density loss (25). Accordingly, because the origin of protein and the dietary composition and dietary patterns in Japan differ from those in the USA, it cannot be concluded that the protein intake of these subjects is excessive. On the other hand, it is recommended for food planning purposes that an appropriate protein allowance could be 12–14% of the total energy intake, for mixed protein sources characteristics of normal diets (2). The values in this study were 16.2% of total energy intake and were slightly high for both sexes (Table 3). The proportion of proteins of animal origin was also significantly higher than recently reported among the Japanese (48.3% in those aged 70 y and older) according to the National Nutrition Survey in 2001, because of the high intake of fresh fish or shellfish and their products (Table 5). Therefore, we find interesting a previous report that the proportion of animal protein to total protein consumed by centenarians was high (men: 59.6%, women: 57.6%), compared with that of contemporary average Japanese (26). Furthermore, we are concerned about serum albumin as an indicator of protein nutritional status, because the body pool of albumin is large, with about 60% being in the extravascular space, and its half-life is relatively long (27, 28). As shown in Table 1, the serum albumin concentration was 4.2 g/dL in the present subjects. From a study of longitudinal changes of serum albumin in elderly people living in the community, Shibata et al. confirmed that the age-associated decline of serum albumin concentration is considered mainly attributable to intrinsic changes in albumin metabolism rather than to nutrient intake (29). Therefore, it is possible that high intake of protein derived from fresh fish confers advantageous effects on the nutritional and health status of elderly people, although the relationship between high protein intake and serum albumin level is not clear.

Intake of minerals and vitamins, and their correlations

Data from the National Nutrition Survey in Japan (16) indicate that the average daily intake of calcium, iron, zinc, and copper in both sexes of adults over age 70 is deficient when compared with the Japanese RDAs (13). The vitamins for which intake is low are vitamins E, B₁, and B₆ and niacin for men, and vitamins E, B₁, and B₆ for women. However, in the present survey, the intake of minerals and vitamins was higher than the RDAs for elderly men and women over age 70 (Table 2). It is generally known that calcium plays an important role in the prevention of osteoporosis in elderly people, especially women. The daily calcium intake of the subjects in this study was higher than the Japanese RDAs (13) by 190 mg/d for men and by 116 mg/d for women. A nutritional survey of elderly Japanese women conducted by Okazaki, reported that the intake of calcium and iron was 828±359 mg/d and 12.1±3.9 mg/d, respectively, although the calculation conducted was based on the Standard Tables of Food Composition in Japan (4th ed.). According to the dietary reference intakes established by the Institute of Medicine in the USA, an adequate intake (AI) of calcium for adults over 50 y is 1,200 mg based on clinical data (30). The cal-

cium intake of the study participants is about 60–65% of this proposed value.

Mertz has listed the essential trace elements that may be involved in aging and that are not well distributed in diets. The list includes magnesium and copper in relation to cardiovascular disease, zinc in relation to immune function, and fluorine in relation to bone health. Furthermore, he has pointed out that the minerals usually adequately provided in the diet were iron, manganese, iodine and molybdenum (2). As shown in Table 4(a), the correlations among mineral intakes were markedly strong between magnesium and iron, zinc, and copper; between iron and zinc and copper; and between zinc and copper. From our results, it seems that magnesium, iron, zinc and copper are appropriately distributed in the consumed foods, especially beans and their products, shellfish and seaweed. Daily use of food groups by the present subjects was characterized by high consumption of vegetables except for dark green and yellow vegetables, fruits and fish including shellfish and their products (Table 5). However, in the data for people over age 70 from Japan's National Nutrition Survey in 2001, the three values were 165 g, 154 g, and 112 g for men, and 156 g, 169 g, and 93 g for women (16). For both sexes in this study, the daily use of fruits was about 2.2 times larger than that of the National Survey and that of milk and its products was 1.4 times. Accordingly, our data suggest that iron intake is an indicator of the intake of magnesium, zinc, and copper in the dietary pattern observed in this study, although it should be considered that the bioavailability of minerals is significantly affected by other dietary constituents, including other minerals, during the processes of digestion absorption, and metabolism.

A number of pieces of evidence indicate that both vitamin E and vitamin C are important for the central nervous system and that a decrease in their concentrations causes structural and functional damage to cells (31). Therefore, the dietary intake of vitamins E and C is essential with regard to aging and age-associated neurodegenerative diseases. Regarding vitamin C, recent studies have shown that 200 mg/d appears to be necessary to increase blood levels maximally and consequently increase tissue levels (31). Furthermore, Martine et al. have proposed that a good diet containing an average of three servings of fresh vegetables and two servings of fruits daily may provide an average vitamin C intake of 105 mg from vegetables and 70 mg from fruits and may provide approximately 20 mg of vitamin E. In spite of the high intake of vegetables and fresh fish reported in this study, vitamin E intake was 12.8± 3.6 mg/d for men and 10.5±3.1 mg/d for women, while vitamin C intake was more than 200 mg/d for both sexes. Although the RDA for vitamin B₆ is given as the value per unit g protein intake, the correlation coefficients between vitamin B₆ and total or animal protein were not especially high compared with vitamin D, vitamin B₂, niacin, and pantothenic acid (Table 4). Ribaya-Mercado et al. have reported that elderly men who ingested about 120 g protein/d required 1.96±0.11 mg

of vitamin B_6 to normalize xanthurenic acid; women who ingested 78 g protein/d required 1.90 ± 0.18 mg of vitamin B_6 to normalize xanthurenic acid (32). If so, the vitamin B_6 intake of the subjects in the present study is insufficient. Although it has been reported that vitamin B_{12} deficiency is common in elderly people and vitamin B_{12} status is associated with bone health in elderly women (33), the mean vitamin B_{12} intake was very high, and the distribution was similar to that of zinc or copper. Strong correlations were found between vitamin B_{12} and zinc or copper because cobalt is included in the structure of vitamin B_{12}.

Payette et al., who evaluated a community-living elderly population, pointed out that "good appetite," as reported by the participants, appeared to be an important indicator for dietary intake of energy ($p<0.01$) and protein ($p<0.05$) (34). Nicolas et al. reported that healthy aging among older French people was associated with a mean energy intake close to the upper limits of the RDAs (35). Although the intake of energy and nutrients is generally lower among elderly people than among younger adults, we concluded that age is not an important determinant of dietary intake among apparently healthy Japanese people aged 74 y. We also concluded that a high intake of energy and protein based upon high consumption of fish and/or shellfish, vegetables and fruits, provide sufficient minerals and vitamins in the Japanese dietary pattern.

