ORIGINAL RESEARCH

Evaluation of Mode of Delivery and Various Postnatal Factors on Acquisition of Oral *Streptococcus mutans* in Infants: A Prospective Study

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Abstract

Aim of the study: The objective of the current study is to assess and establish a relationship between the mode of delivery and postnatal factors in the early colonization of *Streptococcus mutans* (*S. mutans*) in infants' oral cavities.

Materials and methods: The primary goal of the investigation is to assess and compare the oral microflora of newborns immediately after birth and at 3, 6, 9, and 12 months of age in babies born by normal vaginal delivery and lower segment cesarean section and divided into (group III) and (group V), respectively. Around 50 mother–baby pairs in total had their swab samples collected for the identification of *S. mutans* and were monitored for a year. The role of other postnatal factors in the acquisition of *S. mutans* in infants was also evaluated.

Results: Data analysis showed that different postnatal factors like feeding patterns, oral hygiene practices, and socioeconomic factors affected the infant's oral cavity's initial colonization by *S. mutans*.

Conclusion: Infants' first exposure to oral *S. mutans* depends on the delivery method and various postnatal factors.

Keywords: Breastfeeding, Breastfeeding duration, Delivery mode, Dental caries, Feeding methods, Pre- and postnatal infants, Saliva, Saliva sample, Socioeconomic status, *Streptococcus mutans*.

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Introduction

Dental caries is caused by mutans streptococci which is infectious and transmissible. Dental caries takes place because of the acids produced by the bacteria when acting on the fermented carbohydrates in the oral cavity.[1–3] Mother's breast milk has inhabiting factors (immunoglobulin, antibiotics, etc.) to prevent *Streptococcus mutans* (*S. mutans*) colonization in the mouth. This is mainly because the mammary glands are an integral part of the humoral and cellular immune system.[4] The method of delivery has a general impact on the colonization of *S. mutans* as well, compared to reasonably aseptically delivered cesarean babies, vaginally delivered newborns come into contact with more and more intensely with perineum bacteria. However, research has shown that the style of delivery did not significantly affect the prevalence of *S. mutans* in infants.[5]

Another factor that influences the accretion of *S. mutans* is the infant's surroundings which cause oral microflora, generally from the mother as she is the first person that would come in contact.[6] The mouth of predentate children possesses only a mucosal surface that can be exposed to saliva flow. The *S. mutans* persists in the mucosal environment and forms colonies that either adhere to the mucosa or remain free in the salivary flow. The primary method of spreading the microorganism to children in that age-group is vertical transmission.[7]

Tradition, culture, and condition of the mother during pregnancy are also important factors for the transmission of *S. mutans*. The postnatal factors affecting multiple sclerosis (MS) transmission from mother to infant are complications at the time of delivery because of exposure to antibiotics, gestational period of birthweight of infants, and frequency of inoculation. Thus, mother who shares drinks, foods, toothbrushes, utensils, and other items with their kids have the maximum risk of transmission of MS to their children.[8] With their social contacts outside the family, children are more likely to contract MS from nonfamilial guardians and other family members.[9] Compared to children whose moms have low levels of salivary MS, children whose mothers have high concentrations of MS acquire the germs at earlier ages and in greater quantities. Around >50% of moms who have salivary levels of *S. mutans* higher than 106 organisms/mL of saliva transmit the germs to their 10–16-month-old children, compared to 30% of mothers who have just 103 organisms/mL of saliva.[10] The formation of anti-MS immunoglobulins may be induced by the beneficial effects of early MS transmission to the newborn due to natural immunization.[11–13]

The prenatal and postnatal factors of mothers' *S. mutans* level, low infant birth weight, various feeding patterns, early tooth emergence in the oral cavity, poor oral hygiene, education, and socioeconomic conditions will influence the babies' early development of *S. mutans*.[14] The main objective of this study is to

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evaluate the effects of the delivery mode and postnatal factors on *S. mutans* early accumulation in the infant's oral cavity.

Materials and Methods

The descriptive prospective cohort investigations were carried out by the Department of Pedodontics, Rajasthan Dental College & Hospital, Jaipur, Rajasthan. The aim of the current research was to evaluate and determine the consequences of the mode of delivery and postnatal factors on the *S. mutans* early accumulation in an infant's oral cavity. Data was collected from the infants at different time intervals from the selected infant sampling size.

