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ORIGINAL RESEARCH

Evaluation of Total Salivary Secretory Immunoglobulin A and *Mutans*-specific SIgA among Children having Dissimilar Caries Status

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ABSTRACT

Introduction: The occurrence of dental caries has become quite a common phenomenon nowadays. The varying levels of salivary secretory immunoglobulin A (SIgA) usually determine the progression of caries. The present study was aimed to determine the correlation between SIgA and *mutans*-specific antigen SIgA in children having different caries status. Scanning electron microscopic analysis was also completed to correlate the results.

Materials and methods: This study comprised 60 subjects, who were divided into three groups depending on caries status. In all, saliva was collected to determine the level of SIgA and *mutans*-specific antigen SIgA using enzyme linked immunosorbent assay (ELISA). The World Health Organization (WHO) criteria and method were used to evaluate dental caries. Bradford reagent was used to evaluate the levels of protein in the antigen. Furthermore, 20 sections of enamel were randomly obtained to estimate the severity of caries development among groups.

Results: Categorical characteristics among all groups were compared by basic statistical analysis and Chi-squared test. Mean age (years) was found to be 9.214 ± 2.28, 9.5 ± 2.51, and 10.2 ± 2.35 in groups I, II, and III respectively. *Mutans*-specific IgA level (μg/mL) was 34.63 ± 7.46, 28.24 ± 4.52, and 23.56 ± 1.62 in groups I, II, and III respectively. Total SIgA (μg/mL) was 142.53 ± 22.4, 186.10 ± 24.70, and 214.8 ± 27.56 in groups I, II, and III respectively. Caries index was 6.74 ± 2.16, 2.32 ± 0.86, and 0 ± 0 in groups I, II, and III respectively.

Conclusion: Immunoglobulin A is dominantly present in saliva and it plays a significant role in prevention of dental caries. Hence, dental caries is more likely to develop in subjects with low level of salivary IgA (high caries index).

Clinical significance: A low level of IgA may be associated with a high risk of developing dental caries. This association may possibly be useful in predicting the future caries status. Accordingly, suitable caries-preventive measures can be selected and employed.

Keywords: Bradford reagent, Dental caries, Enzyme linked immunosorbent assay, Immunoglobulin, Scanning electron microscopy.

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INTRODUCTION

Dental caries is defined as an irreversible microbial disease of calcified tissue characterized by demineralization of inorganic and destruction of the organic portion of the tooth. It is a common condition in children. It is characterized by the presence of bacteria, such as *Streptococcus mutans, Streptococcus aureus,* etc., on the surfaces of teeth, thus leading to destruction and resulting in dental caries.[1] Various studies have reported on the defensive role of salivary SIgA against dental caries in both children and adults. Dental caries is caused by acid production due to degradation of the carbohydrates present in the food by the microorganisms. Thus, the role of salivary SIgA in preventing progression of dental caries

may be considered. It has also been shown that glucosyltransferase is the main causative agent in caries progression, which is largely prevented by SIgA.[2] Studies have been performed to determine the correlation of dental caries in children with the presence of SIgA. Literature has strong evidence from various studies, such as those evaluating the association between different caries status in children with different levels of SIgA.[3] The production of SIgA specific to *mutans* is highly dependent on the amount of SIgA. It is further determined by the caries prevalence in the mouth. To establish the link between SIgA and *mutans*-specific antigen SIgA with the presence or absence of dental caries, only less number of research studies have been done so far.[4] The ELISA is a diagnostic method helping in the detection of SIgA and *mutans*-specific antigen SIgA. Commonly, the direct, indirect, sandwich, and competitive ELISA methods are used. These methods are widely used to detect antigen and are only possible when the antibody that attaches with the antigen is present in a preconjugated state.[5] The present study aimed to determine the correlations between SIgA and *mutans*-specific antigen SIgA in children having different caries status. We also used SEM to further correlate and substantiate the results.

