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RESEARCH ARTICLE

ACQUISITION OF STREPTOCOCCUS MUTANS IN INFANTS AND ITS CORRELATION WITH MODE OF DELIVERY AND FEEDING PATTERNS OF INFANTS

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ABSTRACT

Introduction: Bacteria as Streptococcus mutans have been implicated to play a key role in causation of dental caries. Reducing the bacterial load can aid in prevention of caries. Further the acquisition of Stretococcus mutans is influenced by numerous maternal factors as mother's SM counts, feeding practices, mode of delivery, oral hygiene, sharing of food and utensils etc.

Objective: To investigate the correlation of streptococcus mutans counts with maternal factors as type of feeding practices and mode of delivery.

Material and methods: A blind longitudinal Study was carried out on 60 full term healthy infants with normal body weights to assess the Streptococcus Mutans colonization pattern. Salivary samples of infants were taken at birth, 1 month, 3, 6, 9, 12 and 15 months and data regarding type of feeding and delivery was noted through questainaires filled by parent. The non-parametric chi-square test was applied from birth till 9 months on obtained results and parametric ANOVA test was applied on all the obtained results at 12 and 15 months of age.

Results: SM counts were seen to increase with increasing age of the infant. Significant association of SM colonization with infants born by C-section upto the age of 12 months was seen. With regards to feeding pattern, significant association at 5% level of significance was seen till 9 months of age in infants taking breast feed or combination of both.

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INTRODUCTION

Dental caries is a transmissible infectious disease that represents a significant oral health problem in our country. Early childhood caries (ECC) is a virulent form of dental caries that can destroy the primary dentition of toddlers and preschool children and affects 54.1% of population in India (Mahejabeen *et al*. 2006). The notion that dental caries is a transmissible disease was first demonstrated by Keyes (1960) who found a group of phenotypically similar bacteria, collectively known as the mutans streptococci (MS) were implicated as the principal bacterial component responsible for dental caries in humans (Gibbons and Van Houte,1975; Loesche *et al*.,1975). Traditionally, streptococci mutans was believed to colonize the mouth only when teeth were present and most of the studies had reported initial colonization only after eruption of the primary teeth (Masuda *et al*. 1979; Caufield *et al.* 1993). However, investigations in the recent past have shown that colonization of S.mutans can occur in predentate infants as young as 3 months of age (Wan *et al*. 1945;2001b;2002;2003) and six-months old children without teeth as well. The cariogenic properties of mutans streptococci (Edwardsson, 1970) and studies related to their relationship with caries (van Houte, 1980) and with sugar intake led to this conclusion (Emilson and Krasse, 1985; Loesche, 1986). Also there are many maternal factors associated with S.mutans colonization as type of delivery and type of feeding practices. Caufield et al. (2005) correlated mode of delivery and other maternal factors to acquisition of SM in infants. The study concluded that the infants born by caesarean section acquired SM earlier than did the vaginally delivered infants. Once established, early colonizing species tend to persist in the mouth (Cole *et al* 1998; Kononen *et al* 1999). Florio Flavia Martao *et al* (2004) showed that MS colonization may occur in edentulous mouths showing significant relationship with breast feeding on demand, presence of oral developmental nodules,

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low socioeconomic status and other feeding habits. Milgroom *et al* (2000) and Habibian *et al* (2001) proved in their studies that prolonged bottle feeding with sugar containing drinks, night feeding of sweetened fluids as well as frequent in between snacking are well known factors which are associated with increased MS levels and development of caries in young children. Thus the aim of this blind longitudinal study was to investigate the correlation of SM counts in children with maternal factors as mode of delivery and type of feeding practices.

MATERIAL AND METHODS

Ethical clearance was obtained from the Ethical Committee of Baba Farid University of Health Sciences vide letter no. 26 dated 6-6-2008 for the study protocol. 100 pregnant mothers were randomly recruited for the study based on family history taken on a proforma (Annexure I). A total of 60 full term healthy infants with normal body weight (2000-3500g) as per normal for Indian Population given by ISP were finally selected at birth from the recruited family sample. Oral hygiene practices of parents and infants were also noted. The families were then enrolled for the study for 15 months.

