

Comparison of the Salivary Immunoglobulin Concentration Levels between Children with Early Childhood Caries and Caries-Free Children

Ali Bagherian¹, Abdullah Jafarzadeh², Mohsen Rezaeian³, Shima Ahmadi¹, Mohammad Taghi Rezaity²

¹Department of Pediatric Dentistry, Dental School, ²Department of Immunology, ³Department of Epidemiology, Medical School, Rafsanjan University of Medical Sciences, Rafsanjan, Iran

ABSTRACT

Background: Early Childhood Caries (ECC) is one of the most common chronic childhood diseases. In spite of the global decrease in dental caries in the past decades, ECC has become a significant problem in many developing countries and also in a few industrialized nations. Saliva as a host factor can play an important role in the process of dental caries. **Objective:** The aim of this study was to compare sIgA and IgG as saliva components between ECC and caries-free groups. **Methods:** In this cross-sectional study, samples of unstimulated saliva of 90 children (45 in ECC group & 45 in caries-free group) were taken with Scully method. Then the concentration levels of sIgA and IgG were measured with Enzyme Linked Immunosorbent Assay and Single Radial Immunodiffusion methods. **Results:** Mean concentration levels of salivary sIgA and IgG were significantly higher among children with ECC ($p < 0.05$). There was also a weak inverse correlation between sIgA level and DMFT index in ECC group but it was not statistically significant ($p = 0.056$). **Conclusion:** The high concentration of salivary immunoglobulin in children with ECC may be associated with an increased antigenic load, leading to high production of antibodies.

Keywords: Dental Caries, Immunoglobulins, Saliva

INTRODUCTION

Dental caries is one of the most common chronic childhood diseases (1). In spite of its global decline in the past decades, early childhood caries (ECC) is still a significant problem in many developing countries and in a few developed nations (2, 3).

The American Academy of Pediatric Dentistry (AAPD) defines ECC as the presence of one or more decayed (non-cavitated or cavitated), missing (due to caries), or filled tooth

*Corresponding author: Dr. Ali Bagherian, Department of Pediatric Dentistry, Rafsanjan University of Medical Sciences, Rafsanjan, Iran. Tel: (+) 98 391 8220013, Fax: (+) 98 391 8220008, e-mail: drbagherian@yahoo.com

surfaces in any primary tooth in a child 71 months of age or younger (1). Similar to other types of caries, ECC is caused by *Streptococcus mutans* that ferments dietary carbohydrates and produces acid on susceptible teeth, leading to caries. Although the general etiology of ECC appears similar to that of other types of caries, the predisposing factors are still unknown (2).

Both genetic and environmental factors influence dental caries and because of genetic differences, certain environmental factors tend to be more cariogenic for some people than others (4). Saliva and its components (immunologic or non-immunologic) as host factors may play important roles in maintaining oral health. However, data published about correlation between salivary immunoglobulin and dental caries especially ECC are few and contradictory (5-8).

The aim of the present investigation was to compare total concentration levels of salivary sIgA and IgG between caries-free children and those with ECC.

MATERIALS AND METHODS

Study Design. This is a cross-sectional study, comparing salivary sIgA and IgG concentration levels in ECC with those of caries-free children. The protocol was reviewed and approved by the Medical Research Ethics Committee of Rafsanjan University of Medical Sciences.

Subjects. Only children fulfilling the following conditions were included in the study: Age between 36 to 70 months, no history of congenital and genetic problems, parental permission and ability to expectorate.

The children were selected by a random sampling procedure from those referred to the pediatric department of Rafsanjan School of Dentistry. Forty five randomly selected ECC and 45 caries-free children matched with respect to age, sex and body mass index [Weight (kg)/Height (m²)] as a criterion for nutrition status (9) were selected to enter the study.

Clinical Examination. Examination for dental caries was carried out using conventional dental chairs, artificial light, flat mirror and explorer; it was done according to the World Health Organization criteria and methods (10). The children were divided into ECC and caries-free groups after the examination. Also determination of decayed, missing and filled teeth (DMFT) for primary teeth was done in ECC group.

Collection of Saliva. Dental examination and saliva sampling were done in different days. At the day of saliva collection, the parents were asked to perform regular oral hygiene procedure after breakfast (1.5 hours before collection) and during this period children were not allowed to eat or drink. Children were seated in dental chairs and 2 cc of unstimulated saliva was collected in especial tubes using the method described by Scully (11), (In this method children were asked to spit in the tubes once a minute for ten minutes). All samples were collected between 10 to 11 a.m., and the time spent on each procedure did not exceed 30 minutes.

Saliva Analysis. Tubes of saliva were stored in the ice box and carried immediately to immunologic laboratory. Saliva samples were centrifuged (centrifugal force: 1000g) to remove bacteria and other extraneous material. The supernatant was used for immunological assays to measure salivary concentration levels of sIgA and IgG.