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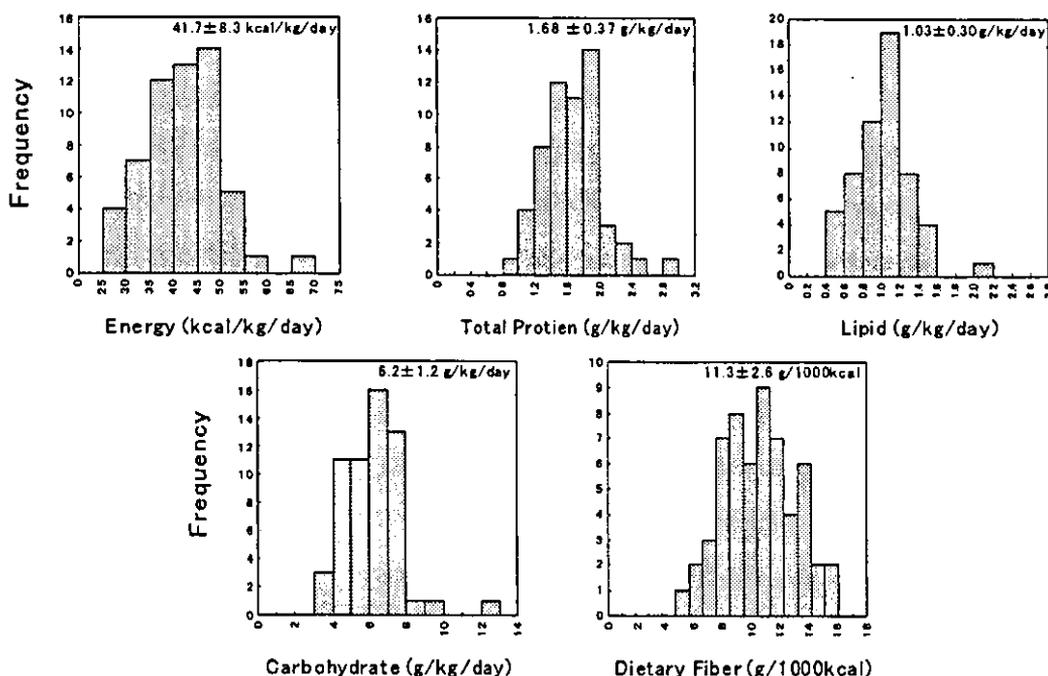
Appendix 1. Frequency of daily intakes of energy, protein, lipid, and carbohydrate per kg body weights, and that of total dietary fiber per 1,000 kcal energy intake by healthy free-living Japanese elderly people (*n*=57). Calculation was conducted using the Standard Tables of Food Composition in Japan (5th ed.).
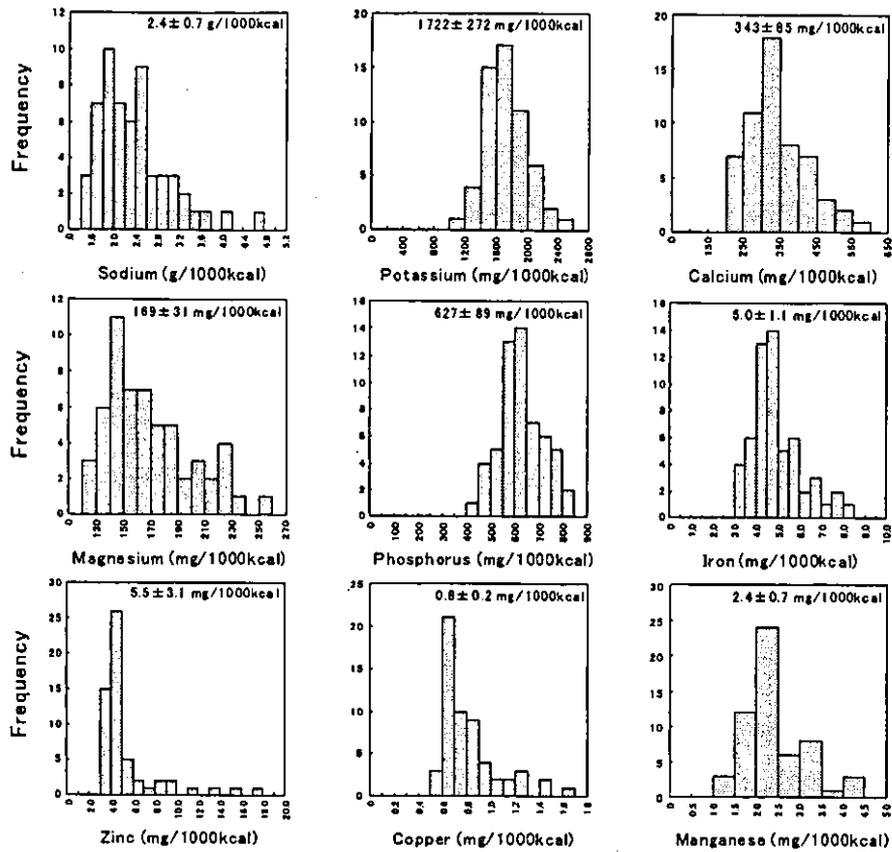
Appendix 2. Frequency of mineral intake per 1,000 kcal energy intake in healthy free-living Japanese elderly people (n=57). Calculation was conducted using the Standard Tables of Food Composition in Japan (5th ed.).
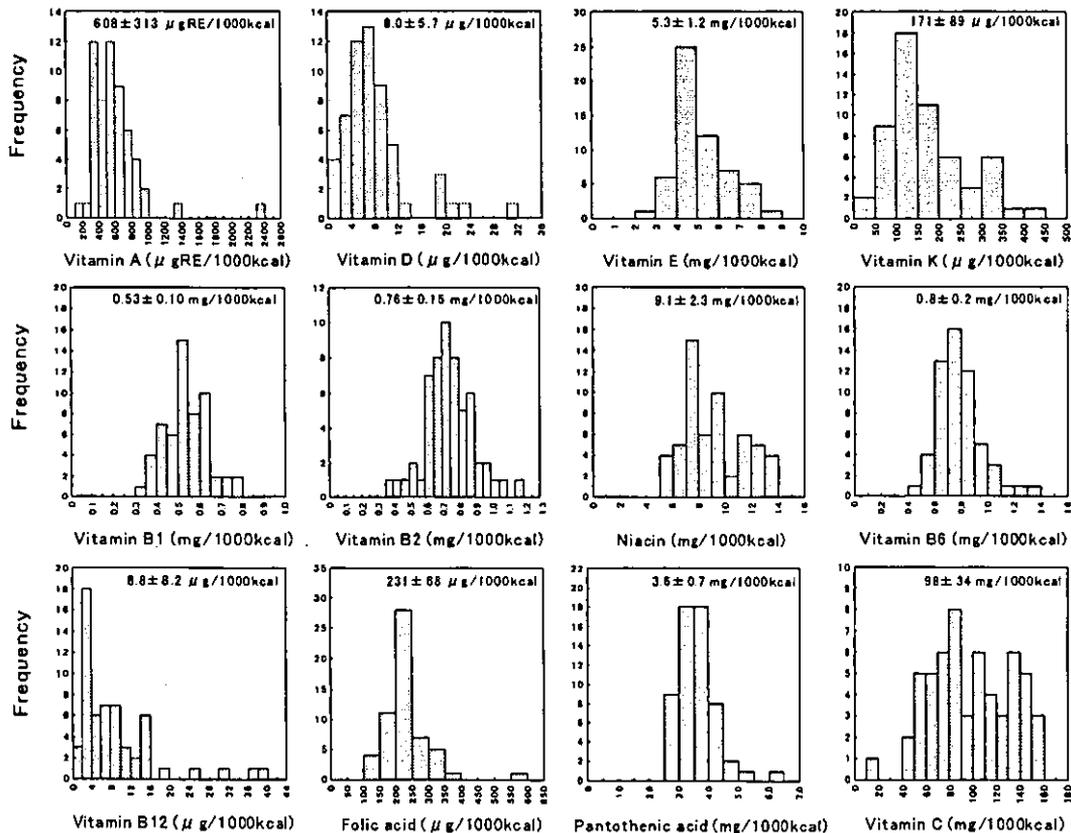
Appendix 3. Frequency of vitamin intake per 1,000 kcal energy intake in healthy free-living Japanese elderly people (n=57). Calculation was conducted using the Standard Tables of Food Composition in Japan (5th ed.).

