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A COMPARATIVE ASSESSMENT OF VARYING LEVELS OF IGA, FIBRONECTIN IN SALIVA AS A BIOMARKER FOR THE IDENTIFICATION OF DENTAL CARIES IN PEDIATRIC POPULATIONS: AN ORIGINAL RESEARCH STUDY

Dr. Kavya Kandula[1], Dr. Manikanta Koyi[2], Dr. Singaraju Lakshmi Lanka Jayasri[3], Dr. Krishna Pavan Sanjay Medida[4], Dr Raj Kishore Jammalamadugu[5], Dr. Bogi Prashanthi[6]

[1]MDS, Assistant Professor, Department of Pediatric and Preventive Dentistry, Nimra Institute of Dental Sciences, Ibrahimpatnam, NTR District, Andhra Pradesh, India
[2]BDS, Masters of Science in Health Informatics
[3]MDS, Pediatric and Preventive Dentistry, Vijayawada, Andhra Pradesh, India
[4]BDS, Masters of Health Administration
[5]MDS, Assistant Professor, Department of Pediatric and Preventive Dentistry, Mamata Dental College, Khammam, Telangana, India
[6]MDS, Reader, Department of Oral Medicine and Radiology, Nimra Institute of Dental Sciences, Ibrahimpatnam, NTR District, Andhra Pradesh, India
***Corresponding Author:** Dr. Kavya Kandula

ABSTRACT

Aim: This study aims to assess varying levels of IGA and fibronectin in saliva as a biomarker for the identification of dental caries in pediatric populations

Materials and Methods: This study examines a cohort of 120 pediatric patients aged 6 to 14, evenly split by gender, who present with various dental issues, primarily dark cavities or mild food impaction, but without severe pain. Participants are divided into two groups: the Caries-Free Group (Group 1) consists of 60 patients with a DMFT/dmft score of zero, indicating no dental caries, while the High Caries Risk Group (Group 2) includes 60 patients with a score of 5 or higher, indicating significant dental problems. A certified dentist performs clinical examinations using standardised tools, and saliva samples are collected after a dietary restriction to minimise variability. The study uses the DMFT/dmft index and Plaque Index to assess dental health and oral hygiene, respectively. Saliva samples are analysed for human secretory Immunoglobulin A (sIgA) and fibronectin using ELISA kits. Data analysis employs advanced statistical methods to identify patterns and significance.

Statistical Analysis and Results: The Caries-Free Group (Group 1) included patients with a DMFT score of zero, indicating excellent oral health, while the High Caries Risk Group (Group 2) had scores of 5 or higher, reflecting significant dental issues. The study aimed to compare levels of two salivary biomarkers: Salivary Immunoglobulin A (IgA) and Fibronectin, both vital for immune response and tissue repair. Results showed that the Control Group had a mean Salivary IgA concentration of 5.58 ± 1.72 µg/ml, while the Study Group had a significantly lower concentration of 2.91 ± 1.61 µg/ml, with a statistically significant t-value of 8.779 and a p-value less than 0.001. For Fibronectin, the Control Group averaged 84.12 ± 26.95 U/ml compared to 67.85 ± 25.40 U/ml in the Study Group, also statistically significant (t-value of 3.403 and p-value of 0.001).

Conclusion: The study concluded that Salivary IgA, crucial for mucosal immunity, decreased markedly, along with lower Fibronectin levels. This decline may suggest a weakened oral defence or increased stress in children with dental caries compared to healthy controls.

