QUANTITATIVE CORRELATION OF SALIVARY STREPTOCOCCUS MUTANS COUNT AMONGST SIBLINGS AND THEIR MOTHERS

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ABSTRACT

Aim: The present study was planned to analyze quantitative correlation of salivary *Streptococcus Mutans* (*S. Mutans*) in siblings and their mothers. **Materials and Methods:** Quantitative analysis of *S. Mutans* in saliva was performed using Dentocult SM strip mutans kit (*Orion Diagnostica, Helsinki, Finland*) in closely related members of the family i.e. siblings along with their mothers. **Results:** *S. Mutans* count between the siblings showed positive correlation which was statistically highly significant. The younger children's *S. Mutans* count was very highly correlated ($r_s = 0.711$) with the mothers' as compared to that of the older children ($r_s = 0.412$). The *S. Mutans* count was found to be associated with caries score and was statistically significant. A statistically highly significant positive association was also found with the plaque score. **Conclusion:** The correlation between the *S. Mutans* count of related individuals has been reaffirmed. *S. Mutans* is positively associated with dental caries.

Key words: Streptococcus Mutans, S. Mutans, siblings, horizontal transmission, quantitative analysis.

INTRODUCTION

Streptococcus Mutans (*S. Mutans*) was first described by J Kilian Clarke (1924)¹ and is one of primary factors causing dental caries. This microorganism metabolizes sucrose in an anaerobic environment to produce lactic acid and provides an acidic environment for dental caries initiation. It thrives under acidic conditions and becomes the dominant bacterium in cultures with permanently reduced pH. It starts to colonize an infant's mouth soon after birth.² Vertical transmission of *S. Mutans* from mother to child is a well known fact, and a discrete window of infectivity exists around 18 months of age.^{2,3} Later on microbial colonization increases as more tooth surfaces are available for flourishing of

the microbes. Maternal salivary level of *S. Mutans* greater than 10^6 CFU/ml has been proposed as a risk factor for vertical transmission.^{4,5} Thus, mothers who share food, drinks, utensils, toothbrushes and other items with their children have the highest risk of transmitting *S. Mutans* to their offsprings.⁶ The current reports point out that vertical transmission is not the only vector by which *S. Mutans* are perpetuated in humans.⁶ Numerous studies have investigated the horizontal or child to child transmission of *S. Mutans*. Frequent close contacts with others seem to be sufficient to transmit *S. Mutans*. Hence, the present study was conducted to evaluate *S. Mutans* count in siblings and their mothers along with various causal factors.

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MATERIALS AND METHODS

Ninety subjects constituted the total sample size. Thirty mothers along with their 2 children belonging to age range of 6-10 years were included in the study. Children were divided into younger and the older child based on their birth order. The study was approved by Ethics Committee of B.R.S. Institute of Medical Sciences. Research has been conducted in full accordance with the World Medical Association Declaration of Helsinki.⁷ Selection of the sample was done from patients visiting the outpatient Department of Paediatric & Preventive Dentistry, B.R.S. Institute of Medical Sciences, Vill. Sultanpur (Panchkula), Haryana, India and children studying in the nearby schools. An inclusion criterion was healthy children and the mothers who were the primary caregivers to the selected children. The subjects who had received any antibiotic medication in the past one month, received prophylaxis, topical fluoride application and use of mouth wash in past 3 weeks, allergic to paraffin wax and undergoing orthodontic treatment were excluded from the study.

Clinical examination

Written consent by translating in local language was obtained after explaining the nature of the study from the parents of selected children and consent procedure was approved by Ethical committee of the Institute. Clinical examination was done by seating the patient in a dental chair. Caries was recorded according to WHO criteria (1997)⁸ and lesions at D₂ and D₃ levels were included in the study. The plaque score was recorded as per Silness and Loe (1964)⁹ Index. Findings were recorded on specially designed proforma for mothers and children, separately. The subjects were given instructions not to eat, brush or use mouthwash 2 hours before sample collection as it would interfere with the actual count of *S. Mutans* in the saliva. Subjects were recalled next day for saliva sampling. Twenty four hour diet recall was also obtained. Not more than three

mothers along with their 6 children were sampled in one day.

