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Salivary microbial levels in relation to periodontal status and caries development

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Background and Objective: Although an inverse relationship between caries and periodontal disease has been suggested, some studies have reported a positive correlation between periodontal disease and the decayed, missing and filled teeth (DMF) index. The aim of the present study was to examine the relationship between caries and periodontal disease.

Material and Methods: We assessed the clinical parameters and salivary levels of *Porphyromonas gingivalis* and *Streptococcus mutans* using real-time polymerase chain reaction in 40 subjects with varying degrees of caries and periodontal disease.

Results: The salivary levels of *S. mutans* were significantly higher in the periodontally healthy group than in the periodontitis group. The salivary levels of *P. gingivalis* were significantly higher in the caries-free group than in the periodontally healthy group with caries. The salivary levels of *S. mutans* were significantly increased after the initial periodontal treatment.

Conclusions: This study showed that an inverse relationship exists between periodontitis and caries in terms of the clinical and bacteriological findings.

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Periodontal disease and dental caries are the most common oral diseases, and an inverse relationship has been suggested to exist between the two (1–3). However, while epidemiological studies showing high periodontal index scores in caries-free populations support this concept (4,5), some studies have reported a positive correlation between periodontal disease and the decayed, missing and filled teeth (DMF) index (6–9).

Periodontal disease and caries are both chronic bacterial infectious diseases. Periodontal disease (10–12), a destructive inflammatory disorder that leads to the loss of tooth support, is initiated by a specific group of gram-negative anaerobic bacteria, which modulate periodontal tissue destruction through complex interactions with host cells (13,14). A great deal evidence points to *Porphyromonas gingivalis* as the key pathogen in periodontal disease (15,16). The ability of this bacterium to form a biofilm and to colonize subgingival sites is a critical step in the initiation of periodontal disease. Moreover, high levels of *P. gingivalis* were observed in subgingival plaque and saliva samples from subjects with periodontitis (17).

Dental caries is the direct result of enamel dissolution by acid-producing bacteria inhabiting the biofilm, especially when the biofilm reaches a critical mass due to poor oral hygiene. *Streptococcus mutans*, a major cariogenic bacterium, produces extracellular polysaccharide that contributes to the formation of the biofilm (18), and the levels *S. mutans* in saliva have been shown to predict caries activity (19,20).

A previous study reported that *S. mutans* is significantly associated with healthy and non-active periodontitis sites compared with active disease sites (21). However, information is lacking on the association between periodontal disease and

dental caries based on sensitive microbial identification techniques. To increase our knowledge of this relationship, this study examined the salivary levels of *P. gingivalis* and *S. mutans* in periodontitis patients, caries patients and healthy subjects using real-time polymerase chain reaction (PCR).

Material and methods

Patients, clinical evaluations and saliva samples

This study was approved by the Nihon University School of Dentistry Institutional Review Committee and all subjects gave written informed consent for study participation. The study population consisted of 40 systemically healthy patients who first visited Nihon University School of Dentistry Dental Hospital for oral examination, periodontal treatment or conservative dental treatment between March 2006 and March 2007, comprising 17 men and 23 women ranging in age from 23 to 78 years (mean age 49.2 years). None of the patients had received any kind of professional dental intervention during the previous year.

The subjects were evaluated at the time of the first visit, and the components of the examination were probing depth, bleeding on probing (BOP), the plaque score and the number of decayed teeth. The probing depth and BOP were recorded with a periodontal probe (CP11; Hu-Friedy, Chicago, IL, USA) and rounded off to the nearest 1 mm. The plaque score was recorded using O'Leary's plaque control record, and caries lesions were assessed according to the diagnostic criteria of the World Health Organization. During the clinical examination, paraffin wax-stimulated whole saliva was collected for 5 min. The saliva samples were measured using a measuring cylinder and stored at −80°C until analyzed.

Clinical recording and saliva sampling were also done after initial periodontal treatment in 10 periodontitis patients. The 2–4 month initial periodontal treatment consisted of oral hygiene instruction and scaling and root planning was carried out between first and second clinical assessment.

Real-time PCR

Saliva samples were boiled for 10 min and then centrifuged at 10,000*g* for 5 min; 5 μL of supernatant was used as the template for PCR. Real-time PCR was performed using the ABI PRISM 7700 Sequence Detection System (Applied Biosystems, Foster City, CA, USA). Each reaction tube contained 50 μL of reaction mixture, including 5 μL of sample, 1× Universal PCR Master Mix (Applied Biosystems), 900 nM of each primer and 250 nM of probe.

