〈Original Article〉

Distribution of periodontal pathogens and lifestyle habits of Japanese mothers and their children

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Summary We analyzed the relationship between the distribution of periodontal pathogens and the lifestyles of Japanese mothers and their children. Dental plaque samples were collected from 93 mother-and-child pairs using a sterile toothbrush. Bacterial DNA was extracted from each specimen, and the presence of 8 bacterial species (*Porphyromonas gingivalis* (*P. gingivalis*), *Treponema denticola* (*T. denticola*), *Tannerella forsythia* (*T. forsythia*), *Prevotella intermedia* (*P. intermedia*), *Prevotella nigrescens* (*P. nigrescens*), *Fusobacterium nucleatum* (*F. nucleatum*), *Aggretibacter actinomycetemcomitans* (*A. actinomycetemcomitans*), and *Campylobacter rectus* (*C. rectus*)) was assessed using the nested polymerase chain reaction method. Furthermore, we investigated the participants' lifestyles, such as the presence of smokers in the home, tooth-brushing habits, and the sharing of food or tableware between the mothers and their children. The detection rates of *P. gingivalis, T. denticola*, *T. forsythia*, *P. intermedia*, *P. nigrescens F. nucleatum*, *A. actinomycetemcomitans*, and *C. rectus* in the children were 10.8%, 5.4%, 88.2%, 10.8%, 51.6%, 100%, 0%, and 100%, respectively, and those in the mothers were 40.9%, 72.0%, 95.7%, 43.0%, 95.7%, 100%, 3.2%, and 100%, respectively. In the children whose mothers were positive for each bacterium, the rates were 100% for *F. nucleatum* and *C. rectus*, 15.8% for *P. gingivalis*, 6.0% for *T. denticola*, 91.0% for *T. forsythia*, 51.7% for *P. nigrescens*, 12.5% for *P. intermedia*, and 0% for *A. actinomycetemcomitans*. The percentage of families who had smokers in the home was 44.1%, and the smokers were mostly the fathers. In addition, 19.5% of the fathers who smoked did so near

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their children. The number of times that tooth brushing was performed per day was 2.1 ± 0.6 times for mothers and 1.7 ± 0.7 times for their children, and there was a significant correlation between the mother's tooth-brushing frequency and their child's tooth-brushing frequency ($r = 0.379$, $p < 0.01$). The rates of the mother sharing tableware or food with their children were 62% and 69% respectively, and there was a significant correlation between the mothers who shared tableware and those who shared food with their children ($p = 0.579$, $p < 0.01$). However, there was no correlation between the distribution of periodontal pathogens and the absence of smokers in the home, tooth-brushing habits, or sharing food or tableware with their mothers. Our results suggest that the presence of *F. nucleatum*, *C. rectus* and *T. forsythia* in children is closely associated with their presence in the children's mothers. Regarding the route of infection of periodontal pathogens, a future longitudinal study is required because some periodontal pathogens were suggested be transient organisms in a previous study.

Key words: Periodontal pathogens, Mothers and their children, Lifestyles

1. Introduction

Periodontal disease is a chronic inflammatory disease of the gums and the nearby tissues of the mouth. Infection is accompanied by periodontal pathogens and chronic poor oral hygiene; bacterial biofilm, i.e., dental plaque, is formed when the number of periodontal pathogens increases. As a result, inflammation develops in the periodontal tissue. There have been many studies on the risk factors of periodontal disease.[1,2] Smoking is one of the major risk factors for periodontitis.[3] Smoking may suppress the host-defense system, which may promote periodontal disease progression.[4] Furthermore, active and passive smoking may have harmful effects on periodontal health in men.[5] Passive smoking has been associated with a number of negative health outcomes in children; it increases the levels of cotinine, a major metabolite of nicotine, in children's saliva concomitant with a lowered clinical attachment level, which is defined as the distance from the cement-enamel junction.[6] Periodontal disease not only causes the loss of teeth but is also related to systemic diseases such as circulatory disease and diabetes.[7-10] Therefore, both the prevention and treatment of periodontal disease are important. The deterioration of periodontal tissue is rarely observed in childhood as periodontal disease mostly develops in adults. In recent years, however, it has been reported that periodontal pathogens are also detected in children without clinical signs of periodontal disease, which suggests the transmission of periodontal pathogens from parent to child.[11-14] In the case of dental caries as an indication of the presence of oral disease, it has been reported that *Streptcoccus mutans* (*S. mutans*), which is a primary pathogen that causes dental caries, propagates to the child via the mother's saliva.[15,16] It was also reported that the frequency of infant infection was significantly greater when maternal salivary levels of *S. mutans* exceed 10^5 colony-forming units/ml.[17] On the contrary, how and when periodontal pathogenic bacteria colonize the oral cavity in childhood remains unknown. Thus, further analysis is required to determine the route of infection of these pathogens.

