



The Detection of *Porphyromonas gingivalis*, *Prevotella intermedia*, and *Actinobacillus actinomycetemcomitans* in the Supragingival Plaque of Children With and Without Caries

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Abstract

Purpose: The present study was undertaken to determine the presence of 3 periodontopathic bacteria in the supragingival plaques of 3- to 16-year-old children with different oral health conditions.

Methods: DMFT and dft, PMA index (P=papillary gingivitis, M=marginal gingivitis, and A=attached gingivitis), OHI (oral hygiene index), and oral malodor of each subject were determined prior to the collection of supragingival plaques. Periodontopathic bacteria (*P gingivalis*, *P intermedia*, and *A actinomycetemcomitans*) in supragingival plaques were detected using an immunoslot blot assay with monoclonal 3 periodontopathic bacteria in the 2 subject groups (children with and without caries). *P gingivalis*-positive subjects, but not their *P intermedia* or *A actinomycetemcomitans* counterparts, were correlated to oral malodor. Oral malodor was also correlated to debris index, a component of OHI.

Results: The group with the higher OHI showed a higher prevalence of periodontopathic bacteria. For the 3 periodontopathic bacteria in the subjects tested, *P gingivalis*-, *P intermedia*-, and *A actinomycetemcomitans*-positive plaques were not age related.

Conclusions: The supragingival plaques in children can harbor periodontopathic bacteria such as *P gingivalis*, *P intermedia*, and *A actinomycetemcomitans*. (*Pediatr Dent*. 2003;25:143-148)

KEYWORDS: PERIODONTOPATHIC BACTERIA, BACTERIA DISTRIBUTION, SUPRAGINGIVAL PLAQUE, ORAL MALODOR, IMMUNOSLOT BLOT ASSAY

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The authors recently reported a relation between the frequency of reactivity for *Porphyromonas gingivalis* and *Prevotella* species in supra- or subgingival plaques and periodontal clinical parameters according to subject age. This finding suggested that the supragingival plaques in 6- to 9-year-old children harbor *P gingivalis*.¹ There are few studies²⁻⁴ about periodontopathic bacteria in children. In contrast, the presence of *P gingivalis* and *Prevotella intermedia* is a well-known etiologic factor for chronic periodontitis. In addition, *Actinobacillus*

actinomycetemcomitans is implicated as a pathogen in early-onset periodontitis.

A relation between chronic periodontitis and periodontopathic bacteria in subgingival plaques has been reported.⁵ In addition, the finding of suspected periodontal pathogens in supragingival plaque samples was described by some investigators.⁶⁻⁸ However, to date few comprehensive studies of the microbial composition of supragingival plaque of children have been carried out. Further, there appears to be no evidence of a relation between the presence of

periodontopathic bacteria in supragingival and subgingival plaque on the same tooth surfaces. In a review of the literature, the majority of recent studies have focused primarily on the microbial composition of the subgingival plaque and not on supragingival plaque, suggesting a role for a number of subgingival microorganisms in initiation of periodontal infections.^{9,10}

Recently, Ximenez-Fyvie et al,⁸ reported that the microbial composition of supra- and subgingival plaques can harbor putative periodontal pathogens. This suggests a possible role for the environment as a reservoir of such species for the spread or reinfection of subgingival sites. The role of supragingival plaque in the initiation of gingivitis and/or periodontal diseases in children is unclear.

The purpose of the present study was to compare and detect the 3 periodontopathic bacteria in supragingival plaques of children with different oral health conditions.

Methods

Sixty children (37 boys and 23 girls between 3-16 years), with or without active caries (open caries), were used at the Meikai University Hospital in Saitama prefecture, Japan. Clinical parameters of tooth number in df and DMF and PMA index,¹¹ OHI,¹² and oral malodor of each subject were determined prior to the collection of supragingival plaques. In addition, the oral malodor for subjects was determined as the quantity of intraoral ammonia using Atein (Mitoleben Co, Japan).¹³ Exclusion criteria included antibiotic therapy in the previous 5 months and any systemic conditions which could influence the course of periodontal diseases or which would require premedication for monitoring procedures. Informed consent was obtained from each child's parents. Ethical clearance for the study was obtained from the local ethics committee.

