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Colonization and Vertical Tranmission 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 prevalance 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 wih 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 sugget that MS require a non-desquamating surface in order to coloniz and thrive, which is present with decidious 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's oral cavities and the genotypic diversity of the strains.

Materıal 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, İstanbul, 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 t oto the assesment. 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 stimuleted saliva samples were collected into sterile tubes. The samples were immediately transported to the laboratory on ice to be cultered with in 2 h. A part of samples were keep 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^{-1} to 10^{-3} dilutions were cultered on mitis salivarius agar plates supplement with 20% sucrose (synth) and 0.2 units ml^{-1} 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 gram staining. All isolates were classified as either *S. mutans* or S. sobrinus using biochemical test and the PCR method. With biochemical test, the isolates were tested for ability to ferment mannitol and sorbitol. *S mutans* ATCC 25175 was used as a reference strain.

Extraction of DNA

DNA from strains was extracted using simple 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/HCI,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 *S. mutans* by PCR using primers designed to amplify a 282 bp sequence of the16S rRNA genes. The sequences of these primers were 5'- GGTCAGGAAAGTCTGGAGTAAAAAGGCTA -3' and 5'-GCGGTAGCTCCGGCACTAAGCC -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 $MgCl_2$, 0.1 mM dNTPs,1.5 U Taq DNA polimerase (MBI Fermantas, Vilnius, Lithuania), 0.2 µM of each primer and 2 µl DNA sample. purified genomic DNA from *S. mutans* ATCC 25175 and distileted 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 95ºC 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 elangation step at 72ºC for 10 min. Amplicons generated by PCR were separeted by electrophoresis 1.5% agorose gel in TBE running buffer and stained in 0.5 µg ml^{-1} ethidium bromide and visualized with ultraviolet light. Gene Ruler DNA ladder mix was run a molecular size marker in the gel.

AP-PCR typing

Strains identified as *S. mutans* were genotyped. AP-PCR fingerprinting was performed to DNA extraction. Bacterial cells were harvested by centrifugation (x1500 g for 10 min) and cells washed with 1 ml of TE buffer (10 mM Tris base, 1 mM EDTA pH: 8.0). Cells were resuspended in 100 µl of TE, 50 µl of 10% sodium dodecyl sulfate (SDS) was added and cells were incubated for 30 min at 65ºC. The suspencion was centrifuged (x2000 g for 5 min) and the supernatant was removed. The ependorf tubes containing the cels were placed in a microwave oven (490W) and heated for 2 min 30s. The pellets were dissoved in 250µl of TE and the ependorf tubes were frozen at -20ºC. Before AP-PCR assay, the suspension was melted, centrifuged and the supernatatnt was used in AP-PCR. AP-PCR was performed by using primer 5'- AGGGGTCTTG-3' (OPA-05). PCR's were carried out in a 50 µl reaction mixture containing the DNA template (2 µl of 1:100 diluted DNA), 0.2 mM of each deoxynucleoside triphophate/liter, 0.4 µmol primer/liter, 3.0 mM $MgCl_2$ and 2.5 U Taq DNA polimerase (MBI Fermantas, Vilnius, Lithuania) in the manifactures' recommnded buffer. DNA amplification was performed in a programmable thermal cycler (Mastercycler personal, Eppendorf, Germany). The cycling parameters used were initial denaturation 94ºC for 5 min, followed by 35 cycles each of 1 min at 94ºC, 2 min at 36ºC and 2 min for 72ºC. After the last cycle, the PCR tubes were incubated at 72ºC for 5 min and held at 4ºC. Amplicons generated by AP-PCR were separeted by electrophoresis 1% agorose gel in TBE running buffer and stained in 0.5 µg ml^{-1} 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). χ^2 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 statisical 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 $x10^4$ and 46 $x10^4$. *S. mutans* 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).

	DMFT	MS (104 cfu/ml)
Mann-Whitney U	5,000	1,000
Wilcoxon W	33,000	29,000
Z	-2,51	-3,003
P	0.011	0,003

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 3th, 4th, 6th and 7th families (Figure 1).

	MS 1st month (10^3 cfu/ml)	MS 4th month (10^3 cfu/ml)	MS 9th month (10^3 cfu/ml)	MS 12th month (10^3 cfu/ml)
1	0	0	3	3,5
2	0	0	2,3	3,4
3	0	0	2,1	2,7
4	0	0	2,2	3,8
5	0	0	2,1	2,9
6	0	0	2,3	3,5
7	0	0	2,2	3,7
8	3	2	2,8	3,6

Table 2: MS (10^3 cfu/ml) levels of children according to months.

Genotype	Number of individuals	Genotype	Number of families
1	13	3	3
2	4	4	1
3	3	5	1
4	2	8	1
		11	1
38 genotype/22 individual (average 1.72 genotype/individual)		37 genotype/7 family (average 5.25 genotype/family)	

Table 3: Genotype types of individuals and families.

Discussion

Studies in using bacteriocin profiles, serotyping and genotyping suggest that the mothers are thr 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”

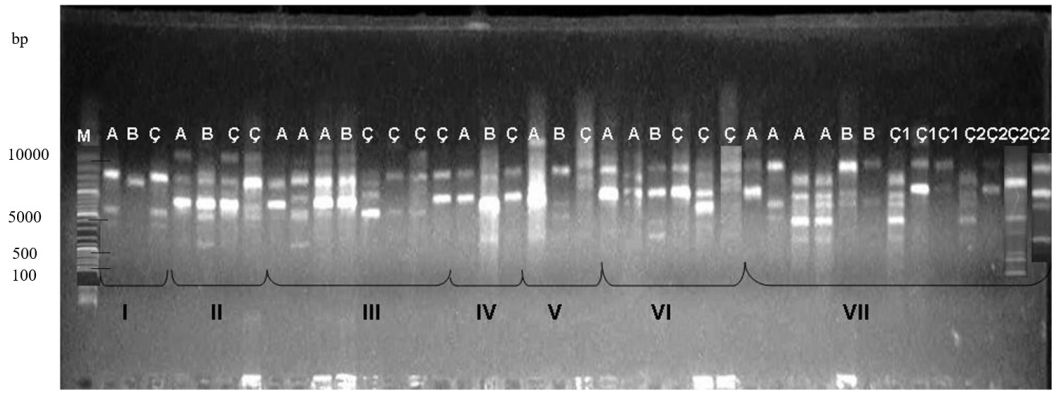

Figure 1: AP-PCR fingerprinting profiles of S. mutans isolated from 7 families.
A: Mother, B: Father, Ç: Children, Ç1: 1st child Ç2: 2nd child, M: Molecular weight, I: 1st family II: 2nd family, III: 3rd family, IV: 4th family, V: 5th family, VI: 6th family, VII: 7th family, bp: base pair.

ranging from 19-31 months of age. Although most studies suggest that MS require a non-desquamating surface in order to colonize and thrieve, which is present with decidous 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.

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