PCR detection of *Streptococcus mutans* and *Streptococcus sobrinus* in plaque samples from Mongolian mother-child pairs

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Abstract Early childhood caries results in a considerable burden of pain and suffering as well as poorer general health. *Streptococcus mutans* (serotypes *c*, *e* and *f*) and *Streptococcus sobrinus* (serotype *d* and *g*) are the species closely associated with dental caries. The exact age at which their colonization occurs in children is controversial. The objective of this study was to detect *S. mutans* and *S. sobrinus* in plaque samples of Mongolian mother-child pairs by PCR and to compare their presence with the caries status. Dental examination and caries risk assessment using the Cariostat® carried out on 320 children aged 6–30 months and their mothers. The presence of *S. mutans* and *S. sobrinus* was checked by PCR. The caries prevalence and DEFT scores of mothers enrolled in the present study were 98% and 11.5 ± 0.7 , respectively. In children, the prevalence and deft scores of the 6–18-month-olds were 29% and 1.3 ± 0.2 while those of the 19–30-month-olds were 59% and 3.4 ± 0.4 correspondingly. Twenty nine percent of the 6–18-months old children of high-risk mothers and 53.1% of the 19–30-months old children of high-risk mothers had high caries risk ($P < 0.001$). There was a statistically significant correlation between caries risk of 19–30 month-old children and their mothers ($P < 0.001$). In mothers, the prevalence of *S. mutans* and *S. sobrinus* was 79% and 33%, respectively; 54% harbored *S. mutans* alone, 8% harbored *S. sobrinus* alone, 25% harbored both strains. In children, 45% were positive for *S. mutans* alone, 9% were positive for *S. sobrinus* alone, 18% were positive for both strains. Either or both strains were detected in 67.3% of 6–18-months old children and 76.5% of 19–30-months old children. In conclusion, our results showed that *S. mutans* and/or *S. sobrinus* first colonized infants' teeth from 6–18 months, and the colonization increased with increasing age, so that by 30 months of age, 76.5% of children harbored the bacteria.

Key words

Caries activity test,
Caries risk,
PCR,
S. mutans,
S. sobrinus

Introduction

Early childhood caries results in a considerable burden of pain and suffering as well as poorer general health¹⁾. The principal causative agents of dental caries are a group of microorganisms collectively referred to as mutans streptococci. Mutans

streptococci possess a number of phenotypic traits such as the synthesis of extracellular polysaccharides, acid production, aciduricity, intracellular polysaccharides synthesis and endodextranase production, which are fundamental to their cariogenicity. Among them, *Streptococcus mutans* (serotypes *c*, *e* and *f*) and *Streptococcus sobrinus* (serotype *d* and *g*) are the species closely associated with human dental caries^{2–8)}.

Serotyping, bacteriocin typing, and genetic

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studies have shown that mutans streptococci strains are commonly transferred from a mother to her infant. The exact age at which mutans streptococci colonization occurs in children is controversial^{9–12}. Mutans streptococci colonize infants' teeth at 19–31 months of age, a period described by Caufield *et al.* as the “window of infectivity”^{13–15}. However, recently it has been reported that *S. mutans* has been found in predeciduous infants^{16,17}. The earlier colonization occurs, the greater the risk of caries development in both deciduous and permanent dentition. Thus, it is very important to assess caries risk of children early in life.

Caries activity testing is very effective in determining the caries risk of children. The Cariostat® test developed by Shimono is a colorimetric test based on the acidogenic properties of dental plaque microorganisms. The Cariostat liquid medium contains 20% of sucrose and pH indicators. After incubation of 48 hours at 37°C, scoring is done according to the color changes of test ampoules^{12,18,19}.

Recent developments in the field of molecular biology have provided a more sensitive means of detection of cariogenic bacteria. DNA assays can be used to identify closely related species that are difficult to differentiate by traditional, culture-based approaches^{20–23}.

The objective of this study was to detect *S. mutans* and *S. sobrinus* in Cariostat-inoculated plaque samples of Mongolian mother-child pairs by PCR and to compare their presence with the caries status.

