Colonization Levels of *Streptococcus Mutans* between Mother and Infant: A Postnatal Prospective Cohort Study

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***Objective**: To investigate the possible association between maternal S. mutans levels and those of the infant during the period between birth and 5 months and evaluate possible risk factors in the S mutans colonization.* ***Study Design**: A prospective cohort study was carried out comprising 62 infants and their mothers, selected at the time of childbirth. For each infant, a sample swab was taken at 0, 15, 30, 90, and 150 days postpartum; on the same days, a sample was obtained from the mothers. TYCSB medium was employed for identifying the microorganism, which was later confirmed by Gram staining, the catalase activity test, and the API strep test.* ***Results**: The final total sample consisted of 60 infants, from which S. mutans was detected in only 2 (3%) at the 150th day of oral sample collection. Of the sample of 60 mothers, 54 exhibited colonization levels.* ***Conclusions**: In the studied sample pairs up to 150 days, it was not possible to demonstrate the presence of a direct relationship between maternal S. mutans oral levels.*

***Keywords**: Colonization levels; Streptococcus mutans; cohort study*

INTRODUCTION

Streptococcus mutans is the most important pathogenic microorganism for initiating dental caries. Colonization of the oral cavity may occur at an early age, even in abundance, depending on the presence and intensity of promoting factors that favor its transmission and settlement in the oral cavity.[1,2] Among the different transmission routes present during the first years of life, the vertical one—from colonized mother to child, through direct contact between both—exhibits the highest relevance for the clinician in pediatric dentistry.[3–5] This pattern of transmission involves close contact, whether by breast feeding, sharing of food and utensils, or the transplacental pass during birth; further, this vertical pattern may be associated with other factors such as low socioeconomic status, poor oral hygiene in the mother, abundance of *S. mutans,* presence of open carious lesions in the maternal oral cavity, narrow interaction with other family members, early solid food consumption, inadequate use of the bottle, or frequent intake of sugary drinks or snacks by the child.[6–8] Data from previous studies have demonstrated that mothers with dense *S. mutans* saliva reservoirs are at high risk for transmitting the infectious agent to their infants; furthermore, it was proved that reducing these high maternal levels of *S. mutans* may inhibit or delay colonization of the microorganism in the oral cavity of their children.[9,10]

It has been commonly believed that oral colonization of *S. mutans* may be initiated after the eruption of the first primary teeth, at around age 6 months.[11] Carlsson *et al* [12] reported that *S. mutans* was detected in 5 of 25 12-to-16-month-old infants; besides, the microorganism could not be detected in any of the examined children during the first year of life. Pearce *et al*[13] and Caufield *et al* [11,14] demonstrated that only *S. oralis* and *S. salivarius* varieties colonized the oral cavity of infants in the first postnatal days, and both *S. sanguinis* and *S. mutans*, installed later, during the first and second years, respectively, after primary tooth eruption. However, other reports indicate that *S. mutans* may be located on the oral mucosa before dental eruption.[8,15–17] Taking into account that the mouth of predentate children possess only mucosal surfaces exposed to saliva flow, a theory has been proposed suggesting that *S. mutans* persists in that environment, forming colonies that either adhere to the mucosa or remain free in the salivary flow, multiplying at a velocity high enough to overcome the washing effect of one's own saliva.[9] On the other hand, some researchers have theorized that there is a time period after the infant's tooth eruption during which the greatest risk of acquiring *S. mutans* takes place and consequently of acquiring carious lesions in a "window of infectivity".[11] Understanding the mechanisms that determine the colonization as well as the identification of the various patterns of transmission of *S. mutans* is essential for comprehending the risk factors that initiate the caries process in preschool children.[8,18]

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It has been demonstrated that early colonization of *S. mutans* considerably increases the risk of initiating dental caries, even the development of carious lesions shortly after eruption of the primary teeth.[19] Therefore, it is important to determine the initial time of transmission and colonization of the microorganism, in order to adequately design a preventive management plan during the first months of life, and in some cases, even before gestation.

