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Presence of *Streptococcus mutans* or *Streptococcus sobrinus* in Cariostat®-inoculated plaque samples from Japanese mother-child pairs

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Abstract The aim of this study was to determine the presence of *Streptococcus mutans* or *Streptococcus sobrinus* in Cariostat-inoculated plaque samples obtained from Japanese mother-child pairs through a conventional PCR technique and to establish the presence of these bacteria and caries risk. Oral examination and caries risk assessment using the Cariostat® were carried out on 168 children, aged 6–31 months, and their mothers. The presence of *S. mutans* and *S. sobrinus* in Cariostat-inoculated plaque samples was checked through PCR and tested for relevance with caries risk. A significant correlation ($P<0.001$) was found between caries risk of mothers and presence of *S. mutans* or *S. sobrinus* in plaque samples from their children in the 19–31-month-old age range. However, no significant relationship found between the presence of either strain in the plaque of younger children (6–18 months) and caries risk of mothers. Likewise, high caries risk was seen in 49.1% of the 19–31-month-old children of high-risk mothers ($P<0.001$) and 27% of the 6–18-month-old children of high-risk mothers ($P<0.05$). The effectiveness of the Cariostat method for prediction of caries risk can be improved by detecting the presence of *S. mutans* and *S. sobrinus* in plaque samples obtained from mothers and their children through conventional PCR techniques.

Key words
Caries risk,
Cariostat,
PCR,
S. mutans,
S. sobrinus

Introduction

Dental caries is an infectious and transmissible disease. It has affected human populations since the prehistoric era and various cultures at different times have exhibited wide variation in their susceptibility to dental caries[1]. Systemic, local, physical, behavioral, and socio-economic factors have been implicated as indicators in caries susceptibility. One area of great concern is the early acquisition and transmission of cariogenic bacteria from mother to child. Mutans streptococci species are thought to have the strongest association with dental caries. Within this group, *Streptococcus mutans* and *Streptococcus sobrinus* are the two strains most commonly isolated from carious teeth. Transmission of *S. mutans* is believed to primarily occur vertically along the mother-child infection route, and a discrete window of infectivity has been found to occur around the age of 2 years[2,3] or earlier[4–7]. In recent studies however, the presence of *S. mutans* has been found in pre-dentate infants[8–10]. In a study by Tanner[11], the presence of *S. mutans* was detected in 70% of samples scraped from the tongues of 57 children aged 6–18 months living in Saipan. It is believed that initial acquisition of *S. mutans* at an early age is associated with caries development at a later age[12–14]. Thus, it is important to establish caries risk assessment early in life. The Cariostat[15] method is a colorimetric caries risk

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assessment test based on color changes produced by acidogenic and aciduric oral bacteria present in a plaque sample. Although analysis is based on visual reading, many bacteriological, behavioral, and epidemiological studies have proved that this method of assessment is a good predictor of caries risk, especially in young children. Recent advances in the field of molecular biology have had a great impact on research concerning oral health. Methods used for strain identification have become faster, more effective and more accurate. Primer pairs for the purpose of specifically identifying *Streptococcus mutans* and *Streptococcus sobrinus* have been developed by Igarashi *et al.*[16–17] These two primer pairs (SD10/SD20 and SOF14/SO1623) were designed on the basis of nucleotide sequence homologies of dextranase genes of specific strains within the mutans streptococcal group. Both pairs can amplify species-specific amplicons with different lengths under the same PCR conditions. This has made strain identification faster and more effective compared with conventional identification techniques such as colony morphology, biochemical and immunological analyses[18–20], which require considerable time and professional skill. With this development, we decided to conduct this study using the recommended molecular biology protocols for mutans streptococcal strain identification in conjunction with the Cariostat® test. Therefore, instead of disposing Cariostat-inoculated plaque samples after incubation, we decided to further analyze the samples to enhance our diagnosis.

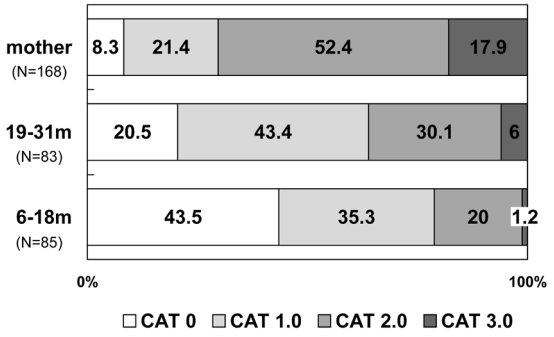

Fig. 1 Cariostat score distribution for mothers and their children

Materials and methods

Participants

The participants in this study were 168 mother-child pairs attending a scheduled medical and dental check-up at the Satosho Town Health Center in Okayama City. The ages of the children ranged from 6 to 31 months. The children were grouped by age range: a 6–18-month-old group (n=85) and a 19–31-month-old group (n=83). All children had at least two teeth present during the examination. The mothers' average age was 28 years. All mothers or guardians gave informed consent for the sampling procedure.