Quantitative analysis of S. Mutans:

The culture medium vials were taken out from the refrigerator 1 hour prior to saliva collection. Two bacitracin discs were added to the medium 15 minutes before sample collection using sterile tweezer and then the medium was gently shaken for activation. Mothers were told to wipe off their lipstick before sample collection as it would have stained the strip. The subjects were told to chew on the paraffin wax pellet provided in the kit for 1 minute and asked to use all teeth during chewing in order to maximize the transfer of *S. Mutans* adhered to the teeth into the saliva. The paraffin pellet was then removed from the mouth and disposed off properly. They were then told to swallow the excess of saliva after 1 minute of chewing. The rough surface of the strip provided in the kit for saliva collection was rubbed 10 times gently against the tongue of the subject which ensured the transfer of *S. Mutans* onto the strip. The strip was then withdrawn from the mouth with lips gently closed to remove the excess saliva.

Microbial culturing:

The strip was immediately transferred to the selective culture vial. It was attached to a groove provided inside the cap of the vial to allow its easy removal after culturing and was then positioned in the special stand provided with the kit and was transferred to the incubator within 1 h. Before putting the vial in the incubator, the cap was opened one quarter to allow elimination of the gases resulting from yeast growth. Strict asepsis was maintained at all levels to avoid any sort of contamination. The vial was incubated at 37 °C for 48 h. In case of no growth, it was further incubated for next 48 h. After incubation, the strips were visually compared for colony density of *S. Mutans* with the chart provided with the kit as shown in figures 1, 2, 3 and 4. The chart contained 4 pictures of strips each

having different scores of *S. Mutans* count for comparison.

Statistical Analysis:

Statistical analysis was performed on the software SPSS version 13.0. Spearman's Rank correlation was used to assess the correlation between the *S. Mutans* count of mother with her children and amongst the siblings as well as between *S. Mutans* count and brushing frequency. Multivariate regression analysis of deft/DMFT, *S. Mutans* count, plaque score, use of fluoridated dentifrice and in-between meals sugar exposures was done.

RESULTS

Out of 30 mothers examined, 76.6% were found to be colonized with *S. Mutans*. Scores '0', 1, 2 and 3 were observed in 23.3%, 20%, 33.3% and 23.3% mothers, respectively. In younger children, out of 30, 60% were found to harbour *S. Mutans*; 40% had score '0'; 13.3% had '1'; 26.7% had '2' and 20% had score '3'. In older children out of 30, 73.3% were found to have *S. Mutans*; thus 26.7% had score '0'; 26.6% had '1'; 20% had '2' and 26.6% had score '3'. Nineteen subjects from the sample were free from caries i.e. 21.1%.

Out of 30 mothers examined, 2 had DMFT score 0 and highest score found was 9. In younger children, highest deft score found was 9 in one child only, score 0 was found in 9 children (30%). Eight subjects in the older child group were caries free (26.7%) and highest deft score was 11 and was found in only one child. The mean DMFT score of mothers was 3.63 (SD $\pm$ 2.38). Mean of deft scores of younger and older children were 2.13 (SD $\pm$ 2.30) & 2.9 (SD $\pm$ 2.80); respectively. Five out of 30 sibling pairs shared their toothbrushes and positive correlation was observed between *S. Mutans* count of these siblings which was statistically highly significant ($r_s = 0.968$, $p = 0.007$; respectively).

DISCUSSION

S. Mutans count of siblings and their mothers was evaluated with the Dentocult SM "Strip mutans" kit (Orion Diagnostica, Helsinki, Finland) with sensitivity

and specificity of 98% and 85%, respectively. Subjects selected were from lower/Upper lower socio-economic status (IV) which was ascertained based on their family income and mother's level of education (below metric).¹⁰ The age group of 6-10 years was selected because the second window of infectivity exists in this age range.¹¹ The presence of *S. Mutans* was evidenced by dark blue to light blue raised colonies on rough surface of the strip. Colonies suspended in the culture broth were excluded from the study.

