A Comparative Study of Mutans Streptococci and Lactobacilli in Mothers and Children with Early Childhood Caries (ECC), Severe Early Childhood Caries (S-ECC) and Caries Free Group in a Low Income Population

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Abstract

Aims and objectives: The aim of the present study was to assess levels of *Mutans Streptococci* and *lactobacilli* in children and mothers of children with early childhood caries (ECC), severe early childhood caries (S-ECC) and a caries free group in a low income population.

Material and methods: The study was designed amongst 3-5 year old preschool children (n=74) and their mothers (n=74).Stimulated saliva samples were collected from each mother and child pair for bacterial assessment .One calibrated examiner carried out a comprehensive dental examination for each subject under optimal light using a mouth mirror and an explorer.

Results: Child dmfs was 2.67 and 10.08 in ECC and S-ECC respectively. Mother DMFS was 21.75 and 24.25 in ECC and S-ECC children respectively. The $\log_{10}$ of total *mutans streptococci* Colony Forming Units (CFU) was 3.51 ± 0.62, 5.8 ± 0.37 and 6.25 ± 0.43 for caries free group, ECC and S-ECC groups respectively. Difference was significantly ($p<0.001$) lower for caries free group when compared with ECC and S-ECC groups, however difference was not found to be significant for ECC and S-ECC groups. *Lactobacilli* $\log_{10}$CFU count was found to be significantly ($p<0.001$) lower for caries free group when compared with ECC and S-ECC groups. Although it was not statistically different for the ECC and S-ECC groups. Total *mutans streptococci* log10CFU count in mothers was 4.40 ± 0.48, 5.1 ± 0.34 and 5.76 ± 0.34 for caries free group, ECC and S-ECC groups respectively. This difference was found to be significant lower ($p<0.001$) for caries free group when compared with ECC and S-ECC groups.

Total *lactobacilli* CFU count in mothers was 2.94 ± 1.19, 3.93 ± 0.99 and 5.27 ± 0.61 for caries free group, ECC and S-ECC groups respectively. This difference was found to be significant ($p<0.001$) for caries free group, ECC and S-ECC groups.

Conclusion: Microbial count was higher in the mothers and children with ECC and S-ECC than caries free group. Mothers can be targeted for intervention, for prevention of early childhood caries.

KeyWords: Early childhood caries (ECC), Severe Early childhood caries (S-ECC), Mutans streptococci, lactobacilli

Introduction

Dental caries is an insidious infection that attacks the hard tissues of the teeth. It cannot be induced in germ free animals. A carious lesion can only occur when a mass of cariogenic microorganisms colonize on tooth surface and forms plaque.

Previous studies on dental caries described the role of salivary *mutans streptococci* as a predictor and marker for caries risk [1]. *S. mutans* and *S. sobrinus* are the *mutans streptococci* present in oral cavity. Much of the research has suggested these species of *mutans streptococci* as the bacterial pathogens of human dental caries [2,3].

Studies suggest strong role of *S. mutans* in the initiation of caries, and *lactobacilli* in the active progression of the cavitated lesion [4].

Several studies suggest salivary *mutans streptococci* in mothers as the major source for the maternal transmission of *mutans streptococci* [5,6]. Studies demonstrated that mothers with high level of *mutans streptococci* in saliva, tend to have early colonization of these organisms in their children, and that a reduction in the level of *mutans streptococci* in mothers can inhibit or delay the progression of caries in their children [7].

The study of dental caries in the primary dentition is important not only for the resulting deterioration in the quality of life of young children, but also because dental caries in the primary dentition is one of the best predictors of caries in the permanent dentition [8,9]. The understanding of these kinds of relationships is important from a preventive perspective, to plan out various public health programs to decrease the risk of Early Childhood Caries (ECC) and severe early childhood caries(S-ECC). This is very important in lower income groups as they share a major burden of the oral disease in comparison to the higher income group in developing countries.

The aim of the present study was to assess levels of *Mutans Streptococci* and *lactobacilli* in children and mothers of children with ECC, S-ECC and a caries free group in a low income population.

Materials and Methods

The study was carried out in Udupi District, Karnataka state, India. The study was designed amongst 3-5 year old preschool children (n=74) and their mothers (n=74) of low socioeconomic status attending Anganwadis in Udupi Taluk. Anganwadis are day care centre for children under the Indian Government ICDS scheme. An Anganwadi Center is the focal point for the delivery of services to children and mothers in their own communities [10]. This program is specifically designed to reach the disadvantaged and low income groups, for effective

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disparity reduction and promotes holistic development of children under six years of age [11].