MATERIALS AND METHODS

The present study included 60 subjects; the age ranged from 5 to 14 years in both the genders. Their parents were informed regarding the study, and written consent was obtained. Ethical clearance was taken prior to the execution of the study. General information, such as name, age, gender, etc., was recorded on case history pro forma. Children were divided into three groups of 20 each, depending upon caries status. Group I had caries index from 5 to 15, group II had caries index from 1 to 4, and group III had 0 caries index. In all children, careful oral examination was performed by a single dentist. The detection of caries was done with the help probe, explorer, mirror, and tweezer under proper illumination. For group I and II subjects, SEM images were also studied in order to observe the changes occurring in the enamel at a microscopic level. Totally, 20 grossly carious primary and permanent molars (those indicated for extraction) were selected randomly for SEM analysis of the changes in the enamel. Teeth were stored in phosphate-buffered saline at 4°C. All attached soft tissue and deposits were removed using hand scalars, and the debris was cleaned with slurry of pumice and water. Enamel samples were obtained by sectioning the tooth through the center of the lesions into two halves using a diamond-impregnated circular disk. Finally, the enamel samples were examined under SEM (Figs 1 and 2).

The WHO criteria and methodologies were used to evaluate dental caries. The presence of discoloration or loss of translucency and catch with explorer tip confirmed the caries. For the collection of saliva, all children were made to sit comfortably on a dental chair and asked to spit in saliva-collecting tubes. All subjects were asked to complete the routine oral hygiene procedure. Soon after breakfast and in between the procedure, children were not allowed to eat or drink. All samples were collected between 10 and 11 am, and the time spent on each procedure did not exceed 30 minutes. Approximately 5 cc of unstimulated saliva was collected and mixed with 5.0 mm phenyl methylsulfonyl fluoride (Sigma, St. Louis, USA). Methylsulfonyl fluoride is added to enhance protein solubilization in order to deactivate salivary proteases. Saliva samples, those stored at −20°C, were taken from the deep fridge and brought to room temperature. The samples were centrifuged for ten minutes at the rate of 2800 rpm. For the measurement of *S. mutans*-specific SIgA and total SIgA, supernatants were equally divided.

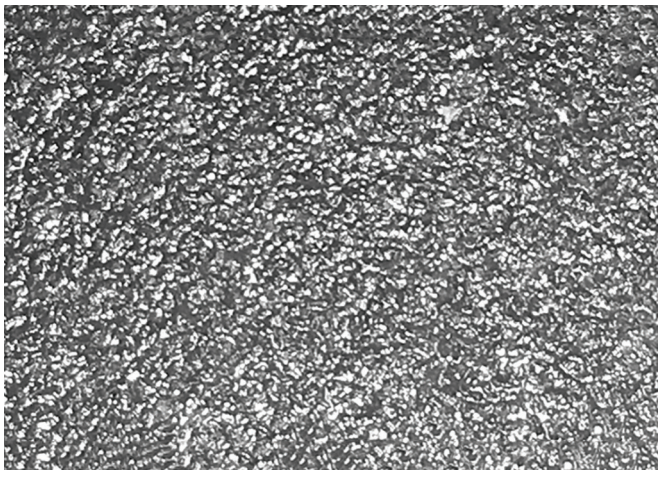


Fig. 1: SEM evaluation of caries occurring in group I subjects (high caries index; larger progressing locules)

