C level on CAL while controlling for other confounding factors. The level of statistical significance was fixed at $P \leq 0.05$.

RESULTS

The current analysis was limited to 413 dentate subjects in whom the data for CAL as well as serum concentrations of vitamin C were available. The CAL of this sample ranged from 1.46 to 7.30mm with a mean of 3.26mm (SD=1.05) while the serum levels of vitamin C ranged from 0.2-22.6 mg/L (results not shown). Table 1 shows the associations between CAL and the independent variables including gender, smoking status, diabetic status, brushing frequency, the number of teeth present and serum vitamin C concentrations. As revealed by Student's *t*-test males had significantly greater CAL (mean=3.54 mm; SD=1.2) than females (mean=2.96 mm; SD=0.8 mm) while those who had 20 or more teeth showed significantly lower CAL (mean=2.92 mm; SD=0.8) compared to the subjects with <20 teeth (mean=3.69 mm; SD=1.2). It is also apparent that CAL was significantly higher in the subjects who brushed their teeth <2/day than in those who used a toothbrush ≥2/day. One-way ANOVA combined with Bonferroni post-hoc test disclosed that current smokers had significantly worse CAL (mean=3.82 mm; SD=1.3) in comparison to both ex-smokers (mean=3.45 mm; SD=1.1) and non-smokers (mean=2.95 mm; SD=0.8) whereas subjects with RBS <140 mg/dL showed significantly lower CAL than those who had RBS ≥ 140 mg/dL. Furthermore, there was an inverse relationship between serum vitamin C concentration and CAL as indicated by Pearson's correlation technique ($r = -0.23$; $P<0.00005$).

All the independent variables that demonstrated significant effects on CAL at bivariate level, namely serum vitamin C, smoking status, diabetic status, gender, toothbrushing frequency and the number of teeth present were included in a multiple linear regression analysis and the variables that

remained significant in the final model are shown in Table 2. Accordingly, it was found that serum vitamin C had a significant effect on CAL (correlation coefficient = -0.04; $P<0.05$), which was independent of the other covariates including smoking and random blood sugar levels. The independent variables in the final model explained 26% of the variance in CAL ($R^2 = 0.26$).

DISCUSSION

The findings of this cross-sectional study suggested that there was a significant association between the level of serum vitamin C and periodontitis as measured by CAL notwithstanding the effect of established risk factors for periodontitis such as smoking and diabetes mellitus in this elderly population. In other words, we observed an inverse independent relationship between serum vitamin C concentration and CAL – the lower the level of serum vitamin C the higher was the periodontal attachment loss. This was indicated by the correlation coefficient of serum vitamin C (coef = -0.04): CAL in subjects with lower serum vitamin C levels would be 4% greater compared to those who had higher serum vitamin C concentrations regardless of other covariates.

Notwithstanding the fact that our study was confined only to the elderly and that we evaluated serum ascorbic acid concentration instead of dietary intake of vitamin C, the present findings may be comparable to those of others (Ismail et al. 1983; Nishida et al. 2000) who observed a weak albeit statistically significant relationship between dietary vitamin C and periodontal disease in the US adults. In particular, the latter (Nishida et al. 2000) found that even after controlling for the effects of age, gender, smoking and gingival bleeding the level of periodontitis in subjects with a lower dietary intake of vitamin C was 1.19 times greater than that of individuals with a higher intake of vitamin C while the former (Ismail et al. 1983) did not adjust for such factors. More recently, Pussinen and co-workers (2003) who investigated the relation between plasma vitamin C levels and serology of periodontitis in Finnish and Russian men observed that the antibody levels to

Porphyromonas gingivalis were inversely correlated with plasma vitamin C concentrations (r= -0.22; P<0.001) and this association remained significant in a linear regression model even after controlling for confounding factors. Accordingly, they concluded that lower concentrations of plasma vitamin C might increase the risk of periodontitis, which is in accord with the present findings.