In this context, the aim of the present study was to investigate the possible association between maternal *S. mutans* levels and those of her infant between birth and age 5 months and to determine whether breast feeding, bottle feeding, mixed feeding (breast and bottle), intake of solid food, or number of siblings are risks factors in *S. mutans* colonization.

MATERIALS AND METHOD

A prospective cohort was carried out in three private maternity hospitals in the city of San Luis Potosi, Mexico. The study included pairs of Mexican mothers and their infants, of either gender and systemically healthy, recruited at birth. Signed informed consent was obtained from the mothers, and the study was approved by the institutional ethics committee. Sampling of the study group was made for convenience during 2009–2011. Infants having any orofacial abnormality; natal or neonatal teeth; receiving antibiotic or immunoglobulin therapy; or being fed by means of a nasogastric tube were excluded.

Each infant's mouth was examined by one experienced pediatric dentist. Details regarding demography, number of siblings, and medical conditions were obtained through personal interviews with each mother. At birth and during the study, the mothers registered the characteristics and evolution of the baby's feeding habits: breast feeding, bottle feeding (with formula or powdered milk), mixed feeding (breast and bottle), and beginning intake of solid food . The variable level of colonization by *S. mutans*, both in the mother and the infant, was categorized according to the number of colony-forming units per milliliter (CFU/mL) calculated in each member of the pair: no colonization, $\leq 2.25 \times 10^2$ CFU/mL; poor colonization, $>2.25 \times 10^2$ CFU/mL to $\leq 5 \times 10^3$ CFU/mL; moderate colonization, $>5 \times 10^3$ UFC/ml to $\leq 5 \times 10^5$; and high colonization, $>5 \times 10^5$ CFU/mL.

Sample collection

Samples for each mother-infant pair were taken at different times: at the moment of birth (day 0, at the hospital) and at 15, 30, 90, and 150 days (at each family's home). Sample collection was carried out by means of vigorous swabbing with a sterile nylon swab (Flocked Swab, Copan, Italy) from the infants' tongue, alveolar ridges, and oral mucosa and from the mothers' teeth (dento-bacterial plaque), tongue, and oral mucosa. Later, samples were placed into sterile tubes containing phosphate buffered saline (0.2 M, pH 7.2). In addition, each mother deposited 0.1 mL of saliva directly into another tube with the same transport medium. Each tube was sealed, labeled, and kept at 4°C for 2 hours until processing and analysis.

Microbiological processing

Samples were homogeneously vortexed at maximum speed for 30 seconds. Aliquots of 50 µL were extracted from each tube and inoculated onto tryptone-yeast extract-cysteine-sucrose-bacitracin (TYCSB) agar plates, a microbiological medium. Later, all samples were cultivated in triplicate and incubated under microaerophilic conditions for 48 to 72 hours at 37°C for bacterial growth. The number of CFU on each plate was enumerated to estimate *S. mutans*; bacterial identification was confirmation by means of Gram staining, catalase activity test, and commercially available biochemical analysis of API 20 Strep kit (BioMérieux Vitek, Marcy-I'Etoile, France). As the confirmatory quality control group, American Type Culture Collection (ATCC, Manassas, VA, USA) *S. mutans* strains was employed.

Table 1. Distribution of mothers according to *S. mutans* level colonization.

Level of colonization	Frequency (percent)
Mothers (N=60)	
High	23 (42.5)
Moderate	10 (16.5)
Poor	21 (38.8)
No colonization	6 (10.0)

Statistical analysis

Frequency patterns for each independent variable (maternal levels of *S. mutans*, type of feeding, and number of siblings) and their association with infant levels of *S. mutans* at different times (15, 30, 90, and 150 days) were established so that a logistic regression analysis could be applied to evaluate the interactions among the different variables in relation to the dependent variable, with a significance level α set at 0.05.

