

Research Article

The Co-relation of Salivary Streptococcus Mutans' Count between Mother and their Neonates within Two Days of Life: An Ex Vivo Microbial Study

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Abstract

Background: Human infants are considered germ free before birth. Immediately after birth, the infant is exposed to millions of microorganisms. During birth and shortly after the birth, epithelial lining of the mouths of neonates are colonized by different microbial species.

Objective: To Determine The Co-relation of Salivary Streptococcus Mutans' Count Between Mother and their Neonates within Two Days of Life.

Study Design: It was an ex-vivo, microbial, double

blinded, interventional, comparative study.

Participants: 42-mothers aged 22-40 years and their 42-neonates born through vaginal delivery.

Intervention: Oral screening of participant mothers was performed to record DMFT/DMFS and Periodontal index. Participants were divided into two groups based on high caries status (DMFT score ≥ 6) and low caries status (DMFT score ≤ 1). Samples were taken from mothers' oral cavity, neonates mouth on day one and within 48 hours by swabbing the tip of the tongue, left vestibule at 1st molar region, buccal

mucosa on left side. Mutans streptococci (MS) were cultivated on Mutans Sanguis Agar.

Outcome: After 48 hours MS count in neonates from Group A was higher than Neonates in Group B which was statistically significant ($p < 0.001$).

Results: Mothers in group A showed more MS count than Group B which was highly significant ($p < 0.001$). The MS count in neonates remained zero immediately after birth irrespective of the MS count in mothers of both the Groups.

Conclusion: As evidence of bacterial transmission has been identified within two days of birth, it can be characterized as two crucial days for oral bacterial infection.

Keywords: Streptococcus Mutans; Dental Caries; Oral Microflora; Colonization; Newborn; Vertical Transmission; Horizontal Transmission; Acquisition; Vaginal Delivery; Perinatal Factors

1. Introduction

Human infants are typically considered free of micro-organisms before birth. Immediately after birth, infants are exposed to millions of micro-organisms, which develop into a small portion of natural oral flora. During birth and shortly after birth, epithelial lining of the mouths of neonates are colonized by different microbial species [1]. Studies have been done in past to check MS count few months/years after birth in infants. However, colony formation over gingiva/epithelium is likely to begin at the first hour or days after birth. Many neonatal bacterial infections are typically acquired at or few hours after birth. Immune mechanisms in neonates is not well distin-

guished at the time of birth, and certain commensals may lead to opportunistic pathogens [2-6]. Infants acquire oral microflora from their surroundings, possibly from the first individuals they have direct contact with. Generally, the first ones to interact with infants are mothers, nurses, and often the hospital setting.

In the mouth of an infant, only oral epithelial surfaces are subjected to salivary fluid, which may produce bacteria [7]. MS can survive in such an environment by forming adhesive clusters on mucosal surfaces or by proliferation in free saliva at a rate exceeding the wash rate caused by the salivary fluid flow [7]. Gram positive bacteria were found in 1 month-old edentulous infants; later gram-negative bacteria were detected on oral mucosa of these infants [8]. Gram-positive microbial commensal *Streptococcus salivarius* is pioneering colonizer of human oral cavity, identified 8 hours after birth [7]. Wide range of colonies remain at this location for the lifespan of the host, this bacterial community constitutes up to 98% of the overall oral microbiota before the teeth eruption. On the first step of life, mucosal cells from normal infants showed a selective attraction for the natural spread of streptococci that would quickly colonize these mucosal surfaces. With the emergence of deciduous teeth, the number and species of the microflora in the oral ecosystem continues to grow [9]. *Streptococcus mutans* and *S. Sobrinus* are important species synonymous with dental caries which colonizes the oral cavity following eruption of teeth. However, *S. salivarius* is found on the surface of tongue as well as buccal mucosa because it has a special tendency to adhere to these surfaces. *S. Sanguinis* colonization of newborns develops within a brief "infectivity window" at about the nine months

of age [1]. Backer et al (2002), proposed that MS species possess a wide variety of traits, such as acidogenicity and acidurance, which confer an ecological gain over other oral carcinogenic bacteria, not all species are similarly pervasive in dental caries [10]. The main virulence factor of MS that is essential for colonization is their capacity to get attached to host surfaces [11]. This encloses a sucrose-independent initial conformity to the salivary pellicle accompanied by sucrose-dependent cellular accumulation.