Various researchers have proposed several plausible biological mechanisms whilst attempting to explain how ascorbic acid could affect the healthy tissues in humans as well as in animals (Alfano et al. 1975, Alvares et al. 1981, Alvares and Siegel 1981, Berg et al. 1983, Boxer et al. 1979, Dallegri et al. 1980, Goetzl et al. 1974, Jacob et al. 1987, Leggot et al. 1986, 1991, Nakamoto et al. 1979). It has been established that ascorbic acid plays a major role in the synthesis of collagen, especially the hydroxylation process, helix formation and cross-linking of collagen molecules (Alfano et al. 1975, Berg et al. 1983). Collagen is undoubtedly an essential component of human tissues including periodontium and required in wound healing as well as periodontal regeneration and maintaining the integrity of the gingival vasculature. Also, there are several lines of evidence to suggest that vitamin C affects chemotaxis as well as phagocytosis of polymorphonuclear leukocytes and thereby influences the host-immune reactions (Alfano et al. 1975, Boxer et al. 1979, Dallegri et al. 1980, Patrone et al. 1982). Moreover, some researchers have hypothesised that ascorbic acid might express an antihistamine effect through direct detoxification of histamine or indirectly affecting the histamine breakdown and this in turn would retard gingival inflammation (Nakamoto et al. 1979) whereas others (Alfano et al. 1975, Alvares and Siegel 1981) reported that the deficiency in vitamin C levels could be linked to increased permeability of gingival mucosa, which allows easy passage of microbial and other noxious products into the periodontium. It has also been shown that ascorbic acid demonstrates antioxidant properties and therefore is considered one of the constituents of antioxidant defence mechanism in human body (Nishida et al. 2000). Tobacco,

especially, cigarette smoke contains various oxidants that cause tissue damage and consequently smokers do require a higher serum concentration of vitamin C than non-smokers do (Kallner 1981, Nishida et al. 2000). Moreover, given that avitaminosis C and diabetes mellitus share some common pathological characteristics such as raising of oxidant stress (Schmidt et al. 1996) and collagen degradation (Kjersem et al. 1988) in gingival tissues it has been hypothesized that vitamin C might play a critical role in the aetiology and/or progression of periodontitis in type I diabetics (Aleo 1981, Nishida et al. 2000,). In this connection, it is also noteworthy that both diabetes as well as smoking, which are regarded as well-established risk factors for periodontitis may contribute to oxidative tissue damage and given the antioxidant properties of vitamin C it might act as a potential moderator in both smoking- and diabetes-periodontal relationships – this would be an interesting hypothesis to be tested in future investigations. Although exploring such biological mechanisms and/or hypotheses was beyond the scope of our study, the association between serum vitamin C levels and CAL that was observed even after controlling for known risk factors such as smoking and diabetes mellitus in the present study could be explained on the basis of these mechanisms. This is further augmented by the fact that such mechanisms could be connected to pathogenesis of periodontal disease, which is of inflammatory nature and which may be mediated through the tissue damage caused by interaction of microbial noxious products and host-immune response. However, it should also be highlighted that these biological phenomena involving vitamin C have neither been clearly understood nor well defined (Leggot et al. 1986; Nishida et al. 2000).

This study population comprised non-institutionalised elderly people who were active, living independently and willing to participate in the survey. It has been shown that the elderly who are institutionalised, less active and dependent are at a higher risk for periodontal disease than those who are active and independent (Hirotomi et al. 2002; Ogawa et al. 2002). In this context, the

current sample might be considered a biased one and therefore the findings should be interpreted with caution.

In conclusion, the results suggest that the serum vitamin C levels in this elderly population may have a significant impact on periodontitis as evaluated by CAL notwithstanding the effects of smoking, diabetes mellitus, gender, oral hygiene practises or the number of teeth present. Nevertheless, not only considering the cross-sectional nature of the study design but also given the relatively low correlation observed between serum vitamin C and CAL in the current analysis we could neither confirm an unambiguous cause-effect relationship between serum vitamin C and periodontitis nor a substantial beneficial effect of vitamin C on periodontal health. Consequently, it warrants further investigations, in particular, longitudinal studies and experimental designs to explore the actual role of vitamin C in the aetiology and/or progression of periodontal disease.