Materials and Methods

Subject selection

The study population consisted of 320 mother-child pairs attending the Health Centers of Songinokhairkhan and Bayangol districts of Ulaanbaatar city. The ages of the children ranged from 6 to 30 months. The children were grouped by age range: 6–18 (n = 160) and 19–30 month-old groups (n = 160). All children had at least two teeth present during the examination. The mother's average age was 29.3 ± 5.59 years. An informed consent was obtained from all parents for the examination and sampling procedures.

Oral examination

A dental examination was done using a mouth mirror and explorer under natural light. The WHO caries

diagnostic criteria were used for determining the deft (decayed, extracted, filled) index.

Plaque sampling and caries risk assessment using Cariostat test

For each mother-child pairs plaque was collected by the swabbing from the buccal surfaces of the maxillary teeth using a sterile cotton swab. Each plaque sample was put into the Cariostat medium (Morita Co., Japan) and incubated at 37°C for 48 hours. After incubation the colorimetric changes were classified into seven grades using the original four grade standard color sample. A score of “0” shows a low caries risk while “3.0” shows a high caries risk. The subjects with Cariostat scores of 0 to 1 (pH 7.2–5.4) were placed in the low-risk group, and those with Cariostat scores of 1.5–3 (pH 5.0–3.8) in the high-risk group. This was done to establish caries risk grouping of the children and their mothers^{12,24,25}. The Cariostat ampoules were shown to the children and mothers in order to give them a visual aid regarding their caries risk. Then, these Cariostat-inoculated plaque samples were further used for the following experiments.

Experimental procedures and PCR

The plaque samples cultured in Cariostat liquid medium from 100 mother-child pairs (200 samples) were randomly selected. The Cariostat ampoules containing the samples were vortexed (Vortex genie-2) to dislodge bacteria attached to cotton swab. Then 1 ml of the sample solution was transferred into a mini centrifuge tube, and harvested by centrifugation at 7,500 rpm for 15 minutes. The supernatant was discarded carefully. Bacterial DNA extraction was performed using the GenElute™ Bacterial Genomic DNA Kit's protocol for purification of DNA from Gram-positive bacteria.

S. mutans ATCC 25175 and *S. sobrinus* ATCC 33478 were used as reference strains. The primer pairs SD1/SD2 and SOF14/SOR1623 were employed^{20–23}. PCR amplification was performed in a reaction mixture (40 µl) that contained 24 µl of distilled water, 4 µl of 10X PCR buffer solution, 4 µl of dNTPs, 0.5 µl of each primer, 1 µl of Taq (AmpliTaq® DNA Polymerase, Roche, Germany), and 5 µl of the template DNA. PCR products were analyzed by gel electrophoresis in a 1% agarose gel containing ethidium bromide (a final concentration 0.5 µg/mL) and visualized by ultraviolet light. The presence or absence of bands was noted and compared within

Fig. 1 Caries risk for mother-child pairs by age group of children ((a) 6-18 months olds, (b) 19-30 months olds)

Fig. 2 The prevalence of mutans streptococci in mothers (a) and their children (b)

each mother-child pair²⁰⁻²²).

Statistical analysis

Data were processed with SPSS 11.5 software. Relationships between caries risk and band detection in mother-child pairs, and differences in caries experience were evaluated statistically using Pearson's correlation coefficient, Chi-square test, and ANOVA.

Results

The caries prevalence and DEFT scores of mothers enrolled in the present study were 98% and 11.5 ± 0.7 , respectively. In children, the prevalence and deft scores of the 6-18 month-olds were 29% and 1.3 ± 0.2 , respectively, while those of the 19-30 month-olds were 59% and 3.4 ± 0.4 , correspond-

ingly. The caries prevalence and deft scores were significantly higher in the 19-30 month-old group compared to the 6-18-month-old group ($P < 0.001$).

As assessed using Cariostat test, 62.2% of mothers had high caries risk, while 37.8% of them had low caries risk. 24.1% of the children aged 6-18 months, and 47.2% of the children aged 19-30 months were at high risk of developing caries ($P < 0.001$).

Figure 1 presents the relationship between caries risk for mother-child pairs. A statistically significant correlation was seen between children's caries risk and their mothers, for children whose age range was 19-30 months ($P < 0.001$). However, no significant difference was seen between 6-18 months children and their mothers probably due to the small number of erupted teeth.