In general, fewer than 10-15 bacterial species are recognized as putative periodontal pathogens, and bacteria known as the red complex, namely *Porphyromonas gingivalis* (*P. gingivalis*), *Treponema denticola* (*T. denticola*), and *Tannerella forsythia* (*T. forsythia*), are the major causative agents of periodontitis and are closely associated with its severity.[18] Previous studies of patients with chronic periodontitis revealed that the red complex

bacterial species are routinely found together at diseased sites in the human mouth.[19,20] *Campylobacter rectus* (*C. rectus*), *Prevotella intermedia* (*P. intermedia*), and *Prevotella nigrescens* (*P. nigrescens*) are also related to periodontal breakdown as the secondary group of periodontal pathogens.[21,22] *Aggretibacter actinomycetemcomitans* (*A. actinomycetemcomitans*) has been reported to be linked to aggressive periodontitis.[23] *Fusobacterium nucleatum* (*F. nucleatum*) in dental plaque plays a significant role in biofilm formation by co-aggregation with other periodontal pathogens.[13]

The purpose of this study was to clarify the infection route of periodontal pathogens in children. In this study, we investigated the distribution of the red complex species and these 5 periodontal pathogens, the frequency of brushing, the presence of domestic smokers, and the sharing of tableware and food. Furthermore, we examined the relationships among these factors and the distribution of periodontal pathogens.

2. Materials and methods

2.1. Participants

Ninety-three children (3.2 ± 1.4 years old) and their mothers (35.1 ± 4.8 years old) participated in the present study. The subjects were selected from those who took part in the events held at the Child Research Center in Higashiosaka College. All children were the first child of their mothers. There were no participants who were undergoing dental treatment. After giving verbal and written explanations about the purpose and content of the study, the participants who agreed to attend the study provided written informed consent. We conducted this study after approval by the ethical review board of the Graduate School of Comprehensive Rehabilitation of Osaka Prefecture University (approval number: 2016-309).

2.2. Plaque sampling and genomic DNA extraction

Dental plaque was collected from the erupted teeth with a sterile toothbrush for 1 min. The plaque adhering to the brush was removed by washing it several times with 5 mL of sterile distilled water in a 15-mL test tube. The plaque was collected by centrifugation at 1,600 × g for 20 min; the supernatant was discarded and the resultant pellets were stored at -20°C until DNA extraction.[24] The genomic DNA of each sample was extracted using a Wizard Genome DNA Purification Kit (Promega, Madison, Wisconsin, USA), and the samples were stored at −20°C until use.

2.3. Detection of periodontal pathogens

The polymerase chain reaction (PCR) primers for detecting the bacterial species used in this study are listed in Table 1. PCR was initially performed using broad-range eubacterial primers based on the bacterial 16S ribosomal-RNA gene.[25] All primers were purchased from Invitrogen, Japan. The PCR-reaction mixture included 0.25 U KOD FX Neo polymerase (TOYOBO, Osaka, Japan), 0.2 mmol/L deoxynucleotide triphosphates (dNTPs), polymerase buffer, 1 mmol/L primers, and 30 ng of DNA solution from the plaque as the template DNA in a total volume of 25 μL. The samples were preheated at 95°C for 2 minutes followed by 25 cycles of amplification under the following conditions: denaturation at 98°C for 10 s, annealing at 55°C for 30 s, and elongation at 68°C for 1 min using a T100 thermal cycler (Bio-Rad, Hercules, California, USA). The 25 cycles were followed by elongation at 68°C for 5 minutes. The PCR products were confirmed by 1% agarose gel electrophoresis and then purified using the Gel/PCR™ DNA Isolation System (Viogene, Taipei, Taiwan) in a unique final solution. A second nested PCR was performed with specific primers for periodontal pathogens designed on the basis of the 16S ribosomal-RNA gene (Table 1), as reported previously.[26] The PCR reaction mixture included 0.25 U KOD FX Neo polymerase, 0.2 mmol/L dNTPs, polymerase buffer, 1 mmol/L primers, and 2 μL of purified first PCR products as the template DNA in a total volume of 25 μL. The PCR conditions were the same as previously described for the broad-range eubacterial primers except for the elongation time, which was 30 s. Subsequently, 5 μL of the obtained PCR products was mixed with 1 μL of Ez-Vision One

