Original Article

# Analysis of Salivary IgA, Amylase, Lactoferrin, and Lysozyme Before and After Comprehensive Dental Treatment in Children: A Prospective Study

**Akhilesh Sharma, Priya Subramaniam, Shebin Moiden**

*Department of Pedodontics and Preventive Dentistry, The Oxford Dental College and Hospital, Bengaluru, Karnataka, India*

## Abstract

**Objective:** This study aimed to evaluate the levels of salivary IgA, amylase, lactoferrin, and lysozyme before and after comprehensive dental treatment in children with early childhood caries. **Design:** Thirty children aged 36–60 months, with a deft score ≥5, were selected for the study. Before dental treatment, paraffin-stimulated whole saliva was collected in a sterile graduated cup for a period of 5 min. The saliva samples were quantitatively analyzed for levels of IgA, amylase, lactoferrin, and lysozyme using enzyme-linked immunosorbent assay. Comprehensive dental treatment was carried out in all the children including caries preventive procedures. A second sample of saliva was collected at 3 months following completion of dental treatment. Data obtained was subjected to statistical analysis using Student's *t*-test. **Results:** The mean levels of salivary IgA was significantly reduced from 59.60 μg/ml to 56.42 μg/ml after dental treatment ($P < 0.05$). There was a significant reduction in the levels of salivary amylase from 115.78 μg/ml to 113.33 μg/ml ($P < 0.001$). Following dental treatment, salivary lactoferrin and lysozyme levels were significantly reduced from 3.76 μg/ml and 10.62 μg/ml to 3.44 μg/ml and 10.27 μg/ml, respectively ($P < 0.001$). **Conclusions:** Levels of salivary IgA, amylase, lactoferrin, and lysozyme were reduced significantly at 3 months following comprehensive dental treatment.

**Keywords:** *Amylase, dental caries, lactoferrin, lysozyme, salivary IgA*

## Introduction

Dental caries is an infectious multifactorial disease caused by interplay of tooth, diet, and microorganisms. The development of dental caries requires the presence of cariogenic bacteria and fermentable sugar to form acid leading to demineralization of enamel.[1] Cariogenic microorganisms enter the dental biofilm early in life and can subsequently emerge, under favorable environmental conditions, to cause disease. Occasionally, children with early childhood caries (ECC) have moderate levels of *Streptococcus mutans*, which are generally acquired from their mothers at an early age.[2] The various adaptive host defenses in response to these infections are expressed in saliva and gingival crevicular fluid in the oral cavity.[3]

Specific immune defense against *S.mutans* is considered to be one of the factors in altering the dental caries process. Protection is provided largely by SIgA antibodies which are generated by the mucosal immune system. The various nonspecific antimicrobial agents in saliva such as immunoglobulins, cystatins, lactoperoxidase, lysozyme, and lactoferrin have also shown an interactive effect on the reduction of bacterial growth and metabolism.

The protective mechanism of saliva is necessary for the reduction and prevention of this infectious disease. Saliva has various protective functions due to its physical characteristics and chemical composition. The immune response in the oral cavity is due to the presence of an extensive and specialized mucosa-associated lymphoid tissue.[4] Antimicrobial proteins in saliva constitute immunoglobulins and nonimmunoglobulins such as lactoferrin, lysozyme, mucins, histatins, and lactoperoxidase. Many of these molecules are present in rather low concentrations in the whole saliva, and it should be considered that their effects are cumulative and/or synergistic, resulting in an efficient molecular defense network of the oral cavity.[5] These salivary immunoglobulins include IgA, IgM, and IgG.



**How to cite this article:** Sharma A, Subramaniam P, Moiden S. Analysis of salivary IgA, amylase, lactoferrin, and lysozyme before and after comprehensive dental treatment in children: A prospective study. Contemp Clin Dent 2017;8:526-30.

