

# Acquisition Of Streptococcus Mutans in Oral Cavity Of Infants In Pre dentate Period And Its Association With Mode Of Delivery- An Original Research

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## Abstract

**Background:** Early initial acquisition of *S.mutans* has become particularly relevant as a predictive marker of future caries activity in primary and permanent dentition as it is seen that *S.mutans* colonization may occur prior to tooth eruption.

**Aim:** This study aims at determining and comparing the presence of *S.mutans* in preterm infants born through Normal delivery and Caesarean section and to correlate their counts at birth and 3 months.

**Methodology:** A total of 154 infants fulfilling the inclusion exclusion criteria were selected and designated as sample. After collecting both the saliva samples using a sterile swab stick from the dorsum of tongue and vestibular mucosa at the age of  $\leq 1$  hour and 3 months, the data was tabulated and was subjected to statistical analysis using Wilcoxon signed-rank test and Mann Whitney U test.

**Results and Observations:** It was found that 3.27% infants born through normal delivery and 1.63% caesarean-born infants acquired *S.mutans* at 0 days of life which increased to 28% of infants born through normal delivery and 13.12% infants born through Caesarean section at 3 months of age.

**Conclusion:** *S.mutans* acquisition in preterm infants is yet to be established, hence this field needs many more research work to determine the presence of *S.mutans* in preterm period, timing of acquisition and its association with dental caries in future, if any, to plan and facilitate the preventive regimen for dental caries for future.

**Keywords:** *S.mutans*, Preterm, Dental caries, Normal delivery, Caesarean section

## Introduction

Dental caries can be considered as a disease of modern times as the occurrence of caries seems to be much higher in the last few generations. It affects both sexes, all races, all socioeconomic strata and every age group, though some people may be more prone to it than others<sup>1</sup>.

Birth is the first environmental event that conditions the allogeneic succession in the oral cavity. In the uterus, the fetus is free from microorganisms and the mouth is sterile. Oral colonization begins when the baby passes through the birth canal and by interaction with the outside world.<sup>2</sup> The establishment of *S.mutans* in the oral cavity of human beings is always being emphasized because they are the principal bacteria responsible for caries. Mouth is an ideal environment for the growth of microorganisms, because it is warm and moist and has constant influx of nutrients through saliva and food intake.<sup>3</sup>

The major virulence factor of *S.mutans* which is important in colonization is their ability to adhere to host surfaces which involves sucrose-independent initial adherence, followed by sucrose dependent cellular accumulation.<sup>4</sup>

Hence, the present study was based on the hypothesis that *S.mutans* colonization may occur prior to tooth eruption. This study was undertaken to assess the counts of *S.mutans* in preterm infants and to determine the effect of

mode of delivery on acquisition of oral *S.mutans* in preterm infants.

## Methods

Ethical clearance was obtained from college ethical committee (SDDHDC/IEC/Mar2014/S11-Annexure-I) and a written consent was taken from the Government multi- specialty hospital authorities, Sector-16, Chandigarh and a private nursing home situated in Panchkula city, prior to commencement of the study.

Also, a written consent was obtained from all the registered full term expecting mothers in the respective hospitals and their husbands for sample collection from their child's mouth.

## Eligibility Criteria

1. Newborn preterm infants  $\leq 1$  hour after birth through normal delivery
2. Newborn preterm infants  $\leq 1$  hour after birth through Caesarean- section.
3. Infants born without any complications like fetal distress, placenta previa, umbilical cord prolapse or compression at the time of delivery.
4. Infants born without natal, neonatal and precocious eruption of teeth.

A total of one hundred and fifty four (154) infants fulfilling the eligibility criteria were

selected and designated as sample and were divided into 2 groups of 77 infants each according to mode of delivery:-

Group I - Infants born through Normal delivery

Group II- Infants born through Caesarean-section

Media preparation was done according to manufacturer's instructions. The saliva sample of infants was collected using a sterile swab stick from the dorsum of tongue and vestibular mucosa within 1 hour of the birth in the presence of a supervisor. The collected salivary sample was transferred into the autoclaved test tube containing tryptone soya broth followed by closing the lid of the test tube and sealing with micropore tape and then it was labeled using a permanent marker. The test tubes were then transported to the microbiological laboratory within 4 hours, where *S.mutans* detection and estimation was done by using serial dilution method. Confirmation of *S.mutans* was done by Gram Staining, Catalase test and **HiStrep™ Identification Kit**. The second sample was collected, estimated and confirmed with the same method at 3 months of age.