Table 1 Primer sets for detection of specfic periodontal pathogens.

Primer pairs	Amplified size (bp)
Eubacterial	
5' – GAG TTT GAT CCT GGC TCA G –3'	
5' – AGA AAG GAG GTG ATC CAG CC –3'	～ 1,500
P. gingivalis	
5' – AGG CAG CTT GCC ATA CTG CG –3'	
5' – ACT GTT AGC AAC TAC CGA TGT –3'	404
T. forsythia	
5' – GCG TAT GTA ACC TGC CCG CA –3'	
5' – TGC TTC AGT GTC AGT TAT ACC T –3'	641
T. denticola	
5' – TAA TAC CGA ATG TGC TCA TTT ACA T –3'	
5' – TCA AAG AAG CAT TCC CTC TTC TTC TTA –3'	316
P. intermedia	
5' – TTT GTT GGG GAG TAA AGC GGG –3'	
5' – TCA ACA TCT CTG TAT CCT GCG T –3'	575
P. nigrescens	
5' – ATG AAA CAA AGG TTT TCC GGT AAG –3'	
5' – CCC ACG TCT CTG TGG GCT GCG A –3'	804
C. rectus	
5' – TTT CGG AGC GTA AAC TCC TTT TC –3'	
5' – TTT CTG CAA GCA GAC ACT CTT –3'	598
F. nucleatum	
5' – TAA AGC GCG TCT AGG TGG TT –3'	
5' – ACG GCT TTG CAA CTC TCT GT –3'	697
A. actinomycetemcomitans	
5' – AAA CCC ATC TCT GAG TTC TTC TTC –3'	
5' – ATG CCA ACT TGA CGT TAA AT –3'	557

(AMRESCO, Framingham, Massachusetts, USA) and electrophoresed through 2% agarose gel. The pathogen-specific bands were visualized under a UV light transilluminator.

2.4. Questionnaire about lifestyle habits

Using a questionnaire, we investigated the tooth-brushing habits of the children and their mothers, the presence of smokers in the home, the presence of smokers who smoked in the vicinity of the child in the houses where a smoker was present, and the sharing of food or tableware between the children and their mothers. The questionnaire was filled out by the mother.

2.5. Statistical analysis

The significance of association between the distribution of the periodontal pathogens in mothers and their children was assessed using Fisher's exact probability test. The relationship between the number of tooth brushings per day and the number of different kinds of periodontal pathogens that were detected was examined by Pearson's correlation analysis. The relationship between the sharing of tableware and the sharing of food was examined by Spearman's rank correlation coefficient. The

Mann-Whitney's *U* test was conducted to compare the presence of food-sharing or tableware-sharing behaviors, the presence of smokers in the home, and the number of different kinds of periodontal pathogens that were detected. Significance was set at 5%. Statistical analyses were performed using statistical analysis software SPSS Ver. 19.0 (IBM Japan, Tokyo, Japan).

3. Results

3.1. Detection of periodontal pathogens by nested PCR

First, we examined the distribution of the 8 periodontal pathogens in the mothers and their children (Fig. 1). The detection rates of *P. gingivalis*, *T. denticola*, *T. forsythia*, *P. intermedia*, *P. nigrescens*, *F. nucleatum*, *A. actinomycetemcomitans* and *C. rectus* in the children were 10.8%, 5.4%, 88.2%, 10.8%, 51.6%, 100%, 0%, and 100%, respectively; and those in the mothers were 40.9%, 72.0%, 95.7%, 43.0%, 95.7%, 100%, 3.2%, and 100%, respectively.