Abhishek Hospital, Jaipur, granted written permission after receiving ethical clearance from the relevant institute committee.

Sample Size

A minimum of 41 newborn babies with their mothers are required for a sample size, which is further enhanced to 50 newborns with their mothers. The final sample size is expected to be 20% dropout or attrition. The sample size has a 95% confidence level.

The formula used for the calculation of the sample is,

$$n = \frac{\left(z_{a/2}\right)^2 \bar{p}(1-\bar{p})}{E^2}$$

Inclusion Criteria

Mothers who delivered in the hospital by either normal or cesarean delivery and willing to participate in the study.

Exclusion Criteria

Mothers with preterm babies (<37 weeks of gestational age), mothers using antibiotics in the last three months before delivery, babies with systemic illness, and the parents without consent.

Collection of Samples

From mother—saliva sample from the dorsum of the tongue.

From the newborn—the saliva sample either from the dorsum of the tongue or from the alveolar ridge using sterile swabs. The swabs were immediately placed in vials containing 0.5 mL of phosphate-buffed saline and were placed on ice containers and transported within 2 hours to the lab.

Preparation of Mitis Salivaris Agar

Mitis salivaris agar is prepared with 1% potassium tellurite to segregate streptococcus from highly contaminated specimens like feces and exudates from body cavities, etc. Casein enzymic hydrolysate and peptic digest of animal tissue in the medium provide the essential growth nutrients.

To prepare agar medium, Mitis salivaris agar base (HIMEDIA M259) powder weight of 90.07 gm was added in 1000 mL of distilled water and heated up to its boiling point for uniform dispersion. This was autoclaved for 15 minutes at 121°C and 15 lbs of pressure. After cooling down to 45–50°C, 1% potassium tellurite solution, 1 mL of sterile, and 0.5 mL of bacitracin were added to the above solution. Further, itraconazole tablets were added to prevent fungal growth. The mixture was combined and then added to sterile petri dishes.

Method of Sample Inoculation

The above sample was vortexed for 30 seconds to disperse bacteria from cotton swabs. Around 20 µL of the sample was taken, to which 2 mL phosphate-buffered saline was further added to increase dilution up to 100 times. Around 20 mL of media was taken in the falcon tube and added with 200 µL of the diluted sample. The sample was mixed in media properly and poured into the plate (pour plate method). Plates were placed in the desiccators and 5% carbon dioxide was maintained in it using a candle jar. The desiccator was placed in the incubator at 37°C. After 48 hours, colonies were counted that appeared on the plate using a digital colony counter.

Colony Count

Following incubating, the colonies of *S. mutans* were identified as either round or spherical, highly convex, raised, with color varying from dark blue to black, and pinpoint to pinhead size with a rough surface. Identification of streptococcus mutans was confirmed with a Gram-stained smear showing gram-positive cocci in the chain. Colonies of different morphology or with doubtful characteristics were excluded. The colony count was carried out by making use of a digital colony counter and was stated as colony forming unit/mL of saliva which served as baseline data/recording for future comparison.

Colonies were Counted Using the Formula

$$CFU/mL = \frac{\text{Number of colonies obtained} \times \text{Dilution Factor}}{\text{Volume of sample inoculated}}$$

Statistical Analysis

Unpaired "*t*-test," one-way analysis of variance test, and Pearson correlation coefficient will be used for the analysis of linear variables while the Chi-squared test and Fisher exact test will be used for the analysis of nominal categorical variables. The p-value < 0.05 will be taken significantly. Medical 16.4 version software will be used for all statistical calculations.

Results

The mean maternal age at the time of delivery was 29 (age range between 24 and 35 years). The average weight of infants at the time of birth was (2.5–3.5 kg). For 44 (88%) of the infants, the mothers were the primary caregivers, and the remaining five (12%) infants, siblings, maids, or grandparents were the secondary caretakers. There were nine (42.9%) females and 12 (57.1%) males in group V compared to 19 (65.5%) females and 10 (34.5%) males in group III. Evaluating the effects on *S. mutans* colonization of other newborn and maternal factors that have been noted. The variations in the incidence of *S. mutans* infections in kids according to gender male six (30.00%) and female 13 (52.00%) were not statistically significant $\chi^2 = 2.204$, $p = 0.224$.