At 3 months, salivary sample of 16 infants born through normal delivery and 12 infants born through Caesarean-section could not be obtained. Hence, *S.mutans* counts from 61 infants were used for data analysis after 3 months. After collecting, both the samples at the age of  $\leq 1$  hour and 3 months, the data was tabulated and was subjected to statistical analysis using Wilcoxon signed-rank test and Mann Whitney U test.

### Results & Observations

While comparing the percentage of infants detected with *S.mutans* in both the groups at two intervals (**Graph 1**), it was seen that at 0 day (within 1 hour after birth), *S.mutans* was detected in 3.27% (2/61) of infants in Group-I (infants born through normal delivery) and 1.63% (1/61) of infants in Group-II (infants born through caesarean section). The percentage of infants with detectable *S.mutans* significantly increased from 0 day to 3 months of age. Percentage of infants with detectable *S.mutans* in Group-I was

28% whereas in Group-II was 13.12%. Thereby, the result revealed that, at 3 months of age a total of 25 out of 122 infants were detected with *S.mutans* which was only present in 3 infants at 0 day (within 1 hour after birth).

The number of *S.mutans* colonies ranged from  $0.00001 \times 10^3$  -  $0.3819 \times 10^3$  in both groups at two different time intervals as shown in **Graph - 2**. At 0 day (within 1 hour after birth), *S.mutans* was detected in Group-I with a mean CFU level of  $0.0006 \times 10^3$  which increased to  $0.3819 \times 10^3$  after 3 months. While in Group-II, mean CFU level of *S.mutans* was  $0.00001 \times 10^3$  at 0 day (within 1 hour after birth), and  $0.0951 \times 10^3$  after 3 months. It was observed in the present study that *S.mutans* counts increase was higher in Group-I i.e. infants born through normal delivery than Group-II i.e. infants born through Caesarean section.

**Graph-3** shows the mean change observed in *S.mutans* colony counts in both groups was between  $0.0951 \times 10^3$  -  $0.3813 \times 10^3$  after 3 months. It was observed that, the changes in mean CFU counts in Group-I was  $0.3813 \times 10^3$  and in Group-II it was  $0.0951 \times 10^3$  which was significant at  $<0.001$  level of significance in Group-I and = 0.012 level of significance in Group-II, when Mann-Whitney U Test was applied. Therefore, it was seen that changes in *S.mutans* counts were higher in Group-I than Group-II.

### Discussion

*S. mutans* is the main causative bacterium of human dental caries and plays an important role in etiology of early childhood caries. Moreover, it is crucial in its initial phase. The initiation of dental caries is preceded by the colonization of dentition by *S. mutans*, usually in early childhood. The early establishment of these bacteria in the mouth of human infants is mainly dependent on the maternal transmission of saliva.<sup>5</sup>

The development and progression of dental caries depend on the presence of cariogenic micro-organisms to metabolise fermentable carbohydrates and produce organic acids that in turn demineralise dental hard tissues (Seow, 1998)<sup>6</sup>.

For paediatric dentists, it is very important to know the changes of the oral microbiota relative to pre-dentate to dentate phase, from breastfeeding or bottlefeeding to eating phase. In particular, the transition of mutans streptococci has received much focus because this change is considered a predictor of future dental caries.<sup>7</sup>

The oral cavity of infant is usually 'germ-free' at the time of birth.<sup>3</sup> Rapid contamination of external and internal surfaces occurs when the fetus is exposed to the flora of the birth canal and to the external environment, especially through contact with the mother and with contaminated milk and water.<sup>8</sup>

Hence, the present study was conducted with the aim and objective to determine the presence of *S.mutans* in newborn predentate infants and to compare the counts of *S.mutans* in the infants born through Normal delivery and Caesarean section at two time intervals i.e. 1 hour after birth and 3 months of age.