Next, we measured the rates of infection-positive mothers and children for the periodontal pathogens (Table 2). The rate of positive children with positive mothers was 100% for *F. nucleatum* and *C. rectus*, and both species were detected in all of the mothers and their children. The percentage of positive children with positive mothers was 91.0% for *T. forsythia*, 15.8% for *P. gingivalis*, 6.0% for *T. denticola*, 51.7% for *P. nigrescens*, and 12.5% for *P. intermedia*. There was a significant association between the distribution of *T. forsythia* among mothers and their children ($p<0.05$).

Furthermore, the number of the 8 species of periodontal pathogens detected was 5.5 ± 1.1 species in the mothers and 3.7 ± 0.8 species in the children. The detection rate for at least one of the three red complex species in mothers and their children was 100%, and 91.4%, respectively. The number of bacterial species from the red complex was 2.1 ± 0.7 species in mothers and 1.0 ± 0.5 species in their children.

3.2. Questionnaire survey

We performed a questionnaire survey on the lifestyles of the mothers and their children. The mean tooth-brushing frequency per day for the mothers was 2.1 ± 0.6 times and that for the children was 1.7 ± 0.7 times. There was a significant correlation between the number of tooth brushings that the mothers performed and the number of tooth brushings that their children performed ($r = 0.379$, $p < 0.01$). Tableware sharing with children was noted for 62% of mothers and food sharing with children was noted for 69% of mothers. There was a

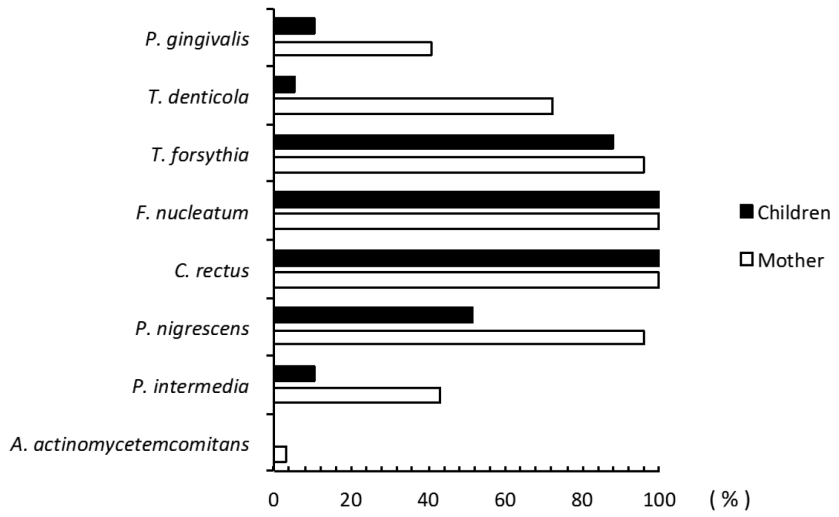

Fig. 1 Distribution of periodontal pathogens in mothers and their children.
Mothers (n = 93), children (n = 93).

Table 2 The distribution of periodontal pathogens in mothers and their children.

		Child (+)	Child (−)	Detection rate of Child (+) in Mother (+) (%)	Fisher's exact probability test
P. gingivalis	Mother (+)	6	32	15.8	p = 0.307
	Mother (−)	4	51		
T. denticola	Mother (+)	4	63	6.0	p = 1.000
	Mother (−)	1	25		
T. forsythia	Mother (+)	81	8	91.0	p = 0.005
	Mother (−)	1	3		
A. actinomycetemcomitans	Mother (+)	0	3	−	NA
	Mother (−)	0	90		
P. intermedia	Mother (+)	5	35	12.5	p = 0.740
	Mother (−)	5	48		
P. nigrescens	Mother (+)	46	43	51.7	p = 1.000
	Mother (−)	2	2		
C. rectus	Mother (+)	93	0	100	NA
	Mother (−)	0	0		
F. nucleatum	Mother (+)	93	0	100	NA
	Mother (−)	0	0		

(+): positive, (−): negative, NA: not available.

