Colonization and Vertical Transmission of Mutans Streptococci in a Group of Turkish Families

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ABSTRACT
Dental caries in babies and toddlers is called Early Childhood Caries (ECC). It is an infectious and transmissible die-to-bacterial disease. Mutans streptococci are important organisms in the initiation and progression of dental caries. The findings of the study demonstrate that these bacteria are found in the mouths of pre-dentate infants and are acquired via vertical transmission from human reservoirs. This information should facilitate the focusing of clinical interventions that prevent or delay infant infection, thereby reducing the prevalence of dental caries (ECC).

Keywords
Mutans streptococci, Transmission, Child, Mother, Father.

Introduction
The mutans streptococci comprise a group of seven species, of which Streptococcus mutans and Streptococcus sobrinus are the predominant species isolated from human saliva and dental plaque [1]. Experiments with gnotobiotic hamsters revealed these to be the main initiator microorganisms in dental caries disease [2]. Dental caries is a common infectious disease world-wide. The aetiology of the disease is multifactorial, life habits and mutans streptococcus infection being the most important factors [3].

Early acquisition of MS contributes to increased caries prevalence in the primary and permanent dentition. The major route of early acquisition of MS indicated the transmission from mother to child. However, other possible transmission routes, such as extra-familial acquisition of MS in children, intra-familial transmission between spouses have also been reported. In most of these reports the individuals with high levels of MS in their mouths and who are in frequent contact with the children were suggested to be the principal source of transmission [4].

MS seem to have a certain optimum colonization period, especially during a discrete time period, called “window of infectivity” ranging from 19-31 months of age, with a median age of 26 months, time of emergence of primary molars. Although most studies suggest that MS require a non-desquamating surface in order to colonize and thrive, which is present with deciduous tooth eruption and the age of about 8 months. There is consistent evidence that MS may be found in pre-dentate mouths or shortly after the tooth eruption [5].

The aim of this study was to evaluate the Streptococcus mutans (S. mutans) and Streptococcus sobrinus (S. sobrinus) colonization profile of individuals’ oral cavities and the genotypic diversity of the strains.

Material and Methods
The subjects were 7 mother-father and 8 children (one twin), who were monitored for 12 months. Unstimulated saliva samples of children were collected on 1st, 4th, 9th and 12th months after birth. And also stimulated saliva samples were taken from mother and father on 1st month only. Experimental procedures were approved by the Ethical Committee of Medical Faculty of Istanbul University, Istanbul, Turkey. All subjects had a similar moderate socioeconomic status. None of the subjects participated in to this study had chronic diseases or antibiotic treatment within the last 1 month prior to the assessment. Children and parents were orally examined with a mirror under daylight. Caries prevalence in the children and the parents were recorded in accordance with the WHO criteria (1997) [6].
Sampling and culture

Five minutes of sugar free chewing gum stimulated saliva samples were collected into sterile tubes. The samples were immediately transported to the laboratory on ice to be cultured with in 2 h. All part of samples were kept at –20ºC for AP-PCR. Samples were dispersed for 1 min in a vortex mixer (FALC Instruments, Italy) and serially diluted. In order to detect mutans streptocci, 50 µl undiluted samples and 10<sup>-1</sup> to 10<sup>-3</sup> dilutions were cultured on mitis salivarius agar plates supplemented with 20% sucrose (synth) and 0.2 units ml<sup>-1</sup> of bacitracin MSB agar. The plates were incubated for 48 h in candle jars at 37ºC.

Isolation of mutans streptocci and strain identification

Each single colony was transferred to 2 ml of brain-heart infusion broth and grown for 18 h. A portion of bacteria was used for DNA preparation modified from Oho et al. [5] which the cells from on overnight 90 µl culture boiled for 10 min with 10 µl TE buffer (100mM Tris/HCl, 10 mM EDTA, pH:8.10% Triton X-100) and then the dry ice for 10 min the debris was pelleted and the supernatant was used for identification by PCR.