The initial study sample size was 154 which was statistically calculated by using the formula by **Fleiss (1980)**.<sup>9</sup> 28 infants were not available after 3 months due to migration of their families. Hence, for data analysis 122 infants (61 in each group) were taken up after 3 months. The number of participating subjects was in close agreement with the number of selected subjects by **Wan et al (2001)**<sup>10</sup>, **Li et al (2005)**<sup>11</sup> in their studies to assess the oral colonization of *S.mutans* in predentate infants.

In the present study, the saliva sample was taken from newborn infants within 1 hour after birth. Saliva sample collection was done at different intervals i.e. at baseline (within 1 hour after birth) and after 3 months. In **2001**, **Wan et al 10** undertook a similar study where they examined newborn infants at birth and collected their salivary samples and repeated the sample after 3 months. **Thakur et al (2012)**<sup>3</sup> observed in their study the correlation between mode of delivery and other associating factors with colonization of oral *S.mutans* in infants. They followed the newborn infants for a period of 1-year after every 3 months. They demonstrated the possible influence of prolonged bottle feeding, socioeconomic status and tasting of food by the mothers on the initial acquisition of *S.mutans* in the oral cavity of infants and concluded that the causes for initial acquisition of oral *S.mutans* in

infants were postnatal factors like feeding and oral hygiene practices. In the present study, for confirmation of *S.mutans*, various biochemical tests were performed like Gram staining, catalase test, VP test, esculin hydrolysis and sugar fermentation tests. For sugar fermentation, VP and esculin hydrolysis tests, **HiStrep™ Identification Kit** (Himedia Laboratories Pvt.Ltd., Mumbai, India) was used. **Duailbe S et al (2007)**<sup>12</sup> and **Patil S et al (2010)**<sup>13</sup> performed Gram staining to confirm the growth of *S.mutans* whereas catalase test and mannitol test was used for the presumptive identification of bacterial isolates by **Duailbe S et al (2007)**<sup>12</sup> in their studies.

In the present study, it was found that 3.27% infants born through normal delivery and 1.63% caesarean-born infants acquired *S.mutans* at 0 days of life which increased to 28% of infants born through normal delivery and 13.12% infants born through Caesarean section at 3 months of age. **Wan et al, 2001**,<sup>10</sup> undertook a study to examine *S.mutans* colonization in 3-month-old predentate infants and showed that *S.mutans* could be isolated as early as 3 months of age in 30% of infants. **Isenberg et al (1988)**<sup>14</sup> reported that Caesarean-born children had significantly decreased numbers of bacterial species and colony-forming units of conjunctivae than did vaginally delivered children, suggesting that, by avoiding passage through the birth canal, C-section infants may be less likely to be exposed to various bacterial species and strains from the mothers.

**Caufield and coworkers (1993)**<sup>15</sup> reported that 25% of their infant population (N=46) acquired MS by 19 months of age. Extrapolations of their data from a figure depicting the cumulative probability of MS acquisition as a function of age indicated that approximately 5% of their study population acquired these organisms by approximately 9 months of age. Approximately 15% of the subjects were colonized by approximately 12 months of age.

The oral colonization of *S.mutans* in saliva from infants born with different modes of delivery was investigated. The findings suggested an increased prevalence of *S.mutans* among infants delivered vaginally compared with those delivered by C- section, which are in accordance with the study by **Barfod et al, 2011**,<sup>16</sup>

These results are in accordance with the studies done by **Wan et al, (2001) 10** who detected *S.mutans* in preterm infants as early as 3 months of age and **Merglova V, 2011, 8** which detected *S.mutans* in newborn infants of 2 days of age.

The fact that early colonization of total microbes in the oral cavity will not influence the colonization of *S.mutans*, which colonize later, suggests that difference in the incidence of *S.mutans* infection of children delivered through different modes is not correlated, which is in accordance with the study by **Wan et al, 2001, 10** and **Thakur et al, 2012. 3**.

