank correlation analyses between mean probing depth (E), mean attachment loss (F), percentage of bleeding on probing (G) and leukocyte counts (H) with serum levels of adiponectin. r_s: Spearmann's rank correlation coefficient.

counts (Fig. 1). While resistin levels did not significantly correlate with mean probing pocket depth and AL (Fig. 1A,B), they were positively correlated with BOP (r_s = 0.198, p = 0.013; Fig. 1C) and leukocyte counts (r_s = 0.233, p = 0.003; Fig. 1D). Adiponectin levels were negatively correlated with mean AL (r_s = −0.212, p = 0.007; Fig. 1F) and leukocyte counts (r_s = −0.316, p < 0.001; Fig. 1H), but not with mean probing pocket depth (r_s = −0.154, p = 0.053; Fig. 1E) nor percentage of BOP (Fig. 1G). Serum levels of resistin and adiponectin were not significantly correlated with IL-6 and TNF-α (data not shown).

Logistic regression analysis was performed using higher resistin levels (≥5.3 ng/mL) and lower adiponectin levels (<11.5 ng/mL) as dependent variables. These cut-off points were determined using the mean serum levels of all subjects (Tables 2 and 3). Sex, smoking, BMI and fasting glucose levels were used as independent variables. Periodontitis was significantly associated with higher resistin levels both in model 1 (odds ratio, 2.0; 95% CI, 1.0–4.0) and in model 2 (odds ratio, 2.9; 95% CI, 1.2–6.9; Table 2). A BMI of ≥25 was associated with higher resistin levels only in model 2 (odds ratio, 3.2; 95% CI, 1.1–9.4). Although higher BMI correlated negatively with adiponectin as previously reported (r_s = −0.245, p = 0.002), it was not significantly associated with decreased adiponectin levels in multivariate logistic regression analysis (Table 3).

In an analysis of covariance for the same variables as above, i.e. sex, smoking, BMI and fasting glucose levels, significantly higher resistin levels were observed in subjects with periodontitis with bleeding than in control subjects without bleeding (6.11 ± 0.47 ng/mL vs. 4.78 ± 0.42 ng/mL; Table 4). Adiponectin levels were slightly decreased in periodontitis and periodontitis with bleeding; however, these differences were not significant.

Discussion

Since all subjects used in this study were elderly (76 years old), most individuals (86.1%) had at least one probing pocket depth site ≥4 mm. In addition, BOP levels (mean, 10.9%; median, 7.0%) appeared to be much lower than in other reports (22). Bleeding on probing is a reliable indicator of activity in periodontal disease (23) and may also indicate the progression of periodontal disease in community-dwelling elderly nonsmokers (20). Therefore, lower BOP levels may indicate that many elderly people with deep probing pocket depth and severe AL have a more stable periodontal condition compared with younger adults.

Among other inflammatory markers that are traditionally used as diagnostic measures to assess infection and inflammation, total leukocyte counts were significantly higher in subjects with periodontitis than in control subjects in this study. Moreover, our

Table 2. Relationship between periodontal conditions and increased resistin level by logistic regression analysis

Model 1

Independent variables	Resistin		p^a	Multivariate odds ratio[b] (95% CI)	p
	< 5.3 ng/mL	≥5.3 ng/mL			
Periodontal condition					
Control	54 (73.0)	20 (27.0)	0.066	1	
Periodontitis	49 (58.3)	35 (41.7)		2.00 (1.20–3.98)	0.046
Sex					
Male	50 (62.5)	30 (37.5)	0.507	1	
Female	53 (67.9)	25 (32.1)		0.43 (0.15–1.21)	0.109
BMI					
< 25	87 (66.4)	44 (33.6)	0.510	1	
≥ 25	16 (59.3)	11 (40.7)		1.57 (0.64–3.85)	0.321
Fasting glucose					
< 110 mg/dL	43 (63.2)	25 (36.8)	0.736	1	
≥ 110 mg/dL	60 (66.7)	55 (34.8)		0.77 (0.39–1.55)	0.466
Smoking habit					
No	56 (64.4)	31 (35.6)	0.867	1	
Yes	47 (66.2)	24 (33.8)		0.51 (0.12–1.42)	0.196

