

Quantification of Plaque *Streptococcus mutans* in 18-36 Months Old Children Following Application of Fluoride Varnish with Xylitol Coated Calcium Phosphate in Mother-Child Pairs

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ABSTRACT

Aim: Dental caries is a chronic infectious disease, with vertical transmission of *Streptococcus mutans* as one of the major risk factors for early childhood caries (ECC). Caries preventive measures can be applied to mothers and their children. This study aimed to assess the levels of *Streptococcus mutans* in dental plaque of children following application of a sodium fluoride varnish with xylitol coated calcium phosphate (CXP™) in mother-child pairs.

Materials and methods: Children aged 18-36 months with ECC were selected. Only those children with mothers having high levels of *Streptococcus mutans* in saliva were included. Sixty mother-child pairs were divided into 3 equal groups. A single application of Embrace™ varnish was carried out in only mother (Group 1) or only child (Group 2) or both mother and child (Group 3). Using PCR technique, the quantification of *Streptococcus mutans* in dental plaque of all the children was done at baseline and at 6 months following varnish application. Data was statistically analyzed using Wilcoxon signed Rank test to compare the mean *Streptococcus mutans* levels between baseline and following 6 months in each group.

Results: A significant reduction in *Streptococcus mutans* levels was seen in Group 2 from $1.19 \pm 1.21 \times 10^7$ cfu/ml to $0.29 \pm 5.76 \times 10^7$ cfu/ml ($p < 0.001$) and in Group 3 from $9.21 \pm 1.36 \times 10^7$ cfu/ml to $1.71 \pm 1.65 \times 10^7$ cfu/ml. ($p < 0.001$).

Conclusion: In mother-child pairs, the application of a fluoride varnish with xylitol coated calcium phosphate (CXP™) to only child and both mother-child was beneficial in reducing *Streptococcus mutans* in dental plaque of the children.

Keywords: Early childhood caries, Dental plaque, *Streptococcus mutans*, Varnish, Fluoride, Xylitol, Calcium phosphate

INTRODUCTION

Dental caries is a common chronic infectious transmissible disease resulting from tooth-adherent specific bacteria, primarily mutans streptococci (MS). *Streptococcus mutans*, a key cariogenic microorganism adheres to the biofilm present on tooth surfaces. There is scientific evidence of vertical transmission of *Streptococcus mutans* from mother to child [1,2]. An association between *Streptococcus mutans* strains in mothers and their children has been found [3,4]. Mothers with salivary levels of *Streptococcus mutans* greater than 10^6 organisms per ml. of saliva have a greater than 50% rate of transmission of the bacteria to their 10 to 16 months old children [3]. Extracellular insoluble polysaccharides, visible dental plaque and cariogenic microorganisms can predict dental caries development, partially explaining the pattern of early childhood caries (ECC) [5]. Development of interventional strategies to prevent or delay the transmission of these microbes can reduce the prevalence of early childhood caries. The American Academy of Pediatric Dentistry recommends the use of professional topical fluoride treatment every 3 months for high risk infants and

children as a caries management protocol [6]. Fluoride varnish is safe and easy to apply, requires very little patient co-operation and is well accepted by parents and children. Earlier studies examined fluoride varnish efficacy in the permanent teeth of school aged-children [7,8]. Reports on the efficacy of fluoride varnish applied to children have been contradictory. Fluoride varnish has been shown to significantly reduce *Streptococcus mutans* count in dental

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plaque and saliva of school children [9,10]. A 2 year community program with semi-annual fluoride varnish applications did not seem to significantly influence the oral microflora in preschool children [11].

A systematic review on sodium fluoride varnish, specifically for ECC concluded that fluoride varnish may be effective to decrease the incidence of dental caries in preschoolers [12]. Fluoride varnishes may be a better alternative to fluoride gels as they are less likely to be swallowed by young children. In comparison to other topical fluoride vehicles, fluoride varnishes have advantages in terms of safety, ease of application and set on contact with intra oral moisture. In addition, the application of fluoride varnishes can be tailored to children who have clinical evidence of high caries attack, such as those with early childhood caries [13]. Caries incidence was found to be lower in preschool children who in addition to caregiver counseling received fluoride varnish application once a year or twice in a year [7]. Simultaneous application of preventive methods in mothers and children could be more beneficial, rather than individual use in either mother or child only. Hence the present study was undertaken to quantify *Streptococcus mutans* levels in dental plaque of children, following application of a fluoride varnish with CXP™ in mother-child pairs.

METHODOLOGY

The study protocol was presented to the Institutional Ethics Review Board and approval was obtained to conduct the study. Written prior permission to carry out the present study was taken from authorities of nurseries and crèches in the city of Bangalore, India. Prior to the study, nature of the study was explained to the parents and their written informed consent was taken to participate in the study. Mother and child pairs were selected. Normal, healthy children aged 18-36 months along with their mothers were screened for the study purpose.

Inclusion criteria for mother

Mothers with high salivary *Streptococcus mutans* levels (>1 million cfu/ml of saliva) [14,15].