***Address for correspondence:***
*Dr. Priya Subramaniam, Department of Pedodontics and Preventive Dentistry, The Oxford Dental College and Hospital, Bommanhalli, Hosur Road, Bengaluru - 560 068, Karnataka, India.*
*E-mail: drpriyapedo@yahoo.com*

**Access this article online**

**Website:** www.contempclindent.org

**DOI:** 10.4103/ccd.ccd_103_17

**Quick Response Code:**

Salivary IgA is the main immunoglobulin in saliva and provides the first line of defense against the pathogens. The decrease or absence of IgA or selective IgA deficiency is significant in immunodeficiency disorders and also has been associated with the occurrence of dental caries.[6] Salivary amylase was earlier considered as a starch digestive enzyme. Salivary alpha-amylase has been identified as a constituent of the acquired enamel pellicle[7] and may act as a receptor for bacterial adhesion to the tooth surface. The enzyme has also been found to interact with several species of oral streptococci, which are among the first to colonize dental plaque.[8] Lysozyme and lactoferrin are proteins produced by the major and minor salivary glands. They contribute to the maintenance of a stable oral ecosystem. These proteins can influence the development of dental biofilms, which inhibit mutans streptococci adherence and modulate planktonic bacterial aggregation to the biofilm.[9]

Diminished levels of salivary proteins have been found to act as a risk factor for the development of dental caries.[10] An antigenic challenge is known to increase the production of these proteins.[11] Oral research has focused on levels of salivary IgA, lysozyme, lactoferrin, and amylase in relation to dental caries. However, there has been no definite association reported between salivary proteins and caries biofilm.[9,12,13]

Immunological basis of dental caries in children has not been extensively studied. There is a paucity of research in the evaluation of immunologic response of specific and nonspecific salivary proteins following complete oral rehabilitation. Furthermore, no studies have evaluated the association of both specific and nonspecific salivary proteins before and after 3 months of complete dental treatment in children. It was hypothesized that increased salivary protein levels would return to normal levels once dental disease is treated and oral health has been restored. Hence, the present study was undertaken to evaluate levels of specific and nonspecific immune defense salivary proteins, namely, IgA, amylase, lactoferrin, and lysozyme following comprehensive dental treatment in children with ECC.

## Methodology

The study was conducted in Department of Pedodontics and Preventive Dentistry. The study was approved by the institution's ethical review board.

The study was carried out from March 2013 to August 2014. Eighty-five children between the age of 36–60 months who were visiting the dentist for the first time were screened in the department. Of these, thirty healthy, cooperative children aged between 36 and 60 months, with only primary dentition with fair oral hygiene and having deft scores >5, were selected for the study. Children having an abscess due to dental caries, children with salivary gland disorders, those with special health care needs, recent exposures to antibiotics (last 3 months), poor oral hygiene, presence of periodontal disease, and children who were chronic mouth breathers were excluded from participating in the study.

Before conducting the study, written consent was obtained from the parents or caretakers of children. Oral examination was performed by a single calibrated examiner using dental mouth mirror and explorer under artificial illumination. WHO criteria (2013) for caries lesions were used to diagnose and record dental caries. Before the start of dental treatment, stimulated whole saliva was collected from each child. The saliva collection was done between 10 AM and 11:30 AM for all the children. It was ensured that the child did not eat or drink anything for 1 h before saliva collection. Each child was instructed to sit motionless and upright and was asked to chew a paraffin wax block of 1 cm diameter for 1 min.[14] The initial pooled saliva was discarded. The child was made to chew on the paraffin wax for an additional 5 min and was told to spit out the pooled saliva into a collecting funnel placed in a sterile graduated jar/cup, over a period of 5 min. The collected saliva was transferred to sterile Eppendorf tubes. To remove any bias, the tubes were randomly coded, refrigerated at 4°C, and transported to the laboratory within 30 min of collection.

