A Comparative Quantitative Assessment of Salivary Iga and Alpha Amylase in Caries Free and Caries Active Children

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Objectives: *The aim of the study was to assess the salivary IgA (immunoglobulin A) and alpha amylase levels in the unstimulated whole saliva samples of caries-free and caries-active children and correlate it with the caries status and age.* ***Study design:*** *The salivary IgA and amylase was investigated in 100 children in the range of 8-12 years divided in two groups, control group (DMFT and/or deft = 0) and study group (DMFT/ deft score ≥5). The salivary IgA was measured using kit based on two-site sandwich enzyme immunoassay principle and amylase was estimated using the vitro amyl slides.* ***Results*** *:The mean salivary IgA and amylase levels in the saliva of the children in the control group was found to be significantly increased (p=.001 and p=.014 respectively) whereas the relationship between salivary IgA and amylase levels in the saliva of the children was found to be insignificant with the age (p=.392 and p=.306 respectively).****Conclusions:*** *The results indicated that salivary IgA and amylase levels in saliva increased significantly in caries free children and the level of salivary IgA and alpha amylase has no significant relation with the age of the children.*

Keywords- *Dental caries, Unstimulated whole saliva, Enzyme linked immunosorbent assay, Salivary IgA, Amylase*

INTRODUCTION

Dental caries is a communicable, infectious disease resulting in demineralization and destruction of tooth structure by acid-forming microorganisms. It is a worldwide public health crisis in which the children and adolescents are considered to be mostly at risk. In Global Burden of Disease Study (2015) it is included as the most prevalent condition ranking first for decay of permanent teeth (2.3 billion people) and 12th for deciduous teeth (560 million children). Untreated dental caries may possibly lead to pain, infections and adverse growth patterns thereby impairing the quality of life of children.[1]

The prevention, initiation, and progression of the caries process to a large extent are managed by a natural protective system inherent within the saliva. Saliva is an imperative intrinsic regulator which provides biological and physical defensive mechanisms to the host. Saliva also contains numerous antibacterial compounds such as lactoferrin, lysozyme, lactoperoxidases, and a variety of immunoglobulins which might control the growth of oral cariogenic microflora. Humoral immunologic response can regulate caries activity, especially salivary immunoglobulin A.[2]

Salivary immunoglobulin A (sIgA) is a prominent immunoglobulin present in whole saliva and is considered as the first line of defense of the host against pathogens which invade the mucosal surfaces. It helps in prevention of dental caries and augment oral immunity by inhibition of bacterial adherence, reduction of hydrophobicity, agglutination of bacteria and inactivation of bacterial enzymes and toxins.[3,4,5] Amylase is a salivary enzyme which was thought to only initiate the digestion of starch in the oral cavity by

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hydrolysis of α 1,4 linkages of polysaccharides to glucose. However, presence of alpha amylase (α amylase) in the acquired enamel pellicle and plaque indicate that it might play an important role in the caries process as well.[6] It has been reported that it has a high affinity to oral streptococci and can bind on the bacterial surfaces and to hydrolyze starch, giving rise to products that are transformed into acids leading to dental caries.[7]

The relationship between sIgA, α amylase and dental caries has not been well established in children. Higher levels of sIgA have been reported in caries resistant subjects in comparison to caries susceptible ones, suggesting an effective protection function of this immunoglobulin.[3,4,5] On the other hand, several other investigations did not observe any association between the presence of dental caries and salivary sIgA levels.[3,8] The relationship between α amylase and dental caries is also unclear.[9,10] Hence, the present study was undertaken to assess the sIgA and α amylase levels in the unstimulated whole salivary samples of caries-free and caries-active children.

MATERIALS AND METHOD

The present study was carried out in the Department of Pedodontics and approved by the institutional ethical committee (IERBC/2016/37). Proper written informed consent was obtained from the children parents/guardians prior to their participation in the present study. One hundred children within the age group of 8 to 12 years, comprising 48 boys and 52 girls attending the outpatient Department of Pedodontics and Preventive Dentistry were included in the study. The children who were medically and physically compromised, history of antibiotics usage in the past two weeks, mouth breathers, presence of any intraoral abscess, draining sinus, cellulitis, moderate to severe gingivitis, periodontitis or any significant soft tissue pathology were excluded from the study. The children included were divided into two equal groups: control group (caries free) with no dental caries (DMFT and/or deft = 0) and study group (caries active) who had DMFT/deft score ≥5. Caries status was assessed according to the WHO recommendations.[11]