Exclusion criteria for mother

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

Samples of unstimulated saliva of the mother were collected in a sterile calibrated vessel for a period of 5 min. The samples were collected in the morning 2 h following breakfasts. The collected salivary samples were transported immediately to the laboratory for quantification of *Streptococcus mutans* using Real time PCR [16].

Inclusion criteria for child (any two) [6]

- (1) Child having visible plaque on teeth.
- (2) Presence of incisors and at least 2 molars.
- (3) Child having dmfs ≥ 3 .
- (4) Child having sugar-containing snacks more than 3 times between their meals.
- (5) Child using a bottle containing natural or added sugar while sleeping.
- (6) Child having one or more white spot lesions or enamel defects.

Sixty mother-child pairs formed the study group. The mother-child pairs were then divided into 3 groups, consisting of 20 pairs each as follows:

Group 1: Only mother received application of Embrace™ varnish

Group 2: Only child received application of Embrace™ varnish

Group 3: Both mother and child received application of Embrace™ varnish.

Initially, dental plaque samples were collected from all the children to assess baseline level of *Streptococcus mutans*. The dental plaque samples were taken from the child before breakfast between 8 AM to 9 AM by means of autoclaved wooden tooth picks passed into interproximal regions and along the cervical margins of all teeth present [10]. The collected samples were transferred to autoclaved eppendorf tubes containing 1 ml of saline and were transported immediately to the laboratory for quantification of *Streptococcus mutans* using Real time PCR [16].

Application of fluoride varnish

A fluoride varnish, Embrace™ varnish PULPDENT™ Corporation, Massachusetts and United States of America, which contains 5% sodium fluoride with additional xylitol coated calcium phosphate (CXP™) was used. Application of varnish to all the surfaces of teeth was done between 10 AM and 11 AM with the help of a small brush after drying the teeth. For children, application of varnish was done in lap to lap position [17]. They were advised not to drink or eat for at least 60 min and not to brush their teeth till the next morning.

Mother-child pairs of all the groups were instructed to follow oral hygiene practices till the end of the study period.

At the end of 6 months, dental plaque samples were again collected from all the children and processed as described earlier and assessed for levels of *Streptococcus mutans* using Real time PCR [7].

The data obtained was subjected to statistical analysis using Wilcoxon signed Rank test to compare the mean *Streptococcus mutans* levels between baseline and at 6 months follow-up period in each group (intra-group). The level of significance was set at $p < 0.05$. Kruskal Wallis Test followed by Mann Whitney Post hoc Analysis was used to compare the mean *Streptococcus mutans* levels between the

3 groups at baseline and 6 months follow-up period (inter-group).

RESULTS

Table 1 gives the mean levels of *Streptococcus mutans* present in dental plaque of children at baseline and at 6 months. In Group 1, where-in only mothers received Embrace™ varnish application; there was an increase in the *Streptococcus mutans* levels of their children from 0.93×10^7 cfu/ml at baseline to 1.67×10^7 cfu/ml at 6 months. In Group 2, where only children received Embrace™ varnish application, the *Streptococcus mutans* levels at the end of 6

months showed a highly significant reduction from $1.19 \pm 1.21 \times 10^7$ cfu/ml to $0.29 \pm 5.76 \times 10^7$ cfu/ml ($p < 0.001$). In Group 3, where-in both mothers and children received the fluoride varnish application, the *Streptococcus mutans* levels in dental plaque of their children significantly reduced from 9.21×10^7 cfu/ml at baseline to 1.71×10^7 cfu/ml at 6 months ($p = 0.001$).

Kruskwal Wallis test found a significant difference in *Streptococcus mutans* levels between the three groups at the end of 6 months ($p < 0.001$) (**Table 2**). Post hoc test found the reduction in *Streptococcus mutans* levels to be significant between Group 2 and Group 3 ($p = 0.009$) (**Table 3**).

Table 1. Comparison of *Streptococcus mutans* levels in dental plaque between baseline and 6 months in each group (Wilcoxon Signed Rank test).

Groups	Time	Levels of <i>Streptococci mutans</i>		p value
		Mean $\pm$ SD (cfu/ml $\times 10^7$)	Difference (cfu/ml $\times 10^7$)	
Group 1	Baseline	0.93 ± 2.03	-0.74	0.033*
	6 months	1.67 ± 1.82		
Group 2	Baseline	1.19 ± 1.21	0.90	<0.001*
	6 months	0.29 ± 5.76		
Group 3	Baseline	9.21 ± 1.36	7.5	0.001*
	6 months	1.71 ± 1.65		

* $p < 0.05$ is significant

Table 2. Intergroup comparison of *Streptococcus mutans* levels following 6 months (Kruskwal-Wallis test).

Groups	Chi Square value	p value
Group I vs. group II vs. group III	21.861	<0.001*

* $p < 0.05$ is significant

Table 3. Comparison of reduction in *Streptococcus mutans* levels between Group I and Group II (Post-hoc test).

