ORIGINAL RESEARCH

Evaluation of Salivary Immunoglobulin A Level and Its Correlation with Severity of Early Childhood Caries: An Original Research

Varsha Sharma[1], Anandamoy Bagchi[2], Brahmananda Dutta[3], Mukul Sharma[4]

Abstract

Background and objectives: Early childhood caries (ECC) remains one of the most prevalent diseases mutilating the primary dentition. It is a multifactorial disease that severely affects the quality of life of affected children. One of the risk indicators reported in the literature is the presence of viable mutans streptococci (MS) and protective factors such as salivary immunoglobulin A (SIgA). Hence, it is important to identify such risks and protective factors associated with ECC using simple yet reliable methods supported by advanced technology and a fully automated platform to improve the results.

Materials and methods: A retrospective analysis was done on 40 children who were divided into two groups: group I (experimental) and group II (control). Group I comprised 30 healthy children who were further divided into three subgroups of 10 children each. Group IA with decayed, missing, filled teeth/decayed, extracted, filled teeth (dmft/deft) = 1–2, group IB with dmft/deft = 3–4, group IC with dmft/deft ≥5, and group II, comprising 10 healthy children having no caries by using World Health Organization (WHO) 2013 Oral Health Survey criteria. Unstimulated saliva was collected by drooling saliva into a sterile container. The samples were transported to the central research laboratory for SIgA by the immunoturbidimetry method by a fully automated Abbott Architect c system. The data obtained was subjected to statistical analysis.

Results: On comparison of SIgA in between varying severities of dental caries and caries-free children between age-group of 3 and 6 years, it was found to be below the grand median 0.20 mg/mL for subgroups IA and control group II. A significant negative statistical correlation ($r = -0.948$) was present between the SIgA and varying severities of ECC and the control group.

Interpretation and conclusion: The low dmft/deft group was found to be relatively closer to the caries-free groups as their mean dmft was 1.50, standard deviation (SD) ± 0.53. A slight change in dmft/deft score and SIgA could be used as a potential biomarker for assessing the severity of ECC in children between age-group of 3 and 6 years.

Keywords: Abbott Architect c system, Decayed, missing, filled teeth/decayed, extracted, filled teeth, Early childhood caries, Salivary immunoglobulin A.

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Introduction

Early childhood caries (ECC) is reported to be the most common chronic disease in childhood, with an increase in newly diagnosed cases of 1.8 billion per year worldwide.[1] The expert panel at the Bangkok Global Summit on ECC defined as "presence of one or more decayed (noncavitated or cavitated lesions), missing (due to caries), or filled surfaces in any primary tooth of a child under age 6."[2]

The concept of the caries initiation process is the presence of Newbrun tetrad-cariogenic bacteria, susceptible host, sugar, and time. Saliva has a well-defined role in the oral cavity, in the origination or neutralization of caries, the cumulation of microbes, the portal of foreign bacteria, viruses, and fungi, and in the equilibrium between these intrinsic and extrinsic factors.[3–6] The antimicrobial element which is the predominant immunoglobulin in the salivary secretions is immunoglobulin A (IgA). The secreted type of IgA is called secretory IgA (sIgA), and secretory IgA in saliva is denoted as salivary immunoglobulin A (SIgA). The SIgA neutralizes the gluing of cariogenic bacteria to the enamel.[7]

Some research probes a pragmatic correlation between the viable count of mutans streptococci (MS) and the SIgA level,[8–10] while others revealed a pessimistic one[11,12] in different classified stages of ECC. However, the interaction between these two variables in the different severities of the ECC is very little explored based on deft/dmft, so the present research is designed to explore this subject further. In the first part of the research, the evaluation of salivary IgA and its correlation with different severities of ECC between age-group of 3 and 6 was found using the immunoturbidimetry method. Later, this was correlated to the second part of the research—evaluation of viable MS colony

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count and its correlation with different severities of ECC in between age-group of 3 and 6 years using colorimetric VITEK® 2 COMPACT automated microbial phenotypic identification system with the specificity of 96.5%. Also, a comparison of salivary IgA level and viable *Streptococci* colony count among 3–6 years old children with varying caries status based on decayed, missing, filled teeth/decayed, extracted, filled teeth (dmft/deft) scoring was evaluated.

