Streptococcus Mutans Strains in Mother-Child Pairs of Children with Early Childhood Caries

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Objective: Dental caries is both an infectious and transmissible disease. Maternal transfer of Mutans Streptococci occurs at an early age and is important in the initiation of dental caries in children. The aim of this study was to identify certain strains of Streptococcus mutans in mother-child pairs, of children with early childhood caries. Study design: Sixty mother-child pairs of healthy children aged 18-36 months were selected. Mothers with high levels of Streptococcus mutans in their saliva and only children with ECC were included. Dental plaque samples were collected from mother-child pairs. The plaque samples were stored, transferred to the laboratory and analyzed for Streptococcus mutans strains c, f, e and k, present in mother-child pairs using Real time Polymerase Chain Reaction (PCR) technique. Data obtained was subjected to statistical analysis for level of similarity in Streptococcus mutans strains present in mother-child pairs. Results: A similar distribution of Streptococcus mutans strains c, f and k was identified in 28 mother-child pairs. Streptococcus mutans strain e was seen in 18 pairs. Conclusion: Less than 50% of mother-child pairs showed similarity in distribution of Streptococcus mutans strains.

Keywords: Early childhood caries (ECC), Streptococcus mutans , maternal transmission

INTRODUCTION

Oral streptococci are divided into five different groups: (1) Mutans group (prominent members are *Streptococcus mutans* and *Streptococcus sobrinus*), (2) Salivarius group (*Streptococcus salivarius*), (3) Anginosus group (*Streptococcus anginosus* and *Streptococcus intermedius*), (4) Sanguinis group (*Streptococcus sanguinis* and *Streptococcus gordonii*), and (5) Mitis group (*Streptococcus mitis* and *Streptococcus oralis*) . Dental caries is a common chronic infectious transmissible disease resulting from tooth-adherent specific bacteria, primarily Mutans Streptococci. Early colonization of Mutans Streptococci in the oral cavity is important for the initiation of dental caries in childhood. Infants acquire Mutans Streptococci from their mothers even as early as the predentate period.[1,2] Children acquire Mutans Streptococci between the age of 19- 33 months, and this discrete period is designated as the "first window of infectivity." [3]

The risk of transmission increases with high maternal salivary levels of Mutans Streptococci and frequent inoculation.[4] The earliest route of transmission from mother to child is referred to as 'vertical' transmission. High maternal salivary Mutans Streptococci challenge is associated with earlier child acquisition of the microorganism.[5] Maternal salivary bacterial challenge was associated with oral infection among children and predicts increased occurrence of childhood caries.[6] Homology of genotypes between mothers and their infants at initial acquisition strongly suggested that Mutans Streptococci strains were transmitted from mother to infant and that this transfer exhibited gender specificity. [7]

Mutans Streptococci have been classified into nine serotypes: *Streptococcus cricetus(a), Streptococcus rattus(b), Streptococcus mutans (c,e,f,k), Streptococcus sobrinus (d,g), Streptococcus downei (h), Streptococcus ferus (c) and Streptococcus macacae (c)*. [8] In the Mutans Streptococci group, *Streptococcus mutans* and *Streptococcus sobrinus*, are the most predominant microorganisms associated with dental caries.

Nakano *et al* reported that *Streptococcus mutans* serotype k is present in the oral cavity in humans. [9] The possibility of maternal transfer of *Streptococcus mutans* from mother to child was initially suggested by Berkowitz *et al* [10] Bratthall classified strains of *Streptococcus mutans* into at least five distinct types, designated a, b, c, d, and e according to their serological specificities.[11] Perch et al demonstrated two additional serotypes f and g.[12]

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Innovative tools for bacterial identification, such as polymerase chain reaction (PCR) techniques and 16s rRNA gene sequencing provide better understanding of the transmission of cariogenic microorganisms between mother and child. *Streptococcus mutans* and *Streptococcus sobrinus* were isolated in higher numbers from caries active pre-school children in Mexico.[13] Using quantitative real-time polymerase chain reaction (qRT-PCR) with specific oligonucleotide primers, *Streptococcus sobrinus* was found to be in higher proportion than *Streptococcus mutans* in the saliva of caries-active Sudanese children when compared to a caries-free group.[14] Serotype specific PCR identified a higher prevalence of *Streptococcus mutans serotype k, e and f* in the saliva of 6-12 year old children with dental caries from South India.[15]