Sample collection

Supragingival plaques from each subject were obtained using an excavator from the tooth surfaces of primary molars or permanent first molars with or without active caries. Thereafter, 1 loopful (Nunk Co, Copenhagen, Denmark) of the collected plaques was transferred to microtubes containing 500 µL of sodium carbonate buffer (pH 9.6). The collected plaque samples from each subject were pooled together at -80°C and then were thawed immediately before being analyzed by immunoslot blot assay. The plaque samples were dispersed by pulsed sonication (Sonifier cell disrupter; Branson Sonic Power Co, Danbury, Conn) for 10 seconds at 40 W. The dispersed samples (25µL) were used for the immunoslot blot assay, which was conducted as described below and described previously.¹⁴

Bacterial strains

P. gingivalis 381, *P. intermedia* ATCC 25611, and *A. actinomycetemcomitans* Y4 were used in this study.

Monoclonal antibodies

The following monoclonal antibodies specific for their respective bacteria were selected for use in this study^{14,15}: monoclonal antibody BGF7 for *P. gingivalis*; BIF6 antibody for *P. intermedia*; and AAY4 antibody for *A. actinomycetemcomitans*.

Immunoslot blot assay

This was performed using the procedure previously described.¹⁴ Briefly, supragingival plaque samples (25 µL) suspended in 500 µL sodium carbonate buffer or each sonicated bacterial extract (25 ng of protein) was blotted onto nitrocellulose paper (Bio-Rad, Tokyo, Japan) in each well of an immunoslot blot apparatus (Hybri-slot manifold, Bethesda Research Laboratories, Gaithersburg, Md). After blotting, the paper was treated for 30 minutes with 3% gelatin and washed with phosphate-buffered saline (PBS) containing 0.02% (vol/vol) Tween 20 (Tokyo Kasei Kogyo Co., Tokyo, Japan; PBS-Tween). After this wash, the paper was treated for 60 minutes with culture supernatants of monoclonal antibodies-producing cells or of control SP2/O- Ag 14 myeloma cells and then washed by shaking for 30 minutes with PBS-Tween.

Next, the treated paper was incubated for 60 minutes with a horseradish peroxidase-conjugated goat antimouse immunoglobulin G (Bio-Rad), washed, and visualized using horseradish peroxidase color development reagent (Bio-Rad). Densitometric analysis of the reactivities was performed with a graphic analyzer (Shoni GA, Showa Denkoh, Tokyo, Japan). The reactivity was evaluated as positive when the intensity of a test sample was more than that assigned to 1×10^6 cells of each periodontopathic bacterium, according to the authors' previous investigation¹⁴ related to the degree of colonization and the reactivity of monoclonal antibody specific for each periodontopathic bacterium. The results were expressed as the percentage of plaque samples that reacted with the species-specific monoclonal antibodies.

Statistical analysis

Clinical parameters were analyzed by the General Linear Models procedure and ANOVA for multiple comparisons. The prevalence of reactivity with the 3 monoclonal antibodies was analyzed using chi-square test software.

Results

The median values of each clinical parameter for subjects with and without active caries are shown in Table 1. As expected, a significant difference between children with and without active caries was observed in df tooth number ($P < .05$) and also in oral malodor ($P < .01$). However, there were no significant differences in other clinical parameters.

The distribution of *P. gingivalis*, *P. intermedia*, and *A. actinomycetemcomitans* in plaques of children with and without active caries are shown in Table 2. The prevalence of *P.*

gingivalis, *P intermedia*, and *A actinomycetemcomitans* in the subjects with caries was probably larger than in those without caries. However, the frequencies of plaques positive for the 3 periodontopathic bacteria in the 2 subject groups tested were different from each other. Overall, 13 of 60 children (22%) harbored *P gingivalis*, 10 (17%) harbored *P intermedia*, and 14 (23%) harbored *A actinomycetemcomitans*. Regardless of whether the subjects had caries, those with a higher debris index and a higher OHI showed a higher frequency of the 3 periodontopathic bacteria.

Table 3 summarizes the percent distribution of the 3 species in children by age. *P gingivalis* and *A actinomycetemcomitans* were detected at almost all ages. The minimum age of subjects positive for *P gingivalis* and *A actinomycetemcomitans* was 3 years, 5 months and 3 years, 11 months for *P intermedia*. The positive percent of the subjects (%) for *P gingivalis*, *P intermedia*, and *A actinomycetemcomitans* (Table 3) in the relation to the 3 to 6, 7 to 12, and 13 to 16 age groups, corresponding to the stage in dental development, is shown in Figure 1. No significant difference in each bacterium between age groups was found, possibly due to the small number of positive subjects in children. Interestingly, the 3 periodontopathic bacteria were found at an early age (3.6 years). *P gingivalis*-positive subjects were detectable at broader age ranges (3-16 years), whereas the *A actinomycetemcomitans*-positive counterparts were detectable at the age range of 3 to 12 years.