Aims and Objectives of the Study

- To determine salivary IgA levels in 3–6 years old children with varying caries status based on deft/dmft scoring.
- To compare the association of salivary IgA levels among 3–6 years old children with varying caries status based on deft/dmft scoring.

Materials and Methods

After screening, 40 healthy children aged between 3 and 6 years from the outpatient department of Pediatrics and Preventive Dentistry Department at Kalinga Institute of Dental Sciences, Kalinga Institute of Industrial Technology (KIIT) (Deemed to be University), Bhubaneswar, Odisha, India, were incorporated in this evaluation based on inclusion and exclusion criteria, after due clinical permission from parents/legal guardian. The study was approved by the Institutional Ethics Committee. Based on inclusion criteria, children were categorized into two different groups, namely group I (experimental) and group II (control).

Group I (experimental):

- Group IA: ECC with deft/dmft score 1–2.
- Group IB: ECC with deft/dmft score 3–4.
- Group IC: ECC with deft/dmft score ≥5.

Group II (control): Caries-free children with deft/dmft 0.

To meet the inclusion criteria, children must have no relevant medical history. Subjects who had been subjected to any tooth restorations, chiefly zinc oxide eugenol or glass ionomer cement in the past 6 months, using chlorhexidine mouthwash, antifungal treatment, under drugs like antibiotics and corticoid therapy in the last 1-month preceding, upper respiratory tract infections, gland infections, congenital, and genetic disorders were excluded from the study.

An alert examiner and a cooperative recorder RECORDED dmft/deft in the World Health Organization (WHO) 2013 format in a dental chair using mouth mirror and blunt explorer. Specimens were cumulated in the morning hours between 8:00 and 11:00 am. Children were Educated not to ingest or sip anything an hour before saliva acquisition to reduce the stimulation of saliva and possible inclusion of food debris in the sample. The dental caries experience status was expressed in terms of decayed dentin (d), indicated for extraction/missing (e/m), and filled (f) teeth for the primary dentition using the deft/dmft index.

For saliva collection for immunological (SIgA) evaluation, each child was made to sit in a coachman position, allowing the saliva to pool on the floor of the mouth, and about 5 mL of unstimulated whole saliva was collected in sterile Himedia Clinicol® wide mouth container by asking them to passively drool the saliva into container (Fig. 1).

Immediately, the samples were stored in the refrigerator at 4°C and then transported to the laboratory in melting ice (0°C) in a transportation box within 1 hour of collection. The samples were centrifuged (1 minute at 3000 rpm, taken as standard) to remove bacteria and other extraneous material; supernatant was used to measure salivary IgA in fully automated immunoassay analyzer (Fig. 2) (Abbott Architect c system, Reagent Kit 9D98 IgA with R1-Buffer, and R2-antibody, Quality Control Lyphochek Bio-Rad, Specific Proteins Multiconstituent Calibrator) using immunoturbidimetric procedure. The samples were run using a 1:5 dilution (0.85–0.90% sodium chloride) (Fig. 3). If any sample result flag "<" or any result error code is generated, the system was configured to automatically rerun the sample undiluted. The sample containing SIgA was incubated with a buffer (R1), and a sample