Model 2

Independent variables	Resistin		p^a	Multivariate odds ratio[b] (95% CI)	p
	< 5.3 ng/mL	≥5.3 ng/mL			
Periodontal condition					
Control without bleeding	45 (75.0)	15 (25.0)	0.024	1	
Periodontitis with bleeding	25 (53.2)	22 (46.8)		2.90 (1.22–6.94)	0.016
Sex					
Male	34 (64.2)	19 (35.8)	0.840	1	
Female	36 (66.7)	18 (33.3)		0.35 (0.11–1.18)	0.091
BMI					
< 25	60 (69.8)	26 (30.2)	0.074	1	
≥ 25	10 (47.6)	11 (52.4)		3.18 (1.08–9.38)	0.036
Fasting glucose					
< 110 mg/dL	30 (61.2)	19 (38.8)	0.422	1	
≥ 110 mg/dL	40 (69.0)	18 (31.0)		0.57 (0.23–1.40)	0.219
Smoking habit					
No	38 (62.3)	23 (37.7)	0.539	1	
Yes	32 (69.6)	14 (30.4)		0.39 (0.12–1.28)	0.119

[a]chi-square test.
[b]Odds rario by logistic regression analysis.

results revealed associations between resistin, adiponectin and other inflammatory variables such as leukocyte counts and BOP. Specifically, resistin levels were siginificantly correlated with BOP and leukocyte counts, indicating an existing inflammation. Therefore, we introduced BOP to the criteria of periodontitis and control in model 2. In addition, serum resistin levels were weakly correlated with average probing pocket depth, but not average AL. These results suggest that resistin levels may be associated with inflammatory variables rather than periodontal destruction such as indicated by AL. Although adiponectin levels were negatively correlated with mean AL, this result was most probably due to the lower adiponectin levels observed in males who had severe AL. Adiponectin levels were negatively correlated with leukocyte counts (Fig.1A), which indicates that adiponectin is an anti-inflammatory mediator, as previously reported (24).

In contrast to the commonly held belief that serum IL-6 and TNF-α levels are increased in periodontitis, we found no significant relationship between either serum IL-6 or TNF-α levels and the severity of periodontitis. Interleukin-6 and TNF-α locally delivered to the gingival tissues influence the pathogenesis of periodontal disease (1,2). However, these cytokines may have little influence on circulating levels of themselves in elderly people. Additionally, TNF-α is produced mainly during the early stages of an acute inflammation, and the production of TNF-α may be decreased in elderly people.

We found that resistin levels, but not adiponectin levels, were associated with periodontal condition and other inflammatory variables. In addition, adiponectin levels were significantly higher in women (12.61 ± 4.95 μg/mL) than in men (10.35 ± 6.04 μg/mL, $p = 0.011$), but resistin levels were not (women, 4.96 ± 2.71 ng/mL; men, 5.53 ± 3.41 ng/mL, $p = 0.24$). Therefore, we analyzed the relationship between serum adiponectin levels and periodontal condition in men and women separately. However, the results of these analyses did not reach statistical significance (data not shown). As in the case of a previous study suggesting that adiponectin does not appear to be influenced by periodontal treatment (19), periodontal conditions were not associated with serum adiponectin levels in our study. Adiponectin levels might not be influenced by LPS stimulation in humans, which is different from the situation for leptin (25) and resistin (17,25). Circulating adiponectin is present in several forms, including low-, middle- and high-molecular weight adiponectin, which may activate different signal transduction pathways and exert distinct effects (26). Some of these specific forms, such as high-molecular weight adiponectin, may be significantly associated with periodontal inflammation. Further studies to examine the effects of high-molecular weight adiponectin are necessary.