Study protocol was reviewed by Kasturba Hospital Ethics Committee, Kasturba Hospital, Manipal**.** Ethical clearance (IEC 25/2011) was obtained from the Kasturba Hospital Ethics Committee, Kasturba Hospital, Manipal. Permission to examine the Anganwadi children was obtained from the teachers and local authorities of the respective schools. Informed consent was obtained from respective parents.

Mother and child pair present on the day of examination, children having ECC and S-ECC and willing to participatewere included in the study. Informed written consent was obtained from the mothers of respective children. Mother and child pair suffering from any infectious or systemic disease or under antibiotic medication in the past 3 months was excluded from the study.

Simple random sampling was done with the help of table of random numbers. Sample size was calculated by taking significance level (α) of 0.01 and power of study (β) to be 90%. Sample size came out to be 64.

The study group consisted of randomly selected 24 children with S-ECC and 24 children with ECC and 26 children who were caries free from anganwadi centres. Children were selected according to the criteria provided by the guide lines of AAPD [12]. Definition of ECC by American Association of Pediatric Dentistry (AAPD) is the presence of 1 or more decayed (non cavitated or cavitated lesions), missing (due to caries), or filled tooth surfaces in any primary tooth in a child 71 months of age or younger, while in children younger than 3 years of age, any sign of smooth-surface caries is indicative of S-ECC. From ages 3 through 5, 1 or more cavitated, missing (due to caries), or filled smooth surfaces in primary maxillary anterior teeth or a decayed, missing, or filled score of 4 (age 3), 5 (age 4), or 6 (age 5) surfaces constitutes S-ECC [12].

Clinical examination

One calibrated examiner (AST) carried out a comprehensive dental examination of each subject under optimal light using mouth mirror and explorer. Intra-observer reliability was assessed using kappa statistics, which was 0.96. The diagnosis of dental caries was based on the modified WHO criteria. Dental caries level was expressed using the DMFS index for permanent teeth and dmfs for primary teeth [13].

Saliva sample

Stimulated saliva samples were collected from both mother and child pair for bacterial assessment. Each subject was given a piece of Paraffin wax (1g) and a calibrated cylinder (10 mL). Participants were asked to chew the paraffin wax and to expectorate the stimulated saliva into the container. The process was carried out in a 20 minute period. Saliva samples were collected before the clinical examination from 9-10 a.m. and the subjects were asked not to eat or drink for one hour prior to sampling. Subjects were also asked to refrain from tooth brushing in the morning before saliva sampling.

Microbiological analysis

Paraffin-wax-stimulated saliva was collected and 1 mL transferred to 5.7 mL of VMG II transport medium (Viability Medium Göteborg II) Next day, the samples were cultured in dilutions of 10^{-1}, $10^{-2}$and 10^{-3}on mitis salivarius bacitracin (MSB) [14] agar differential for *Mutans streptococci* and on a predried surface of Rogosa selective *lactobacillus* agar. The MSB agar plates were incubated for 48 hours at 37°C in 5% CO_2 and 95% N_2, whereas the Rogosa selective *lactobacillus* agar plates were incubated aerobically for 48 hours at 37°C. The numbers of MS and *lactobacilli* CFU per millilitre of saliva were determined. Few colonies of each culture medium were selected for the evaluation of cell morphology by gram staining. After incubation, the number of Colony Forming Units (CFU) was determined on plates containing 10–300 colonies. Microbial counts were performed in duplicate with a digital colony counter. One examiner (DS) who was blinded for the caries status of child and mother pair did the CFU count for *mutans streptococci* and *lactobacilli*; intra-observer reliability was 0.98 using kappa statistics.

Statistical analysis

The analysis of the study was carried out using the Statistical Package for Social Sciences (SPSS version 11.5 version). The cut-off level for statistical significance was taken at 0.01. After application of the Shapiro-Wilk test, data that showed non-normal distribution were -transformed by the Box-Cox method. If fewer than 10 colonies were detected, a value of 2 (or log 10 100 CFU/ mL) was used for analysis. One way ANOVA was used to compare CFU counts between the groups. Pearson correlation was done between mean number of microbial count reported as CFU per mL of saliva in mother and child pair in ECC, S-ECC and a caries free group with mother DMFS and children dmfs.

Results

Table 1 shows the frequency distribution of children and mothers by age, gender and education. The age of children ranged from 36 months to 59 months with a mean age of 45.5 ± 6.2 months. Fifty one percent of the sample was male and forty nine percent of the samples were female. The age of mothers ranged from 25 to 43 years with a mean age of 32.6 ± 3.42.