KEYWORDS: Dental Caries, Salivary IgA, Fibronectin, Biomarker, Secretory Immunoglobulin A, Saliva, Pediatric population

INTRODUCTION

Dental caries, often colloquially known as cavities or tooth decay, is a prevalent, chronic infectious disease caused by bacterial activity in the oral cavity. These bacteria metabolise sugars in the diet, producing acids that gradually erode the protective enamel covering the teeth. Several critical factors contribute to the onset and progression of dental caries. One primary culprit is pathogenic bacteria, particularly Streptococcus mutans. This bacterium can be easily transmitted from caregivers to children through close contact, such as kissing or sharing utensils.[1,2] Once these bacteria establish themselves in the oral microbiome, the risk of dental caries increases. Dietary habits strongly affect dental health. Frequent consumption of sugary beverages such as soft drinks, flavoured milk, and juices fuels bacterial growth when these substances remain in contact with the teeth, especially during extended periods like naptime or nighttime sleep. This prolonged sugar exposure creates an acidic environment that promotes enamel demineralisation. Saliva plays a dual role in oral health: it neutralises acids, washes away food particles, and supplies essential minerals for remineralisation of tooth enamel.[3,4] However, variations in saliva flow can affect the level of protection, with the upper front teeth often more susceptible to decay because they are the first to encounter sugary substances. Preventive measures are essential

to protect against dental caries. It is highly recommended to establish oral hygiene practices early in a child's life. Parents should start by gently wiping the gums with a clean cloth, then transition to brushing the first erupted tooth with a small amount of fluoridated toothpaste.[4,5] Establishing a dental home with a qualified professional is important, and parents should schedule the child's first dental check-up by the first birthday or when the first tooth appears. In addition to early dental visits, parents should limit sugar intake and avoid using bottles or sippy cups filled with sugary liquids at bedtime. This helps prevent night-time sugar exposure, a significant risk factor for cavity development.[6,7] Recent advances in dental research have highlighted the importance of salivary and systemic biomarkers for early identification and risk assessment of dental caries, particularly in children. Salivary biomarkers, including microbial metabolites such as formate and lactate, have shown strong diagnostic potential for detecting early childhood caries.[8,9] Innate immune proteins like statherin and histatins are important for oral health, with higher levels often indicating a caries-free status in children. Key minerals such as calcium and phosphate are essential for tooth enamel, and lower levels are associated with carious lesions. Elevated inflammatory cytokines in saliva can signal an immune response to bacterial threats, linking oral immune status to dental health.[10] Untreated severe caries can also lead to systemic changes that increase inflammatory markers, highlighting the connection between oral and systemic health. Salivary Immunoglobulin A (IgA) helps fight dental caries by targeting harmful bacteria, though studies show mixed results in children regarding its levels relative to caries status. Salivary fibronectin is being investigated as a potential oral health biomarker, but its fluctuating concentrations make it difficult to establish consistent clinical thresholds.[11] This study aims to assess varying levels of IGA and fibronectin in saliva as a biomarker for the identification of dental caries in pediatric populations