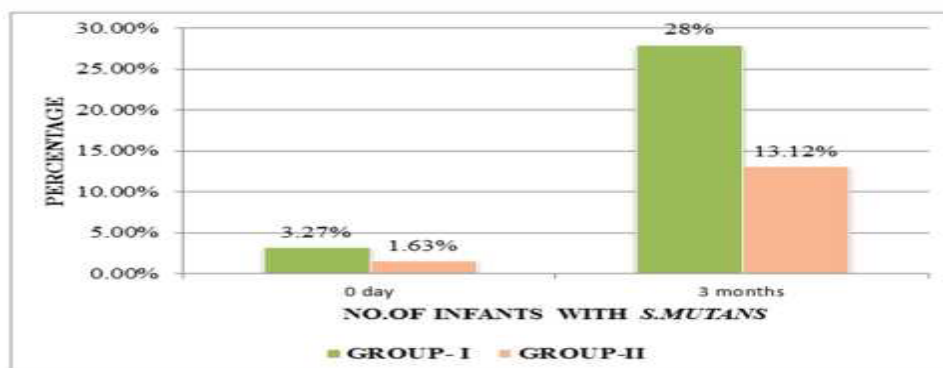
However, in the present study, there has been a significant increase in *S.mutans* counts in both the groups after 3 months which further calls for **FIGURES**

more research work in future to find out preventive regimen for caries association with *S.mutans*.

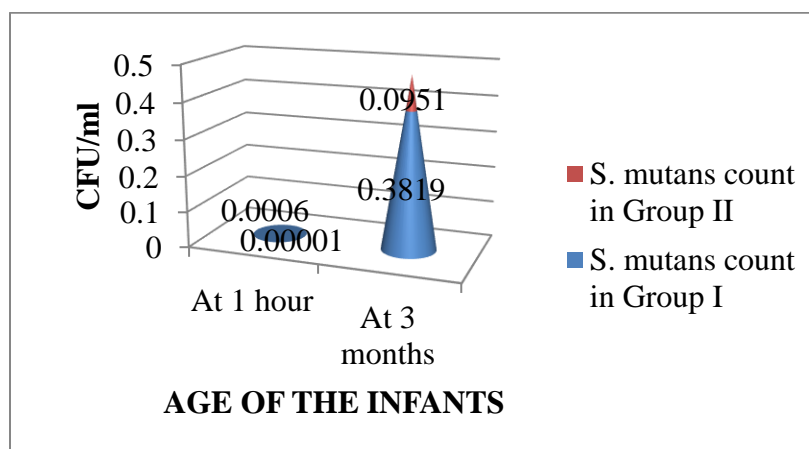
### Conclusion

Based on the results of present study, following conclusions can be drawn:

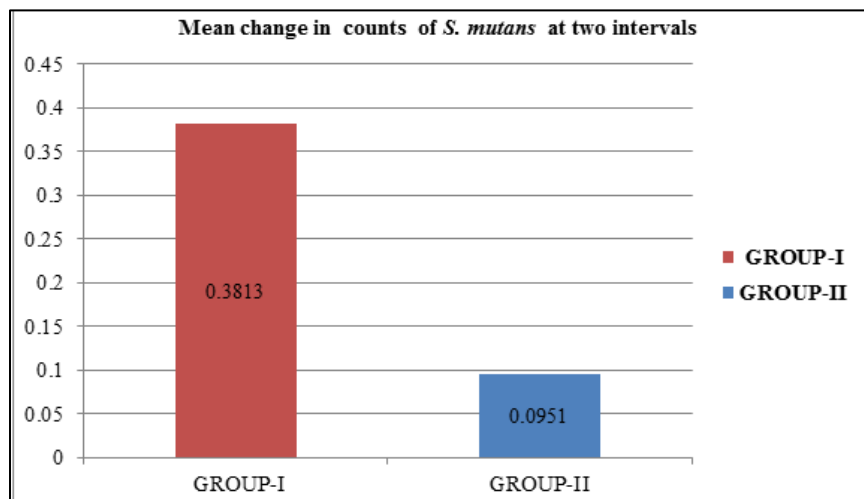
- 1) *S.mutans* was found in a small proportion of infants at birth, which indicates the vertical transmission of the bacterium from mothers to infants.
- 2) *S.mutans* colony counts increased from 0 to 3 months of age.
- 3) No significant association was found between mode of delivery and colony counts of *S. mutans* in infants.



**Graph – 1: Bar diagram comparing percentage of infants in both groups with detectable *S.mutans* at 0 day and 3 months intervals**



**Graph -2: The mean CFU of *S.mutans* in both the groups at 1 hour and 3 months**



**Graph-3: Bar diagram showing mean change in *S. mutans* counts in both groups at two intervals**

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