At first, none of the samples from newborns tested positive for *S. mutans*. However, at 3 months, *S. mutans* was present in three (11.54%) of 26 newborns in group III and four (21.05%) of 19 newborns in group V. By the end of 6 months, 12 (46.15%) of the 26 newborns in group III had *S. mutans*, compared to five (26.32%) of the 19 newborns in group V, and by the end of 9 months, 12 (46.15%) of the 26 newborns in group III had *S. mutans*, compared to six (31.57%) of the 19 newborns in group V. *S. mutans* was found in 13 (50%) of the 26 newborns in group III and six (31.57%) of the 19 newborns in group V by the monitoring period end, or 12 months. the variation in *S. mutans* incidence.

Children in groups III and V were not significantly infected at 3, 6, 9, or 12 months. Weaning (W) is the process of adding complementary foods to breast and bottle feeding after the age of 6 months. At the 6 months, 11 (40.74%) out of 27 infants, and six (33.33%) out of 18 for mixed-fed infants ($\chi^2 = 0.252$, $p = 0.757$) were nonsignificant.

By the end of 9 months, nine breastfed (33.33%) of 27 infants and nine (50.00%) mixed-fed out of 18, were found to be acquitted with *S. mutans* ($\chi^2 = 1.25$, $p = 0.355$), and nonsignificant. At 12 months, eight (29.63%) were breastfed 27 infants and 11 (61.11%) were prolonged mixed fed out of 18 infants. From the aforementioned, it was determined that at 12 months it was statistically nonsignificant ($\chi^2 = 4.388$, $p = 0.064$), whereas the incidence of *S. mutans* colonization was statistically noteworthy for various socioeconomic statuses (SES), which was assessed according to the occupation of the fathers and mothers. By the end of the study, it was found that 10 of 16 infants from low SES, nine of 24 from middle SES, and zero of five from higher classes were *S. mutans* positive ($\chi^2 = 6.57$, $p = 0.037$). For the first 3 months, the association between feeding habits and acquiring *S. mutans* in the breastfed (BR) group was seen in five out of 27 infants (20.83%) and in mixed feed 12 out of 18, (66.67%), that is breast and bottle feeding (BR + mix) with $\chi^2 = 0.451$, $p = 0.684$. This was seen to be nonsignificant. The relationship between the microbial level and other risk factors for general and oral health, and oral hygiene practices was not seen to be statistically significant $\chi^2 = 0.353$, $p = 0.74$. *S. mutans* is present in 16 (39.02%) out of 41 infants and absent in three (75%) out of four. Therefore, the finding that *S. mutans* was present in the oral cavity was not statistically significant ($\chi^2 = 1.934$, $p = 0.295$). If a mother tastes the food before feeding the baby to see its temperature and taste, that will affect the formation of *S. mutans*. Tasting of food by mothers 16 (53.33% out of 30 infants present and for the absent three (20.00%) out of 15 ($\chi^2 = 4.555$, $p = 0.054$). Hence, here it is proved to be statistically significant. Bottle-taking to bed is a common practice. Bottle to bed was seen in nine (60.00%) out of 15 and not seen in 10 (33.33%) infants out of 30, here ($\chi^2 = 2.915$, $p = 0.116$) were not strongly connected to *S. mutans* early colonization of young children's oral cavities.

Discussion

Oral health encompasses much more than healthy teeth. Severe caries can have a significant impact on children's quality of life due to pain and distress and may result in acute and chronic infectious diseases, thus affecting their childhood.[15]

Various maternal variables were studied to understand the early accumulation of *S. mutans* in vaginal birth and cesarean delivery, to ascertain the infant's oral *S. mutans*. A total of 50 newborn children were identified out of which 21 (12 males and nine females) infants were vaginally born and 39 (10 males and 19 females).

Child-nursing practices that enable saliva transfer from parents to children were also substantially linked with *S. mutans* colonization.[16] Literature suggests that mothers and other close relatives who share meals, beverages, utensils, toothbrushes, as well as other objects with their infants face the most significant risk of passing *S. mutans* to their offspring. Mother is regarded as a significant source of *S. mutans* transmitter due to close proximity to the infant during the first 2 years. Close contact such as kissing the child's mouth or fingers, sharing a meal or utensils, and immunological issues may all play a role.