Early childhood caries (ECC) results from an imbalance of multiple risk and protective factors. It is initially recognized as a dull, white band of enamel de-calcification that usually appears on the primary maxillary incisors and rapidly progresses to obvious decay along the gingival margin. Any sign of smooth surface caries in children younger than 3 years is indicative of ECC. Accumulation of dental plaque on tooth surfaces is an early manifestation of dental caries. Factors occurring during the first year of life have an effect on ECC experience.[16]

However, studies on serotypes of *Streptococcus mutans present in mother-child pairs of children with ECC is lacking in India. Therefore, this study was carried out with an aim to identify strains of Streptococcus mutans (c,e,f and k) in mother-child pairs, of children with ECC.*

MATERIALS AND METHOD

The present study was conducted on mother-child pairs, with children selected from various play homes and hospitals of Bangalore. The study protocol was approved by the Institutional Ethics Review Committee of The Oxford Dental College and Hospital, Bangalore.

Normal, healthy children aged 18-36 months along with their mothers were screened for the study purpose. Prior to the study, nature of the study was explained to the parents and their written informed consent was taken.

Oral examination was conducted in natural day-light using a sterile mouth mirror in order to record dental caries according to WHO criteria.[17] Oral examination was carried out by a single examiner in order to avoid any inter examiner variability.

Inclusion criteria:[18,19]

For mothers

Mothers with high salivary *Streptococcus mutans* levels. In order to assess *Streptococcus mutans* levels in the saliva of mothers, Saliva Check-Mutans kit.(GC Corporation, Tokyo, Japan) was used. After a thorough mouth rinse with water, mothers were asked to chew on paraffin and stimulated whole saliva samples were collected in sterile special cups over a period of 5 minutes, and immediately processed as per the manufacturer's instructions. (Using a very specific immuno-chromatography process, the test strip in this kit contains 2 monoclonal antibodies that selectively detect only the *Streptococcus mutans* species with no contamination from other bacteria.) A positive result is shown by a red line in the test window in 15 minutes indicating that the mother has *Streptococcus mutans* levels of ≥ 5 x 105 colony forming units per ml (cfu/ml) saliva.

Mothers with **('Decayed, Missing, Filled Tooth' in permanent dentition) DMFT score** ≥ 2.

For children:

Children with ECC and/or children having any two of the following criteria were selected[20,21] : (1) child having visible plaque on teeth, (2) presence of incisors and at least 2 molars, (3) child having one or more white spot lesions or enamel defects, (4) child having ('decayed, missing, filled surface' of primary teeth) dmfs score ≥ 3, (4) Child having sugar-containing snacks more than 3 times between their meals.

Exclusion criteria

1. Medically compromised mother/ child or both.
2. Mother wearing any removable/fixed prosthesis.
3. Mother wearing any orthodontic appliance.

Identification of *Streptococcus mutans* strains in dental plaque: Dental plaque samples were collected from 60 mother-child pairs. Dental plaque samples were collected from both mother and child. They were instructed not to brush or clean their teeth till the sample collection was done. Dental plaque samples were collected from mother and child before breakfast between 8 am and 9 am using autoclaved wooden toothpicks placed in inter-proximal surfaces and along the cervical margins of all teeth present.[3] At least 2-3mm of the toothpick tip had to be covered with dental plaque. In case of young uncooperative children, the child was made to lie down in a lap-to-lap position for the collection of sample.

The samples of pooled dental plaque were transferred to sterile Eppendorf tubes containing 1ml of buffer solution. All the samples were transported to the laboratory immediately for genotyping using arbitrarily primed PCR technique.[22,23] The samples were stored at -20°C until processed for DNA isolation and downstream application. Specific primers to detect the presence of *Streptococcus mutans* c, e, f and k strains were designed, synthesized and validated on PCR.

Statistical Analysis

The data collected was statistically analyzed by Chi Square Goodness of Fit test and Independent Chi Square test, using IBM Corp. Released 2013.IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY:IBM Corp. A 'p value' of less than 0.05 was considered to be significant.