Relationship between volatile sulphide compounds concentration and oral bacteria species detection in the elderly

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Aim: To evaluate the relationship between oral bacteria species detection and volatile sulphide compounds (VSC) concentration in the elderly. **Design:** Cross-sectional microbiological and clinical VSC examination of elderly. **Participants:** Sixty-seven elderly people aged 75, who were functionally independent and dentate. **Methods:** VSC (H_2S and CH_3SH) concentrations in the mouth air of subjects were measured using portable gas chromatography. Oral bacteria samples were taken from dental plaque and identification of bacteria species was accomplished using standard methods. **Results:** Fewer than 20% of subjects showed more than 10ng/10ml of H_2S (severe odour level). The detection rate of *P. melaninogenica* was significantly higher in elderly people with more than 10ng/10ml ($p=0.043$) levels. *Fusobacterium* had a tendency to be found in those with more than 10ng/10ml, but a significant relation was not found. **Conclusions:** The results suggest that *Fusobacterium* and *P. melaninogenica* may be involved in the production of H_2S in the oral cavity of elderly people.

Key words: Volatile sulphide compounds, oral malodour, oral bacteria

In recent years concerns about oral malodour have been increasing. Oral malodour includes unpleasant smells resulting generally from periodontal disease[1-5], and more rarely from diabetic patients[6] and those with heavy caries[7]. Oral care for elderly persons is important for prevention not only of oral disease but for general disease, for example, aspiration pneumonia[8-10]. Oral malodour is unpleasant for those caring for the elderly and can therefore be a problem in performing oral care smoothly.

Volatile sulphide compounds (VSC) have been considered to be correlated with oral malodour and several VSCs have been used as the indicator of oral malodour[4,11-14]. There have been several reports on the VSC production ability of bacteria species[14-20]. But there have been no reports on the relation between oral bacteria species detection and oral VSC concentration in mouth air, and so an epidemiological study of the elderly to elucidate this was thought to be advantageous.

Dental plaque is known to be a biofilm, a reservoir of bacteria species from which plantonic bacteria disperse and attach to other organs and tissues[21]. We suggested that dental plaque, saliva, the

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tonsils and pharynx harboured similar biofilm bacteria and that these organisms were released from tooth surfaces into saliva and then colonised on the oral cavity surface[22,23]. We hypothesised that bacterial species producing VSC in periodontal pockets and the dorsum of the tongue originate in dental biofilm. Our major concern was the possible association of bacterial species in dental biofilm with VSCs concentrations in the oral cavity.

In the present study, we analysed the relationship between VSCs concentrations and bacterial species detected in the dental plaque of the elderly.

Methods

Subjects

Sixty-seven elderly people aged 75 years (44 males and 23 females) from Niigata prefecture in Japan participated in this study, which was conducted in June, 2002. All of the participants were functionally independent and dentate.

VSC analysis of mouth air

H_2S and CH_3SH concentration were used as the indicator of oral malodour. The VSC concentrations in the mouth air of subjects were measured as follows.

- Prior to each analysis, following a deep breath, the subjects were instructed to keep their mouth closed and breathe through their nose quietly for 30 sec.
- A 1ml-disposable syringe was then inserted into the centre of the oral cavity through the lips and teeth, while the mouth remained closed.
- Thereafter, after aspirating 1ml of oral air with the disposable syringe, 0.5ml of oral air was injected into the GC-SCS for portable gas chromatography (Takasago Electoric Co., Ltd, Japan, 24).
- To avoid inter-operator variation, all gas chromatography

measurements were performed by a single trained individual.

Identification of oral bacteria

Samples were taken from dental plaque on upper molar teeth or upper molar portions of dentures using sterile cotton sticks. The plaque samples were placed in transport fluid (0.4% agar, 0.15% thioglycolate/phosphate buffered saline) and taken to Bio Medical Laboratory (Tokyo, Japan) for analysis. For aerobic bacteria species detection and identification, each sample was poured directly onto chocolate agar, OPA staphylococcus, and drigalski agar plates (Nippon Decton Dickinson Co., Ltd, Tokyo, Japan) using a stick. The plates were incubated in an atmosphere of 5% CO_2 in H_2 at 37°C for 24-48 hours. Representative microbial colonies from each plate were gram stained and isolated by identification of their characteristic appearance, as well as haemolytic, catalytic, and oxidase reactions[9]. Those species found in a majority of the subjects were suspended in 1ml of 0.5% saline, gently shaken, and the results are shown. The following methods were used to confirm identification of the bacteria on the detection plates: *Staphylococcus aureus* [Methicillin sensitive (MSSA) and resistant (MRSA)] by using PS latex, rabbit plasma, and MRSA screening plates (Nippon Becton Dickinson Co); *Pseudomonas* sp. by VITEK [BioMerieux Vitek Japan (BVJ), Tokyo]; *Haemophilus influenzae (H. influenzae)* by a Haemophilus ID4 plate (Nippon Becton Dickinson Co) and *Candida* species by Candida check (Intron Laboratories Inc., Tokyo). For anaerobic bacteria species detection and identification, each sample was poured directly onto an HK agar plate and incubated for 48–72 hour under anaerobic condition using the gas pack system. Representative microbial colonies from each plate were gram stained and isolated by RapID ANAII system. Each colony was

