

## ORIGINAL ARTICLE

# The Correlation between Salivary Mutans *Streptococcus* Count and Dental Caries Experience among Preschool Children and their Parents

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## ABSTRACT

**Introduction:** *Streptococcus mutans* plays a significant role in causation of caries. Transmission of *S. mutans* can be vertical or horizontal from mother to child and mainly occur during the first 2.5 years of age. In this article, we have emphasized on the transmission of *S. mutans* from parents to their 18 months and 3-year-old children. The aim of this study was to find out correlation between salivary mutans *Streptococcus* count and dental caries experience among preschool children and their parents.

**Materials and Methods:** The study comprised randomly selected 50 father-mother-child pairs who were residing at Gandhi Nagar, a village in Indore district. Of 50 children, 25 were of 18 months and the other 25 were of 36 months of age. At early morning, the saliva samples of each pair were obtained before their brushing. The parents were interviewed in accordance with a structured questionnaire. The vital statistics of children and their parents were recorded. The clinical examination of children and their parents was performed to record the caries (DEFS or DMFS scores) and plaque score (Silness and Loe plaque index). The number of salivary *S. mutans* was evaluated in all parents and children.

**Results:** The overall caries experience among the children was 40%. Positive correlation between the high maternal *S. mutans* level and its colonization in children was observed in 91.3%. The association of caries experience in 18-month-old

children did not show statistical significance with the salivary *S. mutans* levels and caries experience in parents. The salivary *S. mutans* levels in 36-month-old children significantly correlated with the salivary *S. mutans* levels in parents. However, its correlation was statistically not significant with the caries experience in both parents.

**Conclusion:** The association between dental caries and salivary *S. mutans* in mother-child and father-child was evaluated in the present study. Both mother and father can transmit the disease to their children.

**Keywords:** Correlation, dental caries, Mutans *Streptococcus*, Parents, Preschool children

**How to cite this article:** Katiyar A, Nayak UA, Mishra N, Dolas A, Verma R, Goel T. The Correlation between Salivary Mutans *Streptococcus* Count and Dental Caries Experience among Preschool Children and their Parents. Int J Oral Care Res 2018;6(2):S64-68.

**Source of support:** Nil

**Conflicts of interest:** None

## INTRODUCTION

Around 700 species are found in the oral cavity, but not all are present in the same mouth.<sup>[1]</sup> Most of these are harmless, but some causes infection such as dental caries and periodontal pathosis.<sup>[2]</sup> *Streptococcus mutans* is the most important organism to cause initiation of caries. This is due to its ability to ferment sucrose to lactic acid and survive in low pH which is required for demineralization of the tooth.<sup>[3]</sup> *S. mutans* and *Streptococcus sobrinus* have a central role in the etiology of dental caries because these can adhere to the enamel salivary pellicle and other plaque bacteria.<sup>[4-6]</sup>

## Aims and Objectives

The aims of this study were as follows:

1. To estimate the salivary *S. mutans* colonization in 18-month-old and 36-month-old children.
2. To evaluate a possible relationship of the salivary *S. mutans* colonization between the children and both their parents.
3. To evaluate the relationship between salivary *S. mutans* count and dental caries in children and also in their parents.

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## MATERIALS AND METHODS

### The Armamentarium used for the Study

The armamentarium used for the study was as follows:

Culture media: Mitis salivarius dehydrated agar - 90 g, 1% potassium tellurite, bacitracin - 0.2 units/ml, 40% D - glucose, and distilled water - 1 l.

The study comprised randomly selected 50 father-mother-child pairs who were residing at Gandhi Nagar, a village in Indore district. Of 50 children, 25 were of 18 months and the other 25 were of 36 months of age.

### Procedure

At early morning, the saliva samples of each pair were obtained before their brushing. The parents were interviewed in accordance with a structured questionnaire. The medical history, use of antibiotics, existing oral hygiene routines, dietary habits, the use of feeding bottles, and evening meals all were included in the study. The vital statistics of children and their parents were recorded. The clinical examination of children and their parents was performed to record the caries (DEFS or DMFS scores) and plaque score (Silness and Loe plaque index). The number of salivary *S. mutans* was evaluated in all parents and children.

### Microbial Sampling, Cultivation, and Evaluation

#### Collection of saliva

An 18-month-old and 36-month-old children and their parents were asked to chew a piece of sterile paraffin wax for 1 min. Expectorate was then collected in the sterilized container and sent to the microbiology laboratory immediately. No transport medium was used as culturing was done within ½ h of the collection of samples. The samples were then subjected to microbiological analysis.

#### Agar plating

About 90 g of Mitis salivarius dehydrated agar was added to 1 l of distilled water and boiled on a Bunsen burner to dissolve completely. This solution was sterilized by autoclaving for 15 min at 15 pounds per square inch at 121°C. After autoclaving, the solution

was allowed to cool at 50°C. To this, 20 g of sucrose per 100 ml was added. Potassium tellurite and bacitracin were added to give a final concentration of 0.1 mg/ml and 0.2 units/ml, respectively. It was mixed well and poured into sterile Petri dish and allowed to solidify.