Figure 2a and 2b show the prevalence of

Fig. 3 Caries status and presence of mutans streptococci in children

Mutans streptococci in mothers

**: $P < 0.005$

Fig. 4 Presence of cariogenic bacteria in mothers and caries risk levels of their children

S. mutans and *S. sobrinus*. In mothers, the prevalence of *S. mutans* and *S. sobrinus* was 79% and 33%, respectively; 54% harbored *S. mutans* alone, 8% harbored *S. sobrinus* alone, 25% harbored both strains, whereas 13% were negative for both strains. In children, 45% were positive for *S. mutans* alone, 9% were positive for *S. sobrinus* alone, 18% were positive for both strains.

Children harboring both *S. mutans* and *S. sobrinus* (88.9%) have a significantly higher prevalence of dental caries than those with *S. mutans* (60%) or *S. sobrinus* (44.4%) alone ($P < 0.01$). The mean dmft of children harbouring *S. mutans* was 3.5 ± 0.59 , that of children with *S. sobrinus* 2.7 ± 1.56 and that of children with both strains was 5.6 ± 1.10 ($P < 0.05$,

Fig. 3).

Moreover, a significant correlation was found between the presence of *S. mutans* or both strains in mothers and the caries risk of children ($P < 0.05$). The caries risk was low in 76.9% of children of mothers negative for both *S. mutans* and *S. sobrinus*, and in 87.5% of children of mothers positive for *S. sobrinus*. Whereas, most of the children of mothers positive for *S. mutans* alone and positive for both strains had high caries risk ($P \leq 0.05$, Fig. 4).

Discussion

Several studies have shown that preventive measures against the early colonization of mutans streptococci

in children are effective in prevention of later dental caries development^{9,11,26}). Thus, this study was done to investigate the relationship between the presence of mutans streptococci in dental plaque samples obtained from Mongolian mother-child pairs and their caries status.

The main finding of our study was a relationship between caries risk of mothers and their children, showing that a high risk of dental caries in mothers leads to a high risk of developing carious lesions in their children as they become older. Twenty-eight percent of the 6–18-months old children of high-risk mothers and 53.1% of the 19–30-months old children of high-risk mothers had high caries risk ($P < 0.001$). There was a statistically significant correlation between caries risk of 19–30 month-old children and their mothers ($P < 0.001$). Also, a significant positive correlation has been found between decayed teeth in mothers and decayed teeth in their children. However, no significant correlation has been seen between caries risk of 6–18-month-olds and their mothers. The lower dental caries risk and caries status in 6–18-month-olds was probably, due to the small number of erupted teeth, dietary and feeding habits, and caregiver behavior at the time of examination. This finding was also in line with the findings of Rodis *et al.*¹²) Furthermore, Fujiwara *et al.* found that the concentration of mutans streptococci correlated with the number of erupted teeth, and the concentration of mutans streptococci was more closely correlated to the caries prevalence of the next year than that of the year when the salivary concentration of mutans streptococci was first evaluated²⁷).

The important finding of this study was that either or both strains were detected in 67.3% of 6–18-months old children and 76.5% of 19–30-months old children. Caufield and his colleagues established a discrete “window of infectivity” period from 19–31 months of age^{13–15}). However, along with findings of other studies^{16,17}), the results of this study suggest that children may be colonized by *S. mutans* before the “window of infectivity” opens.

The results of the present study suggest that there is a tendency for mothers and children to share similar susceptibility to *S. mutans* and *S. sobrinus* colonization. In mothers, the prevalence of *S. mutans* and *S. sobrinus* was 79% and 33%, respectively; 54% harbored *S. mutans* alone, 8% harbored *S. sobrinus* alone, 25% harbored both strains. In children, 45% were positive for *S. mutans* alone, 9% were positive

for *S. sobrinus* alone, 18% were positive for both strains.

The present PCR findings also confirmed that children positive for both *S. mutans* and *S. sobrinus* had significantly higher caries prevalence and caries risk as compared to those positive for *S. mutans* alone which agrees with the results of other surveys^{26,28}).

In conclusion, our results showed that *S. mutans* and/or *S. sobrinus* colonization increased with increasing age, so that by 30 months of age, 76.5% of children harbored the bacteria. These data would be relevant for development of preventive strategies of early childhood caries in Mongolia.

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