Relationship between caries risk and presence of cariogenic bacteria among Japanese pregnant women

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Abstract

Objectives: To analyze the relationship between caries risk and the presence of S. mutans and S. sobrinus from plaques of pregnant women using the Cariostat method and the polymerase chain reaction (PCR) technique.

Materials and Methods: The subjects consisted of 269 pregnant women who were in their 3rd, 4th or 5th month of pregnancy. The presence of S. mutans and S. sobrinus was assessed from their plaques cultured in the Cariostat medium and assessed by PCR.

Results: The correlation between caries risk scores assessed by the Cariostat method and the presence of S. mutans was statistically significant (P<0.05). The tendency of positive correlation was found between the presence of S. sobrinus and caries activity.

Conclusion: This study suggested that pregnant women who have high caries activity should be more aware of the possibility of transmission of cariogenic bacteria to their infants.

Key words
Cariogenic bacteria, Cariostat, PCR, Pregnant women

Introduction

Dental caries is one of the most common infectious diseases in humans. Among the seven cariogenic mutans streptococci species, Streptococcus mutans and Streptococcus sobrinus are most frequently isolated from the human oral cavity and these two species have been implicated as the prime causative organisms of human dental caries. A number of studies have reported that many culture methods, various caries activity tests and DNA technology can be used for caries prediction or detection of cariogenic bacteria.

One of those caries predictive methods, the Cariostat test, developed by Shimono, is a colorimetric test that determines the acidogenicity of oral microorganisms in the dental plaque through changes in pH. The Cariostat test has been widely used in many clinical, epidemiological and fieldwork research.

Furthermore, several investigators recently developed polymerase chain reaction (PCR) methods to detect species of mutans streptococci. One group of these researchers, Igarashi et al. described two primer pairs (SD10/SD20 and SOF14/SOR1623).

Only one study has been conducted until present to determine the correlation between the presence of S. mutans and S. sobrinus and the caries risk of mother and child using the Cariostat method in combination with the PCR technique. Moreover, this study was started in the young children. There have been few studies investigating the caries risk levels, presence of cariogenic bacteria and prevalence of dental caries in pregnant women. It is important to improve the oral health of the women during the prenatal period to prevent the bacterial transmission to their infants.

The purpose of this study was to analyze the relationship between caries risk levels assessed using...
the Cariostat test and the presence of cariogenic bacteria from plaques in pregnant women using the PCR technique.

Methods

Subjects

The subjects consisted of 269 pregnant women who were in the 3rd, 4th or 5th month of pregnancy. These women were patients who were visiting the maternity hospital for pregnancy examination. The mean age of the pregnant women was 29.1 ± 4.2 years old (range: 19–43 years old).

Plaque sampling

For each subject, dental plaque was collected by swabbing from the buccal surfaces of the maxillary teeth using a cotton-tipped applicator. The applicator with the dental plaque sample was put into the Cariostat medium (Sankin Co., Japan) and incubated at 37°C for 48 hours. After incubation, the colorimetric change was evaluated in seven grades: 0, 0.5, 1.0, 1.5, 2.0, 2.5, or 3.0. The subjects with scores of 0, 0.5 and 1.0 were placed in the low-risk group and 1.5, 2.0, 2.5 and 3.0 in the high-risk group.

Preparation of bacterial DNA

An ampoule containing the swab with the dental plaque samples was agitated in a vortex mixer (Ecan Tube Mixer M-2000) for 10 s to disperse the bacteria from the cotton tips. Then, we transferred 1 ml of the sample solution into a 1.5 ml microcentrifuge tube. Plaque samples were harvested by centrifugation at 7,500 rpm for 15 min and the supernatant discarded. Then the pellet was resuspended and lysed in 180μl of lysis buffer solution (10mM Tris-HCl buffer, 5 mM EDTA, and 1% Triton X-100, pH 8.0) containing 10 mg/ml lysozyme (SIGMA®) at 37°C for 30 min. After centrifugation, the supernatant was used for bacterial DNA preparation. The Qiagen DNeasy® Tissue kit was used for extraction and purification of DNA Gram-positive bacteria.