Groups	p value
Group I vs. Group II	0.009*

* $p < 0.05$ is significant

DISCUSSION

Mutant's streptococci can colonize the mouths of infants via vertical as well as horizontal transmission. Mothers with higher levels of salivary *Streptococcus mutans* are at a higher risk of infecting their infants early in life [18]. In mother-and-child pairs, the maternal salivary levels of *Streptococcus mutans* and *Streptococcus sobrinus* were significantly related to MS colonization in plaque as well as dental caries in their children at 2.5 years of age [19]. Thus, determination of maternal levels of both organisms has been proposed to be an efficient method to indicate the risks of maternal transmission of MS and childhood dental caries.

It has been demonstrated that a reduction of MS in the saliva of mothers results in delayed acquisition of MS in children [19-21]. In most studies, preventive measures have been applied to mothers only, with an aim to reduce infection in infants and children. However, studies are lacking on the efficacy of methods applied to mother-child pairs. Therefore, mother-child pairs in which mothers with high salivary *Streptococcus mutans* levels and having children with ECC were selected.

Fluoride varnishes have been used at concentrations of 1% and 5% in the prevention of ECC. The varnish used in our study exhibited high viscosity that facilitated easy

application and allowed it to remain on the teeth surfaces. This allows for high concentration of fluoride to be in contact with tooth enamel and enter bacterial cells and inhibit various cellular processes. In acidic conditions, fluoride acts on the mineral phases of the tooth as well as reduces the acid tolerance of bacteria. Low levels of fluoride can cause complete arrest of glycolysis by *Streptococcus mutans* [22]. A continuous and sustainable effect can be achieved with periodic topical fluoride applications and could reduce the microbial levels in children at high risk for caries.

Apart from sodium fluoride, Embrace™ varnish contains xylitol coated calcium phosphate (CXP™). A disadvantage of adding these salts to fluoride ions in varnishes is the formation of poorly soluble calcium fluoride phosphate phases during storage and can lead to a decrease in the number of bioavailable fluoride ions. On contact with saliva, the xylitol coating dissolves and allows the calcium and phosphate ions, to continuously react with fluoride ions to form protective Fluor apatite on the teeth. Xylitol, a naturally occurring five-carbon sugar polyol cannot be fermented by oral bacteria. *Streptococcus mutans* incorporates xylitol into the cell and converts it to xylitol-5-phosphate through the phosphoenolpyruvate phosphotransferase system, resulting in the development of intracellular vacuoles and cell membrane degradation [23]. *Streptococcus mutans* then dephosphorylates xylitol-5-phosphate and expels this dephosphorylated molecule from the cell, consuming energy leading to bacterial starvation and inhibition of growth [24]. Adhesion of these microorganisms to the tooth surface and their acid production potential are also reduced [25,26]. The xylitol coating also imparts an acceptable taste and helps to gain compliance during application.

Fluoride varnishes containing calcium and inorganic phosphate have shown measurable release of calcium and fluoride ions [27]. A comparative study on fluoride varnishes found Embrace™ with CXP™ to have greater cumulative fluoride release but low substantively, indicating its use in patients with high caries risk and requiring more frequent follow-up or reapplication [28]. Embrace™ varnish with CXP™ has been shown to release ten times more fluoride over a 4 h period than other fluoride varnishes [28]. Therefore, this mechanism can be beneficial in infants and children with oral environments that are exposed to repeated acidic challenges.

Fluoride application can have inhibitory effects on the virulence factors and composition of the cariogenic biofilm formation [29,30]. In the present study, single application of Embrace™ varnish with CXP™ to both mother and child in mother-child pairs showed significant reduction at 6 months in levels of plaque *Streptococcus mutans* in the child. However, this reduction was not as highly significant as compared to the reduction seen following varnish

application only to the child. These findings indicate that vertical transmission from mothers may have made only a minor contribution to their child's *Streptococcus mutans* levels and the children could have acquired *Streptococcus mutans* from other sources via horizontal transmission. Also, varnish application only to the mother, appeared to have no beneficial effect and there was an increase in *Streptococcus mutans* levels of their child. Therefore, preventive measures targeting only maternal transmission is not adequate and there is a need for application of these measures to other individuals that are closely associated with the child.

Pre-school children frequently receive affectionate kisses from family members and care takers. These children are extremely susceptible to bacterial colonization. The sharing of utensils, toys and playing in close proximity with others is a common practice. The outcome of this study draws attention to protecting children in nurseries and crèches, who may be at risk of microbial acquisition and transfer from one child to another. Annual or semi-annual application of fluoride varnish with CXP™ can be beneficial in children with early childhood caries. Involving parents, educating care givers and implementing preventive measures from an early age can help in reducing dental caries in children in the community.

CONCLUSION

In mother-child pairs, application of a fluoride varnish with xylitol coated calcium phosphate to only child and both mother-child significantly reduced *Streptococcus mutans* in dental plaque of the child.

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