MATERIALS AND METHODS

The study meticulously investigates a well-defined cohort of 120 pediatric patients, encompassing an equal representation of both male and female participants aged between 6 and 14 years. These children presented with a range of dental concerns, primarily characterized by the presence of dark cavities or mild food impaction within their teeth. Notably, these patients did not report experiencing severe pain associated with their dental issues, suggesting a unique profile for examination. For the purposes of this research, the participants are meticulously divided into two principal groups based on their dental health status. The first group, referred to as the Caries-Free Group (Group 1), consists of 60 patients who exhibit a Decayed, Missing, and Filled Teeth (DMFT) or dmft score of zero. This score indicates a complete absence of dental caries, thereby highlighting their oral health status. In contrast, the second group, termed the High Caries Risk Group (Group 2), also comprises 60 patients but shows a significantly higher presence of dental issues, with a DMFT or dmft score of 5 or higher. This score reflects a concerning prevalence of carious lesions, marking a stark contrast to the participants in Group 1. The inclusion criteria for this study are rigorously defined to ensure the integrity of the data. Participants must be systemically healthy, free from any chronic conditions or systemic illnesses that could potentially impact their dental health outcomes. Additionally, an essential aspect of participation is behavioral cooperation; children must demonstrate the ability to comply with both oral examinations and saliva collection procedures. Prior to their inclusion in the study, written informed consent is meticulously obtained from the parents or guardians of the participants. Furthermore, verbal assent is sought from the children to ensure they understand their participation. To maintain scientific rigor, the exclusion criteria are carefully articulated to eliminate potential confounding variables. Consequently, children who exhibit systemic illnesses, have recently experienced upper respiratory infections, are on specific medications that may influence salivary flow, or are currently undergoing orthodontic treatments that could affect their dental condition are systematically excluded from the study. All clinical examinations are conducted by a certified dentist who employs standardized evaluative tools and appropriate lighting to systematically assess and document the oral health status of each participant. The study applies the DMFT/dmft index as a primary metric for evaluating the severity of dental caries, complemented by the Plaque Index, which assesses the level of oral hygiene maintained by the children involved in the study. Saliva samples are diligently collected in the morning, following a dietary restriction period of at least 1 to 2 hours. This precaution minimizes any variability in the results, ensuring that measurements are as accurate as possible. Once collected, these samples are processed immediately and stored at a precise temperature of -80°C, preserving their integrity until they can undergo comprehensive biochemical analyses. For the quantitative assessment of the saliva samples, the study employs Enzyme-Linked Immunosorbent Assay (ELISA) kits. These kits are specifically designed to quantify levels of human secretory Immunoglobulin A (sIgA) and fibronectin, which are of considerable interest for their potential roles in oral health. Data analysis is conducted with meticulous care using advanced statistical software, such as SPSS or GraphPad Prism. A variety of statistical tests are applied to explore the relationships within the collected data, helping to identify patterns and determine statistical significance. Ultimately, the overarching objective of this study is to elucidate the levels of sIgA and fibronectin in saliva, with the aim of positioning these biomarkers as valuable indicators that could effectively distinguish between different states of dental caries in pediatric patients. The findings from this research aspire to contribute meaningful insights to the rapidly evolving field of pediatric dental health, potentially informing future preventive measures and therapeutic strategies aimed at improving children's oral health outcomes.

Statistical Analysis

All statistical analyses were conducted using SPSS version 31.0, a robust software application that is widely utilized for statistical computing and data analysis within the social sciences. This software provides a comprehensive range of analytical tools, making it suitable for handling various statistical methods and techniques. In this study, we specifically employed the chi-square test to assess the association between categorical variables, which allowed us to examine the strength and significance of relationships within the data. The chi-square test is particularly useful for determining whether the observed frequencies in our dataset significantly deviate from the expected frequencies under the null hypothesis.

RESULTS

The study involved a comprehensive assessment of a total of 120 participants, carefully organized into two distinct groups: a Control Group and a Study Group, with each group consisting of 60 individuals. Each participant was classified based on their dental health, leading to a meticulous division into two principal categories. The first group, known as the Caries-Free Group (Group 1), is composed of 60 patients who proudly exhibit a Decayed, Missing, and Filled Teeth (DMFT) or dmft score of zero. This score is significant as it indicates a complete absence of dental caries, highlighting their excellent oral health and hygiene practices. In stark contrast, the second group, identified as the High Caries Risk Group (Group 2), also encompasses 60 patients, but this cohort is marked by a considerable prevalence of dental issues. These individuals present with a DMFT or dmft score of 5 or higher, indicating a notable presence of dental caries and a heightened risk of oral health complications. The primary aim of this research was to evaluate and compare the levels of two significant biomarkers found in saliva: Salivary Immunoglobulin A (IgA) and Fibronectin. Both of these biomarkers play essential roles in the body's immune response and tissue repair mechanisms, making them critical components in understanding the connection between oral health and systemic well-being. The insights gleaned from this study may contribute to a deeper understanding of the biological factors influencing dental health and disease.The findings from this study are presented in detail in both Table 1 and Graph 1, offering a thorough comparison of the mean concentrations of Salivary Immunoglobulin A (IgA) and Fibronectin between the two groups, each comprising 60 participants. The results indicate that the Control Group had an impressive average Salivary IgA concentration of 5.58 ± 1.72 µg/ml, highlighting their relatively robust immune profile. In stark contrast, the Study Group exhibited a significantly lower mean concentration of Salivary IgA at 2.91 ± 1.61 µg/ml, suggesting a marked compromise in immune function. A meticulous statistical examination was carried out using an independent t-test, yielding a striking t-value of 8.779 alongside a p-value of less than 0.001. These results strongly signify that the reduction in Salivary IgA levels within the Study Group, when juxtaposed with the Control Group, is not only substantial but also highly significant from a statistical standpoint. When analyzing Fibronectin concentrations, the Control Group reflected an average level of 84.12 ± 26.95 U/ml, indicating a healthy range. Conversely, the Study Group's mean concentration was notably lower at 67.85 ± 25.40 U/ml. The rigorous statistical evaluation for Fibronectin also produced noteworthy outcomes, with a t-value of 3.403 and a p-value of 0.001, reinforcing the conclusion that the diminished Fibronectin levels in the Study Group are significantly meaningful as well. In conclusion, the study's results unveil a prominent decrease in both Salivary IgA and Fibronectin levels within the Study Group compared to the Control Group. Both reductions were determined to be statistically significant, emphasizing potential implications regarding the health status of the Study Group. Additionally, Table 2 offers the results of a one-way ANOVA analysis, which explores the variations across all studied groups, thereby enhancing the context of the observed outcomes.