suspended in 0.6% KCl, 0.05% $CaCl_2$, 0.16mM NaOH. The suspension was inoculated in 10 separate detection medium (1: 0.4% Urea, 2: 0.1% p-Nitrophenyl-β, D-disacharide, 3: 0.1% p-Nitrophenyl-α, L-arabinoside, 4: 0.1% p-Nitrophenyl-β, D-galactoside, 5: 0.1% p-Nitrophenyl-α, D-glucoside, 6: 0.08% p-Nitrophenyl-β, D-glucoside, 7: 0.08% p-Nitrophenyl-α, D-galactoside, 8: 0.08% p-Nitrophenyl-α, L-fucoside, 9: 0.1% p-Nitrophenyl-n-acetyl-β, D-glucosaminide, 10: 0.1% p-Nitrophenyl-phosphate) and incubated in an atmosphere of 5% CO_2 in H_2 at 37° for 4–6 hours (primary test). In a secondary test, 0.01% 3-Phenyl-methylaminoacrolein, 0.1% Hydrochloride acid, 1.0% Acetic acid was added to reaction mixture 3–9 and INNOVA Indole to reaction mixture[10]. Bacterial species were identified by both the results of primary and secondary testing. The levels of detection for each organism were determined according to the manufacturers' instructions.

Statistical methods

In the series of oral malodour measurements, several examiners detected severe odour at a concentration of about 10ng/10ml of H_2S and about 0.5ng/10ml of CH_3SH. Therefore, concentration of H_2S and CH_3SH were classified into two groups ($\leq$10ng/10ml, 10ng/10ml< for H_2S and $\leq$0.5ng/10ml, 0.5ng/10ml < for CH_3SH). The relation between VSC concentration and detection of bacteria species was analysed using a gender-adjusted Mantel-Haenszel test. Difference at the .05 level was considered statistically significant. SPSS for Windows (version 10.0) was used in performing all statistical analyses.

Results

The distribution of VSC concentration

The distribution of H_2S and CH_3SH concentration is shown in *Table 1*.

Table 1 Distribution of subjects in VSC concentration by sex

(1) H₂S

	Men	Women	Total
≤10ng/10ml	38 (86.4)	17 (73.9)	55 (82.1)
10 ng/10ml<	6 (13.6)	6 (26.1)	12 (17.9)

(2) CH₃SH

	Men	Women	Total
≤0.5ng/10ml	28 (63.6)	7 (30.4)	35 (52.2)
0.5ng/10ml<	16 (36.4)	16 (69.6)	32 (47.7)

Table 2 Detection rate of major microorganisms

(1) Aerobic microorganisms

	Number	Percentage
α-Streptococcus	67	100
Neisseria sp.	67	100
Candida sp.	29	43.3
Corynebacterium	5	7.5
E. clocae	4	6.0

(2) Anaerobic bacteria species

	Number	Percentage
Capnocytophaga sp.	67	100
P. melaninogenica	36	53.7
Fusobacterium	18	26.9
P. corporis	15	22.4
P. intermedia	6	9.0

Table 3 The relation between H₂S concentration and oral bacteria detection

	H₂S concentration ≤10 ng/10ml No. (%)	10 ng/10ml< No. (%)	OR	95% CI	p
Aerobic					
Candida sp.	25 (45.5)	4 (33.3)	0.562	0.147–2.118	0.394
Corynebacterium	5 (9.1)	0 (0)	*	*	
Anaerobic					
P. melaninogenica	26 (47.3)	10 (83.3)	5.305	1.052–26.759	0.043
Fusobacterium	12 (21.8)	6 (50.0)	3.506	0.939–13.090	0.062
P. corporis	12 (21.8)	3 (25.0)	1.310	0.298–5.754	0.720
P. intermecdicus	5 (9.1)	1 (8.3)	1.080	0.114–10.242	0.946

*OR and 95% CI cannot be calculated in *Corynebacterium* because this bacteria species was not detected in subjects with more than five.

Less than 20% of subjects showed severe odour levels in H₂S concentration (more than 10ng/10ml of H₂S). A greater percentage of women than men had 10ng/10ml, but no significant difference was found between genders. With regard to CH₃SH, about half the subjects had a recognition level of CH₃SH concentration (more than 0.5ng/10ml of CH₃SH). The concentration of CH₃SH of women was significantly higher than that for men.

The detection of bacterial species

The detection rate of major microorganisms from dental plaque, as determined using the manufactures' instructions, is shown in *Table 2*. Of the aerobic micro-organisms, α-*Streptococcus* and *Neisseria* sp. were detected in all subjects. *Candida* sp. was isolated from 43.3% of subjects. The detection rate of other bacterial species was less than 10%.

Regarding anaerobic bacteria

species, only *Capnocytophaga* sp. were detected in all subjects. *P. melaninogenica* was isolated from about half of subjects (53.7%). *Fusobacterium* species and *P. corporis* were isolated from about quarter of subjects. There was not significant differentiation in the detection rates of bacteria species between both sexes.

The relation between VSC concentration and detection of bacteria species

We analysed the relationship between VSC concentration and detection of each bacterial species by using Mantel-Haenszel test. Subjects with more than 10ng/10ml of H₂S showed a significantly higher detection rate of *P. melaninogenica* than subjects with less than 10ng/10ml (*Table 3*). For *Fusobacterium*, though not statistically significant, the odds of detection amongst those with more than 10ng/10ml were 3.5 times greater than the odds among the less than 10ng/10ml group (OR=3.5 and 95% CI=0.939, 13.090). Regarding aerobic microorganism species, no significant relationships were seen. CH₃SH concentration was not related to micro-organism detection.

Discussion

Studies on the investigation of bacterial species concerned with oral malodour have mainly been performed by measuring VSC production ability of laboratory strains of oral bacteria species[15,16,18]. Recently, Awano et al.[25] reported the relationship between the presence of periodontopathogenic bacteria in saliva and halitosis in adults (average age: 50.0±13.5 years). Kazor et al.[26] found that *Atopobium parvulm*, a phylotype (clone B5095) of *Dialister*, *Eubacterium sulci*, a phylotype (clone DR034) of the uncultivated *Phylum* TM7, *Solobacterium moorei*, phylotype (clone BW009) of *Streptoccocus* were associated with halitosis in adults.

