

Role of natural salivary defenses in the maintenance of healthy oral microbiota in children and adolescents

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ABSTRACT

Aim: The present study served the purpose of assessing the levels of salivary immunoglobulin A (IgA), immunoglobulin G (IgG), proteins, calcium, inorganic phosphorus, and alkaline phosphatase levels in caries-free and caries active children. **Materials and Methods:** Stratified randomized sampling method was used to include 40 subjects in the age group 12–15 years having a full complement of permanent dentition except for third molars. The selected pediatric subjects were further divided into two groups of 20 each based on DMFS score, Group-I - Caries free (DMFS score = 0) and Group-II - Caries active (DMFS score ≥ 10). Unstimulated midmorning saliva samples were collected and analyzed colorimetrically and by radial immunodiffusion method for constituents of saliva under study. **Results:** The mean salivary IgA levels in children in Group-I (caries-free children) was 10.63 ± 2.85 mg/dL which was statistically higher as compared to caries active children in Group-II (8.50 ± 1.43 mg/dL). The mean salivary protein level in children of Group-II was statistically higher at 3.28 ± 0.12 mg/dL as compared to Group-I (2.89 ± 0.11 mg/dL). **Conclusion:** The present study showed decreased levels of salivary immunoglobulin A and high concentration of salivary protein in children with increased caries experience which is indicative of the protective role of salivary constituents in caries-free children.

Keywords: Alkaline phosphatase, dental caries, immunoglobulin A (IgA), immunoglobulin G (IgG), saliva

Introduction

Saliva is one of the most important factors in regulating oral health. Salivary components (immunoglobulins, salivary protein, salivary calcium, and inorganic phosphorus and alkaline phosphatase levels), its flow rate, viscosity, buffering capacity, pH and so on plays a major role in the initiation and progression of dental caries. The current study for the general public aimed at understanding that increased levels of immunoglobulins have a positive effect on maintaining healthy microenvironment in the oral cavity by

reducing the risk of dental caries. Saliva reflects the physiological state of the body including emotional endocrinal nutritional and metabolic variations also known as “THE BODY’S MIRROR”

Saliva can affect the incidence of dental caries in four general ways, firstly as a mechanical cleansing, secondly by reducing enamel solubility by means of calcium, phosphate, and fluoride, thirdly by buffering and neutralizing the acids produced by cariogenic organisms and finally by antibacterial activity.

Saliva is a complex fluid, produced by the salivary glands, whose important role in maintaining the well-being of mouth. Dental caries is a complex multifactorial disease caused by the interplay between a susceptible host, fermentable substrate, microflora, and saliva. Saliva is essential for maintaining the oral

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equilibrium and the effects of saliva and its constituents on the oral microorganisms influence the development of dental caries.^[1] The enzymes found in saliva are essential in beginning the process of digestion of dietary starches and fat. Saliva circulating in the mouth at any given time is termed “the whole saliva”.

Mutans streptococci (MS), gram-positive microorganism, are implicated as the primary causative agent in the formation of dental caries in humans. Early colonization and growth of *Mutans streptococci*, change local conditions, e.g., pH, thereby enabling more (IgG) as well as some monomeric IgA contributing towards host defense against dental caries. IgG are capable of opsonizing bacteria for phagocytosis and thus, intervene in the colonization and pathogenic activity of cariogenic microorganisms.

Saliva also contains various inorganic and organic constituents apart from an immunoglobulin. A major part of the organic component is formed by salivary proteins which play an important role in modulating microbial colonization and formation of enamel pellicle. Salivary proteins bind with calcium and phosphate ions and help to maintain these in a supersaturated state, with respect to enamel and maintain tooth integrity via the common ion effect.^[2,3]

Alkaline phosphatase, an enzyme present in saliva, is active at pH 9–10 and is important for the process of remineralization. A variation in the level of alkaline phosphatase affects the ionic concentration of phosphate and calcium, which, in turn, can alter the equilibrium of demineralization and remineralization. The interplay of the various components of saliva and their protective role against dental caries has been of much interest. Thus, the present study further investigated organisms to further colonize the oral biofilm, forming dental plaque which results in demineralization of tooth structure and consequently, dental caries ensues.^[4] The infective nature of dental caries suggests that the host immunity regulates caries activity.^[5,6] The specific immune defense against mutans streptococci is provided through the common mucosal immune system (CMIS). Immunoglobulin A (IgA) is predominantly released by the common mucosal immune system in the human body secretions including saliva. Naturally occurring salivary IgA antibodies against different streptococcal antigens are present in saliva and constitute major defensive actions against dental caries.^[4,7]

The gingival crevicular mechanism involves the humoral and cellular components of the systemic immune system. The gingival crevicular fluid, serve as a source of secretory immunoglobulin G and estimated the levels of salivary immunoglobulin A (IgA), immunoglobulin G (IgG), proteins, calcium, inorganic phosphorus, and alkaline phosphatase levels in association with presence and absence of dental caries among children.