The obtained saliva samples were then thawed and centrifuged for 15 min at 10,000 rpm at 4°C to remove mucin and debris. The samples were stored at minus 4°C until biochemical analysis was done within 1 week, at Bhat Biotech India (P) Ltd., an ISO 9001 certified laboratory. The concentration of salivary IgA was specifically determined using the competitive indirect enzyme-linked immunosorbent assay (ELISA) technique.[15] A quantitative sandwich enzyme immunoassay technique was used to measure salivary amylase in the samples.[16] A double antibody sandwich ELISA and sandwich ELISA were used for quantification of lactoferrin and lysozyme, respectively.[17,18] The salivary assay for each of the variable was performed in triplicate, and the mean of each was calculated.

Comprehensive dental treatment was carried out for each child in accordance with American Academy of Pediatric Dentistry clinical guidelines.[19] It included oral prophylaxis, topical fluoride application, restorations with glass ionomer cement and composite resin, pulpectomy, and stainless steel crown placement. The treatment was completed for these children within a period of 2 weeks. The children were recalled at monthly intervals and monitored for oral hygiene maintenance and examined for any new or recurrent carious lesions. A second stimulated salivary sample was collected in a similar manner at 3 months following the completion of comprehensive dental treatment and analyzed for levels of salivary IgA, amylase, lactoferrin, and lysozyme. The laboratory personnel analyzing the samples was blind to the purpose for which the samples were estimated. The

data obtained was subjected to statistical analysis using Student's *t*-test and Fischer's exact test.

## Results

Out of thirty selected children, there were 18 boys (60%) and 12 girls (40%). The mean age of the boys and girls were 4.14 ± 0.66 years and 4.13 ± 0.71 years, respectively. The mean of the three calculated values of levels of salivary proteins before and at 3 months following dental treatment are shown in Table 1. The levels of salivary IgA, amylase, lactoferrin, and lysozyme showed a significant reduction ($P < 0.05$, significant; $P < 0.001$, highly significant) at 3 months following dental treatment.

## Discussion

Human saliva acts as a medium for transfer of various protective proteins which act as an important role in the prevention, initiation, and progression of dental caries. Many studies have estimated the levels of these salivary proteins in caries-free individuals and in those with different caries experience.[9,13,20,21] Any alterations in salivary proteins levels due to dental disease could return to the normal range following dental treatment. In the present study, children aged between 36 and 60 months were selected as they can understand, follow instructions, and respond to verbal commands satisfactorily. Furthermore, children in this age group have the capacity to expectorate saliva. Children with any acute signs and symptoms of infection due to caries and those with stress and uncooperative behavior during dental treatment have shown elevated levels of salivary amylase.[22,23] Stress can lead to physiobiochemical alterations in the constituents of saliva. Hence, only children displaying cooperative behavior and without any acute symptoms of pain were selected for the study. Since poor oral hygiene is a predisposing factor for dental caries and periodontal diseases, children selected for study were those having fair oral hygiene without any periodontal pathology. A control group was not included in the study as retaining a group of children with ECC for the purpose of only comparison, without treating them would be unethical and unacceptable. A control group of caries-free children was also not included as the objective of the study was to compare the levels of salivary proteins before and after the complete oral rehabilitation.

**Table 1: Mean salivary protein levels before and 3 months after comprehensive dental treatment**

| Salivary protein | Mean±SD | | Paired *t* | *P* |
|---|---|---|---|---|
| | Before treatment (µg/ml) | After treatment (µg/ml) | | |
| IgA | 59.60±10.85 | 56.42±10.26 | 2.9799 | 0.0058* |
| Amylase | 115.78±13.17 | 113.33±12.53 | 7.4945 | 0.00001** |
| Lactoferrin | 3.76±0.83 | 3.44±0.75 | 7.7791 | 0.00001** |
| Lysozyme | 10.62±1.83 | 10.27±1.81 | 7.8851 | 0.00001** |

*$P$<0.05 is significant, **$P$<0.001 is highly significant.
SD: Standard deviation; IgA: Immunoglobulin A

Dental treatment itself can induce fear and anxiety in children. It has been observed that children had significant increased levels of salivary amylase immediately after dental treatment.[24] Thus, the posttreatment saliva samples were not assessed immediately but collected at 3 months after completion of dental treatment.