Next, the authors examined whether periodontopathic bacteria-positive subjects were related to oral malodor. The authors found that the median values of oral malodor for positive *P gingivalis*, *P intermedia*, and *A actinomycetemcomitans* expression was 32 (range=15-50), 25.5 (range=8-50), and 28 (range=20-68), respectively, and that of negative *P gingivalis*, *P intermedia*, and *A actinomycetemcomitans* expression was 27 (range=3-80), 28 (range=3-80), and 28 (range=3-80), respectively. There was a significant difference between the 2 groups for *P gingivalis* ($P<.05$), but not for *P intermedia* or *A actinomycetemcomitans*. This suggests that oral malodor may be associated with *P gingivalis* colonization. Plots of oral malodor vs the debris index from all subjects with and without caries are shown in Figure 2. As the debris index increased, oral malodor increased. This suggests that oral malodor was responsible for the debris index.

Figure 3 shows the relation of oral malodor between subjects with and without active caries. Oral malodor in children with active caries was significantly higher than in those without active caries.

Discussion

Okada et al,¹⁶ previously detected the presence of *A actinomycetemcomitans* and *P*

gingivalis using a polymerase chain reaction, in dental plaque samples taken with a toothbrush from children. They suggested that *A actinomycetemcomitans* and *P gingivalis* are rarely present in the oral cavity of healthy children. Lamell et al¹⁷ previously reported that *P gingivalis* and *A*

Table 1. Clinical Parameters (median value) in Children With and Without Active Caries

Clinical parameters	With caries (n=27)	Without caries (n=33)	Median test*
df tooth number	10.0 (range: 0-15)	5.0 (range: 0-10)	†
DMF tooth number	5.0 (range: 0-23)	2.0 (range: 0-14)	
PMA index	1.0 (range: 0-7)	1.0 (range: 0-8)	
Debris index	2.2 (range: 0-3.7)	2.1 (range: 0.3-3.3)	
Calculus index	0 (range: 0-0.2)	0 (range: 0-0.2)	
Oral hygiene index	2.2 (range: 0.7-3.7)	2.1 (range: 0.3-3.3)	
Oral malodor	28.0 (range: 12-55)	20.0 (range: 3-80)	‡

*Median test: with caries vs without caries.

† $P<.01$.

‡ $P<.05$

Table 2. Distribution of 3 Periodontopathic Bacteria in Children With and Without Active Caries

Periodontopathic bacteria	No. positive subject/sampled subjects (%)		
	With caries (n=27)	Without caries (n=33)	Total (n=60)
<i>Porphyromonas gingivalis</i>	7/27 (26)	6/33 (18)	13/60 (21)
<i>Prevotella intermedia</i>	5/27 (19)	5/33 (15)	10/60 (17)
<i>Actinobacillus actinomycetemcomitans</i>	8/27 (30)	6/33 (18)	14/60 (23)

Table 3. Percentage Distribution of Periodontopathic Bacteria in Children by Age

Periodontopathic bacteria	Age (y)													
	3	4	5	6	7	8	9	10	11	12	13	14	15	16
<i>Porphyromonas gingivalis</i>	33	25	10	40	0	20	11	0	0	100	43	29	0	0
<i>Prevotella intermedia</i>	66	25	20	20	0	0	11	0	0	100	0	29	0	0
<i>Actinobacillus actinomycetemcomitans</i>	33	25	30	20	0	0	33	50	100	100	14	14	0	0
No. of subjects	3	4	10	5	5	5	9	2	1	1	7	7	0	1

actinomycetemcomitans are common inhabitants of the oral cavity of child subjects of any age and usually colonize only transiently. In addition, they demonstrated that *P gingivalis* apparently becomes more stable in the late teenage years. The authors found in the present investigation that *P gingivalis*, *P intermedia*, and *A actinomycetemcomitans* were detected in the supragingival plaques of children at an early age and *P gingivalis* was detectable at a broader age range. It may be necessary to continue to monitor individuals who are positive for these periodontopathic bacteria since their risk of developing periodontal diseases may be increased in the future. A longitudinal investigation would be particularly important to monitor these bacteria during the transition from periodontal health to disease.

McClellan et al,¹⁸ previously reported that *P gingivalis* was detected in 37% of children and was seen at similar frequencies throughout infancy, childhood, and adolescence. Their findings suggest that *P gingivalis* may be acquired immediately upon exposure, possibly in the first days of life.

