

COMPARATIVE EVALUATION OF ORAL MICROBIOTA OF CHILDREN WITH AND WITHOUT EARLY CHILDHOOD CARIES BORN TO CARIES FREE MOTHERS

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Received: 05 February 2016, Revised and Accepted: 24 March 2016

ABSTRACT

Objectives: To compare the level of mutans streptococci in children with and without Early childhood caries (ECC) born to caries free mothers.

Methods: Twenty children aged between 3 and 6 years were selected depending on their caries experience, and the mother should be caries free in both the groups. The children were divided into two groups. Group I had an active carious lesion and Group II were caries free. Saliva samples were collected from the child and the mother in a sterile tube and bacterial culture was carried out to estimate the colony count.

Results: There was a highly significant difference in the colony forming unit (CFU) between the 2 groups, indicating higher CFU in children with ECC.

Conclusions: Even though there are higher chances of vertical transmission of MS from mother to their child, this study provides a new view that mother alone is not a potential factor for mutans streptococci transmission to their child.

Keywords: Mutans streptococci, Early childhood caries, Colony forming unit.

INTRODUCTION

Oral health of children includes inter-relationship with all aspects of their developmental processes, genetic potential and environmental circumstances. Any alteration in oral health leads to compromised quality of life, masticatory efficiency, and other routine activities [1,2]. The child's oral cavity is usually sterile at birth, but it rapidly acquires a predominance of streptococcal microbiota [3].

Studies have shown that mutans streptococci (MS) are acquired through their mothers during the period of window of infectivity [4], after which colonization of MS does not occur [4-6] and after the age of 5 years when the permanent dentition erupts, which has been termed as a second "window of infectivity" [7].

Association of maternal salivary bacterial challenge of Lactobacilli and MS from pregnancy through 24 months postpartum was evaluated by Chafee *et al.* He concluded that mother with high MS challenge had MS positive children and he also stated that mothers high bacterial MS challenge increases the risk of early childhood caries (ECC) [8].

Acquired oral microflora of newborns during the first 48 hrs of life was evaluated by Rosenblatt *et al.* and he concluded that mother is the major source of microflora during the first 48 hrs of life, and it is possible to control this acquired microflora by maintaining an accurate protocol during an early period of newborn to prevent this early transmission [9].

Even though, studies had proved vertical transmission of MS from mother to their child, several other demographic, and clinical factors are also responsible for the development and progression of caries [2,10]. Carious lesions are produced due to interaction between cariogenic microorganisms, diet, and the host [11]. Time plays a major role for the acid production and for the initiation and progression of caries. Once the transmission of MS occurs, it leads to bacterial adherence and progression of caries [12]. MS possess many characteristic features which facilitate caries progression, including the ability to adhere

to tooth surfaces and to synthesize certain glucans [13]. MS also synthesize intracellular polysaccharides, which leads to continuous acid production, which in turn fosters demineralization of the dental hard tissues [14].

However, identifying the role of MS in the initiation of dental caries is a complex matter, as they are part of normal oral habitant. This study was undertaken to compare the level of MS in children with and without ECC born to caries free mothers.

METHODS

Type of study

The present case-control study was conducted in the Department of Pediatric and Preventive Dentistry, Saveetha University.

Ethical approval

The study protocol received institutional approval from the Ethical Committee of Saveetha Dental College, Chennai, Tamil Nadu.

Selection criteria

The children were chosen from the outpatient clinic and a total of 20 children (10 children with ECC and 10 caries free children) and their mothers were interviewed and examined for the study. Written informed consent was obtained from parents of all the children before the participation in the study.

20 children aged between 3 and 6 years were selected depending on their caries experience and the mother should be caries free in both the groups. The children were divided into two groups. Group I had active carious lesion and Group II were caries free.

The selection criteria for the study include:

1. The decayed, missing, and filled teeth (DMFT) scores of ≥ 5 for the children in Group I.
2. Healthy children without any systemic illness.
3. No antibiotic and analgesics intake in the past 2 weeks.

Techniques

The study comprised three phases. The first phase consists of structured interview with the parents to assess the children's age, gender, pre-natal history, post-natal history, child's feeding habits, history of breast/bottle feeding at night, and child's oral hygiene habits. In the second phase the children and their mothers were examined by the same examiner. The child's caries status was measured by dmft and pulpal involvement, ulceration, fistula, abscess (pufa) scores. In the third phase, the unstimulated whole saliva were collected from the child and the mother in a sterile tube. The participants were asked to provide a 5 ml sample. The participants were instructed to spit into the sterile tube until the required marking without measuring froth. The participants were instructed to place the tip of the tongue behind the front teeth to pool the saliva.

Microbial evaluation

The samples were transferred to the laboratory and bacterial assessment was performed. The samples were diluted in 1:40 and 10 µl of the sample was transferred to Mitis-Sanguis agar. The samples were spread evenly all over the plate and incubated at 37°C over night. Identification of MS was done by observing its colony morphology and the number of colony forming units were counted in each plate (Figs. 1 and 2). The tests were done in triplicate to minimize the test error.

Statistical analysis

The data were coded and entered into the SPSS version 20.0 for the statistical analysis. Descriptive approach was used in the data analysis. Chi-square test was used to determine the relationship between different variables.

