

secretion rate were examined.

Methods

Subjects

Three hundred sixty-eight elderly people aged 75 years old (194 males and 174 females) from Niigata prefecture in Japan participated in this study, which was conducted in June, 2003. All of them were functionally independent and dentate. Prior to the present study, informed consent to all the subjects was carried out and conducting the study was permitted by the Ethic Committee at Niigata University.

Questionnaire

The data on life style concerning dental caries were obtained by using a questionnaire before any oral examinations were undertaken. The life style parameters examined were eating between meals and toothbrushing.

Oral examinations

Oral examinations were performed according to WHO oral examination procedures (WHO 1997). The examiners were calibrated by 18 volunteer patients in the University Hospital before and during the survey. Inter-examiner reliability was assessed for the four examiners. We calculated a kappa score using 5 codes (Sound, Filled, Decayed, Filled with decay, and Bridge abutment: Special crown or Veneer/implant). The kappa values between each pair of examiners were 0.84-0.97. Root surface with gingival recession were recorded, if the surface was clinically visible beyond the cemento-enamel junction. Root decay was defined when a lesion was detected on an exposed root surface and felt soft or leathery when probed. For a single

incidence of decay or for a filling affecting both the crown and the root, the likely site of origin of the lesion was recorded as decayed or filled. For root surface caries status indicators, the number of root surface decayed teeth (RDT), the number of root surface filled teeth (RFT) and the number of root surface decayed teeth and root surface filled teeth (RDFT).

Saliva

Whole saliva was stimulated by chewing a piece of paraffin wax for three minutes. Then, stimulated saliva was collected over 3- min period asking subjects to tilt the head forward and to spilt the saliva into a graduated, preweighted, conical tube.

Microbiological processing

Subjects had been asked not to brush their teeth two hours before sampling. Stimulated whole saliva was collected in and sterile cotton stick was immersed in the saliva for 10 second. The saliva 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. Duplicate samples of 25µl of the appropriate dilutions were placed on mitis salivarius bacitracin (MSB) agar (Gold et al., 1973) for growth of mutans streptococci and in Rogosa selective lactobacillus (SL) agar. The MSB plates were incubated for 2 days at 37°C in 5% CO₂ in N₂, and the SL plates aerobically for 3 days at 37°C. Counts were made of colonies on MSB agar with the morphology typical of lactobacilli in Rogosa SL agar. The number of colony-forming units (CFU) of these

microorganisms per milliliter of saliva was determined. Several representative and atypical colonies on MSB agar were isolated and identified using both specific fluorescent antisera and procedures (Bratthall et al., 1972).

Statistical analysis

To compare the rate of subjects with decayed root surface, with filled root surface, and with decayed root surface or filled root surface between two groups, chi-square test was used. Comparison of RDT, RFT, and RDFT between groups were performed by Mann-whitney U-test or Kruskal Wallis test. Correlation between two variables was tested by Spearman rank correlation. For evaluation of the relationship between caries and bacteria species, linear multiple-regression analysis was performed. The dependent variables were the caries status. As independent variables, gender, frequency of eat between meals (none/lesser than once per day/once or more than once per day), toothbrushing (toothbrush only/ toothbrush and dental floss), stimulated saliva flow (ml/3 min) and bacteria level (CFU/ml) were used. Differences at the 0.05 level were considered statistically significant. SPSS for Windows (Version 10.0) was used for all statistical analyses.

Results

Gingival recession was noted for 356 subjects (96.5 %). Untreated caries on root surfaces were found in 23.0% of the individuals with exposed root surfaces (Table 1). More than 80% of subjects had one or more root with decayed lesion or filling (Table 1). The average value of RDT, RFT, and RDFT of root surface caries were 0.34, 3.61, 3.96, respectively.

The distribution of mutans streptococci level and lactobacilli level were shown in Table 2. Detectable levels of mutans streptococci and lactobacilli were found in 88.2% and 82.6% of the subjects. High counts of mutans streptococci and lactobacilli ($>10^5$ CFU/ml saliva) were found in 31.5% and 17.7% of the subjects.