Materials and Methods

The quantitative determination of constituents in saliva and its relationship with dental caries experience among 40 school

children selected by stratified random sampling were assessed in this study. Ethical approval was obtained from the ethics committee from Hazaribag College of Dental Sciences and Hospital, Hazaribag, Jharkhand and permission were also taken from the school authorities. A written explanatory note was sent to the parents regarding the objectives of the study and written consent was received from them. A total of 150 children between 12–15 years of age, were initially screened. Prior to the commencement of the oral examination, relevant medical, and dental history was elicited from the parents.

Inclusion criteria

- Children in the age group of 12–15 years
- Good general health
- No history of intake of antibiotics or any preventive treatment for the past 6 months
- Regular attendance in school
- Permanent residents of Hazaribag.

Exclusion criteria

- Medically compromised children or children with physical limitations
- Children undergoing orthodontic treatment
- Children with moderate-severe gingivitis or any significant soft tissue pathology.

The children were examined in their classrooms under natural daylight, comfortably seated on ordinary chairs. A thorough oral and soft tissue examination was done with a mouth mirror and CPI probe. The dental caries status was assessed and recorded as per WHO criteria (1997).^[8] The examination revealed 100 caries-active and 50 caries-free children. Based on the inclusion and exclusion criteria of the study, 82 children were selected, 49 caries-active and 33 caries-free children of which 48 were boys and 34 were girls.

The selected children were further divided into 2 groups – Group-I-Caries free children (DMFS score = 0) which included 30 boys and 19 girls and Group-II- Caries active children (DMFS score ≥ 10) with 18 boys and 15 girls. Within each group, the boys and girls were allotted separate sequential numbers. Through a randomized draw of lots done by one of the children, a sample size of 10 boys and 10 girls were obtained (total 20 children) for each group, in the study.

Saliva collection

The unstimulated mid-morning whole saliva samples were collected. The saliva samples were taken one and a half-hour after school had commenced so that enough time had elapsed after breakfast. The children were then asked to allow saliva to drool from the oral cavity into the sterile, labeled disposable containers, to determine the salivary flow rate expressed as mL/min. A total of 5 mL saliva was collected from each child and transported immediately in a thermostat container for further analysis.

Estimation of immunoglobulin A (IgA) and immunoglobulin G (IgG) in saliva

Immunoglobulin A and Immunoglobulin G levels in saliva were estimated by single radial immunodiffusion method described by Mancini *et al.*^[9] It is based on the principle that a quantitative relationship exists between the amount of antigen placed in well of agar antibody plate and the resulting ring of precipitation.

About 2 mL of each salivary sample was centrifuged at 4000 rpm for 20 min, to remove the particulates.^[10] About 5 µL of the supernatant was then placed in each well of the immunodiffusion plate (Diffuplate™ Bioscientifica S. A, batch no. 1017-IgA and 1013-IgG) using a micropipette (CE Biosystems) with disposable tips. After 30 min, 5 µL of the supernatant was added to each well, and the plates were incubated at room temperature for 48 h. The antigen-antibody precipitate formation was observed in agar in the form of a concentric ring around the antigen well and measured with a Tripartigen ruler (Diffuplate™ Bioscientifica S. A) after 48 h.

Estimation of salivary proteins

The level of proteins in saliva was estimated by the procedure described by Lowry *et al.*^[11] The saliva samples were treated with alkaline copper sulphate (CuSO₄) and Folin Ciocalteu reagent. The color change was noted colorimetrically at 660 nm.

Estimation of salivary calcium

Salivary calcium was measured using Trinder's method.^[12] The sample was treated with calcium reagent and the precipitate was mixed with EDTA and treated with ferric nitrate. The reddish-brown color complex was measured colorimetrically at 470 nm. The intensity and color are directly proportional to the calcium content of saliva.