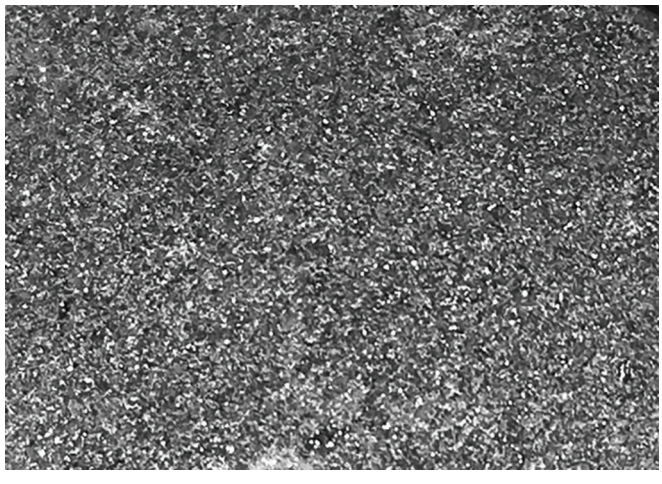
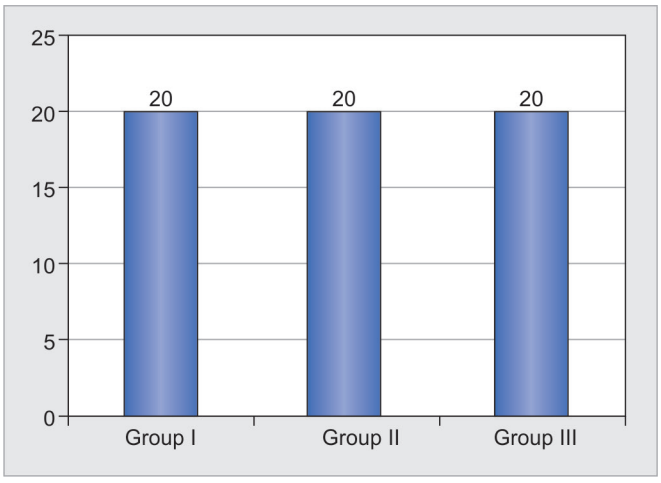


Fig. 2: SEM evaluation of caries occurring in group II subjects (low caries index; smaller progressing locules)

Streptococcus mutans species was used to develop surface antigens, which were stored at a temperature of 20°C. Bradford reagent measured the level of protein in the antigen. The ELISA test measured salivary total SIgA level with the ELISA kit (Human IgG ELISA Kit, Abcam Inc. Cambridge, USA); similarly the *S. mutans*-specific antigen was also calculated. All measurements were done in milligrams. Results thus obtained were subjected to statistical analysis using chi-squared test. A p-value less than 0.05 was considered significant.

RESULTS

Data thus obtained were recorded and tabulated, and sent for statistical evaluation by Statistical Package for the Social Sciences software package version 21 (SPSS Inc., Chicago, Illinois, USA). In groupwise comparison, two-tailed p-values < 0.05 were fixed as statistically significant. Demographic values for all subjects were statistically analyzed. Graph 1 shows that group I (20) had a caries index 5 to 15, group II (20) had 1 to 4, and group III (20) had 0. The difference was nonsignificant (p 1). Table 1 shows that in group I, mean age (years) was 9.214 ± 2.28, *mutans*-specific IgA level (µg/mL) was 34.63 ± 7.46, total SIgA (µg/mL) level was 142.53 ± 22.4, and caries index was 6.74 ± 2.16. In group II, mean age (years) was 9.5 ± 2.51, *mutans*-specific IgA level (µg/mL) was 28.24 ± 4.52, total SIgA (µg/mL) was 186.10 ± 24.70, and caries index was 2.32 ± 0.86. In group III, mean age (years) was 10.2 ± 2.35, *mutans*-specific IgA level (µg/mL) was 23.56 ± 1.62, total SIgA (µg/mL) level was 214.8 ± 27.56, and caries index was 0 ± 0. There are statistical differences existing in mean value of *mutans*-specific SIgA level in groups as shown by the analysis of variance test (p < 0.001, Table 2). Table 3 shows that total SIgA level in groups depicted statistically significant differences (p < 0.001). Graph 2 shows that in group I, *mutans*-specific IgA was 34.6 µg/mL and total IgA level was 142.5 µg/mL. In group II, *mutans*-specific IgA was 28.2 µg/mL and total IgA level was 186.1 µg/mL. In group III, *mutans*-specific IgA was 23.5 µg/mL and total IgA level was 214.8 µg/mL. The difference was statistically significant in all groups (p < 0.05).