In addition, glucan binding proteins enhance the binding of MS to glucans accumulated on hard tissue surfaces, thus leading to the sucrose-dependent adherence of MS and *S. Sobrinus*. These glucans are the components of MS enzymes, glucosyltransferase and fructosyltransferase present in both *S. mutans* and *S. Sobrinus* which increases plaque pH, in-turn results in demineralization of hard tissues. Some clinical trials have found that children typically receive conditions from their mothers that influence the transmission of MS microbiologically, biochemically and at genetic level. Davey (1984) and Berkowitz (1985), showed MS strains isolated from mothers and their children have similar or equivalent bacteriocin profiles [12, 13]. There is now clinical evidence that MS can be detected in pre-dentary children's mouths prior to the first tooth eruption [14, 15]. Wan and co-workers showed that more than 30% of pre-dentated children at 3 months of age were infected with *S. mutans*; around 6 months of age, more than 60% showed bacterial presence [14]. Owing to the absence of longitudinal research, very little is understood about the timing of the arrival of essential microbes in oral biofilm in infants. As clinical trials have demonstrated that the risk of

caries is associated with the age at which initial MS colonization takes place, approaches for the prevention of dental diseases should provide prompt regulation of cariogenic bacteria in young children's mouths.

2. Method

This was an ex-vivo, microbial, double blinded, interventional, comparative study. The study was approved by the institutional ethical committee SVIEC/ON/Dent/BNPG18/D19006 and is registered in Clinical Trial Registry- India (CTRI) code: CTRI/2019/05/019454. The study was conducted among 42 mothers aged 22-40 years and their 42 neonates born through vaginal-delivery who visited the Department of Obstetrics and Gynaecology. Participant mothers aged 22-40 years, neonates born through vaginal delivery, who gave written consent were included in the study. Neonates with premature birth, any physical disability, mother and/or neonates having received any antibiotic therapy were excluded from the study. Mothers were divided into 2 groups based on high caries status (DMFT score ≥ 6) and low caries status (DMFT score ≤ 1).

2.1 Clinical examination

It was done by principal investigator under the guidance of subject expert in order to limit examiner variability. Participant mothers were requested to gargle with water before oral examination. DMFT index was used to determine the occurrence of coronal caries. Periodontal health status was evaluated using Periodontal Index given by Russell AL in (1956) [22].

2.2 Sample collection

Samples were taken from mothers' oral cavity by

swabbing tip of the tongue, left vestibule at 1st molar region, buccal mucosa on left side with sterile transport cotton swabs. Samples were stored at 4°C in peptone water until it was transported to Department of Oral Pathology and Microbiology for microbial culture. MS were cultivated on Mutans Sanguis Agar (HIMEDIA Laboratories, India). Similarly, samples

were taken from neonates’ oral cavity just after birth and another sample within 48 hours after birth. All the samples were coded to avoid bias. (Where, M is mothers’ sample, N₀ is neonates’ sample just after birth and N₁ is neonates’ sample 48 hours after birth) (Figure 1).