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Table 1. Relationships between CAL and independent variables at bivariate level

Independent variables	CAL Mean (SD)	*P*
Gender*		
Male (n=215)	3.54 (1.2)	
Female (n=198)	2.96 (0.8)	<0.00005
Number of teeth present*		
<20 (n=184)	3.69 (1.2)	
≥20 (n=229)	2.92 (0.8)	<0.00005
Brushing frequency*		
<2/day (n=145)	3.40 (1.1)	
≥2/day (n=268)	3.20 (1.0)	<0.05
Smoking status**		
Current smoker (n=71)	3.82 (1.3)	
Ex-smoker (n=137)	3.45 (1.1)	<0.00005[a]
Non-smoker (n=205)	2.95 (0.8)	
Diabetic status*		
RBS<140mg/dL (n=356)	3.22 (0.1)	<0.05
RBS≥140mg/dL (n=57)	3.52 (0.2)	
Serum ascorbic acid***	r = -0.23	<0.00005

*Student's *t*-test

**One-way ANOVA

***Pearson's correlation

[a]Bonferroni test: 3.82>3.45>2.95 (P<0.05)

Table 2. Multiple linear regression model for CAL with significant variables

Independent variables	Coefficient	SE	*P*	95% CI	
Serum ascorbic acid	-0.04	0.02	<0.05	-0.06	-0.005
Current smoker	0.57	0.17	<0.005	0.24	0.92
Gender (Male=0)	-0.30	0.04	<0.05	-0.58	-0.01
Teeth present (<20=0)	-0.73	0.09	<0.0005	-0.92	-0.55
Constant	3.83	0.25	<0.0005	3.46	4.20

R^2=0.26 *P*<0.00005 SE=standard error CI=confidence interval

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E．研究目的

歯周病は，歯周の何組織の破壊に加えて歯槽骨の吸収と特徴づけられている。一方，骨粗鬆症は高齢者に比較的多く見られ，年齢の上昇と伴に増加傾向を示す。歯周病も骨粗鬆症も骨の代謝疾患であることを考えると関連が認められてもおかしくない。しかし，関連ありという報告のある一方で関連の認められない調査もあり，まだ不明確な点が多い。本調査では 70 歳高齢者を対象とし歯周病の進行と骨密度との関連を明らかにすることを目的としている。

F．対象および方法

対象者として，新潟市在住の 70 歳 600 人を選定した。20 本以上現在歯あり，血糖値が 140 以下，を満たす 222 人を分析対象とした。調査項目は，全身状態として，骨密度（超音波式骨量測定装置使用），肥満（BMI）血清値（Albumin，IgG）を測定した。さらに，歯周病の進行として 3 年間の Attachment loss 3 mm 以上の部位数を測定した。分析にあたっては，骨密度を Stiffness で表し，男性の場合は 85%以下，女性の場合は 69%以下を骨量が少ないと評価し，それぞれの歯周組織における 3 年間の Additional Attachment loss を比較した。

G．結果および考察

Stiffness，BMI，Albumin，IgG，3 年間の Attachment loss 3 mm 以上の部位数を男女間で比較した。Stiffness で有意な差が認められた（t 検定，$p<0.01$）。目的変数に 3 年間の Attachment loss 3 mm 以上の部位数を，説明変数に Stiffness，BMI，Albumin，IgG，性別を採用し重回帰分析を行ったところ，統計学的に有意であった変数は，Stiffness（p=0.006）と性別（p=0.014）であった。

骨量の少ないグループ（男性：Stiffness ≦ 69，女性：Stiffness ≦ 85）とそうでないグループで 3 年間の Attachment loss 3 mm 以上の部位数は，それぞれ，男性，7.71 ± 12.30，3.41 ± 2.79，女性，5.45 ± 9.26，3.26 ± 3.02 であった。この差は統計学的に有意であった（p=0.045，分散分析）。