Fig. 1: Sample collection by passive drool

Fig. 2: Abbott Architect c system

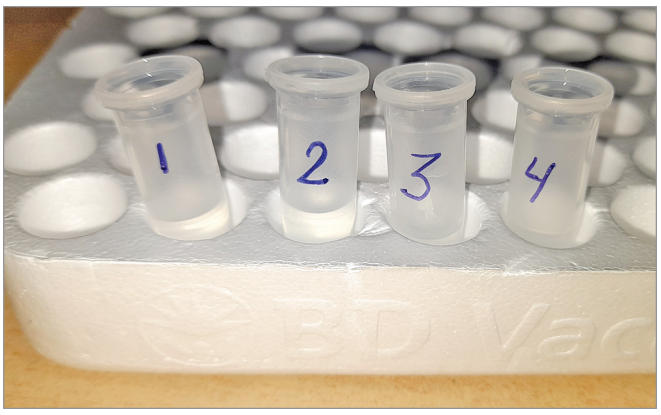

Fig. 3: Sample dilution by 1:5

blank determination was performed prior to the addition of IgA antibody (R2) (Fig. 4). SIgA was then calculated in the presence of an appropriate antibody in excess of the SIgA concentration was measured as a function of turbidity and was expressed in mg/mL.

Experimental Length Estimation

The sample size was evaluated using the software G*Power v. 3.1.9.2. The effect size to be concluded (dz) was 60% for the two-tailed hypothesis, the power of the research was 80% and the limit of error at 5%, the total experimental length needed was 40.

Statistical Analysis

The data was obtained from the applied biosystems—Statistical Package for the Social Sciences for Windows, Version 24.0. Armonk, New York: IBM Corp) for statistical analysis.

Descriptive Statistics

Descriptive analysis of all the explanatory and outcome parameters was done using mean and standard deviation (SD) for quantitative variables' frequency and proportions for categorical variables.

Inferential Statistics

Spearman's correlation test was used to estimate the relationship between SIgA in different severities of ECC groups based on dmft/deft. In the second part of the research, multiple comparisons of mean difference in MS colony-forming unit (CFU) and sIgA levels between different severities of dental caries using Tukey's honest significant difference (HSD) range test.

The level of significance was set as $p < 0.05$, ($p = 0.000$)—significant relationship and ($p > 0.05$)—nonsignificant relationship.

Results

As the laboratory data obtained for SIgA levels followed a skewed distribution, the grand median value was calculated as 0.20 mg/mL for SIgA with SD 0.069 (Fig. 5). The differentiation of the mean salivary IgA levels in mg/mL in the saliva samples among the two main groups, namely ECC (group I) and caries-free control (group II) (Table 1 and Fig. 6) were analyzed using the independent median test. The mean SIgA levels in mg/mL of the saliva of caries-free children were found to be 0.32 mg/mL, while in children with varying severity of ECC, it was found to be 0.19 mg/mL. A statistically significant value $p = 0.000$ was seen, indicating higher SIgA levels in the saliva of caries-free children when compared to ECC children with varying severities based on deft/dmft.

The correlation between the varying severities of ECC and control groups with independent variables (SIgA) was observed