Extraction of DNA

DNA from strains was extracted using simple DNA preparation modified which the cells from on overnight 90 µl culture boiled for 10 min with 10 µl TE buffer (100mM Tris/HCl, 10 mM EDTA, pH:8.10% Triton X-100) and then the dry ice for 10 min the debris was pelleted and the supernatant was used for identification by PCR.

PCR identification

DNA sample from MS isolates were identified as <i>S. mutans</i> by PCR using primers designed to amplify a 282 bp sequence of the 16S rRNA genes. The sequences of these primers were 5’- GGTCAGGAAAGTCTGAGTAAAGGGCTA -3’ and 5’-GCCGTAGTCTCCGGCAGTAAAGGGCAG -3’. The PCR was processed in a 50 µl mixture containing 1x reaction buffer (10 mM Tris/HCl, 50 mM KCl, pH:8.3) 1.5 mM MgCl<sub>2</sub>, 0.1 mM dNTPs, 1.5 U Taq DNA polymerase (MBI Fermentas, Vilnius, Lithuania). 0.2 µM of each primer and 2 µl DNA sample. purified genomic DNA from <i>S. mutans</i> ATCC 25175 and distilled water were respectively used positive and negative controls. PCR amplification was performed using a Eppendorf PCR System (Mastercycler personal, Eppendorf, Germany) under the following conditions: a denaturation step a 94ºC for 5 min, followed by 36 cycles of denaturation at 95ºC for 45 sec, annealing at 55ºC for 30 sec, extension at 72ºC for 45 sec and final elongation step at 72ºC for 10 min. Amplicons generated by PCR were separated by electrophoresis 1% agarose gel in TBE running buffer and stained in 0.5 µg ml<sup>-1</sup> ethidium bromide and visualized with ultraviolet light. Gene Ruler DNA ladder mix (100 bp) was run a molecular-size marker in the gel.

The isolates were rerun on the same gel when the results from the comparison of different gels were dubious. Different patterns in each subject indicated the number of genotypes. If similar patterns were found in different subject, the AP-PCR products were rerun in the same gel to compare the fingerprints.

Statistical analysis

Data was analyzed with SPSS version soft-ware (SPSS Inc., Chicago, IL, USA). χ² and Fischer tests were used for the analysis of the categorical variables. Mann-Whitney U, Friedman and Wilcoxon tests were used for comparisons and correlations among each group. The statistical level of significance was set at p<0.05.

Results

7 mother–father pairs included in the study. The average age of mothers was 30.86 ± 3.93 (years) and the average age of fathers was 37 ± 3.46 (years). The DMFT values of mothers and fathers found 6.86 ± 1.95 and 3.71 ± 1.11. The MS (104 cfu/ml) values of mothers and fathers was 272.86 x10<sup>4</sup> and 46 x10<sup>4</sup>. <i>S. mutans</i> had been isolated from all the mother-father pairs but S. Sobrinus had not been isolated. There were significant differences between DMFT and MS values of mothers and fathers. (p=0,011; p< 0.05, p=0,03; p< 0.01) (Table 1).

<table>
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<tr>
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<th>DMFT</th>
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<tbody>
<tr>
<td>Mann-Whitney U</td>
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<td>1,000</td>
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<tr>
<td>Wilcoxon W</td>
<td>33,000</td>
<td>29,000</td>
</tr>
<tr>
<td>Z</td>
<td>-2.51</td>
<td>-3,003</td>
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<tr>
<td>P</td>
<td>0.011</td>
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Table 1: Comparison of DMFT ve MS (104 cfu/ml) values of mothers and fathers.