Estimation of salivary inorganic phosphorus

The level of salivary inorganic phosphorus was measured by Fiske and Subarow method.^[13] The inorganic phosphorus in a protein-free filtrate reacts with molybdic acid to form a hexavalent phosphomolybdic acid which is further reduced to 1,2,4-aminonaphthol sulphonic acid to give blue colored complex, and the intensities were read at 660 nm using a colorimeter.

Estimation of salivary alkaline phosphatase

The level of alkaline phosphatase was measured by the King-Armstrong method using disodium phenyl phosphate as a substrate.^[14] The reddish-brown color was read colorimetrically at 530 nm.

Statistical analysis

The collected data were tabulated and statistically analyzed by:

- A. Unpaired *t*-test
- B. Chi-square test.

Results

A total of 40 children, 20 in group I (DMFS = 0), and 20 in group II (DMFS ≥10) were selected by stratified random sampling from the municipal school of Hazaribag city.

The mean salivary IgA levels of children in Group-I was 10.63 ± 2.85 mg/dL, which was significantly higher as compared to children in Group-II with 8.50 ± 1.43 mg/dL (*t*-value = 2.600, *P* value = 0.015). The mean salivary IgG levels of children in Group-I was 1.04 ± 0.31 mg/dL as compared to Group-II with 0.87 ± 0.14 mg/dL, which was statistically insignificant with *t*-value = 1.793, *P* value = 0.085 [Table 1].

The mean salivary protein levels in children in Group-I was 2.89 ± 0.11 mg/ml while in Group-II, it was significantly higher i.e. 3.28 ± 0.12 mg/dL with *t*-value = -10.766, *P* value = 0.015 [Table 1]. The levels of salivary calcium in Group-I was 6.81 ± 0.72 mg/dL, while in Group-II, it was 6.39 ± 0.59 mg/dL (*t*-value = 1.966, *P* value = 0.057). Inorganic phosphorus levels for children in Group-I was 17.45 ± 1.34 mg/dL, and levels for Group-II children was 16.74 ± 1.02 mg/dL (*t*-value = 1.896, *P* value = 0.066). The levels of alkaline phosphatase in Group-I was 2.41 ± 0.47 KA units, and Group-II showed 2.70 ± 0.46 KA units (*t*-value = -1.965, *P* value = 0.057). These results were statistically insignificant [Table 1].

The intra-group comparison of salivary IgG levels in girls (Group-I) was significantly higher than boys (*t*-value = -2.327, *P* value = 0.038) but was not statistical significant for IgA (*t*-value = 1.802, *P* value = 0.093) and protein levels (*t*-value = 1.802, *P* value = 0.293). The difference between boys and girls in Group-I with respect to salivary calcium (*t*-value = -0.708, *P* value = 0.488), inorganic phosphorus (*t*-value = 0.989, *P* value = 0.336) and alkaline phosphatase (*t*-value = 0.811, *P* value = 0.335) was statistically not significant [Table 2]. In Group-II, the mean salivary

Table 1: Comparison of immunoglobulin A (IgA) and immunoglobulin G (IgG), salivary protein, salivary calcium, inorganic phosphorus, and alkaline phosphatase levels between Group-I and Group-II

Variables	Mean/SD	Group	
		I	II
IgA (mg/dL)	Mean	10.63	8.50
	SD	2.85	1.43
IgG (mg/dL)	Mean	1.04	0.87
	SD	0.31	0.14
Protein (mg/mL)	Mean	2.89	3.28
	SD	0.11	0.12
Calcium (mg/dL)	Mean	6.81	6.369
	SD	0.72	0.59
Inorganic phosphorus (mg/dL)	Mean	17.45	16.74
	SD	1.34	1.02
Alkaline phosphatase (KA units)	Mean	2.41	2.70
	SD	0.47	0.46

Table 2: Intragroup comparison of immunoglobulin A (IgA), immunoglobulin G (IgG), salivary protein, salivary calcium, inorganic phosphorus, and alkaline phosphatase levels in Group-I and Group-II according to gender