The significant relation of oral malodor between subjects with and without active caries (Figure 3) shows that oral malodor in children with active caries was significantly higher than in those without active caries. Subjects with periodontal diseases frequently suffer from oral malodor^{19,20} and positive correlations have been demonstrated between the severity of periodontitis and the levels of volatile sulfur compounds in mouth air.^{21,22} Ratcliff and Johnson²³ previously demonstrated a relationship between oral malodor and gingivitis or periodontitis and emphasized the potential importance of volatile sulfur compounds in the transition of periodontal tissues from clinical health to gingivitis and then to periodontitis. Recently, studies of oral malodor in children were reported.^{13,24,25} Yosida et al¹³ reported that children with noticeable halitosis and without halitosis had high average concentrations of ammonia in mouth air. However, more studies and clinical approaches concerning halitosis in children are needed.

Experimental evidence strongly suggests that putrefaction of sulfur-containing proteinaceous substrates by predominantly Gram-negative oral microorganisms, such as *Fusobacterium* species and *Bacteroides* species, is a primary cause of oral malodor.^{26,27} Faryavi-Gholami et al,²⁵ previously reported on oral malodor in children and volatile sulfur compound-producing bacteria in saliva. They demonstrated that

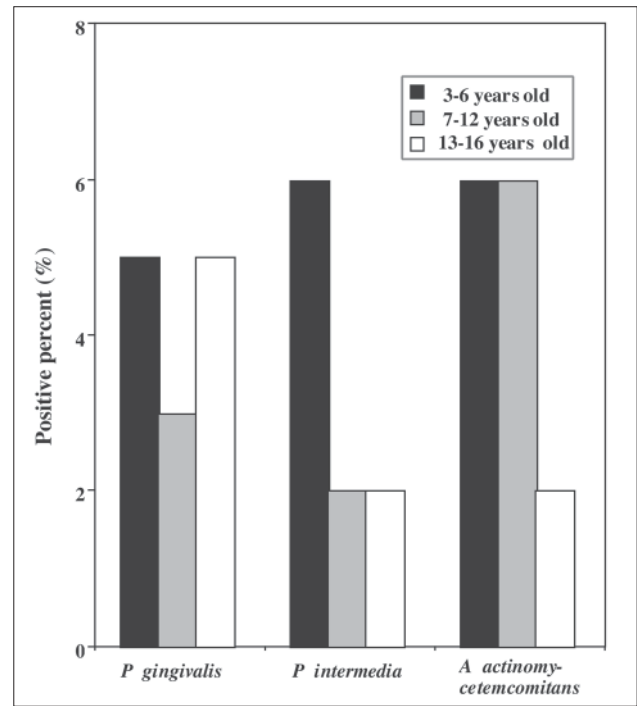


Figure 1. Prevalence (positive %) of periodontopathic bacteria for each age group. No significant difference in periodontopathic bacteria between the age groups was observed.

children with parent-perceived oral malodor exhibited significantly higher concentrations of odorigenic bacteria in saliva than in those without parent-perceived malodor. They also showed that the levels of *Prevotella oralis* were significantly higher in children with oral malodor than those without oral malodor. The present findings suggested that *P gingivalis*-positive subjects were associated with oral malodor.

In the present study, periodontopathic bacteria positive subjects were also correlated to the debris index. This clinically suggests that tooth-brushing plays a crucial role