Fig. 4: Saliva sample run in Abbott Architect c system

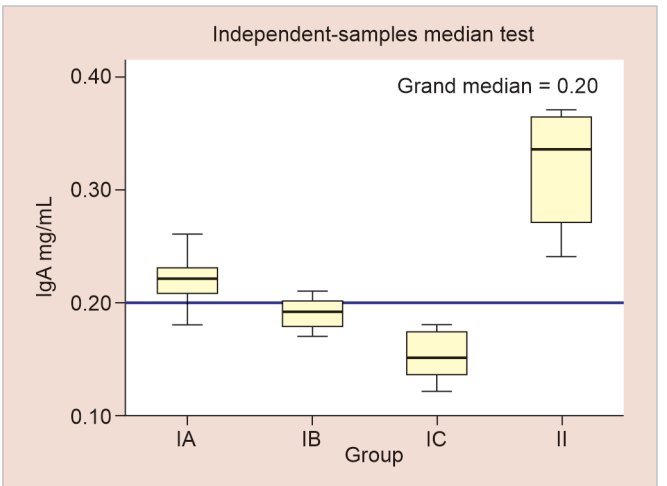

Fig. 5: Skewed distribution of salivary IgA

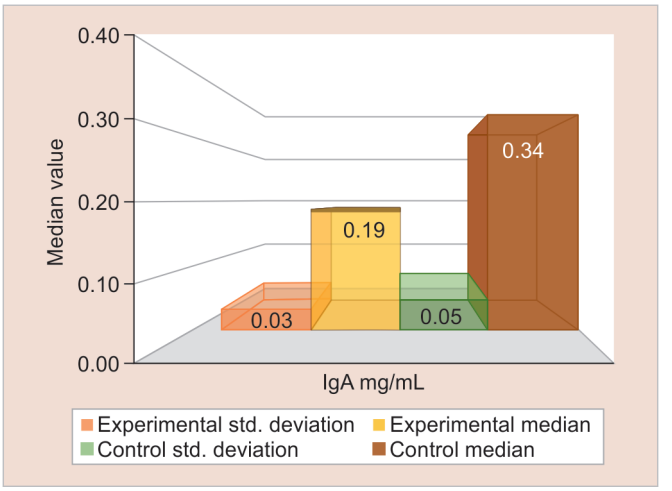

Fig. 6: Bar diagram depicting the correlation of SIgA between experimental groups with different caries status with control group

Table 1: Correlation of sIgA between the experimental group with varying dental caries status with the controlled group by Spearman's correlation (nonparametric) coefficient method

Group		*IgA mg/mL*
Experimental (varying severity of dental caries)	*N*	30
	Mean	0.19
	SD	0.03
	Median	0.19
Control	*N*	10
	Mean	0.32
	SD	0.05
	Median	0.34
	p-value (significant *$p < 0.05$)	0.000*
	r-value **Correlation is significant at the 0.01 level (two-tailed)	–0.948**

Table 2: Comparison of sIgA in saliva samples between varying severities of dental caries and caries-free children between age-group of 3 and 6 years

						95% confidence interval for mean			
Groups		*N*	*Mean*	*SD*	*Standard error*	*Lower bound*	*Upper bound*	*Minimum*	*Maximum*
IgA mg/mL	IA	10	0.2180	0.02098	0.00663	0.2030	0.2330	0.18	0.26
	IB	10	0.1900	0.01247	0.00394	0.1811	0.1989	0.17	0.21
	IC	10	0.1510	0.02234	0.00706	0.1350	0.1670	0.12	0.18
	II	10	0.3180	0.05203	0.01645	0.2808	0.3552	0.24	0.37
	Total	40	0.2193	0.06922	0.01095	0.1971	0.2414	0.12	0.37

using Spearman's nonparametric correlation coefficient method. A significant negative statistical correlation ($r = -0.948$) was present between the SIgA and varying severities of ECC and the control group. Negative *r*-values indicate a pessimistic correlation, where the values of one variable tend to increase when the values of the other variable decrease. This shows when the severity of the ECC increases, there is an exponential decrease in SIgA levels and vice versa.

The mean SIgA levels in three different subgroups of ECC, namely group IA (dmft 1–2), group 1B (dmft 3–4), and group 1C (dmft ≥5) based on varying severity of dental caries were calculated (Table 2) using the independent median test. It was observed that the mean levels of SIgA in children of group I with ECC in three subgroups, namely—group IA was 0.22 mg/mL ranging between 0.18 and 0.26 mg/mL with SD of 0.02, group IB was 0.19 mg/mL ranging between 0.17 and 0.21 mg/mL with SD of 0.012, and group IC was 0.15 ranging between 0.12 and 0.18 mg/mL with SD of 0.022 in contrast to mean of 0.32 ranging between 0.24 and 0.37 mg/mL with SD of 0.052 in children with control group.