The relationship between salivary IgA and dental caries status has been inconclusive.[12,20,25] In these studies, the SIgA levels were determined either before the dental treatment or relied on deft scores. Furthermore, the methodology differed in estimation of IgA. There is a paucity of studies with regard to levels of salivary IgA following dental treatment. In our study, we found a significant decrease in the levels of salivary IgA specific to *S. mutans* and other nonspecific proteins 3 months after completion of dental treatment. The reduced levels of these proteins could be due to combined effect of professional oral rehabilitation and continued home care instructions.

Caries removal leads to reduction in the microbial load and its associated antigenic challenge.[26] Transient reduction in interproximal *S.mutans* levels was observed following professional oral prophylaxis with concurrent practice of chlorhexidine mouth rinses and tongue cleaning by Axelsson *et al*.[27] A transient decrease in salivary IgA following excavation of carious lesions has been reported.[28] In our study, a significant reduction in salivary IgA was seen at 3 months after dental treatment. However, earlier reports found no statistical significant difference in the levels of salivary IgA, pre- and post-dental treatment in children.[11,29] In those studies, the researchers estimated IgA levels in saliva between 1 and 4 weeks, following treatment.[11,29] In the present study, specific and nonspecific proteins were estimated after 3 months. This longer duration and the home care instructions and use of fluoridated dentifrice could be the possible reason for the difference in the outcome of the study. Salivary amylase has a bacterial interactive action with a high affinity to microorganisms such as *Streptococcus* species.[7] This action of amylase can help in bacterial clearance from the oral cavity. Apart from restoration of carious teeth, every child received complete oral prophylaxis, topical fluoride application, and oral hygiene instructions. Further clinical monitoring at monthly intervals was also carried out, and oral hygiene instructions were reinforced. This could have led to reduction in plaque and microbial load, thereby decreasing the antigenic challenge and subsequent decrease in salivary amylase and IgA levels. Previous studies had evaluated the changes after excavation of caries or its restorations. However, in the present study, a comprehensive oral rehabilitation followed by home care instructions and periodic recall visits was done. This could be the reason for statistical decrease in the levels of the salivary proteins.

Many of the defense factors in saliva interact with each other, thereby enhancing their antimicrobial activity. Salivary lactoferrin and lysozyme have been shown to bind to secretory IgA.[28] Furthermore, IgA has been reported to enhance the antimicrobial properties of lactoferrin.[30] Lactoferrin combines with hydroxyapatite and inhibits adherence and biofilm formation which in turn affects the bacterial colonization. The proteolytic action of lactoferrin degrades bacterial virulence factors.[31,32] Lysozyme is an antimicrobial enzyme that catalyzes the degradation of the negatively charged peptidoglycan matrix of microbial cell walls. The decrease in the levels of microorganisms in turn decreases this protein level. The pretreatment levels of lactoferrin and lysozyme in our study were significantly higher than posttreatment levels after 3 months. Dental caries could have been a triggering factor for a nonspecific immune response that may have led to the increase in levels of these proteins.[9] As with the other salivary defense factors, dental treatment could have reduced the antigenic load which in turn led to the decrease in levels of lactoferrin and lysozyme. Although the results indicate a statistically significant reduction in the levels of salivary proteins in response to dental treatment, the changes appear to be small/minor at 3 months. However, there could have been a better understanding of the process/relationship if the levels were assessed at frequent intervals over a period of 3 months or longer. Thus, with the inherent complexities, the hypothesis suggested at the initiation of the study has been proved correct.

The present study showed reduction in the levels of salivary proteins, following 90 days of dental treatment. This outcome indicates that comprehensive dental treatment and reinforcement toward oral hygiene maintenance in children can alter the immunologic response of their oral cavity. Comprehensive dental treatment reduces the levels of oral microorganisms, and as a result the initial immunogenic response could decrease over a period of time.