Variables	Mean/SD	Group I		Group II	
		Boys	Girls	Boys	Girls
IgA (mg/dL)	Mean	11.82	9.43	9.18	7.91
	SD	3.43	1.53	0.83	1.63
IgG (mg/dL)	Mean	0.87	1.20	0.94	0.79
	SD	0.20	0.31	0.15	0.09
Protein (mg/mL)	Mean	2.91	2.86	3.28	3.28
	SD	0.11	0.11	0.14	0.10
Calcium (mg/dL)	Mean	6.69	6.92	6.28	6.51
	SD	0.80	0.66	0.66	0.53
Inorganic phosphorus (mg/dL)	Mean	17.74	17.15	16.87	16.60
	SD	1.18	1.47	0.86	1.20
Alkaline phosphatase (KA units)	Mean	2.49	2.32	2.68	2.72
	SD	0.45	0.50	0.53	0.41

IgA (t -value = 1.866, P value = 0.085), IgG (t -value = 2.191, P value = 0.051), protein levels (t -value = 0.000, P value = 1.000), salivary calcium (t -value = -0.846, P value = 0.409), inorganic phosphorus (t -value = 0.592, P value = 0.561) and alkaline phosphatase (t -value = -0.198, P value = 0.845) was not statistically significant between boys and girls [Table 2].

Discussion

The oral cavity is a distinctive ecosystem, which performs a wide range of functions, harbors a plethora of microorganisms and is unique in accommodating exposed mineralized tissues. The saliva bathes this ecosystem and possesses many components, plays a major role in the etiopathogenesis of dental caries.^[15,16]

The present study included school children in the age group of 12–15 years, as both cell-mediated and humoral immune system are known to be fully functional at this age group.^[17]

The unstimulated midmorning whole saliva samples were collected at least two hours after breakfast as this period has been reported to have fewer diurnal variations in the flow rate and composition of saliva. A total of 5 mL of saliva was collected from each child and transported immediately for the estimation of salivary constituents under study. Prolonged storage of saliva sample should be avoided as it leads to variable loss of protein including immunoglobulins.^[10,11]

In the present study, the mean salivary IgA level in children in Group-I {Cariesfree (DMFS = 0)} was significantly higher than Group-II {caries active (DMFS \geq 10)}, suggesting a possible protective role of IgA in the prevention of dental caries. Lehner *et al.*^[10] reported that subjects with caries had decreased IgA concentrations, as compared to those with no detectable caries and proposed it could be due to the deficient transport mechanism, stimulation of immune system via pulp, deficient local immunoglobulin synthesis and molecular size of

IgA. Challacombe in a study also reported a significant inverse relationship between the IgA secretion rate and dental caries and stated that the raised IgA and IgG levels in serum reflect past caries experience.^[15] Rose *et al.*^[18] compared the IgA levels of the whole saliva and parotid saliva of caries susceptible and caries-resistant children aged 7–11 years using enzyme-linked immunosorbent assay and concluded that whole saliva and not parotid saliva in caries resistant children had a significantly higher IgA levels as compared to caries prone group.

However, Camling *et al.*^[19] have reported a negative correlation between the degree of caries activity and salivary IgA concentration. Bhatia *et al.*^[20] concluded that higher levels of salivary IgA exist in caries susceptible group, which could be due to either a cumulative antigenic or recent antigenic stimulation, with higher caries experience as compared to caries resistant group. Some authors also reported that the saliva of caries-free subjects includes significant IgA antibody against antigen I/II of *Streptococcus mutans*, indicating a protective mechanism.^[21,22] However, microorganisms may protect themselves from host immune attack by forming biofilms and decreasing expression of antigen I/II. The finding of Cogulu *et al.*^[23] tends to support the hypothesis that higher levels of salivary IgA may provide protection against dental caries. The mean salivary IgG levels in the present study were higher in Group-I as compared to Group-II; however, the difference was statistically insignificant. Lehner *et al.*^[10] and Everhart *et al.*^[24] reported that salivary IgG does not seem to play any role in the prevention of dental caries. Bagherian *et al.*^[25,26] reported that the high concentration of salivary immunoglobulin in children with early childhood caries may be associated with an increased antigenic load, leading to high production of antibodies.

Conclusion

Saliva's buffering capability, ability to wash the tooth surface, antibacterial activities, and to control demineralization, and perhaps other mechanisms all contribute to its essential role in preventing caries. An inverse relationship was noticed between the salivary IgA levels and dental caries experience and higher salivary protein levels were associated with high caries experience whereas no significant difference was observed in levels of calcium, inorganic phosphorus, alkaline phosphatase and IgG in saliva samples of children with and without dental caries.

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Conflicts of interest

There are no conflicts of interest.

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