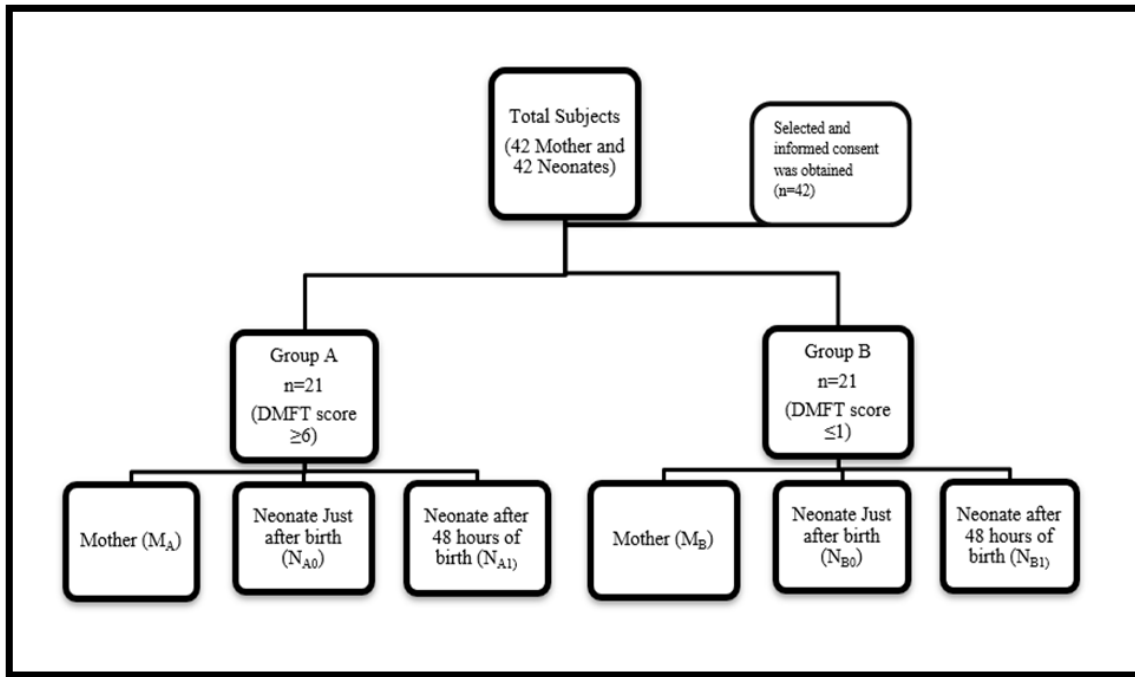


Figure 1: Consort chart showing the flow of participants through each stage.

2.3 Microbial culturing procedure

For microbiological culture 10 µL or 1 loop sample was spread over Mutans Sanguis Agar. Cultural characteristics were observed in presence of 10% CO₂ + 90% H₂, after an incubation at 37°C for 48 hours. S. mutans form rough, stalk up, irregular colonies resembling frosted glass. Mostly friable, colonies can be plucked from the agar that are greyish white or yellow coloured and 0.5 - 2 mm in diameter, can develop a drop of liquid (water-soluble glucan) over the colony or a puddle of polysaccharide

around the colony. Visible S. mutans colonies of grown on these media were counted using a bacterial colony counter (Figure 2-Group A, 3-Group B).

2.4 Statistical analysis

Descriptive and inferential statistical analyses were carried out in the present study. Level of significance was fixed at p=0.05 and any value ≤ 0.05 was considered to be statistically significant. Chi square analysis was used to find out the significance of research parameters on the categorical scale. Mann-

Whitney U test was used to find the significance of study parameters on continuous scale between two groups. Pearson's Correlation coefficient was computed to measure correlation between MS count of mothers and different variables. The Statistical software IBM SPSS statistics 20.0 (IBM Corporation, Armonk, NY, USA) was used for analyses of data and Microsoft word and Excel were used to generate graphs, tables etc.

3. Results

The study was conducted among 42 mothers aged 22-40 years with mean age of 23.14 in Group A and 23.76 in Group B. The mean DMFT score of mothers in Group A was 7.43 and in Group B was 0.48 respectively. Mean DMFS score was 8.33 in group A and 0.86 in Group B. On intergroup comparison of DMFT and DMFS scores ($p < 0.001^{**}$) which was highly significant (Table 1). The mean periodontal index score of mothers was 1.62 and 1.57 in Group A

and B respectively. Table 2 shows periodontal index score in terms of mean standard deviation among both the groups which shows there was no statistically significant difference in periodontal index scores of mothers among both the groups.

The mean streptococcus mutans count of mothers in Group A was 1339.48 with standard deviation 412.327 and Group B was 411.81 with standard deviation of 94.605. On intergroup comparison of streptococcus mutans count of mothers ($p < 0.001^{**}$) which was highly significant. The streptococcus mutans count in neonates remained zero immediately after birth (Table 3). The mean streptococcus mutans count of neonates after 48 hours of birth was 156.81 and 50.10 in Group A and Group B respectively. Table 3 shows intergroup comparison of streptococcus mutans count of neonates ($p < 0.001^{**}$) which was highly significant using unpaired t test.

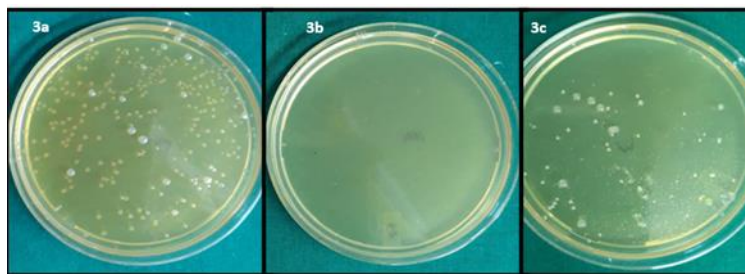


Figure 2 (a,b,c) Group A: 2a. *S. mutans* colonies of mothers' sample; 2b. *S. mutans* colonies of neonates' sample on day 1; 2c. *S. mutans* colonies of neonates' sample day 2.