Table 1. *Frequency distribution of study population by age, gender, and education.*

Age group (in months) of children	Number	Percentage (%)
Less than 48 months	40	54
≥ 48 months	34	46
Gender of children		
Male	38	51
Female	36	49
Age (years) group of mothers		
≤ 30 years	22	30
> 30 years	52	70
Education of mothers		
Middle or less than middle school	10	14
High school	34	46
More than high school	30	41

Table 2. *Frequency distribution of caries status in study and control groups.*

Groups according to the caries status		Child dmfs		Mothers DMFS	
	N	Mean	Std.dev	Mean	Std.dev
Control	26	0	0	0	0
ECC	24	2.67	1.23	21.75	18.38
S-ECC	24	10.08	5.19	24.25	17.93

Table 2 shows frequency distribution of dmft and DMFT in children and mothers respectively.

Table 3 shows mean number (SD) of microbial count reported as CFU per mL of saliva in mother and child pair in ECC, S-ECC and a caries free (control) group. Total *mutans streptococci* CFU count in children was 3.51 ± 0.62, 5.8 ± 0.37 and 6.25 ± 0.43 for control, ECC and S-ECC groups respectively. Difference was significantly (p=0.001) lower for control group in comparison with ECC and S-ECC groups; however difference was not found to be significant for ECC and S-ECC groups.

Similar results were observed for total *lactobacilli* CFU count for children. Mean CFU for *lactobacilli* was lowest for the control group, and was highest in S-ECC group. *Lactobacilli* CFU count was found to be significantly (p=0.001) lower for control group when compared with ECC and S-ECC groups. CFU count was not statistically different for the ECC and S-ECC groups.

Total *mutans streptococci* CFU count in mothers was 4.40 ± 0.48, 5.1 ± 0.34 and 5.76 ± 0.34 for control, ECC and S-ECC groups respectively. This difference was found to be significant lower (p=0.001) for control when compared with ECC and S-ECC groups.

Total *lactobacilli* CFU count in mothers was 2.94 ±1.19, 3.93 ± 0.99 and 5.27 ± 0.61 for control, ECC and S-ECC groups respectively. This difference was found to be significant (p =0.001) for control, ECC and S-ECC groups.

Table 4 shows correlation between the mean number of microbial count reported as CFU per mL of saliva in mother and child pair of three groups with carious lesion prevalence in mother and child. Statistically significant correlation (p=0.001) was observed between dmfs of the child and the DMFS (r=0.53) of mother, *mutans streptococci* level (r=0.78) and *lactobacillus* levels (r=0.66) of mother.

A significant correlation (p=0.001) was observed between the dmfs of the child and the levels of *mutans streptococci* (r=0.62) and *lactobacillus* (r=0.56) in the child. In this study, strong correlation was observed between mother DMFS levels and child *mutans streptococci* (r=0.57) and *lactobacillus* levels(r=0.65).

Discussion

The present study assessed the salivary levels of cariogenic bacteria in relation to the caries experience in children. It also correlated these levels to their mother's levels in an attempt to clarify the mother and child relationship in term of bacterial transmission. Numerous studies have been reported in the literature comparing the microbial levels in ECC and caries free children or S-ECC and caries free children. There have been no studies in our knowledge which are comparing microbial levels in ECC, S-ECC and caries free children.

In the present study, children with S-ECC and ECC showed high salivary *mutans streptococci* levels compared to caries free children. Shukairy et al. [15] also reported higher levels of *mutans streptococci* in S-ECC children when compared with caries free children.

Matee et al. [15] reported that the levels of *S.mutans* in dental plaque were 100 times higher in breast fed children with ECC, than in children without decay. Milgrom et al. [16] and Thorild et al. [17] concluded that children with high levels of *S. mutans* were five times more likely to have dental caries than children with lower *S. mutans*. In the present study, *lactobacilli* count was significantly higher in children with S-ECC and ECC when compared to the caries free group. CFU count was not statistically different for the ECC and S-ECC groups. The results are in accordance with studies by Loesche [18] and Shukairy et al. [14]

This study was an attempt to compare salivary microbial levels in ECC and S-ECC. One of the major finding of our study was that S-ECC and ECC groups were not statistically significantly different to each other with regard to *mutans streptococci* and *lactobacillus* level.

Mothers of children with S-ECC had a higher *mutans streptococci* and *Lactobacilli* count than the mothers in the ECC and caries free group. Significant difference was seen in the *Lactobacilli* and *mutans streptococci* levels between three groups of mothers.