The observation of the current research suggests that 38.1 and 27.6% of the mothers maintained the oral hygiene status of their children in groups V and C, respectively. While exploring the SES of children's families, it reflects more than half of the families belong to the middle class in both delivery groups. Around 10–14% of the families were in high class in both the delivery groups. The findings are similar to the study conducted by Thakur et al.[4] and Sharma P et al.[17]

The current research is compared to the initial acquisition of *S. mutans* in infants and other key variables, including gender, feeding method, bottle to feed, testing of food, oral hygiene practice, SES, and type of contact.

A statistically significant ($p < 0.05$) difference was observed between the presence of *S. mutans* and the type of feeding method (breastfeeding and mixed feed). A statistically significant ($p < 0.05$) difference in *S. mutans* count was reported in children who were both breast and bottle-fed or exclusively breastfed.[17] Breastfeeding may provide an initial barrier against early childhood caries (ECC) due to various reasons like its immunomodulatory features, diverse microbiota, human casein, and secretory immunoglobulin A, which operate as a barrier to pathogen *S mutans*.[18] It has other health benefits of lowering the risk of respiratory and gastrointestinal.

We did not observe a statistically significant difference between *S. mutans* and the bottle-to-bed feeding method in the present study. The study results reflect that the consumption of sugar–sweetened beverages may not be the primary factor responsible for the early colonization of *S. mutans*. Parental influence has a more significant impact on children's oral health.[19]

The family's SES appeared to play an essential role in the early colonization of microorganisms.[18,20] The relationship between ECC and SES is well established.[21] The literature demonstrates that *S. mutans* levels are a substantial risk factor for ECC.[22] ECC is more prevalent in economically disadvantaged children, particularly those of uneducated mothers.[21]

In the present study, we found a statistically nonsignificant ($p > 0.05$) difference between *S. mutans* and the type of contact with children. However, we observed a statistically significant ($p < 0.05$) difference between the presence of *S. mutans* and the testing of children's food by mothers. The study findings are in agreement with other research conducted in the past.[21] On the contrary, the study observations were found to be dissimilar to research conducted by Pattanaporn et al.[19]

Maternal microbes infect the newborn's mouth at birth. The average age at which American children become colonized with *S. mutans* is 26 months. It happens between the ages of 14.9 and 18 months in Latin America. Various studies suggest that *S. mutans* can colonize the oral cavity before tooth eruption. That means that *S. mutans* colonization prevention should begin far before tooth eruption.

In the present study, we evaluate the association of feeding practices (breastfeeding and mixed feeding) with the accumulation of *S. mutans* in the oral cavity of infants at each consecutive visit (3, 6, 9, and 12 months). The study findings suggest no significant ($p > 0.05$) difference between the feeding practices and infants' age. The observation of our study is supported by research conducted by Thakur et al.[4] On the contrary, a study conducted by Wan et al.[23] observed a statistically significant difference between *S. mutans* count with infants age.

Primarily, this research shows that *S. mutans* colonization in newborns is connected with various characteristics, including feeding methods, SES, and food testing for children. The present cross-sectional, descriptive study attempted to explore the limited risk factors associated with early childhood *S. mutans* colonization.

Future studies with larger sample sizes and covering a wider geographic area can explore other risk factors responsible for early childhood *S. mutans* acquisition.

Summary and Conclusion

It is established that the mothers are the main source of bacterial transfer to the infants as per the studies.[18,24,25] This assumption made sense because mothers typically have close contact with their newborns for the first 2 years of life when *S. mutans* are very active and will be transferred for the first time.[26,27] This can be avoided by spreading the awareness of the mothers through instructing parents on how to feed and care for their babies teeth and also by improvising their SES.[28–30]

S. mutans early establishment has improper feeding practices as one of its major contributing factors. Immunoglobulins, antibodies, and other substances found in human breast milk harbor *S. mutans*. According to studies, prolonged bottle feeding of bovine milk and sucrose causes intermittent milk pooling on tooth surfaces, which will lead to the early establishment of *S. mutans* in the oral cavity.

The family's SES appeared to play an essential role in maintaining the oral health of an infant[31] in the early colonization of microorganisms. The relationship between ECC and SES is well established as *S. mutans* levels are a substantial risk factor for ECC. [32]

Primarily, this research shows that *S. mutans* colonization in newborns is connected with various factors including feeding methods, SES, and food testing for children. The present study attempted to explore the limited risk factors associated with early childhood *S. mutans* colonization.

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