Mothers (n = 93), children (n = 93).

significant correlation between tableware-sharing and food-sharing behaviors with children (r = 0.579, $p < 0.01$). Regarding the presence of smokers in the home, "Yes" was answered in 44.1% (51 persons) of the questionnaires and "None" was answered in 55.9% (42 persons). Fifty of the smokers in the present families were the fathers, and the father's smoking rate was 53.8%. In addition, 19.5% of respondents consisted of families where the smoking parent smoked near the child.

3.3. Relationship between the distribution of periodontal pathogens and the results of the questionnaire survey

There was no correlation between the frequency of tooth brushing and the distribution of the number of bacterial species. There was a significant correlation between tableware-sharing and food-sharing behaviors with children (r = 0.579, $p < 0.01$). To investigate the relationships among the number of bacterial species from the red complex, the number of the 8 bacterial species, and food- or tableware-sharing behaviors between parents and their children, the mother group was divided into 2 groups. The results are shown in Table 3. There was no significant difference in the number of bacterial species from the red complex or the presence of food or tableware sharing. We also analyzed the relationship between the number of bacterial species from the red complex, the number of the 8 bacterial species, and the presence of smokers in the home. The presence of smokers in the home was divided into 2 groups and analyzed regarding the number of bacterial species from the red complex and the number of the 8 bacterial species (Table 4). When smokers were in the home, the number of bacterial species from the red complex and the number of the 8 bacterial species in the mothers was significantly higher than those for the mothers without smokers in the home, but there was no significant difference in the children.

4. Discussion

In this study, we investigated the distribution of

Table 3 Comparison of the number of bacteria species in children according to presence of sharing tableware and food with mother.

	Sharing tableware		Sharing food	
	Yes (n = 58)	No (n = 35)	Yes (n = 64)	No (n = 29)
Red complex species	1.1 ± 0.4	0.9 ± 0.5	1.0 ± 0.4	1.1 ± 0.6
Eight species of bacteria	3.7 ± 0.8	3.5 ± 0.8	3.7 ± 0.8	3.6 ± 0.8

Data are means ± SD.

Table 4 Comparison with the number of species by the presence of smokers in the family.

		Smoker in the family	
		Yes (n = 41)	No (n = 52)
Mothers	Red complex species	2.3 ± 0.7	1.9 ± 0.7*
	Eight species of bacteria	5.8 ± 1.1	5.2 ± 1.0*
Children	Red complex species	1.1 ± 0.5	0.9 ± 0.4
	Eight species of bacteria	3.7 ± 0.7	3.6 ± 0.9

Data are means ± SD.

* Significant difference from families with family members who smoke ($p < 0.05$).

8 periodontal pathogens in 93 mother-child pairs. We also conducted a questionnaire survey on the number of tooth brushings per day, the presence of smokers in the home, and food and tableware sharing between mothers and children. We further examined the relationship between their lifestyles and the distribution of periodontal pathogens.

Among the 8 periodontal pathogens, both *C. rectus* and *F. nucleatum* were detected in all mothers and their children. *C. rectus* has been reported to be detected more frequently in childhood.[14,27-29] Ashimoto *et al.*[26] suggested that *C. rectus* is an endogenous pathogen that occasionally leads to the development of periodontitis. *F. nucleatum* is also detected more frequently in childhood.[30] The presence of *F. nucleatum* in dental plaque plays a significant role in biofilm formation due to its co-aggregation with other periodontal pathogens.[31] It was suggested that *F. nucleatum* is present in the oral cavity from an early stage, as it was detected in the oral cavities of all the children and mothers in this study.

The highest detection rate of the red complex species was for *T. forsythia*. *T. forsythia* was detected from the samples of 95.7% of the mothers and 88.2% of the children. In addition, the rate of positive children with positive mothers was 91.0%. Kimura *et al.*[32] found that *T. forsythia* was the most frequently detected species in the red complex in Japanese mothers and their children. Umeda *et al.*[14] found that *T. forsythia* was detected more frequently in the oral cavities of Japanese children whose parents already harbored the bacteria. The result of our study was similar to these reports.[14,32]