In the present study 56.6% mothers had a high count of *S. Mutans* (scores 2 & 3) in their saliva which increase the chances of transmission. Score 2 and 3 of *S. Mutans* was found in 46.6% siblings. Mother to child transmission of *S. Mutans* was first reported by Berkowitz in 1975.¹² It was evidenced from several studies showing that isolates of *S. Mutans* harboured by mothers and their children exhibit identical plasmid or chromosomal DNA patterns.^{12,13} Several studies have indicated that the mutans streptococci are transmitted vertically, along familial lines, from mother to child.¹²⁻²⁰ The present study evidenced that the younger child's *S. Mutans* count was very highly correlated ($r_s = 0.711$) with the mother's count as compared to that of the older child ($r_s = 0.412$) as shown in Table 1. The high

Table 1: Correlation of *S. Mutans* count of mothers with that of younger and older children

		<i>S. Mutans</i> count of younger children	<i>S. Mutans</i> count of older children
<i>S. Mutans</i> count of mothers	Spearman's Rho (r_s)	0.711	0.412
	p – value	0.000	0.024
	N	30	30
<i>S. Mutans</i> count of mothers who shared eatables	Spearman's Rho (r_s)	0.708	0.418
	p – value	0.000	0.024
	N	29	29

correlation of *S. Mutans* count might have existed because of higher frequency of sharing with the younger child as compared to the older child. Antony &

Munshi²¹ also reported quantitative correlation between the number of CFU/ml of *S. Mutans* in the mother and her second child only. Many authors have concluded that there is a clear correlation of the *S. Mutans* levels between mother and her children.^{17,21} On the contrary, Caufield et al (1993)² and Aaltonen et al (1994)¹⁹ found that mothers' and childrens' *S. Mutans* levels were not significantly associated.

Sharing of eatables or utensils is a major mode of transmission of bacteria. Out of 30 sibling pairs, 29 were found to be sharing eatables with their mothers. Twenty nine sibling pairs who shared eatables with their mothers (Table 1) had a significant positive correlation of *S. Mutans* count with their mothers. However, the reported maternal transmission rates range from 24% to 100%.²²⁻²³ This wide range in transmission rates clearly demonstrates that mothers are not the only source of *S. Mutans* transmission, other individuals who are potential sources, such as siblings, grandparents, and non family caretakers who share responsibility for caring for the child might be the reason. In the present study, *S. Mutans* count of siblings was highly correlated ($r_s=0.470$; Table 2) which indicated possibility of horizontal transmission between the siblings. Various authors have reported transmission between family members. Domejean S (2010)²¹ reported horizontal transmission in non-related individuals. Horizontal transmission among nursery cohorts with prolonged exposure to an environment that favours the spread of infectious agents was reported by Mattos-Graner et al (2001).²⁵ In the present study, all 29 sibling pairs who shared eatables with their mothers, also shared eatables with each other and demonstrated a significant positive correlation ($r_s=0.447$; Table 2) between their *S. Mutans* count. All the subjects were from low socio-economic status and the financial constraints might have resulted in the children sharing utensils and eatables which might be the reason for a significant positive correlation of their *S. Mutans*.

Table 2: Correlation of *S. Mutans* count between the siblings

		<i>S. Mutans</i> count of older children
<i>S. Mutans</i> count of younger children	Spearman's Rho (r_s)	0.470
	p – value	0.009
	N	30
<i>S. Mutans</i> count of younger children who shared eatables	Spearman's Rho (r_s)	0.447
	p – value	0.015
	N	29

Figure 1: Score 0 (<10,000 CFU/ml)

Figure 2: Score 1 (<100,000 CFU/ml)

Figure 3: Score 2 (100,000- 10,00,000 CFU/ml)

S. Mutans can be transferred readily when a toothbrush is shared between 2 persons increasing the risk of dental caries.²⁶ Five out of 30 sibling pairs also shared their toothbrushes. The *S. Mutans* count of these 5 sibling pairs showed a very high positive correlation ($r_s = 0.968$). Van Houte & Green (1974)²⁷ pointed out that successful transmission of bacteria between human hosts is complex and depends on a variety of interrelated factors, including bacterial affinity to potential colonization sites and number of infecting bacterial cells available for attachment. The vehicle could be the domestic objects contaminated with saliva, such as spoons or toothbrushes. Warren DP et al²⁸ have shown that contaminated toothbrushes harbour and transmit viruses and bacteria. They found that toothpaste use reduces the residual microbial contamination as in our study, An inverse relationship existed between brushing frequency and *S. Mutans* count (Table 3).