Special diet was prescribed to the children 24 hours prior to saliva collection by a dietician. Children were instructed not to drink or eat anything for at least 1 hour before the collection of saliva samples. Unstimulated saliva samples were collected in the morning time (9 am to 10 am) after routine dental brushing to minimize the effect of diurnal variation. The children were asked to sit comfortably on the dental chair with open eyes, head bent a little forward with minimal oro-facial movements and instructed to relax for 5 minutes. Saliva was allowed to collect in the floor of mouth and children were asked to spit the accumulated saliva into sterile saliva collection vial. Approximately 2-3 ml of unstimulated whole saliva was collected directly into vial, placed in a hermetically sealed ice box and immediately transported to laboratory and stored at -20ºC till further analysis.

The salivary IgA was measured using Total IgA EIA kit (XEMA Co. Ltd., Moscow, Russia). This kit is based on two-site sandwich enzyme immunoassay principle. The sIgA concentration in saliva is directly proportional to the absorbance change on the spectrophotometer at 450 nm caused by antigen antibody reaction. The corresponding concentration of sIgA in the salivary samples was calculated from the known IgA calibration curve. The estimation of salivary alpha amylase was done using the VITROS AMYL slides (Ortho-Clinical Diagnostics Inc., NY, USA). The amylase in the saliva sample catalyzes the hydrolysis of the dyed starch present in the slides into smaller dyed saccharides. The reflectance spectrophotometry measured the reflection density of the dyed saccharides in the reagent layer at 2.3 and 5 minutes. The difference in the slide's reflection density between the two readings was proportional to salivary α amylase level.

The data obtained was systematically compiled, tabulated to a computer from a pre-coded proforma and subjected for statistical analysis. The statistical software namely SPSS 19.0 was used to analyze the data and Chi – square, independent 't' test and Pearson's correlation tests were used to find the significance of study parameters on categorical scale and ordinal scale between two or more than two groups. Significance level for the statistical test utilized in the study was set at $p< 0.05\%$.

RESULTS

The comparison of mean salivary IgA (sIgA) and alpha (α) amylase levels in salivary samples of control group and study group is shown in table 1. The mean sIgA and α amylase level in saliva is significantly increased in the study group when compared to the control group. The mean sIgA levels in the study and control group with the age is shown in Figure 1. In the study as well as the control group, the mean sIgA level was found to have no statistical significance with age ($p = 0.112$ and $p = 0.312$ respectively). When the total mean sIgA levels were correlated with age, irrespective of the caries status no statistical significance was found between age and mean sIgA levels ($p = 0.392$). The mean salivary amylase levels in study and control group with the age is shown in Figure 2. In the study as well as the control group, the mean α amylase level was found to have no statistical significance with age ($p = 0.324$ and $p = 0.603$ respectively). When the total mean salivary α amylase levels were correlated with age, irrespective of the caries status no statistical significance was found between age and mean α amylase levels ($p = 0.306$).

Table 1: Comparison of mean salivary IgA and alpha amylase concentration among the two groups

	Group	N	Mean ± SD	T	p-value
IgA levels (µg/ml)	**Control Group**	50	5.62± 1.77		
	Study Group	50	2.98± 1.66	6.880	**0.001***
Alpha amylase (U/ml)	**Control group**	50	83.53± 27.61		
	Study Group	50	68.42± 26.28	2.505	**0.014***