The primer and probe sets for the two bacterial species are listed in Table 1 (22). To quantify total bacteria, conserved sequences in reported 16S genes were selected. Total bacterial rRNA was amplified in a separate reaction, simultaneously, in the same conditions as those used for specific amplification of the two bacteria. The probes were labeled at the 5′-end with the reporter dye 6-carboxyfluorescein (6-FAM) and at the 3′-end with the quencher dye 6-carboxytetramethylrhodamine (TAMRA). The data were analyzed using Sequence Detection System software from Applied Biosystems. The number of bacterial cells was determined using DNA from known amounts of bacterial strains *P. gingivalis* ATCC 33277 and *S. mutans* GS-8 grown in appropriate culture conditions. Bacterial levels were expressed as a percentage of the total bacteria.

Patient classification

At first, 40 patients were classified into two groups according to the mean probing depth. Patients with a mean probing depth < 3 mm constituted the periodontally healthy with dental decay group (group A). Patients with a mean probing depth ≥ 3 mm were subdivided into two groups according to the presence of dental decay (group B, with dental decay; and group C, no dental decay; Table 2). None of these subjects was periodontally healthy with no dental decay.

Table 1. Sequence of the oligonucleotide primers and probes

	Sequence (5′–3′)
Total bacteria	
Forward	GGATTAGATACCCTGGTAGTC
Reverse	TACCTTGTTACGACTT
Probe	TGACGGGCGGTGTGTACAAGGC
P. gingivalis	
Forward	TACCCATCGTCGCCTTGGT
Reverse	CGGACTAAAACCGCATACACTTG
Probe	GCTAATGGGACGCATGCCTATCTTACAGCT
S. mutans	
Forward	TATCATGGTCAAAGGACTACTACAAGAG
Reverse	AATCCCAGTGGTTTCTTTGATCA
Probe	TGCAGAAGGAAGTTGTTGTACGGTTGGTTTA

Table 2. Patient characteristics of the study population

	Group A	Group B	Group C	Total
No. of subjects	13	10	17	40
Males/females	4/9	5/5	8/9	17/23
Mean age (years)	50.2 ± 19.5	52.8 ± 19.5	45.9 ± 11.5	49.2 ± 16.2
Age range (years)	23–75	32–78	31–73	23–78

Group A, patients with mean probing depth < 3 mm and dental decay.
Group B, patients with mean probing depth ≥ 3 mm and dental decay.
Group C, patients with mean probing depth ≥ 3 mm and no dental decay.

Statistical analysis

Differences in the clinical parameters and bacterial levels between groups were analyzed using the Mann–Whitney *U*-test followed by Bonferroni correction. Differences between before and after initial periodontal treatment were analyzed by Wilcoxon signed-rank test. Statistical analyses were performed using SPSS® software (SPSS Inc., Chicago, IL, USA).

Results

The clinical characteristics and salivary bacterial levels are described in Fig. 1. Both the average number of *P. gingivalis* and the salivary levels of *P. gingivalis* were significantly lower in group A than in group C. The mean number of caries, however, was significantly higher in group A and B than in group C. The average number of *S. mutans* in saliva and the salivary levels of *S. mutans* were both significantly higher in groups A and B than in group C. No significant differences were detected between groups in the amount of saliva, O'Leary's plaque control record or total bacterial number.

The mean probing depth was reduced following the initial periodontal treatment (Table 3). The average number of *P. gingivalis* in saliva was significantly reduced after the treatment. In contrast, the average number of *S. mutans* was significantly increased.

Table 3. The effect of initial periodontal treatment on salivary bacterial levels

Variable	Baseline	After initial treatment	*p* value
Saliva level (mL)	6.63 ± 2.47	7.13 ± 3.15	0.463
Probing depth (mm)	4.20 ± 1.16	2.77 ± 0.57	0.005*
No. of total bacteria/mL	$9.93 \pm 21.2 \times 10^{9}$	$8.61 \pm 22.0 \times 10^{8}$	0.028
No. of *S. mutans*	$2.14 \pm 2.86 \times 10^{4}$	$2.85 \pm 7.61 \times 10^{6}$	0.005*
S. mutans levels (%)	$3.28 \pm 4.86 \times 10^{-3}$	1.20 ± 2.49	0.005*
No. of *P. gingivalis*	$1.27 \pm 3.40 \times 10^{6}$	$6.64 \pm 8.23 \times 10^{3}$	0.005*
P. gingivalis levels (%)	$7.55 \pm 7.30 \times 10^{-3}$	$1.77 \pm 2.42 \times 10^{-2}$	0.721

All values are expressed as means ± SD.
*$p < 0.01$.