Occurrence of decayed root surface was compared between subjects with and without bacteria species examined in this study (Table 3). Lactobacilli carrier showed significantly higher rate of decayed root surface than lactobacilli non-carrier, but mutans streptococci carrier did not show significantly higher rate of decayed root surface than mutans streptococci non-carrier. Subjects with mutans streptococci >20200 CFU/ml saliva showed significantly higher rate of decayed root surface than those with mutans streptococci ≤ 20200 CFU/ml saliva. Significant differences on the rate of having filled root surface and the rate of having decayed root surface or filled surface were not found

between carrier and non-carrier of lactobacilli or mutans streptococci.

Correlation analyses showed that RDT was positively correlated with mutans streptococci level ($p=0.006$, Table 4) and lactobacilli level ($p<0.001$, Table 4). RFT showed significantly negative correlation with Lactobacillus level. RDFT was not correlated with mutans streptococci level and lactobacilli level. Salivary flow was significantly correlated to RFT ($p=0.035$).

The relationship between caries status and life style was shown in Table 5.

Subjects who use toothbrush alone showed significantly higher RFT and RDFT than subjects who use toothbrush and dental floss. Significant difference in RDT was not seen among three groups concerning eating between meals and between two groups concerning toothbrush.

Stepwise multiple regression analyses are presented in Table 6. As mutans streptococci level and lactobacilli level were significantly correlated each other ($p<0.001$), these were introduced into regression models separately. The number of instances of untreated root caries was significantly associated with mutans streptococci level and lactobacilli level.

Discussion

In the present study, all subjects were 75-years old. Because of uniformity of age, the affect of age can be excluded. Lundgren et al have performed epidemiological research on caries prevalence and oral microbial conditions of 88-year-old people (Lundgren et al., 1996, 1998). Subjects of the present were 13 years younger than the subjects of Lundgren's study, so physiological features of two groups are thought to be different. All subjects were functionally independent, which has merit of preventing complication by influence of functional status on oral condition (Beighton et al., 1991). Moreover, sample size of the present study is large enough for epidemiological study. Therefore, the present study will provide valuable model to elucidate of dental caries occurrence of elderly. In the present subjects, almost all (96.5%) had exposed root surface and thus could be at risk of root caries.

Significant difference in the rate of decayed root surface was seen between subjects with lactobacilli and without lactobacilli. This suggests that having decayed root surface is correlated to lactobacillus detection. Mutans streptococci detection was not correlated with having root decayed surface. Our results suggest that lactobacillus detection may contribute to root surface decay more than mutans streptococci detection. This is characteristic for root surface decay. More than 20200 CFU/ml of mutans

streptococci detection was correlated. Mutans streptococci are prominent components to synthesize glucan and play a significant role as pathogenic colonizers to dental caries in the development of oral biofilm. That the level of mutans streptococci is about 20000 CFU/ml saliva may be a sign of having pathogenic biofilm and decayed root surface. The number of decayed root surface was correlated to mutans streptococci level and lactobacilli level. Our result is supported by previous studies which have shown that mutans streptococci and lactobacilli in carious surfaces are found in higher proportions from soft carious root surface than from sound or incipient carious root surface (Keltjens et al., 1987; Fure et al., 1987; van Houte et al., 1994; Bowden 1990; Bowden et al., 1990). Furthermore, Lundgren has reported that decayed root surface caries correlated to *S. sobrinus* and *S. mutans*, which also support our result (Lundgren et al., 1998). The suitable environment for the settlement of lactobacilli which does not have ability to adhere tooth surface may be provided on the root surface by biofilm formation of mutans streptococci (more than 20200 CFU/ ml in saliva) to incorporate lactobacilli.