However, oral bacterial flora depends on age and oral condition. Furthermore, oral malodour of elderly people has been noticed by carers. From this point of view, the present study provides useful data to identify bacterial species relating oral malodour in the elderly.

The results of the study indicated that *P. melaninogenica* was significantly correlated with H$_2$S concentration and *Fusobacterium* had a tendency to be correlated with H$_2$S concentration. It has been reported that *Fusobacterium* has a higher H$_2$S production ability than other bacteria species[16,18]. These reports support our results. *Fusobacterium* are also isolated from periodontal pockets and the tongue[27-29] and are thought to be distributed in various parts of the oral cavity by means of saliva, causing oral malodour. It has been discussed that *Fusobacterium* produce H$_2$S in periodontal pockets and in tongue coating, as well as in dental plaque. With regard to *Prevotella* species, Paryavi-Gholami[30] reported that *P. oralis* are related to VSC levels in children. *P. oralis* was not isolated from the subjects. Oral bacteria flora changes with age, which may explain differences in bacteria species relating to oral malodour among subjects of various ages. *Fusobacterium* and *P. melaninogenica* are known to contribute greatly in forming biofilm[31]. There is a possibility that biofilms incorporate various bacterial species including some producing H$_2$S. Furthermore, pathogenic bacterial species are found in biofilm[22,32,33]. Oral care procedures for removing these bacterial species must be useful for preventing oral malodour as well as general disease.

No bacterial species showed significant association with CH$_3$SH concentration. The result may be concerned with less CH$_3$SH production of bacteria species than we examined, or with lower sensitivity of the detection system to the CH$_3$SH products.

In the present study, we have suggested which bacteria species are related to oral malodour in the elderly, as a useful indicator to help decrease oral malodour. The prevention of odour requires the removal of the biofilm from the tooth surface as well as dorsum of the tongue. For further investigation of the relationship between these bacterial species and oral malodour, establishment of a system for quantitative analysis of these bacterial species is required.

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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 affect or modulate the process of both diseases. The purpose of this study was to evaluate the relationship between systemic bone mineral density (BMD) and periodontal disease among older people.

Materials and Methods: Among all 4542 inhabitants aged 70 years according to a registry of residents in Niigata city in Japan, 600 people were selected randomly. One hundred and eighty-four subjects who did not have diabetes mellitus, whose blood sugar was < 140 mg/dl, who had more than 20 teeth, who were non-smokers, and who did not take medication for osteoporosis, were included in the study. Four dentists performed clinical evaluations on probing attachment level (PAL). We also utilized the data on BMD of the heel, which we measured using an ultrasound bone densitometer. Follow-up clinical surveys were done by measuring PAL after 3 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 BMD and the number of progressive sites which had ≥3 mm 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, respectively, were 4.65 ± 5.51 and 3.26 ± 3.01 in females and 6.88 ± 9.41 and 3.41 ± 2.79 in males. Two-way analysis of variance was performed to discriminate among effects of gender, BMD, and gender–BMD interaction. A significant effect of BMD (OG or NOG, $p = 0.043$) with a significant interaction ($p = 0.038$) was observed. Furthermore, BMD was associated with the number of progressive sites which had ≥3 mm 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 BMD.

Periodontal destruction is frequently experienced by elderly people (Slade & Spencer 1995, Brown et al. 1996) and it contributes to as much as 40% of tooth extraction (Johnson 1993). Periodontal disease is characterized by absorption of alveolar bone as well as by loss of the soft-tissue attachment to 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 affect 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 (BMD) 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 dentists 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 (Von Wowern et al. 1994, Mohammad et al. 1997, Tezal et al. 2000), 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 BMD was more significant in females than in males, the role of factors involved in the regulation of BMD in males as well as in post-menopausal 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 BMD 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 BMD and periodontal disease.

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

Materials and Methods
Subjects and clinical assessment

Initially, questionnaires were sent to all 4542 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% ($N = 3695$). 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 20 g and graduated at 1 mm intervals (VIVACARE TPS PROBE®, Schaan, Liechtenstein), 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.

Seventeen 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 κ ranged from 0.62 to 1.00 for PAL.

The four examiners did not have any information on BMD of the subjects.

The subjects' height, weight and grip power were measured to the nearest 1 mm or 0.1 kg, respectively, to calculate the body mass index (kg/m², BMI) or grip power/body weight (kg/kg). We also utilized the data on BMD of the heel, which we measured using an ultrasound bone densitometer (Lunar Achilles™, GE Medical Systems, Madison, WI, USA). The ultrasound signal is sent to os calcis. Ultrasound densitometry enables the measurement of the physical properties of bone, specifically BMD. The ultrasound measurement contains two criteria, the velocity (speed of sound (s); SOS) and frequency attenuation (broadband ultrasound attenuation (dB/MHz); BUA) of sound wave as it travels through bone (Langton et al. 1984, Rossman et al. 1989). 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 <140 mg/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. As at the baseline examination, 97.3% of the subjects received the follow-up examination by the same four dentists.

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 3 mm 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, PAL at baseline and the number of sites with $\geqslant 3$ mm additional attachment loss during the 3 years between males and females.

Furthermore, we evaluated the relationship between stiffness at the baseline and the number of sites with $\geqslant 3$ mm additional attachment loss during the 3 years by two-way analysis of variance (ANOVA) for discriminating among the effects of gender, stiffness and gender-stiffness interaction. After controlling for serum albumin concentration, serum total cholesterol concentration, grip power/body weight, serum IgG concentration, gender, BMI and PAL at baseline, a multiple linear regression analysis was performed to assess the relationship between stiffness at the baseline and the number of sites with $\geqslant 3$ mm additional attachment loss during the 3 years. The level of significance was set at $p < 0.05$ for these tests.

Results

The mean number of teeth present was 25.37 ± 2.91. The average PAL was 2.61 ± 0.76. Table 1 shows the stiffness, BMI, serum albumin concentration, serum total cholesterol concentration, grip power/body weight, serum IgG concentration, PAL and the number of sites with $\geqslant 3$ mm additional attachment loss during the 3 years between males and females. The stiffness was 74.19 ± 10.65 for males and 59.42 ± 8.87 for females. A significantly greater loss of stiffness was found in females ($p < 0.001$). The serum total cholesterol concentration was significantly lower, and grip power/body weight and PAL were significantly higher in males.