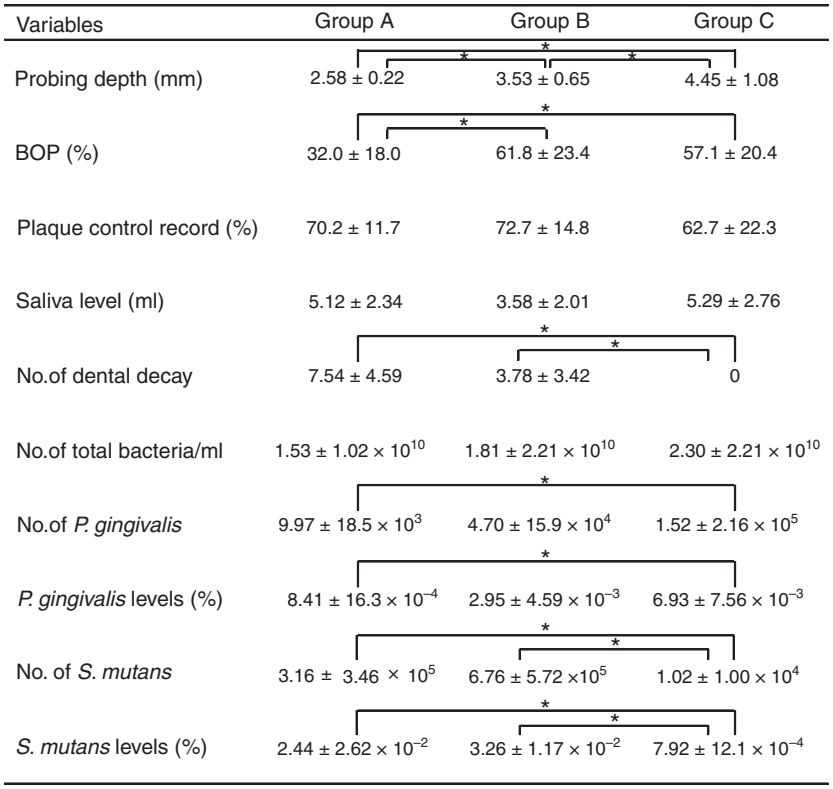

Fig. 1. Clinical characteristics and salivary bacterial levels of the study population.

Discussion

This study assessed the salivary levels of *P. gingivalis* and *S. mutans* using real-time PCR, in addition to the clinical parameters, in a comparison of periodontal disease and dental caries. Saliva represents an easy and non-invasive means of obtaining samples containing bacteria from all oral sites, e.g. the mucosa and the supra- and subgingival plaque (23–25). The real-time PCR measures not only living bacterial cells, but also dead cells in clinical samples. However, the number of *P. gingivalis* calculated by the culture method was almost the same as that calculated by the real-time PCR (17). Our data indicated that an inverse relationship exists between periodontitis and caries in terms of the clinical and bacteriological findings, and the bacteriological data indicated a negative correlation between *P. gingivalis* and *S. mutans*. Bacterial interactions, including synergism, commensalism and antagonism, are likely to play an important role in the ecology of the microbiota of the oral cavity. These phenomena may govern the population shifts observed during the development of periodontal disease. As an example of antagonism, the production of antagonistic substances may be important in the interaction between indigenous and non-indigenous organisms (26,27). Several species of bacteria inhabiting the oral cavity produce antimicrobial compounds, which may be non-protein metabolites, such as organic acids (28,29) and hydrogen peroxide (30), or proteinaceous compounds, such as bacteriocins (31).

The observed inhibition of the growth of *P. gingivalis* by *S. mutans* could be related to the ability of *S. mutans* to produce large amounts of acids, which consequently decrease the pH (32). Bacteria generally have a relatively narrow pH range for growth that may influence their intraoral distribution (33,34). Experimental studies have shown that a sudden drop in pH can shift the balance of oral microbial communities markedly, with periodontopathic bacteria growing poorly and acidophilic bacteria, such as *Lactobacillus casei* and *S. mutans*, predominating (35). Moreover, *P. gingivalis* cannot be maintained stably at a pH below 6.5, and its growth is only reproducible over a pH range of 6.7–8.3. This change in the local environmental pH caused by *S. mutans* could explain the negative correlation between *P. gingivalis* and *S. mutans*.

The results of this study showed that the number of *S. mutans*, in contrast to *P. gingivalis*, increases significantly after the initial periodontal treatment. This observation is in accordance with previous studies using culture methods (36,37). A precise mechanism of the shift from periodontal pathogens toward cariogenic species is not known. One might speculate that the changes in microbial composition after periodontal treatment resulted in more favorable growth conditions for *S. mutans*.

Based on the observations in this bacteriological pilot study, one can conclude that a negative correlation exists between periodontal disease and dental caries. Nevertheless, the following factors are also associated with increased caries risk: low salivary secretion rate, frequent intake of fermentable carbohydrates and low exposure to fluoride. Further study, with a much larger number of patients, is needed to reach more conclusive results.

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