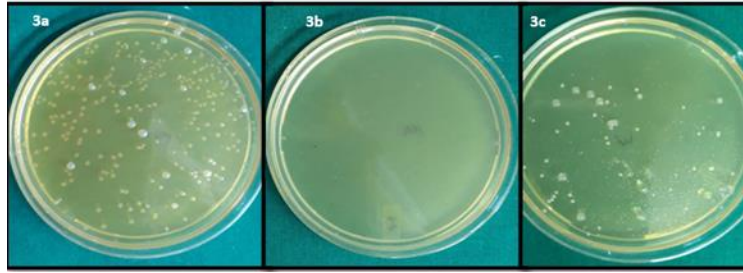


Figure 3(a,b,c) Group B: 3a. *S. mutans* colonies of mothers' sample; 3b. *S. mutans* colonies of neonates' sample on day 1; 3c. *S. mutans* colonies of neonates' sample day 2.

Parameters	Group	N	Mean	Std. Deviation	Z value	P value
DMFT score	Group A	21	7.43	1.326	5.66	<0.001**
	Group B	21	0.48	0.512		
DMFS score	Group A	21	8.33	1.39	5.617	<0.001**
	Group B	21	0.86	1.062		

Table 1: Comparison of DMFT and DMFS score of mothers among both the Groups (p < 0.05 - Significant*, p < 0.001 - Highly significant**)

Parameters	Group	N	Mean	Std. Deviation	t value	P value
Periodontal index score	Group A	21	1.62	0.921	0.178	0.86
	Group B	21	1.57	0.811		

Table 2: Comparison of periodontal index score among both the Groups

Streptococcus mutans count	Group	N	Mean	Std. Deviation	t value	P value
Mothers	Group A	21	1339.48	412.327	10.049	<0.001**
	Group B	21	411.81	94.605		
Neonates at birth	Group A	21	0	0	NA	NA
	Group B	21	0	0		
Neonates after 48 hours	Group A	21	156.81	50.703	9.33	<0.001**
	Group B	21	50.1	13.281		

Table 3: Comparison of streptococcus mutans count of mothers, neonates immediately after birth and after 48 hours (p < 0.05 - Significant*, p < 0.001 - Highly significant**) (NA: Not applicable).

4. Discussion

Dental caries is a widespread infectious and multifactorial disease. These factors include the presence of cariogenic bacteria, fermentable sugars in the diet, anatomy of the tooth, host as well as time. The incidence of caries is witnessing a decline in developed countries due to the adequate availability of fluoride containing materials, improved oral health facilities and understanding of caries aetiology; whereas the situation in developing countries is contrast to this because of poor healthcare services in rural areas and awareness of the same. Caufield et al. (1993) proposed that the development of mutans streptococci in young children is most likely to occur from 19 to 31 months of age during "window of infectivity" [16]. Infants acquire oral microflora from their surroundings, possibly from the first individuals they have direct contact with. Generally, the first ones to interact with infants are mothers, nurses, and often the hospital setting. Bacterial colonization occurs within a few hours of birth. Makhoul (2000) [17] and Davey (1984) [12] have mentioned sophisticated technology using chromosomal DNA patterns or similar plasmids has provided more convincing data to support the principle of vertical transmission.

A systematic review by Douglass (2008) [18] indicates that clear evidence has been found that mothers are the primary source of their children's MS colonization. It also emphasized on the fact that the role of other factors influencing transmission is unclear, and thus the purpose of our study was to determine the co-relation of the count of salivary streptococcus mutans among mother and their neonates within two days of life. Dental caries has been reported in several populations, with women

usually showing higher incidence and more infected teeth [19, 20]. Lukacs J (2006) reported that hormonal variations can have a dramatic impact on women's oral health and are an important causal factor in understanding gender difference in caries rates [21]. The Decayed-Missing-Filled Teeth (DMFT) index given by Klein, Palmer and Knutson in 1938 has been used since decades and is today the definitive population-based index of worldwide caries experience. This index gives the sum total of the decayed- missing, and filled surfaces of permanent teeth of an individual (DMFS) [22, 23].

In present study the mean DMFT score of mothers in Group A was 7.43 and DMFS score was 8.33. Mean DMFT score of mothers in Group B was 0.48 and DMFS score was 0.86. On intergroup comparison of DMFT and DMFS scores ($p < 0.001^{**}$) which was highly significant terms of mean standard deviation among both the Groups using Mann Whitney U test. The mean periodontal index score of mothers was 1.62 and 1.57 in Group A and B respectively with standard deviation of 0.921 in Group A and 0.811 in Group B. The periodontal index score in terms of mean standard deviation among both the groups using unpaired t test ($t=0.178$) and ($p=0.860$) which shows there was no statistically significant difference in periodontal index scores of mothers among both the groups. Mutans Streptococci plays key role in pathogenesis of carious lesions in humans; acquisition and dissemination of this bacterium has gained considerable attention in different populations. Generally, the levels of MS count and lactobacilli in children have been correlated with the levels of their mothers. In the mothers and infants, genotyping of the majority of colonies of MS and Lactobacillus strains found variation, and each

organism displayed a distinct genotypic pattern [24].