After dividing the subjects into the osteopenia group (stiffness $\leqslant 69$ for females, $\leqslant 85$ for males, OG) and the non-osteopenia group (NOG), we evaluated the number of progressive sites which had $\geqslant 3$ mm additional attachment loss during the 3 years. The mean number of progressive sites for the OG

Table 1. Comparison of stiffness, body mass index (BMI), biochemical values, grip power/body weight, probing attachment level (PAL) and additional attachment loss between males and females

Variables	Subjects ($n = 179$)		p-value
	males	females	
stiffness (%, mean $\pm$ SD)*	74.19 ± 10.65	59.42 ± 8.87	<0.001
BMI (kg/m^2, mean $\pm$ SD)*	22.56 ± 2.59	22.69 ± 2.78	0.752
albumin (g/dl, mean $\pm$ SD)*	4.30 ± 0.28	4.33 ± 0.24	0.500
total cholesterol (mg/dl, mean $\pm$ SD)*	194.42 ± 26.90	213.37 ± 29.12	<0.001
grip power/body weight (kg/kg, mean $\pm$ SD)*	0.67 ± 0.10	0.48 ± 0.08	<0.001
IgG (mg/dl, mean $\pm$ SD)*	1515.61 ± 262.88	1566.19 ± 336.75	0.269
PAL (mean $\pm$ SD)*	2.77 ± 0.80	2.46 ± 0.68	0.005
number of sites with $\geqslant 3$ mm additional attachment loss (mean $\pm$ SD)[†]	5.99 ± 8.36	4.37 ± 5.11	0.116

IgG, immunoglobulin G.
*At baseline.
[†]During the 3 years.

and the NOG, respectively, were 4.65 ± 5.51 and 3.26 ± 3.01 in females, 6.88 ± 9.41 and 3.41 ± 2.79 in males (Fig. 1). Two-way ANOVA was performed to discriminate among effects of gender, stiffness and gender-stiffness interaction. As shown by the data in Table 2, significant effects of stiffness (OG/NOG, $p = 0.043$) with a significant interaction ($p = 0.038$) were observed. The number of progressive sites was significantly higher in the OG. Furthermore, we evaluated the mean number of teeth present at baseline and tooth loss during the 3 years. The mean number of teeth present at baseline for the OG and the NOG, respectively, were 24.91 ± 2.71 and 25.05 ± 3.10 in females, 25.80 ± 2.96 and 25.95 ± 3.15 in males. There was no significance between the OG and the NOG in females and in males. The mean number of teeth lost during the 3 years for the OG and the NOG, respectively, were 0.84 ± 2.32 and 0.74 ± 1.41 in females and 0.52 ± 1.17 and 0.73 ± 0.83 in males. There was no significance between the OG and the NOG in females and in males as well.

The results of multiple linear regression analysis are presented in Table 3. Stiffness and gender were associated with the number of progressive sites which had $\geqslant 3$ mm additional attachment loss during the 3 years (stiffness: correlation coefficient $= -0.199$ ($p = 0.001$), gender: correlation coefficient $= -4.412$ ($p = 0.020$)).

Discussion

The results showed that the subjects in the OG had a higher number of progressive sites with $\geqslant 3$ mm additional attachment loss during the 3 years than the subjects in the NOG. This 3-year longitudinal study clearly demonstrated that BMD is a risk predictor for periodontal disease progression in an older population.

Some systemic factors which contribute to loss of bone mass and periodontal progression have been identified (Cummings et al. 1985, Genco & Löe 1993). There were some common factors such as smoking, nutritional deficiencies, age, intake of medications and immune dysfunction (Wactawski-Wende et al. 1996). Considering these facts, it is reasonable that this study showed a significant relationship between BMD and periodontal disease progression. Maybe, systemic factors of bone remodeling also modify local tissue response to periodontal disease.

The relationship between BMD and progression of periodontitis is difficult to establish because there were many potential confounding variables, including local factors. In our previous study of an older population, we found that the subjects who had more than 20 remaining teeth were less susceptible to periodontal disease (Hirotomi et al. 2002). The results of that study prompted us to evaluate the relationship between systemic BMD and periodontal progression after controlling for teeth present, in addition to other factors, such as gender, diabetes mellitus, smoking habits and intake of medications in this study. Likewise, we restricted the age of subjects to 70 years to eliminate the influence of age on periodontal disease progression.

Various researchers have proposed several plausible findings. Kribbs et al.
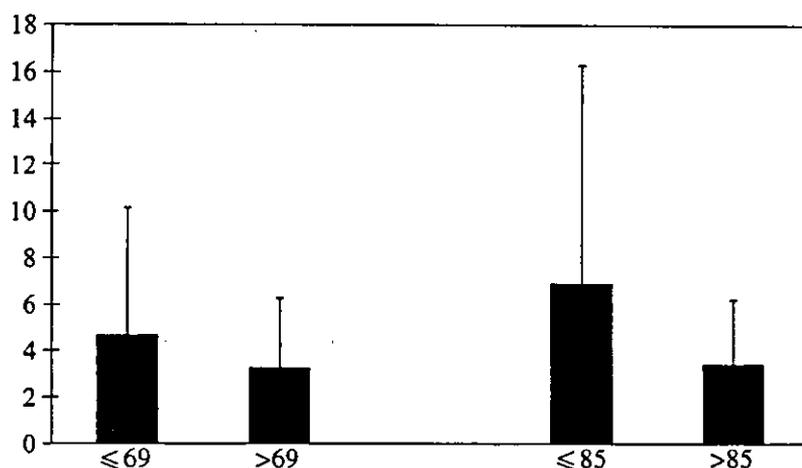
Fig. 1. Relationship between the number of progressive sites with ≥3 mm additional attachment loss and stiffness by gender. The number of subjects: stiffness ≤69 ($n = 74$) and >69 ($n = 19$) for females, ≤85 ($n = 64$) and >85 ($n = 22$) for males. a, stiffness (%)

Table 2. The results of analysis of variance for the evaluation between additional attachment loss and bone mineral density and gender

Variables	Sum of squares	df	Mean square	F	p-value
stiffness (osteopenia/non-osteopenia)	191.67	1	191.67	4.140	0.043
gender (males/females)	136.30	1	136.30	2.940	0.088
stiffness × gender	309.34	2	154.67	3.340	0.038
residual	8148.89	176	46.30		
total	8458.22	178	47.52		

Table 3. Multiple linear regression and associated p-values

Independent variables*	Dependent variable				
	number of sites with ≥3 mm additional attachment loss[†]				
	Coef.	Std. Err.	p-value	[95% CFI]	
stiffness (%)	− 0.199	0.060	0.001	− 0.317	− 0.080
albumin (g/dl)	− 4.286	2.200	0.053	− 8.633	0.061
total cholesterol (mg/dl)	0.003	0.021	0.899	− 0.039	0.044
grip power/body weight (kg/kg)	0.001	0.204	0.763	− 0.341	0.464
IgG (mg/dl)	0.001	0.002	0.494	− 0.002	0.005
gender (1: males, 2: females)	− 4.412	1.881	0.020	− 8.129	− 0.695
BMI (kg/m^2)	0.195	0.231	0.401	− 0.262	0.651
PAL[‡]	0.153	0.801	0.849	− 1.431	1.736
_cons	35.687	12.544	0.005	10.896	60.479

$p = 0.033$, $R^2 = 0.106$. Coeff., coefficient; std. err., standard error; CFI, confidence interval; BMI, body mass index; IgG, immunoglobulin G.
*At baseline.
[†]During the 3 years.
[‡]Mean value of probing attachment level (PAL) at baseline.