Chi Square test applied *Significant

Figure 1: Distribution of mean salivary IgA levels according to age

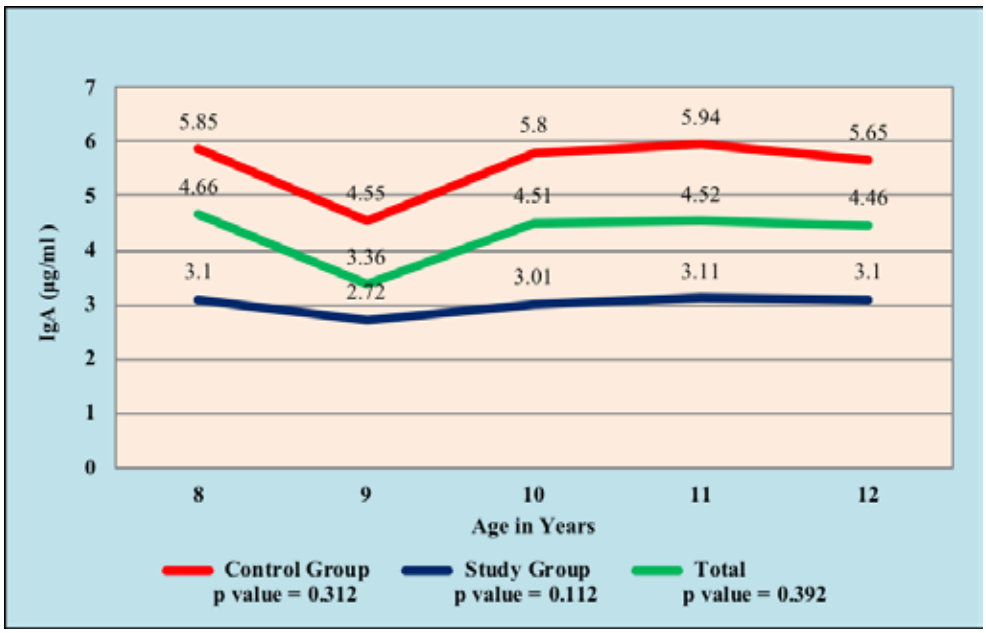
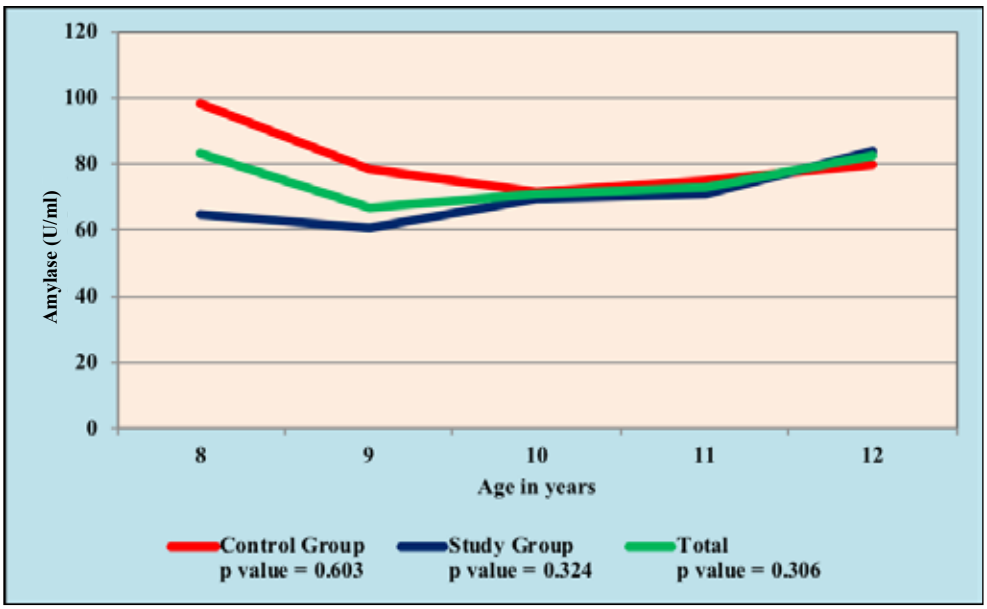


Figure 2: Distribution of mean salivary amylase levels according to age



DISCUSSION

Dental caries is a prevalent oral disease of multifactorial origin resulting from an imbalance of many risk and protective factors which negatively affects the health of the population over a period of time.[12] Extensive clinical researches have been carried out in past to identify and quantify the factors influencing the progression of the disease process.[13] Human saliva is one such innate factor which not only make oral functions such as swallowing and speaking possible, but also lubricates and shields oral tissues, teeth and mucosal surfaces in many ways. Salivary components like secretory IgA, salivary peroxidase, lysozyme, lactoferrin and its properties such as flow, viscosity, buffering capacity, play a major role in the prevention, initiation, and progression of caries.[3] The infectious and microbial nature of dental caries leads to the assumption that some form of host immunity would exist to control caries progression and then as secretory IgA offers protection in other body secretions, it must be able to do so in saliva also.[5]