Graph 1: Distribution of children

DISCUSSION

As already discussed by various authors, dental caries is a frequently seen disease of the oral cavity. In children, due to the habits of eating candies, sugar-rich food items, and malnutrition, the prevalence of caries is higher when compared with any other dental disease. The other common reason for the higher occurrence of dental caries in children is the inadequate and ineffective brushing habit. Dental caries has varied appearance in children. The principal host factors associated with caries include tooth factors, salivary factors, and body or general factors. Other related factors comprise diet (high sugar intake), oral hygiene-related factors, salivary flow, humidity of oral cavity, salivary pH, and glucose content. The oral microbial factors must be imperatively considered in terms of their type, amount, motility, and toxicity.[6] It is a well-known fact that demineralization and remineralization are balanced processes that usually

Table 1: Studied variables in all groups

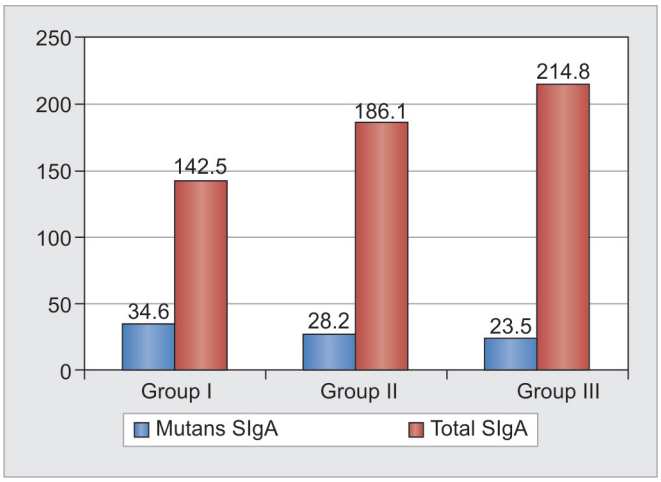
Group I				*Group II*				*Group III*			
Mean age (years)	*Mutans-specific IgA level (µg/mL)*	*Total SIgA (µg/mL)*	*Caries index*	*Mean age (years)*	*Mutans-specific IgA level (µg/mL)*	*Total SIgA (µg/mL)*	*Caries index*	*Mean age (years)*	*Mutans-specific IgA level (µg/mL)*	*Total SIgA (µg/mL)*	*Caries index*
9.214 ± 2.28	34.63 ± 7.46	142.53 ± 22.4	6.74 ± 2.16	9.5 ± 2.51	28.24 ± 4.52	186.10 ± 24.70	2.32 ± 0.86	10.2 ± 2.35	23.56 ± 1.62	214.8 ± 27.56	0 ± 0

Table 2: *Streptococcus mutans*-specific SIgA level in groups using ANOVA test

Source	*Degree of freedom*	*Sum of square*	*p-value*
Method (in between)	5	780	0.001 (p < 0.05)
Method (within)	50	1024	
Total	55	1804	

Table 3: Total SIgA level in groups using ANOVA test

Source	*Degree of freedom*	*Sum of square*	*p-value*
Method (in between)	5	38,410	0.001 (p < 0.05)
Method (within)	50	29,874	
Total	55	68,284	



Graph 2: Comparison of mean values of *mutans*-specific SIgA level and total SIgA level in all groups

occur in the oral cavity. However, remineralization is further assisted by the buffering action of saliva that allows exchange of calcium and phosphate. Literature has proven that the application of SEM in the study of dental caries has greatly enhanced our understanding of the underlying structural changes occurring at crystalline regions (enamel).[7] The microscopy also provides us with an opportunity to clarify the complex carious etiologies.[8] Majority of SEM photomicrographs of group I samples showed larger progressing locules (high caries index), whereas the locules were smaller and relatively inactive in group II samples (low caries index).

Streptococcus mutans is gram-positive anaerobic cocci bacteria commonly encountered as a causative factor of dental caries. It is otherwise a normal commensal of the oral cavity. For initiating decay on tooth, there should be proper adhesion between teeth and causative bacteria. *Streptococcus mutans* attaches to tooth surfaces through its fimbriae. These bacteria promote dental plaque formation on tooth surfaces.[9,10] This formation causes breakdown of sucrose into lactic acid, which lowers the pH of the oral cavity. This, in turn, leads to demineralization of inorganic portions and destruction of organic portions of teeth. Research has shown significant reduction in the *Streptococcus* count in individuals immunized against it.[11] The present study was conducted to determine the relation between SIgA and *mutans*-specific antigen SIgA in children having different caries status. The estimation of total SIgA and *mutans*-specific antigen SIgA was performed by ELISA test. In this study, children were divided into three groups. Group I was the high caries group with caries index ranging from 5 to 15 and consisted of 20 children. Group II (20) had moderate caries with caries index ranging from 1 to 4, while group III children had no caries. Salivary IgA is an important immunoglobulin found in saliva. It is commonly found in the minor salivary gland and shows diurnal variation with the level decreasing as the day progresses. Patients with high caries have low levels of salivary IgA, whereas those with low caries index have high salivary IgA levels. The IgA plays an important role in prevention of dental caries. The level of SIgA is inversely proportional to caries prevalence. These findings may be directly correlated and substantiated with the observations of SEM analysis.