Sampling and experimental procedures

Oral examination was done using a mouth mirror under sufficient lighting for taking notes on oral conditions. One dentist was assigned to do caries risk assessment using the Cariostat method by taking plaque samples from the mothers and their children using a sterile cotton swab that was inoculated into the Cariostat medium and incubated at 37°C for 48 hours. After incubation, color changes were noted, scores were assigned and preparation was done immediately for DNA extraction. One clinician was responsible for scoring by separating samples obtained from the children from those obtained from the mothers. Scoring was initially done using the 7-scale Cariostat scoring system which was later simplified using the original 4-scale system. Scores of 0 were scored as 0, while 0.5 and 1.0 as 1.0, 1.5 and 2.0 as 2.0, and 2.5 and 3.0 were scored as 3.0. The ampoules containing the samples were agitated intermittently using a vortex machine (Ecan Tube Mixer M-2000) for about 10 seconds to dislodge bacterial particles attached to the cotton swab. Then 1 m*l* of the sample solution was transferred into a 1.5 m*l* mini centrifuge tube. A bacterial pellet was prepared by centrifugation at 4,700 × g (7,500 rpm) for 15 minutes, after which careful extraction of the supernatant was done without disturbing the bacterial pellet. Bacterial DNA extraction was done following Qiagen's protocol for purification of DNA from Gram-positive bacteria.

PCR

Oligonucleotide primers were used to amplify species-specific amplicons (1270 bp and 1610 bp) from fragments on the dextranase DNA sequence of both *S. mutans* and *S. sobrinus*, respectively[16,17]. PCR was performed with a 20μl reaction mixture with a designated minimum (2.0μl) and maximum (10.0μl) sample DNA template quantity and was repeated three times for verification. Briefly, the

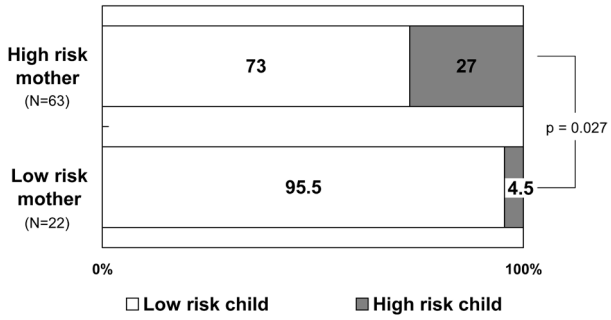

Fig. 2 Caries risk for mothers and their children (6–18 months)

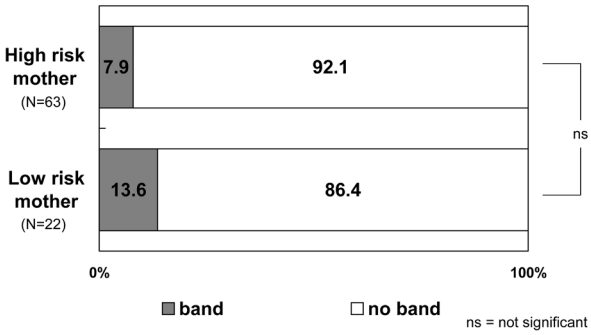

Fig. 4 Mothers caries risk and band detected in their children (6–18 months)

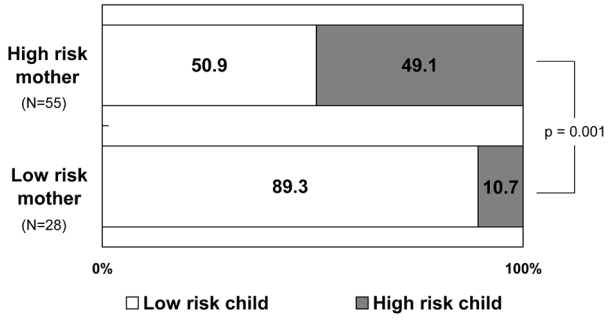

Fig. 3 Caries risk for mothers and their children (19–31 months)

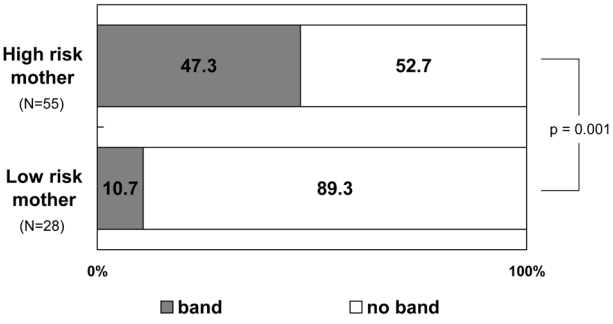

Fig. 5 Mothers caries risk and band detected in their children (19–31 months)

mixture was denatured at 95°C for 3 min followed by 26 cycles of amplification: denaturation at 95°C for 1 min, annealing at 55°C for 1 min and extension at 72°C for 1 min. The final cycle was 94°C for 1 min, 55°C for 1 min and 72°C for 5 min. The PCR products were then subjected to electrophoresis on a 1.0% agarose gel and stained with ethidium bromide for UV viewing. The presence or absence of bands was noted and compared within each mother-child pairing. A positive control (*S. mutans* ATCC 25175 and *S. sobrinus* ATCC 33478) for both strains was also included in each procedure.