This study correlated the level of *mutans streptococci* and *lactobacilli* in child- mother pair of caries free children with ECC and S-ECC children. Statistically significant correlation was observed in between the dmfs of the child with DMFS,

Table 3. *Mean number (SD) of microbial count reported as log10 (CFU) per mL of saliva in mother and child pair in early childhood caries (ECC), severe early childhood caries (SECC) and a caries free group.*

Bacterial count	Groups	N	Mean	Std. Deviation	p-value	Post hoc tukey test
Total child mutans streptococci count	Control (a)	26	3.51	0.62	<0.001***	a<b* a<c*
	ECC (b)	24	5.80	0.37		
	S-ECC (c)	24	6.25	0.43		
	Control (a)	26	3.51	0.62		
Total child lactobacilli count	Control (a)	26	2.89	0.62	<0.001***	a<b* a<c*
	ECC (b)	24	4.63	1.05		
	S-ECC (c)	24	4.82	1.12		
Total mother mutans streptococci count	Control (a)	26	4.40	0.48	<0.001***	a<b* a<c* b<c*
	ECC (b)	24	5.00	0.0		
	S-ECC (c)	24	5.76	0.34		
Total mother lactobacilli count	Control (a)	26	2.94	1.19	<0.001***	a<b* a<c* b<c*
	ECC (b)	24	3.93	0.99		
	S-ECC (c)	24	5.27	0.61		

Anova test and post hoc tukey test. *p<0.01 level of significance.

Table 4. *Correlations among mean number of microbial count reported as log10 (CFU) per mL of saliva in mother and child pair in early childhood caries (ECC), severe early childhood caries (SECC) and a caries free group with carious lesion prevalence in mother (DMFS) and children (dmfs).*

	Correlation coefficient	mothers' DMFS	Log_{10} child mutans streptococci	Log_{10} mother mutans streptococci	Log_{10} child lactobacillus	Log_{10} mother lactobacillus
Child dmfs	Pearson Correlation	0.53**	0.621**	0.78**	0.564**	0.661**
	Sig. (2-tailed)	0.001	0.001	0.001	0.001	0.001
Mothers' DMFS	Pearson Correlation	1	0.57**	0.482**	0.65**	0.509**
	Sig. (2-tailed)	.	0.001	0.003	0.001	0.001
Log_{10} child mutans streptococci	Pearson Correlation	0.569**	1	0.770**	0.602**	0.575**
	Sig. (2-tailed)	0.001	.	0.001	0.001	0.001
Log_{10} mother mutans streptococci	Pearson Correlation	0.482**	0.770**	1	0.626**	0.794**
	Sig. (2-tailed)	0.003	0.001	.	0.001	0.001
Log_{10} child lactobacilli	Pearson Correlation	0.647**	0.602**	0.626**	1	0.674**
	Sig. (2-tailed)	0.001	0.001	0.001	.	0.001
Log_{10} mother lactobacilli	Pearson Correlation	0.509**	0.575**	0.794**	0.674**	1
	Sig. (2-tailed)	.001	0.001	0.001	0.001	.

** Correlation is significant at the 0.01 level (2-tailed).

mutans streptococci and lactobacillus levels of the mother. A significant correlation was observed in between the Mothers' level of *mutans streptococci* and their caries prevalence (DMFS). These results are in accordance with various studies which concluded that levels of *mutans streptococci* in saliva is positively correlated to decay status of individual [19,20].

Strong correlation was observed between mother DMFS levels and child *mutans streptococci* and *lactobacillus* levels. Similar results were reported by Caufield et al. [21]. They reported that higher levels of S. mutans count in mother saliva was the most significant factor for *S.mutans* detection in the children. Smith et al. [22] also reported similar results. They observed that high level of *mutans streptococci* in mothers, maternal active carious lesions are strong caries risk indicators for the child.

Soderling et al. [23] study demonstrated that xylitol consumption decreased plaque *mutans streptococci*. Various investigators have reported beneficial effects on *mutans streptococci* establishment and future caries incidence in children if preventive interventions are targeted to the mothers [24,25]. These studies further strengthen the concept that mothers should be the target of choice for intervention for prevention of early childhood caries.[21-23]

Gomez and Weber [26] reported that the children, whose mothers had participated in preventive dental programs, were more caries free as compared to the children whose mother did not participate in any preventive program.

Levels of *lactobacilli* showed high counts among the mother-child pair in the S-ECC group. However, it showed significantly low counts among the pairs belonging to the caries free group. The correlation of mother and child with respect to their *lactobacilli* level which appeared clearly in the caries free group supports the role of *lactobacilli* in dental caries.

However, our results are limited by the sample size and the bias associated with microbiological data obtained at a single point of time, and no information regarding effects of fluoride application, abnormal saliva secretion rate and other such factors. However, further studies are required to determine the precise process of the maternal transmission leading to caries incidence in children, as the present findings are limited by the cross-sectional nature of the study and the potential bias described above.

Conclusion

Children with S-ECC and ECC had a statistically higher count of *mutans streptococci* and *lactobacilli* than caries free children. Mothers of the children with S-ECC had higher *mutans streptococci* than mothers of caries free children. Significant correlation exists between mothers and children with S-ECC with respect to *mutans streptococci.*

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