Table 1: Comparison of Mean Salivary IgA and Fibronectin Concentration Between Groups (n=60)

Parameters / Biomarkers	**Control Group (n=60) (Mean ± SD)**	**Study Group (n=60) (Mean ± SD)**	**t-value**	**p -value**
Salivary IgA levels (μg/ml)	**5.58±1.72**	**2.91±1.61**	**8.779**	**<0.001**
Fibronectin concentration (μg/ml)	**84.12±26.95**	**67.85±25.40**	**3.403**	**0.001**

Graph 1: The chart below displays the significant drop in both biomarkers from the Control Group to the Study Group

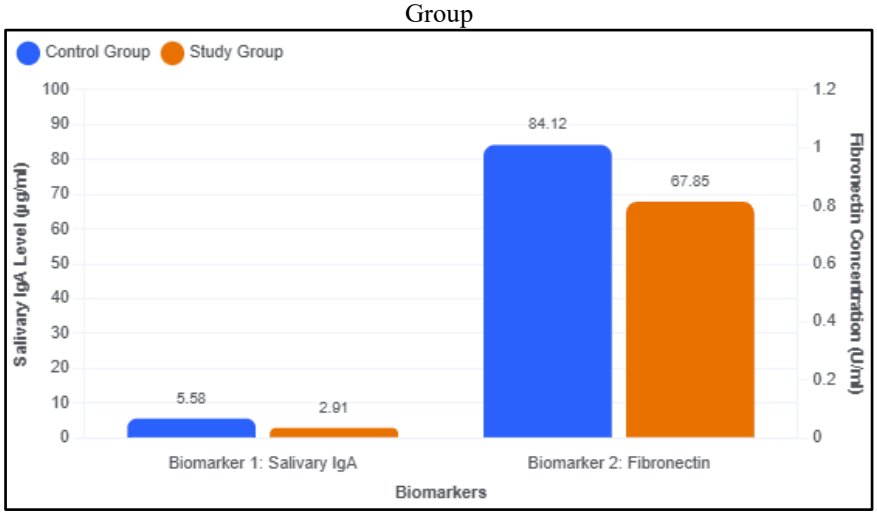

Table 2: Estimation amongst all studied groups using one-way ANOVA

Variables	Degree of Freedom	Sum of Squares ∑	Mean Sum of Squares m∑	F	Level of Sig. (p)
Between Groups	5	3.893	2.045	1.2	0.01*
Within Groups	17	2.732	1.242	_	
Cumulative	182.15	83.067	*p<0.05 significant		

Table 3: Detailed Statistical Description for Studied Parameters for Control Group