Data Collection

After having taken a signed consent from the parents to carry out the study. the first salivary samples of infants were taken at birth. The salivary samples of the subjects were subsequently collected at regular intervals of 1 month, 3months, 6 months, 9 months, 12 months and 15 months respectively and results of Streptococcus Mutans count were noted each time. Information regarding type of delivery and feeding practices was collected through questionnaire filled by the parents. (Annexure I)

Collection of Salivary Samples for Microbiological Analysis

The salivary samples were obtained by swabbing the dorsum of tongue and gum pads of the infants with sterile cotton tips soon after birth. From each swab, 0.1ml of saliva was placed into a calibrated sterile vial containing phosphate buffered saline and was transported to the microbiology laboratory at 4°C where it was processed within 4 hours. Similarly, the samples were taken at 1, 3, 6, 9, 12 &15 months of age.

Isolation of Streptococcus mutans from Saliva

Samples were vortexed for 30 seconds to disperse bacteria. 50-µL aliquots of ten-fold dilutions were placid onto Streptococcus mutans-selective tryptone – yeast – cysteine – sucrose–bacitracin agar (TYSCB). The media was poured into Petri dishes and these were inoculated in duplicate and incubated at 37°C for 72 hrs. Colonies were counted by colony counter by two different individuals at different time to reduce the interpersonal bias and the mean colony-forming units/Ml saliva (CFUs/mL) were calculated for Streptococcus. The growth obtained was subcultured on blood agar to see alpha hemolysis. Colonization was considered positive when 2 consecutive salivary samples showed the growth. After incubation, the colony characters like colour, size, shape, convexity, surface margins, consistency, opacity, haemolysis on blood agar, pigmentation etc. were studied. Smears were made from the colony and were subjected to gram staining to study morphology and staining characters. All gram positive cocci arranged in short chains and pairs were presumed to be streptococci and were confirmed by carrying out Mannitol and sorbitol fermentation test, Aesculin hydrolysis and Catalase test.

The information regarding type of feeding practices and mode of delivery was obtained through questionnaires filled by the parents. These were noted as follows

- Type of delivery-whether Normal or Caesarean Section.
- Type of Feeding Practices i.e. Breast fed, Bottle fed or both from birth till 15 months of age

The obtained results were correlated with SM counts, statistically analysed and parametric/nonparametric tests were applied on them. The non-parametric chi-square test was applied from birth till 9 months of obtained results and parametric ANOVA test was applied on all the obtained results at 12 and 15 months of age.

RESULTS

The study was conducted on 60 full term infants born with normal body weights as per normal for Indian population given by ISP. Salivary samples of the selected infants were taken at birth, 1month later and at regular intervals of 3 months till 15th month of age (Table 1 & 2). SM colonization was absent at birth and one month age interval in all the subjects. At 3 months of age, Streptococcus mutans colonization was present in 1.7% subjects and absent in 98.3% of subjects. At the age of 6 months, 53.3% of subjects were found to have Streptococcus mutans colonization while at the age of 9 months the percentage increased to 76.7%. At the age of 12 and 15 months, all the infants were colonized with Streptococcus mutans.

Table 1. Showing distribution of total 60 subjects according to presence of streptococcus mutans at different age intervals

	Strep Mutans Present (%age)	Strep Mutans Absent (%age)
At birth	0(0%)	60(100%)
At 1 months	0(0%)	60(100%)
At 3 months	1(1.7%)	59(98.3%)
At 6 months	32(53.3%)	28(45.7%)
At 9 months	46(76.7%)	14(23.3%)
At 12 months	60 (100%)	0(0%)
At 15 months	60 (100%)	0(0%)

Table 2. Showing mean streptococcus mutans counts in subjects at different age intervals