Statistical methods

Data were processed with SPSS 11.0 statistical software. Relationships between caries risk and band detection in mother-child pairs were calculated using Pearson's correlation coefficient. Mothers and their children were grouped as low risk for CAT scores of 0 and 1.0 or high risk for CAT scores of 2.0 and 3.0. This was done to establish caries risk grouping of the participants. Values of "0" and "1" were assigned to the low risk and the high risk groups, respectively. Likewise, a value of "1" was assigned for band detection and a value of "0" was assigned for no detection.

Results

The Cariostat score distribution for all groups is shown in Figure 1. Only 1.2% of the children in the 6–18-month old group had a Cariostat score of 3.0 and 43.5% had a score of 0. However, in the 19–31-month-old group, 6% had a score of 3.0, while the percent of children with a score of 0 was less than half of that in the 6–18-month-old group. Almost 70% of the mothers had Cariostat scores of 2.0 to 3.0. As assessed using the Cariostat scores, a statistically significant correlation was seen between children's caries risk and their mothers, for children whose age range was from 6–18 months ($P<0.05$) (Fig. 2) and 19–31 months ($P<0.001$) (Fig. 3). Moreover, a significant positive correlation existed between high-risk mothers and their children ($P<0.001$) in the 19–31-month-old group. Approximately 49% of the children of high-risk mothers had high caries risk

(Fig. 3). Surprisingly, no significant difference was seen between the high-risk and low-risk mothers and the presence of *S. mutans* or *S. sobrinus* in their 6–18-month-old children (Fig. 4). This may be partly due to the number of teeth present when the plaque sampling was done. However, a significant correlation ($P<0.001$) was found for the presence of *S. mutans* or *S. sobrinus* in 19–31-month-old children and high-risk mothers. Either or both strains were detected in 47.3% of children of high-risk mothers (Fig. 5).

Discussion

This study was carried out to determine the relationship between the presence of *S. mutans* or *S. sobrinus* in Cariostat-inoculated plaque samples obtained from mother-child pairs and caries risk through a conventional PCR protocol designed for identifying both strains. Normally, samples are discarded after incubation and scoring. However, we decided to take a further step by analyzing the samples based on current trends in strain identification through molecular biology techniques. Scores were grouped into low-risk group and high-risk group based on the 4-scale scoring system. Studies regarding Cariostat and caries risk in children have suggested that scores of 0–1.0 may be considered as low risk group and scores higher than 1.5 are considered as high risk group[21]. Two oral bacterial strains commonly isolated from carious teeth are *Streptococcus mutans* and *Streptococcus sobrinus*. In studies on these two strains, both have been found in young children and have been implicated in caries risk, especially when their mothers also have high caries levels or have high bacterial counts[22]. Since the Cariostat has been used for caries prediction, we found it appropriate to check the presence of both strains directly from samples after incubation. This would be beneficial for enhancing the usefulness of the Cariostat method for the prediction of caries risk.

The main finding of this study was a correlation between caries risk of mothers and their children, indicating that a high risk of caries in mothers leads to a high risk in their children as they become older. Also, children less than 18 months of age tended to show no correlation for band detection and caries risk of their mothers probably, due to the small number of teeth present, dietary and feeding patterns, and caregiver at the time sampling was done. From 6 months to about 18 months of age, many changes occur in a child's oral cavity. There is a successive eruption of primary teeth, and other local factors of growth and development also occur. As the child becomes older, however, for a time, the primary dentition stabilizes and feeding patterns and behavior become established. Caufield and his colleagues established a discrete window of infectivity period from 19–31 months of age and suggested the importance of transmission patterns[2]. This was the reason why the children's age range was grouped into 6–18 months and 19–31 months. Mothers, who usually act as the primary caregivers, may have frequent salivary contact with their children. This may demonstrate the vertical transmission of oral bacteria occurring from mother to child. Recent studies have revealed that there are sources of infection other than the mother[23,24] or other members of the family circle. Yet, several studies[25,26] have indicated that preventive measures against the early establishment of *S. mutans* in children are effective in preventing later caries development. Thus, along with results of other studies, the findings reported have suggested that there is a strong tendency for mothers and their children to share similar susceptibility to *S. mutans* or *S. sobrinus* colonization. Interestingly, since caries risk in this study was established using the Cariostat score grouping, the test could have much more predictive capacity in children younger than 18 months due to its capability to screen not just mutans streptococci but also lactobacilli strains. PCR techniques are very sensitive but still depend greatly on the amount of starting material for this purpose. Using the Cariostat in conjunction with the PCR method may enhance caries prevention, diagnosis and treatment plan. Further studies on the Cariostat method and PCR techniques are needed.

Conclusion

The effectiveness of the Cariostat method for prediction of caries risk can be improved by detecting the presence of *S. mutans* or *S. sobrinus* directly in plaque samples obtained from mothers and their children through conventional PCR techniques. It is important to identify and educate mothers at high risk for caries for control of caries risk for their children.

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