A test tube was taken filled with 5 ml normal saline. 50 µl of expectorated saliva was transferred to the test tube (containing 5 ml of normal saline). The solution was mixed thoroughly and 50 µl of this solution was transferred into the culture media plate. This was placed in an anaerobic jar for incubation.

### Incubation and Counting

The anaerobic jar was incubated at 37°C for 48 h in the presence of 5% carbon dioxide and 95% of nitrogen. The number of colony-forming units (CFUs) on a predetermined area was counted manually with the help of magnifying lens. The colonies of *S. mutans* were identified as round or spherical, raised, convex, black in color, ranging from a pinpoint to pinhead size with a rough surface [Figure 1]. The colony count of each agar plate was recorded and the mean CFUs per milliliter were determined after multiplying the colony count of each plate with its respective dilution factor.<sup>[7]</sup>

## RESULTS

The data obtained were subjected for statistical analysis for the tabulation of results.

Among the total mothers (50), six mothers had low salivary *S. mutans* colonization. 44/50 mothers had high salivary *S. mutans* colonization. Among the total fathers (50), four fathers had low salivary *S. mutans* colonization. 46/50 fathers had high salivary *S. mutans* colonization [Table 1].

Among the children who had early childhood caries (ECC), 100% (20/20) of them had high salivary *S. mutans* levels. Among the children who were caries free, 80% (24/30) of them had higher salivary *S. mutans* level. However, this difference was statistically non-significant ( $P = 0.069$ ) [Table 2].

Univariate analysis of variables associated with oral salivary *S. mutans* colonization in children was tabulated. Positive correlation between the high maternal *S. mutans* level and its colonization in children was

**Table 1:** Salivary *S. mutans* levels in children and parents

Group	n (number)	Salivary <i>S. mutans</i> levels		
		Low<10 <sup>4</sup>	Medium 10 <sup>4</sup> -0 <sup>5</sup>	High>10 <sup>5</sup>
Mother	50	6	0	44
Father	50	4	0	46
18-month-old children	25	1	2	22
36-month-old children	25	3	0	22

*S. mutans*: *Streptococcus mutans*

observed in 91.3%. The positive correlation also exists between the high paternal *S. mutans* level and *S. mutans* colonization in children (91.3%). 100% of the children showing negative salivary *S. mutans* colonization had high paternal salivary *S. mutans* level. However, the difference between the two groups was not significant [Table 3].

A 36-month-old children ( $n = 15$ ) had significantly more ECC than the 18-month-old children ( $n = 5$ ) ( $P = 0.01$ ). The median DEFS score in 18-month-old

children was 0, with the interquartile range of 0.0 and that of 36-month-old children was 1 with the interquartile range of 0 and 1 [Table 4].

The salivary *S. mutans* levels in 18-month-old children significantly correlated with the salivary *S. mutans* levels in both parents and caries experience in fathers [Table 5].

## DISCUSSION

In the present study, the children with ECC showed higher salivary *S. mutans* level than the caries-free group ( $P = 0.069$ ). The data support previous study done by Haneen Al Shukairy, Najlaa Alamoudi, Najat Farsi, Abdullah Al Mushayt, and Ibrahim Masoud who reported high salivary *S. mutans* level of children with ECC as compared to caries-free children (80% and 16.7%, respectively).<sup>[8]</sup> The most critical time for *S. mutans* colonization have been suggested to occur between 19 and 31 months, a period designated as “the window of infectivity” with a median age of 26 months, the time of the emergence of primary molars.<sup>[9]</sup> In the present study, even the 18-month-old children showed high levels of salivary *S. mutans* colonization. According to Karn *et al.*, evidence of MS colonization was seen as early as 10 months of age. For children 12 months old or younger, 25% had shown detectable levels of MS; in the 15-month age group, 60% were colonized, whereas in the reported study, 96% of the 18-month-old and 88% of 36-month-old children showed positive *S. mutans* colonization. However, lower prevalence of *S. mutans* in 18-month-old and 36-month-old children has been reported in the literature.<sup>[10-15]</sup> A study showed that *S. mutans* colonization increases with increasing age so that by 24 months of age, 84% harbored the bacteria.<sup>[16]</sup> In the given study,

**Table 2:** Relationship between salivary *S. mutans* levels and caries status in children

Caries status	<i>S. mutans</i> levels in children		Total (%)
	<10 <sup>5</sup> CFU (%)	≥ 10 <sup>5</sup> CFU (%)	
ECC	0 (0)	20 (100)	20 (100)
Caries free	6 (20)	24 (80)	46 (100)
Total	6 (12)	44 (88)	50 (100)
<i>P</i>	0.069		

*S. mutans*: *Streptococcus mutans*, ECC: Early childhood caries, CFUs: Colony-forming units

**Table 3:** Univariate analysis of variables associated with oral salivary *S. mutans* colonization in young preschool children