本調査から骨密度と歯周病の進行の間には弱いながらも有意な関係が認められ，全身の骨代謝が歯周病の進行にも影響していることが示された。しかし，腫種骨と歯槽骨では骨の成り立ちが違うことから，今後は，顎骨等他の部位との関連も見ていく必要があるだろう。

A longitudinal study of the relationship between periodontal disease and bone mineral density in community-dwelling older adults

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Abstract

Objective: Bone loss is a common feature of periodontitis and osteoporosis. Both diseases may share common etiologic agents which may either directly influence or modulate the process of both diseases. The purpose of this study was to evaluate the relationship between systemic bone mineral density and periodontal disease among elderly people.

Materials and methods: Among all 4,542 inhabitants aged 70 years according to a registry of residents in Niigata city in Japan, 600 people were selected randomly. 184 subjects who did not have diabetes mellitus, whose blood sugar was <140mg/dL, who had more than 20 teeth, who were non-smokers, who did not take medication for osteoporosis, were included in the study. Four dentists performed clinical evaluations on probing attachment level. We also utilized the data on bone mineral density of the heel, which we measured using an Ultra-Sound Bone Densitometer. Follow-up clinical surveys were done by measuring probing attachment level after three years. Finally, 179 subjects who could participate in both the baseline and the follow-up examinations were included in the analysis. After dividing the subjects into an osteopenia group (OG) and non-osteopenia group (NOG), we evaluated the relationship between bone mineral density and the number of progressive sites which had ≥3mm additional attachment loss during 3 years after controlling the known confounding factors.

Results: The mean number of progressive sites for the OG and the NOG were 4.65±5. 51 and 3.26±3.01 in females, 6.88±9. 41 and 3.41±2.79 in males, respectively. Two-way ANOVA was

performed to discriminate among effects of gender, bone mineral density, and gender-bone mineral density interaction. A significant effect of bone mineral density (OG or NOG, $p=0.043$) with a significant interaction ($p=0.038$) were observed. Furthermore, bone mineral density was associated with the number of progressive sites which had ≥3mm additional attachment loss during the 3 years ($p=0.001$) by multiple linear regression analysis.

Conclusions: This study suggested that there was a significant relationship between periodontal disease and general bone mineral density.

Key words: Bone loss, Periodontal disease, Etiology

Introduction

Periodontal destruction is frequently experienced by elderly people (Slade & Spencer 1995, Brown et al. 1996) and it contributes to as much as 40 percent of tooth extraction (Johnson 1993). Periodontal disease is characterized by the absorption of the alveolar bone as well as by the loss of the soft tissue attachment to the tooth. On the other hand, 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. Because bone loss is a common feature of periodontitis and osteoporosis, both diseases may share common etiologic agents which may either directly influence or modulate the process of both diseases. Given that the final expression of periodontitis is predicated by the complex interactions occurring within an intricate mosaic of host, microbial and environmental factors, it was felt that the contribution of bone mineral density as a risk factor might be worthy of investigation (Offenbacher 1996). The clinical consequence of these findings suggest that physicians should be encouraged to send their osteoporotic patients to the dentist for a periodontal examination and dentists should be encouraged to send their patients with severe periodontal disease for a medical examination for osteoporosis.

However, the relationship between osteoporosis and periodontal disease has been suggested in a limited number of studies. The results of some previous studies have indicated a relationship between periodontal disease and osteoporosis (Tezal et al. 2000, Mohammad et al. 1997, Von Wowern et al. 1994), while others have not shown any significant

relationship (Elders et al. 1992, Klemetti et al. 1994, Lundstrom et al. 2001). All of these studies used the cross-sectional study design, and examined bone loss and periodontal condition in females. Even if the loss of bone mineral density was more significant in females than in males, the role of factors involved in the regulation of bone mineral density in males as well as in postmenopausal females needs to be evaluated further with reference to oral bone loss and periodontal disease. In addition, it is necessary to evaluate the relationship between bone mineral density and progression of periodontitis in longitudinal studies.