RESULTS

The total study group consisted of 62 mother-infant pairs, from which 2 pairs were eliminated due to their dropping out during the 5-month follow-up period. Of the 60 studied mothers, 54 (90%) exhibited detectable oral colonization by *S. mutans*, of which 23 (42.5%) showed high levels of colonization; 10 (16.5%), moderate levels; and 21, (38.8%) low levels; while the remaining 6 (10%) had undetectable levels of the microorganism (Table 1). On the other hand, only 2 (3%) of the total sample of 60 infants showed some level of colonization by *S. mutans*, classified in both cases as low; those babies had mothers with high levels of the microorganism that were detected during the 150-day examination when their mandibular primary incisors were partially erupted. The remaining babies exhibited undetectable levels of colonization during the various sampling times (0, 15, 30, 90, and 150 days) (Table 2).

Relative to the babies' feeding type, 6 (10%) were fed only by breast, 53 (88%) by mixed feeding, and 1 (2%) by bottle only. As to the number of siblings, 22 infants (37%) had no siblings, 28 (46.6%) had 1 sibling, and 10 (16%) had 2 or more siblings. Finally, in relation to the introduction of solid foods (ablactation), only 4 babies were ablacted (7%) during the entire study, while the other 56 (93%) did not receive solid food during the same period (Table 3). Additionally, 5 babies (8%) exhibited partial eruption of their mandibular primary central incisors at the time of the last examination at age 5 months. A direct association between maternal levels of *S. mutans* colonization and the other independent variables with their infants' levels could not be demonstrated ($P > 0.05$).

DISCUSSION

Currently, *S. mutans* is considered the main etiologic agent in dental caries. Early childhood caries, or nursing caries, is a variety of this highly destructive disease, which is characterized

Table 2. Distribution of children according to *S. mutans* colonization.

Time (days)	Colonization
0 (At hospital)	No colonization
15	No colonization
30	No colonization
90	No colonization
150	Poor (2 children)

No colonization, ≤2.25 x 10^2 CFU/mL; poor colonization, >2.25 x 10^2 CFU/mL to ≤5 x 10^3 CFU/mL; moderate colonization, >5 x 10^3 UFC/ml to ≤5 x 10^5; and high colonization, >5 x 10^5 CFU/mL.

Table 3. Description of characteristics within study group.

Characteristics	Frequency (percent)
Feeding type	
Breast	6 (10)
Mixed feeding	53 (88)
Bottle	1 (2)
Siblings	
None	22 (37)
One	28 (47)
Two or more	10 (16)
Type of feeding	
Solid food	4 (7)
Liquid	56 (93)

by demineralization and cavitation of most of the primary teeth, mainly the maxillary incisors and the maxillary and mandibular first molars.[20] In children with this type of dental caries, *S. mutans* can be so dominant that the plaque above the carious lesions consists almost entirely of this microorganism in relation to other pathogenic bacteria.[21] That is why one of the crucial indicators of caries risk and high scores of decayed, missing, and filled surfaces (DMF indexes) in young children is the early colonization of *S. mutans* on the primary teeth.[1]

It is very important for the dentist who treats young children to know the timing, besides identifying and understanding, of the determining factors in the transmission and initial colonization of S. mutans in the oral cavity so that the ideal time for starting, and later reinforcing, preventive and interceptive strategies can be established. There has been great interest in the past several decades in ascertaining how children become colonized with S. mutans and whether this colonization can be delayed or reduced to decrease their caries risk.[8] The exact mechanism in mother-to-infant vertical transmission of the microorganism during the first months of life is not clear; however, it has been suggested that intimate contact, like kissing the child's mouth or fingers, sharing food or utensils, and immunological issues are contributing factors.[22] This kind of transmission may be a factor in early S. mutans colonization even before primary tooth eruption.[8,15–17,23] Other studies have identified non-maternal and maternal mutans streptococci transmission.[24]