(1990) observed a significant correlation between several skeletal bone mass measurements and the number of remaining teeth in 85 osteoporotic women between 50 and 80 years of age. Some other reports showed that mandibular bone mass was significantly correlated with skeletal bone mass as well (Klemetti et al. 1993, Von Wowern et al. 1994). Furthermore, the BMD of the mandible is affected by the mineral status of skeleton and also by general disease that causes generalized bone loss (Klemetti et al. 1993). On the contrary, Mohajery & Brooks (1992) found there was no correlation between

skeletal and mandibular bone measurements. The results of these studies should be interpreted with caution since the number of subjects might be small, the age of subjects might have not been restricted, and the oral or skeletal bone loss might have been measured only in females.

In our study with adequate control of confounding variables, a weak relationship between BMD and periodontal disease progression existed although it was statistically significant. General BMD might not influence the alveolar bone loss directly in some cases. The skeleton is heterogenic, and bone density, bone turnover rate and bone remolding ability differ in some parts of the skeleton, suggesting that those regions, although related to each other, have some degree of independence. In addition, some bias such as local oral factors for alveolar bone loss might blur a clear relationship between systemic BMD and periodontal progression.

As our study was aimed at older subjects aged 70 years who had more than 20 teeth present, the subjects whom we examined might have been periodontitis-resistant. Therefore, it was difficult for PAL to contribute to inter-individual difference in resistance to periodontitis. This might be a reason for not having a significant relationship between periodontal disease condition such as PAL at baseline and additional attachment loss during the 3 years in this study. In addition, there was no significance in the number of teeth present at baseline, and tooth loss during the 3 years between the OG and the NOG in males and females. Therefore, the selection bias by the number of teeth present might be eliminated.

Likewise, ultrasonic bone density measurement was performed to evaluate BMD of the heel in this study. The ultrasound methods assess both bone volume and bone quality accurately and safely (Heaney et al. 1989). Some researchers have evaluated BMD by ultrasonic bone density measurement (Heaney et al. 1989, Resch et al. 1990). Ultrasound densitometry of the os calcis is highly reproducible and has a high correlation with BMD measured by dual-energy X-ray absorptiometry (DEXA) in different parts of the skeleton such as the spine or femur (Yamazaki et al. 1994).

In conclusion, this study suggested that there was a significant relationship between periodontal disease and general BMD in the present study.

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Serum vitamin C–periodontal relationship in community-dwelling elderly Japanese

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Abstract

Objective: To determine the relationship between serum vitamin C and periodontitis as estimated by clinical attachment loss (CAL) in community-dwelling elderly Japanese.

Material and Methods: This analysis was confined to 413 Niigata citizens aged 70 years in whom the data for serum vitamin C and CAL were available. High-pressure liquid chromatography method was used to ascertain the serum vitamin C levels while CAL was assessed on six sites of all teeth present including third molars by means of pressure-sensitive probes. Other variables included gender, smoking, toothbrushing frequency, number of teeth present and random blood sugar levels.

Results: Serum vitamin C concentration was inversely related to CAL ($r = -0.23$, $p < 0.00005$) at bivariate level. Multiple linear regression analysis showed that CAL was 4% greater in subjects with lower serum vitamin C levels than in subjects with higher serum vitamin C levels notwithstanding smoking, diabetes, oral hygiene, gender or number of teeth present.

Conclusion: The findings suggested that serum vitamin C might have relatively weak but a statistically significant relationship with periodontitis in this elderly population.

Investigations into vitamin C (ascorbate/ascorbic acid)–periodontal relationship go as far back as the 18th century when a British naval physician revealed that scurvy, which was accompanied by putrid gums could be successfully treated with oranges and lemons (Rubinoff et al. 1989). Since then numerous experimental as well as epidemiological studies in both humans and animals have attempted to address this issue but the findings have been rather incoherent: some have failed to suggest any significant relationship between vitamin C and periodontal disease (Waerhaug 1958, Barros & Witkop 1963, Russel et al. 1965, Enwonwu & Edozien 1970, Woolfe et al. 1980, 1984) while others reported that the deficiency of this vitamin could not be correlated with severe periodontitis but with gingival inflammation

or acute necrotizing ulcerative gingivitis (Enwonwu 1972, Shannon 1973, Leggott et al. 1986, Melnick et al. 1988). Still others have observed a weak association between vitamin C and periodontitis (Ismail et al. 1983, Nishida et al. 2000). However, the majority of workers who have looked into ascorbic acid–periodontal relationships in humans estimated the dietary intake of vitamin C (Ismail et al. 1983, Nishida et al. 2000) in comparison to the few who have assessed serum or plasma ascorbic acid levels (Leggott et al. 1986, Melnick et al. 1988, Pussinen et al. 2003), which might provide much reliable information than the former method (Simon & Hudes 2001). On the other hand, little or virtually nothing has been reported in the literature on the association between vitamin C and periodontal status of the elderly who may be at a higher risk of

developing vitamin C deficiency compared with other age groups (Rubinoff et al. 1989, Pussinen et al. 2003). In view of these facts, it is important to explore the link between serum ascorbic acid levels and periodontitis in an elderly population. Accordingly, the main purpose of the present investigation was to ascertain the relationship between serum vitamin C levels and periodontitis as measured by clinical attachment loss (CAL) among senior citizens in Japan.

Material and Methods

This was a part of the ongoing oral and general health survey, which has been carried out in senior citizens of Niigata city, Japan since 1998. The study methodology has been described in

detail elsewhere (Hirotomi et al. 2002, Ogawa et al. 2002). A written invitation was sent to all individuals aged 70 years ($n = 4542$; 2099 males and 2443 females) who were registered as the citizens of Niigata city, Japan in 1998 to take part in this survey once the Ethical Committee of the School of Dentistry, Niigata University, approved the research protocol of the survey. After sending a second request, 79.5% (3695) of the population consented to participate in the survey. Having considered the resources available, out of the positive respondents 600 individuals were randomly recruited into a cross-sectional community-based study so as to have an approximately equal number of males (306) and females (294). Informed consent was obtained from all subjects prior to the investigation.