Studies have also indicated an abundance of resistin in peripheral blood mononuclear cells and macrophages, suggesting an important role of resistin in the process of inflammation (8,10). Circulating resistin levels are elevated in patients with rheumatoid

Table 3. Relationship between periodontal conditions and decreased adiponectin level by logistic regression analysis

Model 1

Independent variables	Adiponectin ≥11.5 μg/mL	Adiponectin <11.5 μg/mL	p^a	Multivariate odds ratio[b] (95% CI)	p
Periodontal condition					
Control	34 (45.9)	40 (54.1)	0.336	1	
Periodontitis	32 (38.1)	52 (61.9)		1.38 (0.68–2.80)	0.374
Sex					
Male	19 (23.7)	61 (76.3)	<0.001	1	
Female	47 (60.3)	31 (39.7)		0.21 (0.07–0.61)	0.004
BMI					
<25	57 (43.5)	74 (56.5)	0.395	1	
≥25	9 (33.3)	18 (66.7)		2.21 (0.83–5.92)	0.114
Fasting glucose					
<110 mg/dL	39 (57.6)	29 (42.6)	0.001	1	
≥110 mg/dL	27 (30.0)	63 (70.0)		2.57 (1.26–5.24)	0.009
Smoking habit					
No	48 (55.2)	39 (44.8)	<0.001	1	
Yes	18 (25.4)	53 (74.6)		1.04 (0.36–3.04)	0.942

Model 2

Independent variables	Adiponectin ≥11.5 μg/mL	Adiponectin <11.5 μg/mL	p^a	Multivariate odds ratio[b] (95% CI)	p
Periodontal condition					
Control without bleeding	27 (45.0)	33 (55.0)	0.321	1	
Periodontitis with bleeding	16 (34.0)	31 (66.0)		1.62 (0.67–3.92)	0.290
Sex					
Male	13 (24.5)	40 (75.5)	0.002	1	
Female	30 (55.6)	24 (44.4)		0.25 (0.08–0.83)	0.023
BMI					
<25	37 (43.0)	49 (57.0)	0.321	1	
≥25	6 (28.6)	15 (71.4)		2.20 (0.69–7.04)	0.182
Fasting glucose					
<110 mg/dL	28 (57.1)	21 (42.9)	0.001	1	
≥110 mg/dL	15 (25.9)	43 (74.1)		3.11 (1.30–7.43)	0.011
Smoking habit					
No	31 (50.8)	30 (49.2)	0.011	1	
Yes	12 (26.1)	34 (73.9)		1.00 (0.31–3.25)	0.997

[a]chi-square test.
[b]Odds ratio by logistic regression analysis.

Table 4. Adjusted mean value of serum resistin and adiponectin in the subjects with each periodontal condition

Periodontal status	n	Resistin (ng/mL)	P^a	Adiponectin (μg/mL)	p^a
Model 1					
Control	74	4.86 ± 0.36[b]	0.138	12.00 ± 0.63[b]	0.248
Periodontitis	84	5.59 ± 0.34		11.00 ± 0.59	
Model 2					
Control without bleeding	60	4.78 ± 0.42	0.037	11.74 ±0.76	0.551
Periodontitis with bleeding	47	6.11 ± 0.47		11.05 ±0.86	

[a]ANCOVA was performed adjusting for sex, BMI, fasting glucose, and smoking.
[b]mean ± standard error of mean

arthritis (27), cardiovascular disease (28,29) and chronic kidney disease (30). In a previous study, increased resistin promoted endothelial cell activation by endothelin-1 release and upregulated chemokines (28), suggesting that increased resistin is related to cardiovascular disease. Increased resistin levels caused by periodontal inflammation may mediate the relationship between periodontitis and cardiovascular disease.