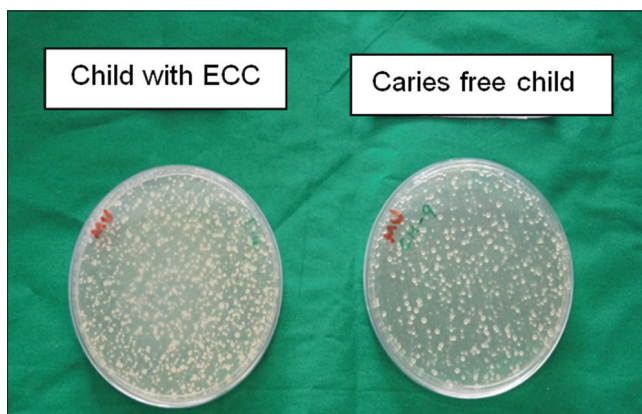


Fig. 1: Comparison of caries free child and child with early childhood caries

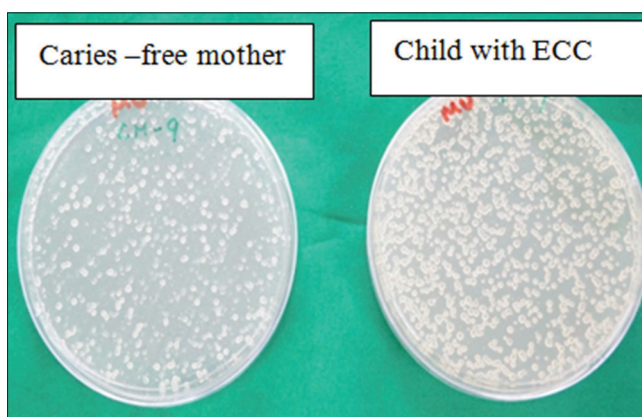


Fig. 2: Comparison of caries-free mother and child with early childhood caries

RESULTS

Tables 1 and 2 represent the severity of decay based on dmft and pufa scoring criteria. Analysis using chi-square test indicated no statistically significant association between age and severity of decay ($p>0.05$).

Distribution of children according to their duration of feeding practices is given in Table 3. Duration of bottle feeding was high compared to breast feeding in caries group. However, the difference was not statistically significant ($p>0.05$).

MS colony forming units (CFU/ml) among children who were caries-free and with ECC given in Table 4. There was a highly significant difference between the 2 groups, indicating higher CFU in children with ECC.

Comparison of CFU of caries-free mothers and their children (children with ECC) is given in Table 5. MS count was drastically low in mothers, showed a significant difference between caries-free mother and their children. This clearly explains, that mother alone is not a potential source of MS transmission to their child.

DISCUSSION

The purpose of this study was to compare the level of MS in children with and without ECC born to healthy, caries-free mothers, since many studies have proved maternal MS levels were associated with the colonization of infants by MS and there is a positive correlation between maternal levels of MS and the likelihood of infection being found in the infant [15,16]. This study provides a new insight that, even though the mothers were caries-free, several other factors play a major role for their child to experience a high caries challenge.

Association between age and severity of decay based on dmft score and pufa score is presented in Tables 1 and 2. Although there was no significant difference between age and severity of dental caries based on this scoring criteria, but most of the teeth had pulpal involvement indicated by pufa scoring index. It clearly explains the demerits of dmft index, that it has failed to explain the progression of dental caries [17,18].

In this study, it was found that the duration of breast feeding or bottle feeding did not have significant influence on the occurrence of dental caries (Table 3). In this study, there was significant difference between the CFU of MS between children with and without ECC and the

Table 1: Association between age and severity of decay based on dmft score

dmft score	Age (in years)				Total (%)
	<3	4	5	6	
1-5	0	0	1	1	20
6-10	2	2	1	1	60
11-15	0	0	1	0	10
16-20	1-0	1	0	0	10

*Pearson Chi-square-7.778 (>0.05), dmft: Decayed, missing, and filled teeth

Table 2: Association between age and severity of decay based on pufa score

pufa score	Age (in years)				Total (%)
	<3	4	5	6	
1-5	2	2	3	2	90
6-10	0	1	0	0	10
11-15	0	0	0	0	0
16-20	0	0	0	0	0

pufa: pufa-pulpal involvement, ulceration, fistula, abscess, Pearson Chi-Square-2.593 (>0.05)

Table 3: Distribution of children according to their duration of breast feeding and bottle feeding

Duration of breast feeding (in months)		
Months	Group I (%)	Group II (%)
<6	20	60
6-12	0	20
13-18	10	10
19-24	30	0
>24	40	10
Pearson Chi-square-9.000 (>0.05)		
Duration of bottle feeding (in months)		
Months	Group I (%)	Group II (%)
<6	0	0
6-12	20	30
13-18	20	10
19-24	10	40
>24	50	20
Pearson Chi-square-4.235 (>0.05)		

Table 4: CFU (CFU/ml) in unstimulated saliva of caries-free and ECC children

Caries free (CFU/ml)	Severe ECC (CFU/ml)
<60,000	>2,00,000
CFU: Colony forming units, ECC: Early childhood caries, p<0.000-highly significant	

Table 5: Comparison of CFU (CFU/ml) in unstimulated saliva of caries free mothers and ECC children

Healthy mother (CFU/ml)	Severe ECC (CFU/ml)
<46,000	>2,00,000
p<0.000 highly significant, CFU: Colony forming units, ECC: Early childhood caries	

children born to caries free mothers. This is very important to provide a new insight, because apart from environmental, dietary and maternal factors, several other factors such as protective role of immunoglobulin to dental caries, genetic variation of bacterial strains, and different serotypic bacterial strains could also be responsible for variation in rate and progression of dental caries in children [19-21].

The limitations of this study include smaller sample size, single-centered study conducted at Saveetha dental college and hospital and the final limitation includes the lack of isolation of bacterial strains for genetic analysis.

CONCLUSION

Even though there are higher chances of vertical transmission of MS from mother to their child, this study provides a new view that mother alone is not a potential factor for MS transmission to their child. Apart from environmental, dietary and maternal factors, importance should

be given for new approaches to identify the genomic isolation of species to improve the diagnostic criteria in the future.

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