Four calibrated dentists conducted the intra-oral examination involving assessment of CAL, probing depth (PD) and bleeding on probing (BOP) using mouth mirrors and pressure-sensitive TPS Probe® (Vivacare, Schaan, Liechtenstein) under artificial light. All teeth present including third molars were probed at six sites per tooth, namely, mesio-buccal, mid-buccal, disto-buccal, disto-lingual, mid-lingual and mesio-lingual and the recordings were rounded up to the nearest whole millimetre. Calibration of the examiners was carried out before and during the survey and the examiner consistency ranged from 0.56 to 0.92 as indicated by κ statistic. Information pertaining to smoking habits as well as oral hygiene practices was obtained by means of a personal interview, whereas blood samples were sent to the laboratory in order to evaluate the serum vitamin C levels using high-pressure liquid chromatography (HPLC) method and random blood sugar levels.

Statistical analyses were carried out by means of STATA statistical software package. Throughout the analysis, CAL, the dependent variable, was considered as a continuous variable and the unit of analysis was the subject. Among independent variables, serum vitamin C level was a continuous variable while gender (male:female), smoking status (current smoker: ex-smoker: non-smoker), diabetic status (random blood sugar (RBS) < 140 mg/dl: $\geqslant 140$ mg/dl), frequency of tooth cleaning (<2/day: $\geqslant 2$/day) and the number of teeth present (<20 teeth: $\geqslant 20$ teeth) were treated as categorical variables. To

compare the difference between two means Student's t-test was employed while one-way ANOVA combined with Bonferroni's test was used where necessary to compare more than two means. Moreover, the association between two continuous variables was determined by means of Pearson's correlation technique. Finally, having excluded the presence of multicolinearity, the independent variables that showed significant relationships with CAL at bivariate level were included in a multiple linear regression analysis to identify the independent effect of serum vitamin C level on CAL while controlling for other confounding factors. The level of statistical significance was fixed at $p \leqslant 0.05$.

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 serum

Table 1. Periodontal characteristics of the sample

Variable	Mean	SD
CAL (mm)	3.26	1.05
PD (mm)	2.10	0.58
BOP (%)	7.30	8.60

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

levels of vitamin C ranged from 0.2 to 22.6 mg/l with a mean of 7.21 (results not shown). Table 1 shows the periodontal characteristics of the sample. Accordingly, the mean CAL was 3.26 mm (SD = 1.05) while the mean PD was 2.1 mm (SD = 0.58). The mean percentage of BOP per person was 7.3 (SD = 8.6). It was also observed that 62.6% of subjects had at least one site with CAL of $\geqslant 6$ mm, whereas almost one-third (33%) of the sample exhibited PD of $\geqslant 6$ mm at least on one site (results not shown). Indeed, there was no significant relationship between serum vitamin C levels and either PD or BOP (results not shown) and consequently, it was decided to confine the present analysis to explore the relation between the independent variables including serum vitamin C concentration and CAL. Table 2 depicts 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 with the subjects with <20 teeth (mean = 3.69 mm; SD = 1.2). It is also apparent that CAL was significantly higher in the

Table 2. Relationships between CAL and independent variables at bivariate level

Independent variables	CAL Mean (SD)	p
Gender*		
male ($n = 215$)	3.54 (1.2)	<0.00005
female ($n = 198$)	2.96 (0.8)	
Number of teeth present*		
<20 ($n = 184$)	3.69 (1.2)	<0.00005
$\geqslant 20$ ($n = 229$)	2.92 (0.8)	
Brushing frequency*		
<2/day ($n = 145$)	3.40 (1.1)	<0.05
$\geqslant 2$/day ($n = 268$)	3.20 (1.0)	
Smoking status†		
current smoker ($n = 71$)	3.82 (1.3)	<0.00005‡
ex-smoker ($n = 137$)	3.45 (1.1)	
non-smoker ($n = 205$)	2.95 (0.8)	
Diabetic status*		
RBS <140mg/dL (n = 356)	3.22 (0.1)	<0.05
RBS $\geqslant 140$ mg/dl ($n = 57$)	3.52 (0.2)	
Serum ascorbic acid§	$r = -0.23$	<0.00005

CAL, clinical attachment loss; RBS, random blood sugar. *Student's t-test.
†One-way ANOVA.
‡Bonferroni's test: $3.82 > 3.45 > 2.95$ ($p < 0.05$).
§Pearson's correlation.

Table 3. 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.

subjects who brushed their teeth <2/ day than in those who used a toothbrush ≥2/day. One-way ANOVA combined with Bonferroni's post hoc test disclosed that current smokers had significantly worse CAL (mean = 3.82 mm; SD = 1.3) in comparison with 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 3. 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 weak but 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 indi-

cated by the relatively smaller correlation coefficient of serum vitamin C (correlation coefficient = − 0.04): CAL in subjects with lower serum vitamin C levels would only be 4% greater compared with 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 et al. (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 while attempting to explain how ascorbic acid could affect the healthy tissues in humans as well as in animals (Goetzl et al. 1974, Alfano et al. 1975, Boxer et al. 1979, Dallegri et al. 1980, Alvares et al. 1981, Alvares and Siegel 1981,

Berg et al. 1983, Leggot et al. 1986, Jacob et al. 1987, Nakamoto et al. 1984). 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 leucocytes 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 hypothesized 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. 1984) 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 et al. 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 1 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 the observed relationship is rather weak and that these biological phenomena involving vitamin C have neither been clearly understood nor well defined (Leggot et al. 1986, Nishida et al. 2000, Pussinen et al. 2003).

This study population comprised non-institutionalized elderly people who were active, living independently and willing to participate in the survey. It has been shown that the elderly who are institutionalized, 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). Besides, the mean serum concentration of vitamin C in this sample was rather high and the serum vitamin C level in only about 4% of subjects (results not shown) was below the reference range for Japanese elderly (Sakai et al. 1998). 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 weakly correlate with periodontitis as evaluated by CAL notwithstanding the effects of smoking, diabetes mellitus, gender, oral hygiene practices or the number of teeth present. Moreover, considering the cross-sectional nature of the study design it was almost impossible for us to ascertain lifetime changes in either the vitamin C intake or serum vitamin C levels in this population. Because of these facts and 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. All in all, the association observed here could not be a straightforward one but it would be plausible that the serum vitamin C might be inflicting a moderating influence on periodontitis through the established risk factors such as smoking and diabetes, as it was mentioned hitherto. 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. It should also be highlighted that this elderly cohort will be followed up for several years and thus, we intend to analyse the serum vitamin C–periodontal relationship prospectively, in the same population.

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