When BOP was introduced as one of the selection criteria of periodontal condition, the association between periodontitis and resistin became stronger. Additionally, serum resistin levels were associated with periodontitis in middle-aged Japanese women in the Hisayama study (unpublished data). It remains unknown whether periodontal inflammation influences circulating resistin levels in humans; however, inflammatory cells such as monocytes and macrophages present in the periodontal tissue appear to be the major source of resistin. Since inflammatory cytokines such as IL-6, TNF-α and interleukin-1β have an effect on the expression of resistin *in vitro* (31), it is possible that periodontal inflammation influences resistin expression. Resistin expression also increases in concert with the maturation of monocytes into macrophages. Resistin may play a significant role in monocyte–macrophage function (10). In our study, adding monocytes as an independent variable in the logistic regression analysis did not affect the result. This result suggests that total monocytes may have a limited impact on resistin levels. However, when total leukocyte counts were added, the odds ratio of periodontitis was reduced (data not shown). Total leukocyte counts, including macrophages, may be a causal intermediate existing between periodontitis and increased levels of resistin. Indeed, total leukocyte counts were significantly correlated both with mean probing pocket depth (r_s = 0.212, p = 0.007) and with percentage of BOP (r_s = 0.205, p = 0.01). Recent data indicate that stimulation of macrophages *in vitro* with LPS or proinflammatory cytokines leads to a marked increase in resistin production (17). Furthermore, administration of LPS to humans is associated with dramatically increased circulating resistin levels (25). Therefore, it is possibile that LPS from periodontal pathogenic bacteria influences adipose

tissues and macrophages through inflammatory cytokines.

In summary, here we report that serum resistin is associated with periodontal condition independent of sex, smoking, fasting glucose and BMI. Additionally, this association becomes stronger when BOP is included in the model. It is not clear how serum resistin is associated with periodontal inflammation. Further studies are required to clarify these mechanisms.

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ORIGINAL ARTICLE

Relationship between mandibular inferior cortex and general bone metabolism in older adults

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Abstract
Summary The purpose of this study was to investigate whether a link exists between the jawbone and general bone metabolism. The results of our study indicate that a mandibular inferior cortical erosion finding on dental panoramic radiographs is significantly associated with increased biochemical markers of bone turnover.
Introduction The purpose of this study was to investigate whether a link exists between the jawbone and general bone metabolism.
Methods We measured values of serum bone-specific alkaline phosphatase (S-BAP) and urinary N-telopeptide cross-links of type I collagen (U-NTX). To evaluate the jawbone, we used mandibular inferior cortex (MIC) classification on dental panoramic radiographs. After 134 subjects were divided into three groups (C1: normal cortex, C2: mildly to moderately eroded cortex, C3: severely eroded cortex), we evaluated the relationship between S-BAP or U-NTX and MIC classification by Scheffe's multiple comparison test.
Results A significant correlation was found between MIC classification and S-BAP (C1 vs. C2: $p<0.01$, C1 vs. C3: $p<0.01$, C2 vs. C3: NS). A significant correlation was found between MIC classification and U-NTX (C1 vs. C2: $p<0.01$, C1 vs. C3: $p<0.001$, C2 vs. C3: $p<0.01$).
Conclusions The results of our study indicate that the mandibular inferior cortical erosion finding on dental panoramic radiographs is significantly associated with increased S-BAP and U-NTX levels. We suggest that there is an association between the jawbone and general bone metabolism.

Keywords Bone metabolism · Cortical erosion finding · Dental panoramic radiograph · Elderly · Mandibular inferior cortex · Osteoporosis

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Introduction

Many investigations about the relationship between oral and general health have been reported. Among them, some studies have examined the association between jawbone mineral density and skeletal bone mineral density (BMD) [1–3], and between skeletal BMD and alveolar bone loss in periodontitis [4–6]. We hypothesized that the jawbone might be affected by general bone metabolism. However, although some investigators [1–6] demonstrated the significant association between skeletal BMD and jawbone or periodontal condition, other studies [7, 8] failed to find this association. The results of these studies should be interpreted with caution, since the age of the subjects might not have been restricted, and oral and skeletal bone loss might have been measured only in women. Further studies are needed in both men and women restricted by age.

In addition, BMD of the spine, leg, etc. was used to evaluate bone condition in previous studies. However, the skeleton is heterogenetic, and BMD differs in each area. When we evaluate the general bone condition, serum and