It could be, therefore, concluded that a slight change in deft/dmft score and individual immunological marker—SIgA in saliva could applied as a prospective biomaker for the assessing severity of ECC in children between age-group of 3 and 6 years.

Discussion

Early childhood caries gravely influence the quality of life of affected children and their blood and have been found to be directly related to socioanthropological variables, including minimal education, sucrose-rich dietary habits, and poor oral hygiene.[13]

Saliva plays a cardinal role in the oral cavity through its immunological and nonimmunological constituents, which reflects an illustration of the body secretion, the immunity in children developed with age, and susceptibility to several microbiomes and immunogens.[14,15] Various investigators have attempted to relate the concentrations of IgA in whole or glandular saliva to the variety of dental caries.

The current research was undertaken in two parts, respectively, to investigate the salivary IgA levels and salivary MS count in children in varying severities of ECC between age-group of 3 and 6 years. According to Nanda, all the milk teeth surfaces were supposed to populate by MS at 3 years of age and secondary teeth at about 6 years of age. Therefore, caries risk assessment, prevention, and provocation of children should be stimulated in this age-group.[16,17] Therefore, the age-group of 3–6 years was selected for this research. The research incorporated a mean age of 4.58 years, and their dmft/deft score ranged between 0 and 16.

The IgA concentration decreases with increasing saliva flow rate; hence, unstimulated saliva by passive drool method was used for collection of samples for salivary IgA estimation.

Current methods used for the estimation of salivary IgA concentration include immunoturbidimetry, radioimmunoassay, and enzyme-linked immunosorbent assay.[18] Shifa et al. used an immunoturbidimetry assay to assess and measure the SIgA levels from the saliva.[19] Immunoturbidimetry uses a single IgA antibody, which, when mixed with a specimen, consists of IgA form insoluble complexes. These complexes cause a change in the optical density of the specimens, which could be measured in a spectrophotometer at a wavelength of 340 nm. The immunoturbidimetry method is superior in methodology as it uses only one antibody; thus, the cross-reactivity from the secondary antibody and background is also eliminated.

On collating the present research results with other studies, the low dmft/deft group was found to be relatively closer to the caries-free groups as their mean dmft was (1.50, SD ± 0.53). The role of salivary IgA against various species of bacteria, viruses, and other types of microbes by impeding bacterial adherence, neutralizing effect, and agglutination. The mechanism of salivary IgA is agglutination with the surface antigens of microorganisms in saliva, thereby promoting its swift abolition and preventing the occurrence of dental caries.[20] Even so, some researchers have propounded that microorganisms could save themselves from host immune assault by forming biofilms and reducing the expression of antigens.[21,22]

In the study done by Kuriakose et al.,[23] they found diminished salivary IgA levels in rampant caries children and higher salivary IgA levels in caries-free children, linking its contribution to reducing caries in children. A controversy was found in the systematic review framed by Fidalgo et al.,[24] who concluded that increased levels of salivary IgA in caries active groups reflect as preceding exposure of the host to cariogenic microbes.

Interestingly, due to the effective sample size in the present study, we were able to establish a significant correlation between caries-free children—a control group and three different subgroups of caries-active children with *p*-value = 0.000, respectively, for the variable.

Thus, our research adds significant value to the existing body of literature by either concluding that MS count or SIgA levels could alone be used as a prospective biomarker for caries diagnosis. Therefore, in the second part of the research, multiple comparisons of mean differences in MS CFU and SIgA levels between different severities of dental caries will be made using Tukey's HSD range test.

Conclusion

The following conclusions were drawn from the first part of the present study:

- The mean SIgA mg/mL in the exploratory grouping was found to be less than that of the control group.

- A significant negative correlation was found between the varying deft/dmft scores of experimental groups and their SIgA levels in comparison to the control group.

Our research showed that there was a significant correlation with slight variation in deft/dmft scores and varying severities of ECC. It further supports the correlation between SIgA levels and ECC.

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