Saliva is composed of an array of analytes from systemic sources that reach the oral cavity through different pathways.[14] The research regarding saliva has always been an interesting aspect as it represents all the ions usually present in body fluids. Samples from the stimulated saliva were accepted to be used in caries research for the analysis of its composition, bacteriological investigation (such as counting Streptococcus mutans and lactobacilli spp.), initial and final pH and its buffering capacity because of its expected functional role during food intake. However, recently, Bardow *et al* emphasized the importance of the composition of unstimulated saliva in caries process.[15] Moreover, Mandel and Khurana[16] found that the IgA concentration decreased with increased salivary flow from the parotid and submaxillary salivary

glands and postulated that stimulated saliva could have decreased the concentration of the IgA. It has also been reported that the levels of antimicrobial agents in saliva of children, depends on the sample collection technique as it is also present in nasal and lacrimal secretions, which can easily contaminate saliva in restless children.[17] Moreover, Dodds *et al* [18] reported that the unstimulated state is the predominant condition in terms of the salivary gland activity, and the unstimulated salivary flow is the critical determinant of salivary clearance. Therefore, in the present study the levels of sIgA and α amylase levels were measured in unstimulated saliva. Recently it has been reported that enzyme linked immunosorbent assay (ELISA) is an immunoassay for antibody detection that provides a combination of sensitivity, specificity, detection limit and precision with advantage of not using radioisotopes.[19] Therefore, in the present study, ELISA technique was used for the estimation of sIgA antibodies.

It is well recognized that saliva plays a special role in the maintenance of oral health due to its immunological and non-immunological components such as antimicrobial proteins and specific enzymes.[20] Secretory immunoglobulin has been shown to be the predominant immunoglobulin found in human secretions and at mucosal surfaces. Naturally occurring sIgA antibodies to various antigens (oral, ocular and respiratory microorganisms) are present in mucosal fluid and can also serve as a foremost immunological defense against disease like dental caries. Salivary IgA contributes to 60% of the total immunoglobulin count in the saliva and is the first line of defense of the host against pathogens which invade mucosal surfaces. The distinctive specificities and intensities of sIgA antibody responses to antigens of streptococcus mutans may influence colonization by these microorganisms. The sIgA mechanism of the action is thought to be based on the obstruction of sucrose dependent and sucrose independent attachment of mutans streptococci to the hydroxyapatite and possibly on inhibition of metabolic activities. Exposure to cariogenic microorganisms leads to the secretory immune components against several microorganism epitopes, and this binding is responsible for instigating the immunological response. One hypothesis proposes that the binding decreases the free sIgA levels in saliva from caries active subjects in contrast with caries resistant ones, while other suggest that this binding generates the immune response resulting in the increase of the free sIgA levels.[21] So far, many attempts to relate the salivary concentrations of these antibacterial factors to the prevalence of caries have been made.[22] Yet the relationship between sIgA and dental caries has not been well established.

In the present study, the means IgA levels in children in the caries free group was significantly higher than the caries active group, suggesting a possible protective role of IgA in prevention of dental caries. This finding can be due to the local immune protection provided by the secretory immune system against cariogenic microorganisms in the oral environment resulting in the prevention of dental caries in caries free group. This difference may be because of the greater number of mutans streptococci present in the whole saliva of caries active children which might adsorb greater amounts of whole sIgA antibody to mutans streptococci as compared to caries free children. In addition, it can also be postulated that the caries free children produced a greater amount of sIgA antibodies to mutans streptococci in minor, submandibular or sublingual salivary glands than caries active children. Some authors have reported that saliva of caries free subjects shows significant IgA antibodies against streptococcus mutans, indicating a protective mechanism.[22] The present results are in agreement with the several studies that have reported an increase in the level of sIgA with the decrease in caries activity.[17,22,23,24,25] However, the present results are in disagreement with the many studies who have reported an increase in sIgA level with increase in caries activity.[3,4,5,26] Moreover, authors have also reported that there was no correlation between dental caries and sIgA levels.[27]

Lehner *et al* reported that subjects with caries had decreased sIgA concentration, as compared to those with no caries and proposed that it could be due to the deficient transport mechanism, stimulation of immune system via pulp, deficient local immunoglobulin synthesis and molecular size of IgA.[23] Previously, it was reported that the degree of caries status and total sIgA concentration were negatively correlated and tends to support the hypothesis that higher levels of sIgA may provide protection against dental caries.[24] Rose *et al* compared the IgA levels of whole saliva and parotid saliva of caries susceptible and caries resistant children aged 7-11 years using ELISA and concluded that whole saliva and not parotid saliva in caries resistant children had a significantly higher IgA level as compared to the caries prone group.[25] Omar *et al* reported significantly higher levels of sIgA in caries free subjects which might be due to IgA increase in response to mild exposition of dental caries as a form of a protective mechanism of the body against caries attack and concluded that the levels of sIgA can be seen as a risk factor for upper respiratory infection, periodontal disease and caries.[28] Pal *et al* [5] studied the relationship between mutans specific sIgA and total sIgA and its impact on the caries status of children. They reported that high caries group showed high mutans specific sIgA and less total sIgA levels compared to low caries group. Kuriakose *et al* [29] also reported that caries resistant children had significantly higher mean IgA levels when compared to caries active children; however, the study was done in the children afflicted with rampant caries. Patel *et al* [30] also reported a correlation between low rates of caries activity and higher levels of sIgA antibodies, although the study was done in adults as compared to present study which was conducted in children aged 8 to 12 years.