In our study we have assessed few variables like breast feeding, kissing, contact with anyone else other than mothers. We have found that 83.4% mothers gave history of kissing newborn on first day of birth. 92.1% mothers breastfed their babies and all 42 newborns babies came into contact with someone else than mother like doctors, nurses, relatives as well

as hospital environment. So, there was definitely a chance of bacterial transmission from mothers as well as from other variables. Rosenblatt R (2015) [8] assessed the same conditions as contact with the baby was tested and 96% of the mothers mentioned touching their infants, 86% kissed them, and a relative also had some contact with the baby in 96% of the cases. Much of the infants were breast fed (73%).

Bacterial factors Transmission	Increased numbers of MS in mothers/close contacts
	Increased frequency of contact with MS Carriers
MS strains	Virulent strains of MS
Biofilm	Little competition with other species
	Ecological sites available for colonization
Host factors Hereditary	HLA genes with unfavourable immunological, salivary,
	Tooth, mucosal effects
Surfaces for microbial adherence	Increase tooth surfaces
	Altered mucosal surfaces
Saliva	Reduced quantity and quality of saliva
Immunological	Reduced oral immunity from congenital and acquired conditions
Diet	Frequent ingestion of sweet snacks and drinks
Oral hygiene	Lack of oral hygiene

Table 4: Factors which influence Mutans Streptococcus colonization [9].

Some clinical trials have found conditions that influence the transmission of streptococci mutans-microbiological, biochemical and molecular-that children typically receive from their mothers. Davey (1984) [12] and Berkowitz (1985) [13], showed S. Mutan strains isolated from mothers and their children have similar or equivalent bacteriocin profiles. The mean streptococcus mutans count of mothers in Group A was 1339.48 with standard deviation 412.327 and Group B was 411.81 with standard deviation of 94. 605. On intergroup

comparison of streptococcus mutans count of mothers ($p < 0.001$) which was highly significant. The streptococcus mutans count in neonates remained zero immediately after birth irrespective of the streptococcus mutans count in mothers of both the groups. The mean streptococcus mutans count of neonates after 48 hours of birth was 156.81 and 50.10 in Group A and Group B respectively with standard deviation of 50.703 in Group A and 13.281 in Group B. The intergroup comparison of streptococcus mutans count of neonates with ($p < 0.001$) which was

highly significant using unpaired t test.

Similar results were published by Rosenblatt R (2015) [8] who stated there was complete lack of any of the oral microorganisms soon after birth. The first appearance of oral microorganisms was expected to appear two days post birth. A positive correlation among the bacterial colony counts of the mothers and infants can be appreciated. Mothers play a major important part in the first transmission of the oral microflora in the newborn seen after his first 48 hours of life. These results focus on the need to control and thereby change the microflora that has been acquired, at the start of a neonate's life, which can further help in creating a less cariogenic flora. Thus, a protocol to examine the avoidance of initial transmission between the mother and the newborn can help reduce the occurrence of caries in future. Douglass et al., (2008) [25] stated that consumption of xylitol in mothers resulted in reduced caries incidence in neonates otherwise expected to be at high caries risk. For the reduction of transmission of bacteria that is likely to cause caries in their infants, Xylitol gums and chlorhexidine rinses can be used as additional treatment protocol in mothers with high caries risk in early postpartum period. The prenatal dental care of the expecting mothers during the course of pregnancy can lead to reduced incidence of infant caries.

Key Message: The adoption of strict hygienic measures by the mother and the nursing staff should be emphasized to delay the occurrence of infections caused by microorganisms in newborns. In addition, hospital procedures must be aseptic and invasive interventions must be minimized as much as possible. Establishment of 'Dental Home' would help spread awareness of oral hygiene measures among mothers

and accompanying personnel.

What is already known: As evidence of bacterial transmission has been identified within two days of birth, it can be characterized as two crucial days for oral bacterial infection. It is possible to monitor and modify the acquired microflora at the very beginning of human life, producing a new, but less cariogenic flora.

What this study adds: If we could find ways to stop this initial propagation, there are possibilities to limit caries in the future. One way of reinforcing the value of oral hygiene for mothers during the last months of pregnancy and shortly after birth of infant, before touching and handling the infant, to clean breast and nipple area before feeding the baby.

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