Variations in salivary proteins may play an important role in determining their protective features toward dental caries.[33] Longitudinal studies involving a larger sample of children with oral disease could be carried out to estimate salivary levels of proteins with increasing age and changes in the oral cavity.

## Conclusions

In children with ECC, complete oral rehabilitation followed by oral home care reinforcement showed a significant reduction in the levels of salivary IgA, amylase, lactoferrin, and lysozyme at 3-month follow-up.

### Financial support and sponsorship

Nil.

### Conflicts of interest

There are no conflicts of interest.

## References

1. Lenander-Lumikari M, Loimaranta V. Saliva and dental caries. Adv Dent Res 2000;14:40-7.
2. Chawda JG, Chaduvula N, Patel HR, Jain SS, Lala AK. Salivary SIgA and dental caries activity. Indian Pediatr 2011;48:719-21.
3. Bowen WH, Koo H. Biology of *Streptococcus mutans*-derived glucosyltransferases: Role in extracellular matrix formation of cariogenic biofilms. Caries Res 2011;45:69-86.
4. Czerkinsky C, Anjuere F, McGhee JR, George-Chandy A, Holmgren J, Kieny MP, *et al.* Mucosal immunity and tolerance: Relevance to vaccine development. Immunol Rev 1999;170:197-222.
5. Fabian TK, Fejerdy P, Csermely P. Saliva in health and disease. Wiley Encyclopedia of Chemical Biology. 1st ed., Vol. 4. Hoboken, NJ, USA: John Wiley & Sons Inc.; 2008. p. 1-9.
6. Rose PT, Gregory RL, Gfell LE, Hughes CV. IgA antibodies to *Streptococcus mutans* in caries-resistant and -susceptible children. Pediatr Dent 1994;16:272-5.
7. Al-Hashimi I, Levine MJ. Characterization of *in vivo* salivary-derived enamel pellicle. Arch Oral Biol 1989;34:289-95.
8. Scannapieco FA, Torres G, Levine MJ. Salivary alpha-amylase: Role in dental plaque and caries formation. Crit Rev Oral Biol Med 1993;4:301-7.
9. Felizardo KR, Goncalves RB, Schwarcz WD, Poli-Frederico RC, Maciel SM, de Andrade FB. An evaluation of the expression profiles of salivary proteins lactoferrin and lysozyme and their association with caries experience and activity. Rev Odonto Cienc 2010;25:344-9.
10. Vibhakar PA, Patankar PR, Yadav MR, Vibbhakar PA. Salivary total protein levels and their correlation to dental caries. Int J Oral Maxillofac Pathol 2013;4:13-6.
11. Gregory RL, el-Rahman AM, Avery DR. Effect of restorative treatment on mutans streptococci and IgA antibodies. Pediatr Dent 1998;20:273-7.
12. Al Amoudi N, Al Shukairy H, Hanno A. A comparative study of the secretory IgA immunoglobulins (s.IgA) in mothers and children with SECC versus a caries free group children and their mothers. J Clin Pediatr Dent 2007;32:53-6.
13. Kuriakose S, Sundaresan C, Mathai V, Khosla E, Gaffoor FM. A comparative study of salivary buffering capacity, flow rate, resting pH, and salivary immunoglobulin A in children with rampant caries and caries-resistant children. J Indian Soc Pedod Prev Dent 2013;31:69-73.
14. Navazesh M, Kumar SK, University of Southern California School of Dentistry. Measuring salivary flow: Challenges and opportunities. J Am Dent Assoc 2008;139 Suppl:35S-40.
15. Rashkova M, Baleva M, Toneva N, Peneva M, Perenovska P, Koprivarova K. Secretory immunoglogulin A (S-IgA) in the saliva of children with type 1 diabetes, asthma and systemic health but wearing removable orthodontic appliances. OHDMBSC 2009;8:16-24.
16. Chaudhuri B, Rojek J, Vickerman MM, Tanzer JM, Scannapieco FA. Interaction of salivary alpha-amylase and amylase-binding-protein A (AbpA) of *Streptococcus gordonii* with glucosyltransferase of *S. Gordonii* and *Streptococcus mutans*. BMC Microbiol 2007;7:60.
17. Beltzer EK, Fortunato CK, Guaderrama MM, Peckins MK, Garramone BM, Granger DA, *et al.* Salivary flow and alpha-amylase: Collection technique, duration, and oral fluid type. Physiol Behav 2010;101:289-96.
18. Zitka O, Skalickova S, Krizkova S, Vlkova M, Adam V, Kizek R. Separation of lactoferrin from human saliva using monolithic disc. Chromatographia 2013;76:611-9.