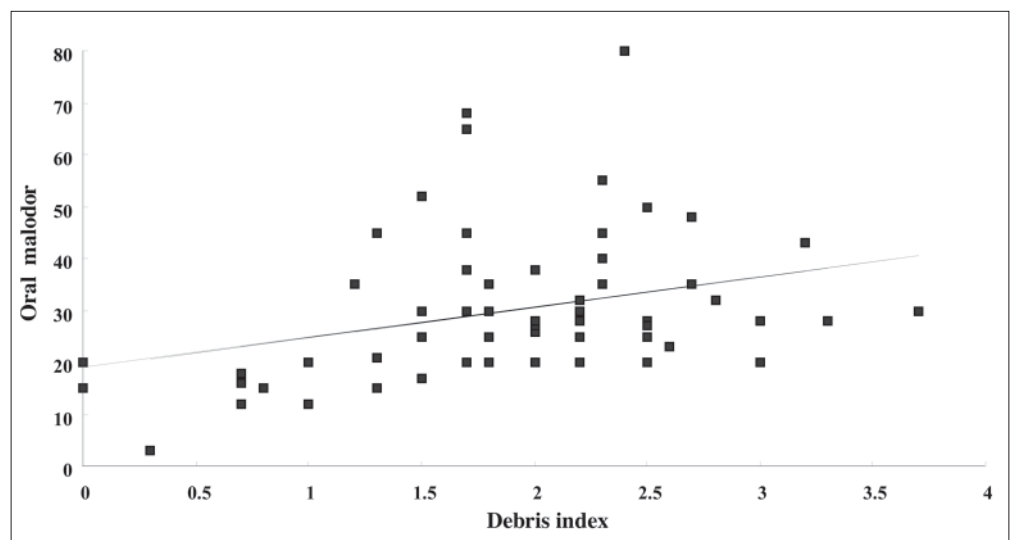


Figure 2. Comparison of oral malodor and debris index from 60 subjects. The regression line for oral malodor vs debris index is shown ($r=0.324$, $P<.05$).

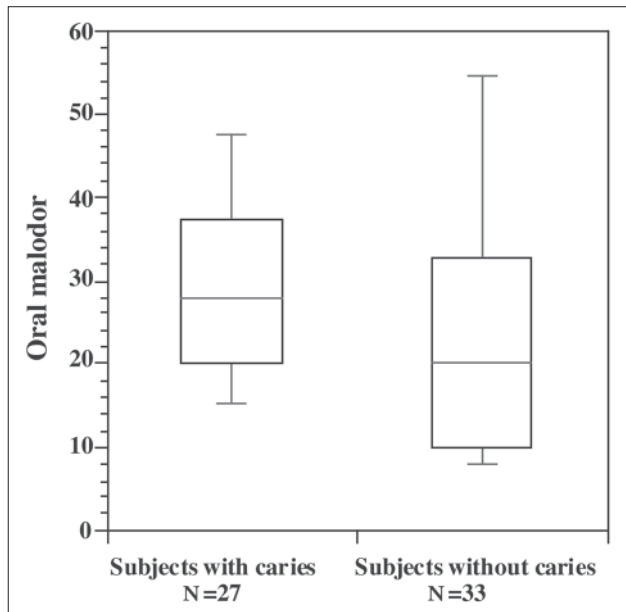


Figure 3. The distribution of oral malodor in relation to subjects with caries or without caries. The oral malodor for group A (subjects with caries) and group B (subjects without caries) were significantly different ($P < .05$). Values of oral malodor were determined as the quantity of intraoral ammonia 5 minutes after gargling with 20 mL of 1% urea solution, using Atein. The horizontal line in the box corresponds to the median. The top and bottom of the box are 75 and 25 percentiles, respectively. Vertical lines correspond to the range from 10 (bottom line) to 90 (top line) percentiles.

in preventing caries and periodontitis as well as gingivitis in children.

Conclusions

1. *P. gingivalis*, *P. intermedia*, and *A. actinomycetemcomitans* were detected in the supragingival plaques of children with and without active caries.
2. The supragingival plaques must be capable of harboring these periodontopathic bacteria.
3. Oral malodor in subjects with active caries was significantly higher than that without active caries. In addition, oral malodor in *P. gingivalis*-positive subjects was significantly higher than that of negative subjects.
4. Oral malodor was significantly correlated to the debris index, a component of OHI.

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ABSTRACT OF THE SCIENTIFIC LITERATURE



UPRIGHTING IMPACTED SECOND MOLARS

Nonsurgical management of impacted second molars can be challenging, as access for regular bonding techniques may be minimal. This article presents cases and describes modification of a preformed transpalatal arch (TPA) as an effective treatment option that affords the requisite anchorage. A custom-modified TPA is inserted in the lingual sheath of the abutting first molar, with the coffin/omega loop extending distally to the retromolar pad and occlusally to the impacted tooth. A button is bonded as mesially as clinically possible on the offending tooth and a power chain is attached to the loop at the distal marginal ridge region, with force vectors directing the tooth occlusally and distally. The first case demonstrates third molar removal, partial appliance therapy with sequential button bonding, and tube placement on the impacted tooth of 4 months duration. The second case presents a younger patient with completed uprighting in 2 months. Unfavorable impactions may be best treated with surgical techniques, while several orthodontic techniques are riddled with undesirable side effects and financial and time expenses. Advantages of the illustrated method include ease of technique, short treatment time, minimized clinical and laboratory work, and minor side effects.

Comments: Although multiple practitioners may have designed such an appliance, it is fitting that the technique is presented in the literature. The pictorial contribution of the authors is helpful. **AW**

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