RESULTS

On comparison of the distribution of *Streptococcus mutans* strains in mothers, strain f was seen to be the highest (61.7%). Among the strains, a significantly lower percentage of mothers (33.3%) had *Streptococcus mutans* strain e. (p=0.01)(Table 1) Whereas, in children the *Streptococcus mutans* strain c was the highest (55%) and followed by *Streptococcus mutans* strain f (53%). A significantly lower percentage of children (33.3%) had strain e (p=0.01) (Table 2). The percentage of mother-child pairs with identical distribution of *Streptococcus mutans* strains was highest for strains c and f (46.7%) and significantly lower for strain e (30%) (p=0.002) (Table 3). On further analysis, it was found that in 9 mother-child pairs, *Streptococcus mutans* strain f was present only in the mothers. Also, in 5 mother-child pairs, *Streptococcus mutans* strain c and in 4 mother-child pairs *Streptococcus mutans* strain k were present only in the children. (Table 4) Using Independent

Chi Square test, comparison of distribution of different strains of *Streptococcus mutans* between mother and child did not show any significant difference.(Table 5)

Table 1: Comparison of distribution of S.mutans strains in mothers (Chi square Goodness of Fit test)

S mutans Strain	Present n(%)	Absent n(%)	X^2 value	p value
c	31(51.7)	29(48.3)	0.067	0.80
f	37(61.7)	23(38.3)	3.267	0.07
e	20(33.3)	40(66.7)	6.667	0.01*
k	28(46.7)	32(53.3)	0.267	0.61

Table 2: Comparison of distribution of S.mutans strains in children (Chi square Goodness of Fit test)

S mutans Strain	Present n(%)	Absent n(%)	X^2 value	p value
c	33(55.0)	27(45.0)	0.600	0.44
f	32(53.3)	28(46.7)	0.267	0.61
e	20(33.3)	40(66.7)	6.667	0.01*
k	31(51.7)	29(48.3)	0.067	0.80

Table 3: Number of mother-child pairs with identical distribution of S mutans strains (Chi Square Goodness of Fit test)

S mutans Strain	Identical n(%)	Non-identical n(%)	X^2	p value
c	28(46.7)	32(53.3)	0.267	0.61
f	28(46.7)	32(53.3)	0.267	0.61
e	18(30.0)	42(70.0)	9.600	0.002*
k	27(45.0)	33(45.0)	0.600	0.44

Table 4: Distribution of *S mutans* strains in mother-child pairs

S mutans Strain	Mother-child pairs		
	Present in both mother and child n(%)	Present in mother only n(%)	Present in child only n(%)
c	28(46.7)	3(5.0)	5(8.3)
f	28(46.7)	9(15.0)	3(5.0)
e	18(30.0)	2(3.3)	2(3.3)
k	27(45.0)	1(1.7)	4(6.7)

Table 5 : Comparison of distribution of different strains of S mutans between mother and child (Independent Chi Square test)

Strains	Category	Mother		Child		X^2	p value
		n	%	n	%		
c	Absent	31	51.7	33	55.0	0.134	0.71
	Present	29	48.3	27	45.0		
f	Absent	37	61.7	32	53.3	0.853	0.36
	Present	23	38.3	28	46.7		
e	Absent	20	33.3	20	33.3	0.000	1.00
	Present	40	66.7	40	66.7		
k	Absent	28	46.7	31	51.7	0.300	0.58
	Present	32	53.3	29	48.3		

DISCUSSION

High maternal salivary levels of *Streptococcus mutans* was found to be a significant factor for colonization of *Streptococcus mutans* in Swedish pre-school children. The outcome of their study supported the concept of vertical transmission from mother to child.[24] There is scientific evidence of vertical transmission of *Streptococcus mutans* from mother to child as there was an association between *Streptococcus mutans* strains in mothers and their respective children.[25] In this study, initially saliva was collected only from the mothers in order to assess their salivary *Streptococcus mutans* levels using a simple chair side test. This was done to include only those mothers with high *Streptococcus mutans* levels.[18,19] Dietary sugar experience has been seen to influence the microbiological composition of dental plaque in children with ECC.[20] The initial stage of dental caries is highly affected by visible dental plaque on maxillary incisors. Extracellular insoluble polysaccharides, visible dental plaque and cariogenic microorganisms could predict dental caries development, partially explaining the pattern of ECC.[21] Therefore, these factors were considered only for purpose of inclusion of children with ECC in our study. *Streptococcus mutans*, a key cariogenic microorganism adheres to the biofilm present on tooth surfaces. Dental plaque samples were collected from both mother and child in order to identify the distribution of *Streptococcus mutans strains* present in mother-child pairs. An epidemiological survey suggested that serotype c strains were most prevalent in dental plaques of Japanese children.[26] The most common serotype isolated was serotype c from almost all individuals who developed caries.[27]

There is a genetic diversity of *Streptococcus mutans* in preschool children being associated with dental caries.[28] Therefore, the present study was carried out to investigate the distribution of *Streptococcus mutans* strains c, e, f and k in the dental plaque samples collected from mother-child pairs of children having ECC.