	N	Range	Mean ± SD	SEm	95% Confidence Interval
At birth	60	-	-	-	-
At 1 months	60	-	-	-	-
At 3 months	60	0-300	5.00 ± 38.73	5.00	0.00 – 15.00
At 6 months	60	0-700	178.67 ± 209.40	27.03	124.57 - 232.76
At 9 months	60	0-840	440.67 ± 278.30	35.93	368.77 - 512.56
At 12 months	60	600-1000	791.33 ± 128.37	16.57	758.17 - 824.50
At 15 months	60	1000-1800	1283.33 ± 190.99	24.66	1234.00 - 1332.67

Where N-Total no. of subjects
SEm-Standard error of Mean
SD-Standard Deviation.

Table 3. Showing association of streptococcus mutans colonization in subjects with type of delivery at different age intervals

Time	Type of Delivery	No.of subjects showing S.Mutans Presence	No.of subjects showing S.Mutans Absent	x^2 value	P value
At birth	Normal	-	25	-	-
	Caesarian	-	35		
At 1 month	Normal	-	25	-	-
	Caesarian	-	35		
At 3 month	Normal	0	25	0.726	0.394^{NS}
	Caesarian	1	34		
At 6 month	Normal	5	20	19.133	$<0.001^{***}$
	Caesarian	27	8		
At 9 month	Normal	14	11	10.232	0.01^{**}
	Caesarian	32	3		

Time	Type of Delivery	N	Range	Mean ± SD	F value	P value
At 12 months	Normal	25	600-1000	737.00± 123.87	8.458	0.005**
	Caesarian	35	600-1000	829.71± 118.85		
At 15 months	Normal	25	1000-1500	1240.00±156.631	2.253	0.139NS
	Caesarian	35	1000-1800	1314±208.838		

NS -Not Significant

**p<0.01 Significant at 1% significance level ; *** p<0.001 Highly Significant

Table 4. Showing asociation of streptococcus mutans colonization in subjects with type of feeding at different age intervals

Time	Type of Feeding	No.of subjects showing S.Mutans Presence	No.of subjects showing S.Mutans Absent	x^2 value	P value
At birth	Breast fed	-	23	-	-
	Bottle fed	-	17		
	Both	-	20		
At 1 month	Breast fed	-	23	-	-
	Bottle fed	-	17		
	Both	-	20		
At 3 month	Breast fed	1	7	0.726	0.394^{NS}
	Bottle fed	-	14		
	Both	-	38		
At 6 month	Breast fed	2	0	6.244	0.044^{*}
	Bottle fed	11	18		
	Both	19	10		
At 9 month	Breast fed	2	0	8.599	0.013^{*}
	Bottle Fed	13	10		
	Both	31	4		

Time	Type of Feeding	N	Range	Mean ± SD	F value	P value
At 12 months	Breast Fed	1	700-1000	787.50±99.103	2.374	0.102NS
	Bottle Fed	32	600-1000	744.00± 155.001		
	Both	27	600-1000	821.88 ±109.587		
At 15 months	Breast Fed	1	1000-1400	1160.00±167.332	1.366	0.263NS
	Bottle Fed	34	1000-1800	1274.78±200.201		
	Both	25	1000-1800	1308.75±184.91		

NS -Not significant

* p<0.05 Significance at 5% significance level

Factors related to colonization of SM were also noted as type of delivery and feeding practices. Association SM counts in infants with the type of delivery at different time intervals is shown in Table 3. On statistical evaluation, highly significant association of SM colonization with subjects born by cesarean delivery was seen at 6 months which became significant at 9 and 12 months interval. At 15 months of age, results were not significant. The association of S. Mutans colonization with type of feeding i.e. Breast fed, Bottle fed or both was statistically evaluated at different age intervals. A significant association of MS colonization with type of feeding was found at 5% significance levels at 6 and 9 months of age with more no. of cases having mutans counts who were exclusively breast fed or both (breastfed +bottle fed). At 12 and 15 months of age, no significant association with type of feeding habit was seen.