In contrast to results of the present study, many authors have reported that the presence of dental caries was associated with an increase of total sIgA levels.[3,4,26] Parisotto *et al* found high concentrations of sIgA in children with caries and suggested that caries stimulates the production of sIgA and also that specific antibodies could play a role in oral homeostasis.[31] Arafa *et al* [32] in a study reported a significant inverse relationship between the IgA secretion rate and dental caries in asthmatic children and stated that the raised levels of IgA in serum reflect past exposure of the host to the cariogenic microorganisms. Bhatia *et al* found that higher levels of sIgA concentration in caries susceptible group and postulated that it could be either due to a cumulative or recent antigenic stimulation, with higher caries experience as compared to caries resistant group.[33] Bagherian *et al* reported that the high concentration of sIgA in children with caries may be associated with an increased antigen load, leading to high production of antibodies.[26] These contradictory results can be explained by the fact that the

infective nature of caries amounts to high streptococcus mutans in the oral cavity resulting in the immune system response to the high antigenic load and eventually leading to the greater production of immunoglobulins. It can also be postulated that sIgA might be the first line of defense but when the force of microbial load is high, more sIgA production cannot help in prevention of disease. Another reason for an increase in sIgA levels might be the cumulative antigenic stimulation of repeated attacks of caries or more recent antigenic stimulation or both of the above factors. Interestingly, some studies also show that there was no correlation with the dental caries status and sIgA levels.[3,27] Smith *et al* reported that the level of whole sIgA antibodies to mutans streptococci were relatively equal in caries free children as compared with the caries active children and postulated that this might be due to the shift in actual proportion and concentration of secretory IgA in children from birth to adulthood.[21] The contradictory results seen in all these studies may be due to the differences in sampling methods, study design and populations, different criteria for patient groups, sample collections, assay methods, and statistical techniques between the studies.

The immunological system matures with age. Previous researches demonstrated that secretory IgA levels increase with age.[34] It has been reported that the salivary sIgA antibodies to mucosal bacteria begin to appear as early as the first week of life[21], and approach adult levels by 1 to 2 years of age and most children over 3 years of age already have salivary sIgA antibodies reactive with antigens of mutans streptococci.[33] Parisotto *et al* performed an sIgA longitudinal evaluation and suggested that the increased sIgA levels over time are explained by the mixed dentition transition and the growth process that could be related to maturation of salivary glands as part of the general development of systems of the body.[31] Koga-Ito *et al* also found increased concentrations of sIgA in young adults in comparison to children.[27] Tenovuo *et al* observed that immunoglobulins did not show any clear age dependency in children aged 0.8 to 3.8 years.[35] Jafarzadeh et al reported that sIgA levels increased with the age up to 60 years and then slightly decreased in subjects aged 61 to 70 years.[36] In the present study, there was no correlation found between the age and sIgA levels in children which might be due to the narrow age range (8-12 years) included in the present study.

Amongst the numerous salivary electrolytes and enzymes, amylase is notably the most abundant enzyme present in human saliva and is considered to be causing caries by binding its alpha fraction to the bacteria.[13] Alpha-amylase is one of the major components of saliva and one of the striking features of this enzyme is that it is exclusively of salivary origin when compared with other enzymes of saliva which are of both bacterial and salivary origin. Salivary amylase plays an important role in the colonization and metabolism of streptococcus, leading to the formation of dental plaque and caries in human beings. It has been identified as a component of the acquired pellicle and also acts as a receptor for microorganism adhesion on tooth surface. It has the capability to bind on the bacterial surfaces and to hydrolyze starch, thereby giving rise to products that are transformed into acids leading to dental caries.[7] The close relation of α-amylase with carbohydrate digestion and oral microbial flora complicates its action in the dental caries process.[37]