19. American Academy of Pediatric Dentistry. Guideline on Pediatric Restorative Dentistry. Pediatr Dent 2010;32:187-93.
20. Bagherian A, Jafarzadeh A, Rezaeian M, Ahmadi S, Rezaity MT. Comparison of the salivary immunoglobulin concentration levels between children with early childhood caries and caries-free children. Iran J Immunol 2008;5:217-21.
21. Mojarad F, Fazlollahifar S, Poorolajal J, Hajilooi M. Effect of alpha amylase on early childhood caries: A matched case-control study. Braz Dent Sci 2013;16:41-5.
22. Rohleder N, Wolf JM, Maldonado EF, Kirschbaum C. The psychosocial stress-induced increase in salivary alpha-amylase is independent of saliva flow rate. Psychophysiology 2006;43:645-52.
23. El-Saied S, Kaplan DM, Zlotnik A, Abu Tailakh M, Kordeluk S, Joshua BZ, *et al*. A comparison between amylase levels from peritonsillar, dental and neck abscesses. Clin Otolaryngol 2014;39:359-61.
24. Noorani H, Joshi HV, Shivaprakash P. Salivary alpha amylase as a noninvasive biomarker for dental fear and its correlation with behavior of children during dental treatment. Int J Clin Pediatr Dent 2014;7:19-23.
25. Ranadheer E, Nayak UA, Reddy NV, Rao VA. The relationship between salivary IgA levels and dental caries in children. J Indian Soc Pedod Prev Dent 2011;29:106-12.
26. Wright JT, Cutter GR, Dasanayake AP, Stiles HM, Caufield PW. Effect of conventional dental restorative treatment on bacteria in saliva. Community Dent Oral Epidemiol 1992;20:138-43.
27. Axelsson P, Kristoffersson K, Karlsson R, Bratthall D. Six month longitudinal study of the effects of some oral hygiene measures on *Streptococcus mutans* and approximal dental caries. J Dent Res 1987;66:761-5.
28. Kugler J, Breitfeld I, Tewes U, Schedlowski M. Excavation of caries lesions induces transient decrease of total salivary immunoglobulin a concentration. Eur J Oral Sci 1996;104:17-20.
29. Priya PR, Asokan S, Karthick K, Reddy NV, Rao VA. Effect of dental treatments on salivary immunoglobulin a of children with and without dental caries: A comparative study. Indian J Dent Res 2013;24:394.
30. Rogers HJ, Synge C. Bacteriostatic effect of human milk on *Escherichia coli*: The role of IgA. Immunology 1978;34:19-28.
31. Ward PP, Paz E, Conneely OM. Multifunctional roles of lactoferrin: A critical overview. Cell Mol Life Sci 2005;62:2540-8.
32. Jenssen H, Hancock RE. Antimicrobial properties of lactoferrin. Biochimie 2009;91:19-29.
33. Banderas-Tarabay JA, Zacarías-D'Oleire IG, Garduño-Estrada R, Aceves-Luna E, González-Begné M. Electrophoretic analysis of whole saliva and prevalence of dental caries. A study in Mexican dental students. Arch Med Res 2002;33:499-505.