The correlation between root surface decay and mutans streptococci or lactobacilli suggests two aspects: (i) These bacteria species are cause of root surface caries (ii) Root surface decay lesions provide nidus for these bacteria species. Mutans streptococci level and lactobacilli level showed significant correlation each other and

both were detected in 74% of subjects. Mutans streptococci and lactobacilli are thought to be indicator bacteria for root surface caries. In pit and fissure caries lesion, mutans streptococci and lactobacilli were very often found together (Weerhijim et al., 1992; Klock and Krasse 1978; de Soet et al., 1995). Saliva flow did not show significant correlation with root surface decay. Root surface may not be ready to be influenced by saliva flow, which is convenient for the settlement of lactobacilli which does not have ability to adhere tooth surface. Life style factors (eating between meals, and toothbrushing) were not correlated with root surface decay. These results suggests that mutans streptococci and lactobacilli must be mainly took care for the prevention of root surface caries of elderly.

Filled root surface did not show positive significant correlation with mutans streptococci or lactobacilli. It is thought that not all subjects with RFT had root caries experience because to eliminate wedge-shaped defect from filled root surface is impossible.

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Table 1 Distribution of subjects by RDT, RFT, and RDFT

	0	1-5	6-10	11-
RDT	274 (77.0)	81 (22.8)	1 (0.3)	0 (0)
RFT	92 (25.8)	170 (47.8)	77 (21.6)	17 (4.8)
RDFT	70 (19.7)	180 (50.6)	86 (24.2)	20 (5.6)

Number (%)

Table 2 Distribution of subjects by bacteria species level

	ND	<10 ⁵	10 ⁵ -10 ⁶	>10 ⁶
Mutans Streptococci	42 (11.8)	202 (56.7)	99 (27.8)	13 (3.7)
Lactobacilli	62 (17.4)	231 (64.9)	50 (14.0)	13 (3.7)

Number (%)

Table 4 Correlation coefficients between caries RDT, RFT and RDFT and mutans streptococci level , lactobacilli level and saliva flow

	RDT	RFT	RDFT
Mutans Streptococci	0.144**	-0.050	-0.017
Lactobacilli	0.264***	-0.133*	-0.093
Saliva flow	-0.033	0.110*	0.092

*:p<0.05 **:p<0.01 ***:p<0.001

Table 5 RDT, RFT and RDFT in subjects categorized life style

	RDT	RFT	RDFT
Eating between meals			
None (122)	0.35±0.69	3.14±3.17	3.49±3.17
lesser than once per day (189)	0.33±0.79	3.58±3.97	3.92±3.93
once or more per day (44)	0.36±0.87	4.82±4.55	5.18±4.59
Toothbrushing			
toothbrush only (214)	0.28±0.74	4.03±3.94***	4.31±3.96**
toothbrush and dental floss (129)	0.31±0.74	2.63±3.31	3.00±3.30

*:p<0.05 **:p<0.01 ***:p<0.001

missing data were excluded

Table 6 Effects of various parameters on the number of decayed root surface
(1) model 1

Variable	β	T	P value
Sex	-0.040	-0.716	0.475
Mutans streptococci	0.119	2.185	0.030
Toothbrushing	0.052	0.944	0.346
Eating between meals	-0.006	-0.118	0.906
Saliva flow	-0.066	-1.180	0.237

(2) model 2

Variable	β	T	P value
Sex	-0.028	-0.492	0.623
Lactobacilli	0.125	2.256	0.025
Toothbrushing	0.041	0.734	0.463
Eating between meals	-0.003	-0.056	0.956
Saliva flow	-0.054	-0.971	0.332

Table 3 Root caries prevalence in oral bacteria carrier and non-carrier

	RDT		RFT		RDFT		P
	0	1 \leq	0	1 \leq	0	1 \leq	
Mutans Streptococci							
Non-carrier	34 (81.0)	8 (19.0)	12 (28.6)	30 (71.4)	9 (21.4)	33 (78.6)	0.759
Carrier	240 (76.4)	74 (23.6)	80 (25.5)	234 (74.5)	61 (19.4)	253 (80.6)	
MS level \leq 20200	129 (82.2)	28 (17.8)	38 (24.2)	119 (75.8)	30 (19.1)	127 (80.9)	0.815
MS level > 20200	145 (72.9)	54 (27.1)	54 (27.1)	145 (72.9)	40 (20.1)	159 (79.9)	
Lactobacillus							
Non-carrier	56 (90.3)	6 (9.3)	17 (27.4)	45 (72.6)	12 (19.4)	50 (80.6)	0.946
Carrier	218 (74.1)	76 (25.9)	75 (25.5)	219 (74.5)	58 (19.7)	236 (80.3)	