Salivary sIgA level was quantified by ELISA method (12), using a commercial sIgA ELISA kit (Immunodiagnostik, Benshiem, Germany). Salivary sIgA level was calcu-

lated using standard samples with known level of sIgA provided by the manufacturer and was expressed in milligrams.

Salivary IgG concentration level was determined using IgG-LC kit (Biogene, Mashhad, Iran) with Single Radial Immunodiffusion method (13), using specific antibodies to form precipitation rings in agarose gels. The diameter of the ring formed is quantitatively related to the concentration of the salivary parameter analyzed.

Statistical Analysis. Mean age, BMI, sIgA and IgG (\pm SD) were calculated for the two groups. The statistical significance of the difference between the means was tested by Student's t-test. The lack of difference between the two groups in terms of sex ratio was tested by chi-square test. Furthermore correlation of DMFT index with salivary sIgA and IgG levels was assessed by Pearson correlation test. P-values less than 0.05 were considered as statistically significant.

RESULTS

Since the two groups were matched, Table 1 indicates that there were no statistically significant differences between mean age and BMI in ECC and caries-free groups.

Table 1. Comparison of age and BMI (mean \pm SD) between caries-free and early childhood caries groups

Variables	Groups	Mean \pm SD	Number	Statistical Test	P-value
Age (months)	ECC	60.9 \pm 8.8	45	t-test	0.48
	Caries-free	59.4 \pm 12.09	45		
BMI (kg/m ²)	ECC	16.9 \pm 5.7	45	t-test	0.09
	Caries-free	15.2 \pm 3.2	45		

The sex ratios in two groups were nearly the same (60 % male in ECC group versus 62.3 % male in caries-free group) and there was no statistically significant differences between them ($X^2= 0.047$ and $p=0.829$).

In the ECC group, the minimum of DMFT was 3, the maximum was 20 and the mean \pm SD was 9.3 \pm 3.6.

Table 2 indicates that salivary concentration levels of sIgA and IgG were significantly higher among children with ECC ($p<0.05$).

Table 2. Comparison of salivary sIgA and IgG concentration levels between caries-free and ECC group

Variables	Groups	Mean \pm SD	Number	Statistical Test	P-Value
sIgA (mg/dl)	ECC	196.14 \pm 100.07	45	t-test	0.015
	Caries-free	148.45 \pm 81.16	45		
IgG (mg/dl)	ECC	9.78 \pm 3.26	45	t-test	0.046
	Caries-free	8.49 \pm 2.75	45		

There was also a weak inverse correlation between DMFT and salivary sIgA concentration level (Pearson correlation= -0.287) but it was not statistically significant ($p=0.056$). No correlation was found between DMFT and salivary IgG levels (Pearson correlation= $+0.046$).

DISCUSSION

Early childhood caries is a major public health problem due to the high prevalence in all regions of the world (3). Since microorganisms like *S. mutans* are one of the main etiologic factors of dental caries (14), immune system and immunological factors can interfere the disease process. Although inappropriate feeding patterns and *S. mutans* infection may cause a disease, they are not sufficient for initiating ECC. Host genetic differences and their effect on saliva characteristic and composition may be an answer to the question as to why some children develop ECC while others do not.

The role of salivary immunoglobulin in the protection against dental caries has been investigated in several studies, but making this association would be complicated since there are different sampling methods, different criteria for patient group and different laboratory tests between the studies. The concentration of salivary immunoglobulin may change depending on salivary flow rate, hormonal factors, emotional states, physical activity, etc (15); and their control is impossible in any study.

According to our results, there was a significant positive association between concentration of salivary sIgA and IgG and the presence of ECC. Similar findings were observed in other studies on ECC (5, 6), and in adult dental caries (16).

This finding can be explained by infective nature of ECC and dental caries. Since children with ECC show high amounts of *S. mutans* in the oral cavity, their immune system responds to the high antigenic load leading to a high production of immunoglobulins. Therefore, it seems that sIgA is at the first line of defense. However, when the load of microorganisms is high, more sIgA production is not able to prevent the disease.

Since gingival crevicular fluid (GCF) is the main source of salivary IgG, the researcher agrees with Gran's idea (16) that more IgG concentration in dental caries group can be due to the presence of more gingival inflammation. ECC patients have certainly poorer oral hygiene and more cavities in their mouth that increase plaque accumulation and cause more localized gingival inflammation.

Some studies in both children and adults have indicated different results. They have shown that caries-free or caries-resistant children had higher levels of immunoglobulins in their saliva which was correlated with a low risk of dental caries (7, 8, 17-19).

In this study, we also indicated a weak inverse correlation between salivary sIgA level and DMFT index. This agrees with the study of Camling et al. (20), who found higher levels of IgA in the saliva of children recently colonized (<6months) with *S. mutans* than those who had harbored *S. mutans* for a longer period of time (24 months). Since caries score measure takes time to develop, children with less DMFT, which are probably in the early stage of the disease, have shown higher salivary sIgA levels.

The contradictory findings in the literature and the results of this study suggest that in order to prevent early childhood caries, it is advantageous to study the role of salivary constituents in young children.

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