Recent advances in molecular diagnostics for Mutans Streptococci strain identification include multilocus sequence typing[29] ; chromosomal DNA fingerprinting[30] ; pulsed-field gel electrophoresis **(PFGE)**[31,32] and arbitrarily primed-polymerase chain reaction **(AP-PCR).**[33,34]

A PCR method used to determine serotypes of *Streptococcus mutans* in Japanese preschool children found serotype c to predominate (84.8%), followed by serotype e (13.3%), and rarely

serotype f (1.9%). [35] Since PCR is highly sensitive and more effective in detecting the target molecule, real time PCR was utilized in this study.

A systematic review and meta-analysis demonstrated evidence of vertical transmission of *Streptococcus mutans* from mother to child because there was an association between *Streptococcus mutans* in mothers and their respective children.[36] In our study, distribution of serotypes c, f and k of *Streptococcus mutans* was seen to match in 45 to 46.7% of mother-child pairs, probably indicating maternal or vertical transmission to be the source. In comparison to mothers, *Streptococcus mutans* strains c and k were seen to occur in more number of children.

Maternal transmission was found to be 33% in Japanese children in a day care setting, followed by paternal transmission(8%). There was evidence of horizontal transmission from playmates in 58% of the children.[37] The findings of the present study are similar to that of Kohler's study, wherein 50% of children had non-maternal strains.[38] Although Klein et al reported evidence of maternal transmission of *Streptococcus mutans* in 81% of children, strains that could not be identified as maternal were found in 75% of children.[39] In children with severe ECC, maternal transmission of Mutans Streptococci was seen in 41% of mother-child pairs. Majority of children(74%) had Mutans Streptococci genotypes that did not match the maternal strains.[40]

The results of this study implied that more than 50% of children received other serotypes of Mutans Streptococci or strains of *Streptococci mutans*, probably through horizontal transmission from non-maternal sources. Most of the children were enrolled in play homes and day-care centers, where-in they spent several hours in close proximity to other children as well as the care-givers. Sharing of snacks, spoons and toys is quite common in such places. Many care-givers lack awareness about the consequences of sharing of utensils and other play items. Other members in the family, including siblings could have been a source of horizontal transmission. There could also be other serotypes of Mutans Streptococci that were responsible for dental caries in these children.[8]

Our findings are similar to a previous report that indicated *Streptococcus mutans* may be transmitted horizontally between children during the initial phases of colonization in nursery environments.[23]

A longitudinal study showed an increase in genotypic diversity of *Streptococcus mutans* in the oral cavity during a follow-up period. *Streptococcus mutans* and *Streptococcus sobrinus* acquired from maternal or alternative sources may show effective persistence in the oral cavity and/or transitory detection in the children's mouths, reflecting the continuous development of oral microbiota in children.[39] In our study, a diverse population of *Streptococcus mutans* appears to have been acquired at a relatively early age (18-36 months) through both vertical and horizontal routes of transmission.

The clinical significance of identifying the *Streptococcus mutans* strains is important because the virulence of the microorganisms is varied. The virulence affects the rate of dental caries development, being more or less aggressive.[36] Knowledge of genotype diversity of *Streptococcus mutans* could help in the development of new preventive and treatment strategies in combating dental caries.[41] The focus of caries prevention has to shift from mothers to all individuals and settings that are associated with children and should begin at an early age.

In the present study, samples were not collected from the children at the time of initial acquisition. Therefore, the original, maternally acquired strains could have been substituted with newer strains. It is also equally possible that the strains detected were the original strains acquired initially by the child, from a non-maternal source. Since less than 50% of mother-child pairs in our study showed identical strains of *Streptococcus mutans*, future investigations including more diverse strains of *Streptococcus mutans* and other serotypes of Mutans Streptococci could be done.

CONCLUSION

In mothers, *Streptococcus mutans* strain f was seen to be the highest (61.7%) and in children, *Streptococcus mutans* strain c was the highest (55%).

The percentage of mother-child pairs with identical distribution of *Streptococcus mutans* strains was highest for strains c and f (46.7%)

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