Identification of the source of transmission is necessary to improve preventive strategies for caries. According to the findings of the present study, we suggest that there is no direct association between *S. mutans* colonization in the maternal oral cavity and that of her infant until at least the first 150 days after childbirth. Our results indicate that only 2 of the 60 infants studied exhibited detectable numbers of the microorganism. These data—although not clinically or statistically significant—suggest that the variables considered as contributing factors in infant colonization (i.e., maternal colonization level, type of feeding, start of ablactation, and number of siblings) were not associated with infant *S. mutans* levels during the first 5 months of life. In another cross-sectional investigation, also on a Mexican sample, Revuelta and Díaz-Romero[25] included 71 mothers with their children between 4 and 24 months old. The authors chose as a cutoff for the high infectivity level of CFU to be >10^5. An association between high levels of mothers' *S. mutans* and high levels in their infants was found, although most of the infants already had some primary teeth; the infectivity window of *S. mutans* in children of that sample was situated around age 14.9 months, that is, when several teeth were already erupted and the type of feeding was different from that of our study group.

Likewise, Carletto *et al* [18] researched the transmission and genetic identity of S. mutans strains in 17 mother-infant Argentines with the aim of establishing whether the mother was the main infective source of the babies, whose age was around 18 months with some primary teeth present. They confirmed that vertical transmission is the main mechanism of transmitting the microorganism to children of that age group, based on the identical or closely comparable genetic similarity between the S. mutans strains of the pairs. Li and Caufield[26] reported that the two most important factors increasing the probability of vertical transmission of S. mutans are poor maternal oral hygiene and inadequate infant feeding habits. In this sense, Agarwal *et al* [27] reported significant differences in mother´s caries activity, high levels of S. mutans, educational level, socioeconomic status, frequency of maternal sugar consumption, and their child´s caries experience. Although we could not confirm these hypotheses or a direct association between maternal S. mutans levels and those of their babies, we nevertheless recommend the implementation of rigorous preventive programs of oral hygiene and nutrition for the mothers—even after the pregnancy period—to include the first professional dental examination of the infant during its first month of life.[28] Baca *et al*[29] analyzed possible horizontal transmission patterns of *S. mutans* among 6- to 7-year-old children by identifying genotypes and their diversity and relationship with caries, and the authors suggested a horizontal transmission.

Our technique of vigorously swabbing the tongue, alveolar ridges or teeth, and oral mucosa, employing rigid nylon swabs, was justified in order to yield the maximum quantity of *S. mutans*, as reported in other similar studies.[16] In addition, we used a microbiological culture medium (TYCBS), which other authors found to be the most sensitive and selective for the recovery and growth of *S. mutans* compared with other selective media, such as mitis-salivarius-bacitracin agar (MSB).[30,31]

Finally, regarding the employment of cross-sectional or one-time sampling designs, as in some of the above-mentioned studies, Douglas *et al* [8] mentioned that this kind of design limits the ability to control the variability of oral microbiota over time, This makes it difficult to confirm our microbiological findings; therefore,

we are considering using longitudinal designs to overcome these restrictions when planning studies similar to the present one. Here, we used a cohort study with a closed follow-up. To our knowledge, this is the first study in a Mexican population that indicates the necessity to identify the main cariogenic genotypes of S. mutans and its transmission from mothers to their children[32,33] and the need to design programs to reduce mother-to-child transmission of S. mutans and initiate dental caries prevention in early childhood.[34-36] We are currently designing a follow-up study of these children in order to determine whether they acquire significant levels of *S. mutans* in the future and, by sampling their DNA, what proportion of the of *S. mutans* strains are from the same phylogenetic family as those found in their mothers.

CONCLUSIONS

According to the results of the present research, it was not possible to find significant detectable levels of *S. mutans* collected from the oral cavity of the studied infants during the five pre-established intervals (0, 15, 30, 90, and 150 days), regardless of the presence of the revised risk factors: maternal *S. mutans* level of colonization, type of feeding (breast, bottle, mixed or solid), and the infant´s number of siblings. Our findings have not demonstrated any transmission of *S. mutans* from mother to child from early infancy to age 5 months, before tooth eruption.

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