DISCUSSION

Since dental caries is an infectious disease, a plausible method of prevention is the removal of cariogenic bacteria from the mouth. In this regard, knowledge of the time when Streptococcus Mutans colonizes the mouth of infants is important for the determination of optimal period for preventive and interceptive treatments. A total of 60 full term new born infants with normal body weights were included in this study with similar socio-economic status and ethical background. Differences in caries prevalence between different racial/ethnic groups have been reported with some frequency (Barnes *et al.* 1992; Infante *et al*. 1975)**.** However such differences can be confounded as a result of socio-economic factors. In the present study differences in socio-economic

status have been mitigated by selecting a sample of population with similar ethical/socioeconomic status as given by Kuppuswamy in updated scale for 2007. In a country like India with diverse ethical population, the eating patterns show equal amount of diversity. Although no Indian studies exist regarding the correlation between S.Mutans colonization in different ethnic groups, the study design included North Indian Punjabi families with similar dietary patterns as was obvious from dietary history of parents and siblings. The analysis of longitudinal data of the current study showed that out of a total of 60 subjects, the cumulative prevalence of streptococcus mutans colonization in all infants was 0% at birth and one month, 1.7% at 3 months, 53.3% at 6 months,76.7% at 9 months and 100% at 12&15 months of age (Table1). The colonization rates in the present study showed a gradual increase in rate and were generally higher than those reported by other investigators (Wan and Seow, 2003). The sensitivity and selectivity of TYCSB agar media was already confirmed in the clinical studies carried out by Wan *et al* (2001b) and Wan *et al* (2003). Hence TYCSB agar media was selected to investigate the Streptococcus mutans colonization in this study.

Type of delivery

In the present study 35 infants out of 60 were delivered by caesarian section and 25 by vaginal delivery. The SM colonization was seen in one infant at three months of age who was delivered by caesarean section whereas 25 vaginally delivered infants did not show colonization at the same time. Although this result was not statistically significant at 3 months but at 6,9 and 12 months of age, p value of <0.01 was seen showing a statistical association of SM colonization with type of delivery at 1% significance level (Table 3).

Type of Feeding Practices-Breast fed, Bottle fed or both

Mohan *et al* (1998) stated MS colonization was associated with feeding practices & significant relationship between consumption of sweetened beverages & MS colonization. Also Florio *et al* (2004) concluded that MS colonization may occur in edentulous mouths showing significant relationship with breast feeding on demand, presence of oral developmental nodules, low socioeconomic status and other feeding habits. In the present study, at 6 and 9 months of age, a significant association of SM colonization with breast fed and both (breastfed+bottlefed) was found to be present at 5% significance levels. However, at the age of 12 and 15 months, the results obtained were not significant. The mean CFU/ml range was slightly higher in combination practice but not significant when compared with the other two (Table 4).This could be attributed to the small sample size of exclusive breast feeding subjects as compared to others. The change in level of significance at 12 and 15 months could also be related to the presence of teeth in the oral cavity and other contributing factors such as snacking, sharing of foods, liquids and utensils with parents and siblings.

Once colonized, SM counts tend to increase due to their association with number of factors (Masuda *et al*. 1979; Mohan *et al.* 1998, 2003; Gibbons *et al*. 1971) which may have nothing to do with the window of infectivity. However, following the same subjects over a longer period till all primary teeth erupt would further establish the factors which would primarily be responsible for maintaining the trend of Streptococcus Mutans colonization, as was seen in the present study.

Conclusion

Significant association of SM colonization with infants born by C-section upto the age of 12 months seen at 1% level. With regards to feeding patterns, significant association at 5% level of significance was seen till 9 months of age in infants taking breast feed or both fed (Breastfed+Bottlefed). Thus it can be concluded that initial colonization of Streptococcus mutans is associated with the above mentioned factors. Streptococcus mutans starts initial colonization as early as 3 to 6 months irrespective of the presence or absence of teeth and vertical and horizontal transmission plays an important role in mutans colonization. Thus, instructions in oral hygiene and feeding practices, avoidance of in between meal snacking are few practices that can help prevent caries in children.

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