MS levels of children were shown in Table 2. S. mutans colonization began at 1st month for only one infant (number 8) and for the other infants S. mutans colonization began after 4th month. S. sobrinus species had not been isolated. Individuals harboured 1 to 4 distinct genotypes of S. Mutans; and for the families maximum 11 distinct genotypes had been isolated (Table 3). The subjects were 7 mother-father pairs and 8 children (one twin), who were monitored for 12 months. Genotypes of S. mutans appeared identical in 4 mother-5 child and 1 mother-father pair. Twenty nine different genotypes were identified totally, and there were 2 different genotypes of S. mutans (genotype 1 and genotype 12) isolated repeatedly among specific families. Genotype 1 found common for 1st and 7th families and genotype 12 found common for 3rd, 4th, 6th and 7th families (Figure 1).

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Number of individuals</th>
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<tr>
<td>1</td>
<td>13</td>
<td>3</td>
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<td>4</td>
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<td>38 genotype/22 individual (average 1.72 genotype/individual)</td>
<td>37 genotype/7 family (average 5.25 genotype/family)</td>
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</table>

Discussion

Studies in using bacteriocin profiles, serotyping and genotyping suggest that the mothers are the principal source of MS to their children because of the fact that the mothers are in frequent and intimate contact with their infants in the first year of life [7-9]. However it has been suggested that there were examples of fathers who may have been the source and the possibility of extrafamilial transmission [9]. In the present study 5 of 8 children harbored genotypes of S. mutans identical to those of their mothers and none of the children harbored the identical S. mutans genotypes with their fathers.

In this study, one of the mother-father pairs showed identical S. mutans genotypes. This result was not in accordance with the reports of Redmo Emunelsson et al. [7] who found no transmission of S. mutans among the spouses.

Oho et al. indicated that various methods have been used to differentiate and identify S. mutans and S. sobrinus, including colony morphology on mitis-salivarius agar, biochemical tests, immunological methods and genetic methods with DNA probes [5]. According to their results, PCR method developed in this study was useful for detecting S. mutans and S. sobrinus in saliva.

Loyolo-Rodriguez et al. used PCR for investigating the distribution of MS infection of caries-free and caries-active preschool Mexican children. They indicated that PCR is a useful tool in molecular epidemiology or dental caries studies; it was effective in detecting and identifying MS from saliva in children. In this study the same PCR method with Oho et al. was used and all the strains were identified by AP-PCR [10].

Kozai et al. reported that the number of MS strains harbored in a person can range from one to four. Another genotypic analysis by Kulkarni et al. [11] showed from one to five strains. In this study, mothers harbored at one to three different strains, fathers harbored two and children harbored at least four different strains [9].

MS seem to have a certain optimum colonization period especially during a discrete time period called "window of infectivity"
ranging from 19-31 months of age. Although most studies suggest that MS require a non-desquamating surface in order to colonize and thrive, which is present with deciduous tooth eruption at the age of about 8 months. Instead of this, some studies suggest that MS might colonize the children before the “window of infectivity” period [12,13]. The findings of this study reinforce this information. For 1 of 8 children, MS colonization confirmed at 1st and 4th months after the birth.

S. mutans and S. sobrinus differ in properties which should influence their survival and persistence in vivo and which are believed to potentiate the initiation and progression of dental caries. Thus, S. sobrinus strains are more acidogenic, more aciduric, produce more water-insoluble polymer from sucrose, and exhibit a greater degree of cariogenicity in gnotobiotic animals than S. mutans strains. Despite possessing these properties, S. sobrinus is isolated from human populations far less frequently than S. mutans and, when isolated, is almost invariably present in lower numbers than S. mutans [14-16]. In this study, S. mutans had been isolated from all the mother-father pairs but S. sobrinus had not been isolated.

Li et al. [17] hypothesized that several maternal factors, including the mode of delivery, influence the initial acquisition of S. mutans in infants. A prospective cohort study was conducted in 156 mother-infant pairs. Among infants who became infected, those delivered by Caesarean section acquired S. mutans 11.7 more earlier than did vaginally delivered infants (p = 0.038). C-section infants harbored a single genotype of S. mutans that was identical to that of their mothers (100% fidelity). In this study, all the infants delivered by Caesarean section and acquired S. mutans before 12th month.

References
6. https://apps.who.int/iris/handle/10665/41905