Likewise, the results may easily be confounded by other factors such as intake of medications, smoking, race and age. Many of the studies conducted to date have been plagued by relatively small sample sizes and lack of adequate control of potential confounding variables. Larger studies are needed to better define the relationship between bone mineral density and periodontal disease.

The purpose of this study was to evaluate the relationship between systemic bone mineral density and periodontal disease, controlling the known confounding factors.

Material and Methods

Subjects and clinical assessment

Initially, questionnaires were sent to all 4,542 inhabitants aged 70 years according to a registry of residents in Niigata City in Japan, and they were informed of the purpose of this survey. The response rate was 81.4% (N= 3,695). Among them, after dividing into male and female groups, 600 people were selected randomly in order to have approximately the same number of each gender for the study (screened population). The subjects for the study agreed to undergo medical and dental examinations, and signed informed consent forms regarding the protocol, which 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 clinical evaluations on the following items: (1) number of teeth present, (2) probing attachment level (PAL). Mouth mirrors with a light, and pressure-sensitive plastic periodontal probes, set to give a constant probing force of 20g and graduated at 1mm intervals (VIVACARE TPS PROBE®), were used. All functioning teeth, including third molars, were assessed, except for partially erupted teeth. PALs 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), PAL was measured taking account of the anatomical features of the teeth and, if present, the CEJ of the adjacent tooth/teeth.

17 volunteer patients were examined by each of the four examiners in the Faculty Hospital of Dentistry, Niigata University and their results were compared. The percentage of agreement ranged from 70.0 to 100% for PAL. The kappa ranged from 0.62 to 1.00 for PAL. The four examiners did not have any information on bone mineral density of the subjects.

The subjects' height, weight and grip power were measured to the nearest 1mm or 0.1kg, respectively, to calculate the body mass index (kg/m^2, BMI) or grip power/body weight (Kg/Kg). We also utilized the data on bone mineral density (BMD) of the heel, which we measured using an Ultra-Sound Bone Densitometer (Lunar, Achilles ^{TM}). The ultrasound signal is sent to os calcis. Ultrasound densitometry enables the measurement of the physical properties of bone, specifically BMD. The ultrasound measurment contains two criteria, the velocity (speed of sound (sec); SOS) and frequency attenuation (broadband ultrasound attenuation (dB/MHZ); BUA) of a sound wave as it travels through a bone (Rossman et al. 1989, Langton et al. 1984). The 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 biologically relevant ranges. Stiffness is indicated in the monitor of the bone densitometer as the percentage for the value of the normal younger generation. Osteopenia was defined as a stiffness ≤85 for 70-year-old males, and ≤69 for females (Lunar Corporation 1991). Furthermore, a personal interview was performed to obtain the bulk of information regarding smoking habits, diabetes mellitus and the intake of medications for osteoporosis. To monitor the general health condition,

serum or plasma levels of disease markers were also investigated. These disease markers were immunoglobulins (serum IgG concentration), nutritional factors (serum albumin concentration and serum total cholesterol concentration) and blood sugar. Among the screened population, 184 subjects who did not have diabetes mellitus, whose blood sugar was <140mg/dL, who had more than 20 teeth, who were non-smokers, and who did not take medication for osteoporosis, were included in the study.

Follow-up clinical surveys were done by measuring PAL after 3 years. 97.3% of the subjects received the follow-up examination by the same four dentists as at the baseline examination.

Finally, 179 subjects who could participate in both the baseline and the follow-up examinations were included in the analysis.

Statistical analyses

Mean and standard deviation (SD) were used to characterize the continuous variables. Following Brown et al. (1994), a change in the attachment level of 3mm or more was set as a conservative estimate of actual change taking place. Using the *t*-test, we compared stiffness, BMI, serum albumin concentration, serum total cholesterol concentration, grip power/body weight, serum IgG concentration, probing attachment level at baseline and the number of sites with ≥3mm additional attachment loss during the 3 years between males and females.

Furthermore, we evaluated the relationship between stiffness at baseline and the number of