Variables	Salivary <i>S. mutans</i> colonization in children		
	Yes <i>n</i> =46%	No <i>n</i> =4	<i>P</i>
High maternal salivary <i>S. mutans</i> level	42 (91.3)	2 (50)	0.066
High paternal salivary <i>S. mutans</i> level	42 (91.3)	4 (100)	1.000
Night feeding	40 (87.0)	4 (100)	0.446
Addition of sugar to bottle	14 (30.4)	0 (0)	0.317
Brushing in a day	44 (95.7)	4 (100)	1.000
Sweets in between meal	12 (26.1)	4 (100)	0.560

*S. mutans*: *Streptococcus mutans*

**Table 4:** Relationship of ECC, DEFS, plaque score, and salivary *S. mutans* between 18-month-old and 36-month-old children

Age of children in months	<i>n</i>	Factors			
		Number of children with ECC	DEFS	Plaque score	Salivary <i>S. mutans</i> count
18	25	5	0 (0.0)	0.03 (0.005, 0.136)	12353 (552, 19,232)
36	25	15	1 (0.1)	0.025 (0.010, 0.050)	4640 (680, 26,784)
<i>P</i>		0.01	0.01	0.434	0.522

\*Using Mann–Whitney U-test, *S. mutans*: *Streptococcus mutans*, ECC: Early childhood caries

**Table 5:** Correlation of salivary *S. mutans* levels and caries experience of parents and children

Variables	<i>S. mutans</i> levels in 18 months	Caries experience in 18 months	<i>S. mutans</i> levels in 36 months	Caries experience in 36 months
<i>S. mutans</i> levels in mothers	0.733 <i>P</i> <0.001	-0.323 <i>P</i> =0.229	0.564 <i>P</i> =0.003	0.085 <i>P</i> =0.685
<i>S. mutans</i> levels in fathers	0.641 <i>P</i> =0.001	-0.373 <i>P</i> =0.066	0.422 <i>P</i> =0.036	-0.213 <i>P</i> =0.306
Caries experience in mothers	0.3 <i>P</i> =0.146	0.202 0.334	-0.094 <i>P</i> =0.653	0.278 <i>P</i> =0.178
Caries experience in fathers	0.433 <i>P</i> =0.031	0.067 <i>P</i> =0.075	0.206 <i>P</i> =0.323	0.338 <i>P</i> =0.099

*S. mutans*: *Streptococcus mutans*

*S. mutans* colonization was observed to be more in 18-month-old children rather than in 36-month-old children.<sup>[17,18]</sup> In this study, *S. mutans* was detected in 24 of 25 children of 18 months old.<sup>[19,20]</sup> A longitudinal investigation done by Carlsson *et al.*<sup>[21]</sup> reported that MS were detected in 5 of 25 (20%) infants 12 months–16 months old. These organisms were not detected in any of the 25 subjects during their 1<sup>st</sup> year of life. Berkowitz *et al.* reported that MS were detected in 9 of 40 (22%) infants who had only primary incisor teeth.<sup>[22]</sup> Although in certain other studies, mothers are recognized as the main source of *S. mutans* transmission.<sup>[23-26]</sup>

Bacterial and host factors have been related to the transmission, including high number of *S. mutans* in maternal saliva, frequent salivary contacts after the eruption of teeth, the presence of non-shedding hard surfaces in the recipient, and the availability of substrate for the *S. mutans* to thrive once attached.<sup>[27-29]</sup> The evidence for this concept comes from several clinical studies in which *S. mutans* strains isolated from mothers and their babies exhibited similar bacteriocin profile and an identical chromosomal pattern.<sup>[29,30,31,32]</sup>

Sugito *et al.* found no correlation between breastfeeding and the severity of caries.<sup>[33-36]</sup> Their findings correspond with the study done by Iida *et al.* He found no correlation between the duration of breastfeeding and ECC.<sup>[37]</sup> Sweetened condensed milk contains sucrose, which is the most cariogenic form of sugar. While formula milk which contains lactose is less cariogenic, formula milk which contains sucrose is more cariogenic.<sup>[38]</sup>

Breastfeeding can prevent ECC.<sup>[36,39]</sup> Correlation between breastfeeding frequency and using bottle until the child falls asleep with the severity of ECC is still a controversy.<sup>[40,41]</sup> According to Nanda *et al.*, mutans streptococci CFUs in caries-active subjects were higher in both saliva and plaque samples as compared to that found in caries-free subjects.<sup>[42]</sup> Mutans streptococci play an important role in ECC formation in preschool children and low socioeconomic status.<sup>[43]</sup>

## CONCLUSION

- The overall caries experience among the children was 40%. The caries experience among 18-month-old and 36-month-old children was 20% and 60%, respectively.
- The overall caries experience among the mothers and fathers was 92% and 88%, respectively. 91% of mothers and fathers in whom salivary *S. mutans* were present, had caries.
- The caries experience among 18-month-old and 36-month-old children was 20% and 60%, respectively. DEFS score of 36-month-old children was significantly more than that of 18-month-old children.

- The salivary *S. mutans* levels in 18-month-old and 36-month-old children significantly correlated with *S. mutans* levels in parents.
- Positive correlation was also observed between the high maternal or paternal salivary *S. mutans* levels and its colonization in children. However, it was statistically not significant.

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