In the present study, an inverse relation between caries status and α amylase concentration of saliva and a statistically significant difference between the levels in the control group and study group was seen. These results are in agreement with the results obtained by Prabhakhar *et al* [9] and Mojarad *et al*[38]. The low caries status in children with high α amylase concentration can be because of the starch clearance action of amylase. Prabhakar *et al* [9] reported that the mean α amylase concentration in the caries free group was found to be high when compared to the high caries group. However, they had evaluated α amylase concentration in stimulated saliva as compared to unstimulated saliva evaluated in the present study. Mojard *et al* [38] concluded that the low levels of alpha amylase may promote caries in young children. On the other hand, dental caries may consequently decrease the level of salivary alpha-amylase and this vicious cycle can promote and accelerate dental caries among susceptible subjects with low level of salivary alpha-amylase. The results of the present study disagree with the results obtained by Singh *et al* [10] who reported higher amylase concentration in caries active group when compared to caries free group and showed a direct relationship between amylase and carious surfaces on teeth. It can be explained by the reason that in high caries group having high α amylase concentration, there is increased degradation of dietary starch into disaccharides in close proximity to tooth structure. The utilization of these carbohydrates by oral bacteria results in increased acid production and increase in the tooth demineralization process. However, there was no strong correlation of the amylase activity to the presence of dental caries found in studies done by Kargul *et al.*[39] *Jacobsen et al* [7] found no correlation between the amount of amylase in the plaque samples and saliva samples and concluded that salivary amylase does not contribute in any considerable extent to the formation or accretion of dental soft deposits. Scannapieco *et al* reported that alpha amylase can promote catalyzing of the dietary starch hydrolysis by binding on the surface of cariogenic bacteria. The amylase-binding bacteria present in plaque may accumulate salivary alpha-amylase inside the plaque matrix to provide more glucose from dietary starch in proximity to the tooth surface and might play an important cariogenic role in the presence of starch-containing foods.[37] Liang et al indicated that the composition of salivary alpha-amylase isoenzymes are related to the occurrence of dental caries.[40] Juan *et al* [41] revealed a considerable difference between the level of salivary alpha-amylase in caries free and caries active subjects and concluded that there was a relationship between salivary alpha-amylase and dental caries.

During early childhood the non-immune salivary factors like lysozyme, salivary peroxidase etc. work already at almost full capacity. However, it has been reported that lactoferrin, myeloperoxidase and total protein are still significantly less abundant in the mixed saliva.[17] It has been reported that in newborns the salivary alpha amylase activity is detected very less and climbs to the adult levels within the first 3 years. Over the adulthood until older ages the salivary alpha amylase activity seems to remain unchanged. Lehner *et al* observed that amylase activity did not show any clear age dependency in children aged 0.8 to 3.8 years.[42] The present study also showed that there was an insignificant relationship between the age and saliva α amylase which might be due to the narrow age range (8-12 years) included in the present study.

Dental caries is a slowly progressing disease which takes a long time for clinical manifestation to be observed. The defense system of the host becomes active as soon as the pathogens start invading. In contrast, the level of antibodies is usually determined on one occasion only, making it difficult to establish a correlation between the level of antibodies and number of carious lesions. In view of the multifactorial nature of the caries process, it is apparent that a certain level of antibodies to S mutans, protective in one individual, might not confer protection in another because of pattern of sugar consumption, differences in oral hygiene, plaque levels of cariogenic bacteria, and exposure to fluoride. In future, an increase in sample size along with the investigation of the IgA specific to streptococcus mutans might give a clear correlation. Interestingly, many of the salivary antimicrobial agents interact with one another in a synergistic or additive way. For example, such interactions have been reported between sIgA and peroxidase, lactoferrin and peroxidase, lactoferrin and lysozyme.[3,4,5] Although, these interpretations are from *in vitro* experiments, it is probable that such concerted effects may also exist in-vivo, where all the components are simultaneously present providing the local host defense system against caries. The complexity of the relationship between dental caries and sIgA and alpha amylase levels needs to be confirmed in more extensive studies.

CONCLUSIONS

From our results, it can be concluded that the level of salivary IgA and alpha amylase increases in children who were caries free as compared to the caries active children. The level of salivary IgA and alpha amylase has no significant relation with the age. Further future longitudinal studies with an increase in the sample size along with the analysis of other salivary factors might establish the true relationship between salivary immunoglobulin, alpha amylase and the dental caries.

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