Role of anti-PAc (361-386) peptide antibody for periodontal disease status in elderly

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Abstract

Aim: We attempted to confirm the relationship between the concentration of anti-PAc (361-386) peptide antibody, which inhibits the adhesion of *S. mutans* to the tooth surfaces, and periodontal status.

Methods: Eighty-seven functionally independent elderly subjects were enrolled and their dental calculus deposition, attachment loss, and anti-PAc (361-386) peptide antibody titer assessed. The subjects were divided into 2 groups according to anti-PAc (361-386) peptide antibody concentration.

Results: Subjects with an anti-PAc (361-386) peptide antibody titer greater than 2^2 (High group) showed lower levels of dental calculus deposition than those with a titer less than 2^2 (Low group). Further, attachment loss was significantly smaller in the High group than in the Low group.

Conclusion: Our results suggest that *S. mutans* may be indirectly involved in periodontal disease occurrence and anti-PAc (361-386) peptide antibody titer may be an indicator of periodontal disease.

Text

Streptococcus mutans (*S. mutans*) has been reported to have an association with dental caries (Hamada & Slade 1980, Loesche 1986). The function of the cell surface protein antigen of *S. mutans*, known as PAc (Okahashi et al. 1989), Ag I/II (Russell & Lehner 1978), PI (Forestor et al. 1983), or B (Russell 1979), is essential for colonization of the bacterium on tooth surfaces and its interaction with the salivary pellicle that coats dental enamel (Demuth et al. 1990, Russell and Masson-Rahemtulla 1989, Senpuku et al. 1996). The alanine-rich repeating region (residue 219-464, A-region) of the PAc molecule, which is important for the interaction of *S. mutans* with the salivary film (Brady et al. 1992, Nakai et al. 1993, Yu et al. 1997), has a strong immunogenicity in humans (Senpuku et al. 1996) and may be a candidate antigen for inducing the production of inhibiting antibodies against the adherence of *S. mutans* to tooth surfaces.

PAc (amino acid residue 361 to 377, 365-377) in the A-region has been shown to induce an antibody to inhibit *S. mutans* colonization and, therefore, is considered important for the adherence of *S. mutans* to the tooth surface (Senpuku et al. 2001, Takeuchi et al. 2001). The overlapped PAc (370-386) peptide to PAc (361-377) peptide includes a multiple binding motif (L - - V - K - A) that reacts with human leukocyte antigen (HLA)-DRB1*0802, *1101, *1402, and *1405 genotypes (Senpuku et al. 1998),

and is also recognized in the A-region. Therefore, the coupled PAc (361-386) peptide from residues 361-377 and 370-386 may be a minimum antigen of PAc that induces the inhibiting antibodies in humans.

The presence of the anti-PAc (361-386) peptide antibody has been suggested indicate an ability to prevent dental caries in humans and it is also speculated to prevent the adhesion of dental calculus to tooth surfaces, leading to the prevention of periodontal disease, because dental calculus harbors microorganisms that are involved with periodontal disease formation.

In the present study, we analyzed the relationship between anti-PAc (361-386) peptide antibody titer and periodontal status in 87 elderly people (average 75 years old, 60 males and 27 females) from Niigata prefecture in Japan in June 1999. All were functionally independent and had full dentition. Prior to the study, informed consent was obtained from all subjects and the study was approved by the Ethics Committee of Niigata University.

Four calibrated dentists assessed the periodontal conditions in the subjects based on 6 point measurements (mesio-buccal, buccal, disto-buccal, mesio-lingual, lingual, disto-lingual) of each tooth. Intra- and inter-examiner reliability was confirmed by a kappa statistic ($k=0.56-0.92$ for attachment loss). To estimate periodontal status,