S. Mutans is strongly associated with human dental caries and has been supported by many epidemiological studies.^{18,21} In the present study, multivariate regression analysis was done in which caries score was taken as dependent variable (Table 4) and independent variables were *S. Mutans* count, plaque score, use of fluoridated dentifrice and number of sugar exposures. The regression analysis of the

Table 3: Correlation between brushing frequency and *S. Mutans* count of mothers, younger and older children

		<i>S. Mutans</i> count of mothers	<i>S. Mutans</i> count of younger children	<i>S. Mutans</i> count of older children
Brushing Frequency	Spearman's Rho(r_s)	-0.381	-0.214	-0.145
	p - value	0.038	0.257	0.444
	N	30	30	30

Table 4: Multivariate regression analysis for carious score of mothers and siblings

Dependent variable: Caries Score		<i>S. Mutans</i> count	Plaque score	Fluori- dated dentifrice	Sugar exposures
Mothers	t - value	4.568	1.473	-0.134	0.546
	p-value	0.000	0.153	0.895	0.590
Younger children	t - value	2.725	2.085	-0.055	1.601
	p-value	0.012	0.047	0.956	0.112
Older children	t - value	2.414	1.433	-1.620	1.600
	p-value	0.023	0.164	0.118	0.122

Figure 4 : Score 3 (> 10,00,000 CFU/ml)

mother found that *S. Mutans* count was positively associated with mothers' caries score and was statistically significant. Plaque score and number of sugar exposures were also positively associated but statistically insignificant and use of fluoridated

dentifrice was negatively associated and it was also statistically insignificant. In younger children, both *S. Mutans* count and plaque score were statistically significant and positively associated with caries score, whereas, use of fluoridated dentifrice and number of sugar exposures were statistically insignificant. In older children, only *S. Mutans* count was statistically significant and positively associated and plaque scores, use of fluoridated dentifrice and number of sugar exposures were statistically insignificant. This demonstrates the infectious nature of dental caries. Very few studies like those on Danish preschool children and British school children; however reported no significant relationship between *S. Mutans* and the initiation of dental caries.²⁹⁻³⁰

When *S. Mutans* count was taken as dependent variable (Table 5) and caries score, plaque score, use of fluoridated dentifrice and number of sugar exposures as independent variable, it was seen that in mothers' *S.*

Table 5: Multivariate regression analysis for *S. Mutans* count of mothers and siblings

Dependent variable: <i>S. Mutans</i> count		Caries Score	Plaque score	Fluoridated dentifrice	Sugar exposures
Mothers	t-value	4.568	1.740	-0.694	0.112
	p-value	0.000	0.094	0.494	0.912
Younger children	t-value	2.725	1.549	-0.297	0.689
	p-value	0.012	0.134	0.769	0.497
Older children	t-value	2.414	2.838	-1.801	1.216
	p-value	0.023	0.009	0.084	0.235

Mutans count was very highly associated with caries as well as other factors like plaque and number of sugar exposures but were statistically insignificant. The use of fluoridated dentifrice was negatively associated with *S. Mutans* count but was statistically non significant. In younger children, it was only significantly associated with caries score, while plaque scores, use of fluoridated dentifrice and number of sugar exposures were statistically insignificant. In older children, it was statistically significant and positively associated with caries score and had highly significant association with plaque score but no statistically significant association

was found with the use of fluoridated dentifrice and number of sugar exposures.

CONCLUSION

In the present study, quantitative analysis of *S. Mutans* showed positive correlation between mothers and their children. It showed the possibility of vertical and as well as horizontal transmission but it could not be proved whether this actually happened as the study lacked in procedures which prove homology of genotypes of *S. Mutans* between mothers and their children and also between siblings. In spite of these drawbacks, the conclusion drawn from the present study reinforces their certainty that *S. Mutans* is closely associated with dental caries. Also the correlation between the *S. Mutans* counts of related individuals has again been affirmed. Moreover, the results of the study once again call for the need for education and awareness of low socioeconomic strata where oral health measures still remains one of the most neglected aspect of general health especially in developing nations like India.

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