PCR experiments

*S. mutans* ATCC 25175 and *S. sobrinus* ATCC 33478 were used as reference strains. The primer pairs (SD10/SD20 and SOF14/SOR1623) were used in this study, because they amplified the species-specific amplicons with different lengths. This indicated that the present PCR method is useful for the detection and identification of these two human cariogenic species, *Streptococcus mutans* and *Streptococcus sobrinus*. PCR detection of *S. mutans* and *S. sobrinus* was performed following the procedure by Igarashi et al. Each PCR mixture (20μl) consisted of 2μl of 10×PCR buffer, 1.6μl of dNTP mixture, 0.1μl of Taq DNA polymerase (*Takara Taq™*), 5.9μl of distilled water (*GIBCO™*), 10μl template solution and 2μl each of the primer pairs. The PCR conditions were denaturation at 95°C for 3 min, followed by 26 cycles of denaturation at 95°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 1 min. The last cycle was comprised 94°C for 1 min, 55°C for 1 min and 72°C for 5 min. After amplification, the PCR products were analyzed by gel electrophoresis in a 1% agarose gel containing 10 mg/ml ethidium bromide and visualized by ultraviolet light. The presence or absence of bands was noted.

Statistical analysis

Statistical analysis was performed using SPSS version 11.5. Chi-square analysis was used to determine the relationship between the distribution of cariogenic bacteria and risk levels evaluated by the Cariostat method.

Results

Figure 1 shows the relationship between caries risk levels as assessed by the Cariostat method and the presence of *S. mutans* of in 131 pregnant women. *S. mutans* was detected in 36.8% of the low-risk group and 55% of the high-risk group. The detection of *S. mutans* increased significantly with increasing caries risk levels (*P*<0.05). The detection of *S. sobrinus* also had an increasing trend with
increasing caries risk levels (Fig. 2). However, it was not statistically significant ($P > 0.1$). Table 1 shows the grouping (G1, G2, G3, and G4) by PCR detection of *S. mutans* and *S. sobrinus*. The groups were as follows: G1 (no detection for both *S. mutans* and *S. sobrinus*), G2 (detection for *S. mutans* alone), G3 (detection for *S. sobrinus* alone), and G4 (detection for both *S. mutans* and *S. sobrinus*). The results showed that 45.0% (121/269) had neither *S. mutans* nor *S. sobrinus*, 42.7% (115/269) had either *S. mutans* or *S. sobrinus* and 12.3% (33/269) had both *S. mutans* and *S. sobrinus*. Figure 3 shows the percentage of caries risk levels in each group (G). We did not find any statistically significant differences among groups.

**Discussion**

Many studies suggest mothers as the principal source of mutans streptococci to their infant [18–21], and poor maternal oral hygiene and dietary habits increase the likelihood of transmission of the infection from mother to child. Furthermore, some data [22,23] show that child-rearing habits which facilitate saliva transfer from adults to the child, such as sharing of food and utensils, and habits which involve close contact, such as breast feeding and sleeping beside the mother, were also significantly associated with infection and colonization of *S. mutans*. The main finding of this study was that high-risk group of pregnant women had more *S. mutans* detection, compared with low-risk group. This may demonstrate the importance of maintaining mothers’ oral hygiene. If mothers know their own caries risk during their pregnancy, they could pay better attention to their diets, receive professional tooth brushing instructions and maintain good oral hygiene in order to be highly effective in preventing or delaying mother-child transmission of cariogenic bacteria.

In this study, although we did not find statistically significant difference between the detection of *S. sobrinus* and caries risk levels or caries risk levels and groups (G1–4), we saw a positive trend with increasing caries risk levels. The lack of statistically significant difference is probably due to the fact that our sample size is too small, as well as that *S. mutans* is more prevalent than *S. sobrinus* in dental plaque samples [24]. One previous study [17] suggested that using the Cariostat in conjunction with the PCR method may enhance caries prevention, diagnosis and effectiveness of the treatment plan. In the future, we should conduct more studies to investigate behavioral factors of children and transmission between mother and child in early infancy, using the conventional Cariostat method and PCR techniques. We believe that it is important to predict and prevent dental caries in children in the early stages of their lives.

**Conclusion**

This study showed that caries risk and the presence
of *S. mutans* were correlated and that caries risk and the presence of *S. sobrinus* were possibly correlated. This study suggests that pregnant women should be more aware of their own caries risk in order to prevent or delay mother-child transmission of cariogenic bacteria.

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**References**