The detection rates of another two species of the red complex (*P. gingivalis* and *T. denticola*) in children were 10.8% and 5.4%, respectively. According to Kimura *et al.*[32], the detection rate of *P. gingivalis* and *T. denticola* in Japanese children whose mean age was 4.6 years was 13.1% and 0%, respectively. Okada *et al.*[27] reported detection rates of *P. gingivalis* and *T. denticola* in children with

healthy gingiva of 4.8% and 0%, respectively. In accordance with these reports,[27,32] *P. gingivalis* and *T. denticola* are rarely the cause of cavity infections that occur in early childhood, in particular, in children with healthy gingiva. The detection rates of *P. nigrescens* and *P. intermedia* in children were 51.6% and 10.8%, respectively. Kobayashi *et al*.[13] reported detection rates of *P. nigrescens* and *P. intermedi* a in Japanese children of 38.5% and 19.2%, respectively. The result of our study was similar to these results.[13] However, Ooshima *et al*.[29] found that *P. gingivalis*, *P. intermedi* a, and *T. denticola* were likely transient organisms in their longitudinal study. Therefore, a future longitudinal study is required to establish the chronology of the infection and the probable direction of pathogen transfer.

In this study, we also analyzed the relationship between periodontal disease and the participants' lifestyles. Smoking is a major risk factor for periodontal disease.[3] The percentage of homes with smokers in the present study was 44.1%, and the smokers were mostly the fathers. The fathers' smoking rate was 53.8%. Currently, the percentage of fathers who are habitual smokers in Japan is approximately 29.4% for men.[33] Compared with the national average[33], the fathers of the children in this study had higher smoking rates. Smokers have more severe periodontal disease than non-smokers.[34,35] Ueno *et al*.[5] found that passive smoking may have harmful effects on periodontal health in men. In this study, when there were smokers in the home, the number of bacterial species that were detected in the mothers was larger than the number of species detected in the mothers without smokers in the home. Furthermore, the detection rates of the red complex species in the mothers were higher than those in the previous studies.[13,32] Therefore, the mothers living with smokers may have been infected by them. Although there was no significant correlation between smoker presence in the home and the number of bacterial species detected in children, passive smoking may negatively influence periodontal attachment in children.[6] Thus, it is important to convey to parents of children exposed to passive smoking that there is the possibility of a negative impact on the teeth of their children.

Saliva contains microorganisms, particularly bacteria, originating mainly from exfoliation from oral surfaces. Yahfoufi *et al*.[36] found that periodontal pathogenic infection was higher in families using the same plate for eating. Wan *et al*.[37] found that contact with an adult's saliva, such as the sharing of food and tableware with other individuals or having their food pretested, was a possible method of transmission of *S. mutans* from mother to child. In this study, there was no significant correlation between the distribution of periodontal pathogens and food or tableware sharing. This is likely because eating habits in Japan are not the same as those in other countries. In addition, *S. mutans* is a facultative anaerobic bacterium, but most of the periodontal bacteria are anaerobic.[38] Therefore, further investigation is needed to clarify whether the sharing of food or tableware increases the possibility of infection with periodontal pathogens.

On the other hand, the number of tooth brushings that the mothers and children performed was significantly correlated. In a previous study,[39] after 9-12 days without undergoing oral hygiene, 11 subjects with previously excellent oral hygiene and healthy gingiva exhibited marked accumulation of plaque and generalized mild gingivitis. Furthermore, there was a significant correlation between tableware-sharing and food-sharing behaviors. The present study suggests that education is needed for mothers to make them conscious of these issues (e.g., oral care is important for the prevention of periodontal disease, the cause of which is bacterial infection).

In some cases, the mother was negative for periodontal pathogens but her child was positive. This suggested that the transmission route in the child was not the mother. For example, the child of a heavy smoker had a similar infection profile of periodontal pathogens to their grandmother (unpublished data). Taken together, the transmission among family members other than the mother and in the children's community should be investigated in order to clarify the infection route of periodontal pathogens. In addition, a future longitudinal study is

essential to examine periodontal disease bacterial infections, including families and children's communities, from early childhood.

One of the limitations of this study was that the mothers' subjectivity in answering the questions may have influenced the results of the questionnaire. In addition, it was difficult to examine the subject's periodontal status from a clinical point of view because we did not perform the dental clinical examinations. Thus, clinical information will need to be collected, and further analysis should be performed in the future.

Conflicts of interest

The authors have no conflicts of interest.

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