A study by Gregory et al[12] on salivary SIgA and serum antibodies to *S. mutans* ribosomal preparations in dental caries-free and caries-susceptible human subjects found that patients with high levels of salivary SIgA had a lower prevalence of dental caries and those with low levels of salivary SIgA had more number of dental caries. Everhart et al[13] conducted a study and evaluated the dental caries and salivary SIgA levels in children aged 3 to 7 years. They found that children with a low caries index had higher amounts of salivary SIgA level and vice versa. In this study, we found that in group I, the total SIgA (µg/mL) level was low (142.53 ± 22.4), whereas the caries index was high (6.74 ± 2.16). In group II, total SIgA (µg/mL) was more than in group I (186.10 ± 24.70); however, relative caries index was observed to be lower (2.32 ± 0.86) in group II than in group I (6.74 ± 2.16). In group III, total SIgA (µg/mL) level was the highest than all the other groups (214.8 ± 27.56), while caries index was none. We found that the *mutans*-specific IgA level was higher in group I as compared with groups II and III. Ranadheer et al[14] conducted a study to establish the relation between salivary IgA levels and dental caries in children, and found low salivary SIgA levels in children with a high caries index, while high salivary IgA levels were present with low caries index. Similar results were seen in our study. However, Watanabe et al[15] found no relationship between salivary flowrate or SIgA with dental caries in children. Bratthall et al[16] studied the IgA reaction to oral *Streptococci* in the saliva of subjects with different combinations of caries and levels of mutans streptococci. Their study results showed low incidence of carious lesions in subjects with higher IgA concentrations. It was in accordance to our study results. Cogulu et al,[17] in their study of evaluation of the relationship between caries indices and salivary SIgA, salivary pH, buffering capacity, and flowrate in children with Down's syndrome, found a positive correlation between salivary SIgA, salivary pH, and presence of dental caries. Rose et al,[18] in their study of IgA antibodies to *S. mutans* in caries-resistant and susceptible children, concluded that salivary SIgA is predominantly occurring in the saliva and active against caries-causative bacteria, *S. mutans*. The author found higher bacterial counts in children with low salivary IgA level and they were seen having more number of carious lesions as compared with children with high salivary IgA levels. However,

Smith et al[19] found different concentrations of total IgA level in different age grouped children. There were variations in the occurrence of bacterial levels in caries-susceptible subjects and caries-resistant subjects. Many of the other researchers also found a negative correlation between IgA antibodies to *S. mutans* antigens in human saliva and breast milk and the numbers of indigenous oral *S. mutans*.[20-24]

CONCLUSION

An increased level of salivary SIgA in children with low caries index literally highlights the role of immunoglobulin in the prevention of dental caries. Similarly, children with high caries had lower levels of salivary SIgA. Though our study lacked specifying the resources for SIgA, it could exactly indentify cariogenic bacteria. It has also been revealed that salivary SIgA does not have a direct involvement in the prevention and progression of dental caries. Nevertheless, our study results should be interpreted as suggestive. Authors also recommend some future long-term studies with concurrent microbiological correlations so as to precisely observe the "caries progression patterns" that can affect the treatment longevity and outcomes.

CLINICAL SIGNIFICANCE

Our study results underline the importance of salivary immunoglobulins in being associated with caries in the oral environment. Measurement of salivary SIgA in children can effectively help in assessing the future caries status. A low level may be alarming and may indicate a high risk for developing dental caries. Since the secretory IgA is associated typically with *S. mutans* and its enzymatic antigens, protective action of SIgA against dental caries might not be so efficient sometimes due to the steady self-cleansing actions of saliva.

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