Parameters / Biomarkers	Std. Dev.	Std. Error	95% CI	Pearson Chi-Square Value	df
Salivary IgA levels (µg/ml)	2.24	2.156	2.125	2.36	2.126
Fibronectin concentration (µg/ml)	2.08	2.012	1.038	1.23	1.108

Table 4: Detailed Statistical Description for Studied Parameters for Study Group

Parameters / Biomarkers	Std. Dev.	Std. Error	95% CI	Pearson Chi-Square Value	df
Salivary IgA levels (µg/ml)	1.01	1.09	1.013	1.02	1.004
Fibronectin concentration (µg/ml)	1.13	2.102	2.090	2.12	2.112

DISCUSSION

Grier A et al reported in their study that dental caries, or tooth decay, is the most common chronic disease affecting children globally. In pediatric dentistry, managing cavities focuses on early risk assessment, dietary changes, and conservative treatments to preserve primary teeth until they naturally fall out. Primary teeth are more susceptible to decay due to thinner enamel and larger nerve chambers. The development of dental caries results from a combination of cariogenic bacteria like Streptococcus mutans, a diet high in fermentable sugars, and the vulnerability of tooth surfaces. Common types include Early Childhood Caries (ECC), often seen in infants and toddlers exposed to sugary liquids, and pit and fissure caries in the grooves of back molars.[12,13] Prieto-Regueiro B et al reviewed in their study that preventive strategies include regular fluoride therapy, dental sealants, and appropriate toothpaste userice-sized for children under three and pea-sized for ages three to six. Treatment can involve Silver Diamine Fluoride (SDF) for halting decay, composite fillings for minor cavities, or more invasive options like pulpotomies and pediatric crowns for severe cases. Biomarkers, particularly through saliva analysis, play a crucial role in identifying caries in pediatric patients. Monitoring biological indicators helps clinicians predict and detect decay before it causes significant damage.[14,15] Raji SAH et al included in their study that the primary biomarkers for assessing dental caries in children fit into four main categories. First,

microbial biomarkers and virulence factors are crucial indicators. High counts of bacteria like Streptococcus mutans and Lactobacillus significantly correlate with increased caries risk and disease progression. Glucosyl transferases, enzymes from S. mutans that help form cariogenic biofilms, also highlight caries presence. Second, metabolic and chemical markers reveal changes in the oral environment. Children with active ECC exhibit elevated levels of lactate, formate, and glycerophosphocholine in their saliva.[16,17] Seni AG et al reported in their study that additionally, salivary nitrite levels decrease with tooth decay, suggesting a shift in the protective redox environment, while reduced salivary pH and buffering capacity indicate increased susceptibility to enamel demineralization. The third category encompasses salivary proteins and innate immunity markers. Variations in secretory immunoglobulin A (sIgA) reflect mucosal immune defense activation against cariogenic pathogens.[18,19] Seni AG et al reviewed in their study that lactoferrin, an antimicrobial peptide, varies with caries severity and serves as an early risk predictor. Protective proteins like statherin and histatins help maintain calcium and phosphate levels, and caries-free children show significantly higher statherin concentrations. Salivary calcium and phosphate levels also inversely correlate with caries severity. Finally, systemic inflammatory biomarkers are relevant when untreated caries lead to chronic inflammation, measurable through blood tests. Elevated levels of pro-inflammatory cytokines such as TNF-α and IL-6, along with increased neutrophil gelatinase-associated lipocalin (NGAL) and changes in hematologic ratios like the Neutrophil-to-Lymphocyte Ratio, indicate severe untreated cases. Salivary immunoglobulin A (IgA) and fibronectin show potential as non-invasive biomarkers for detecting dental caries in children, but their clinical use is limited due to variable research results and individual differences.[20,21] Many of the recent researches have also studied and proposed the similar outcomes and prospects in these regards.[22-28] Angarita-Díaz et al showed in their study that salivary IgA is the main antibody, helping combat cariogenic bacteria and preventing tooth decay. Research on sIgA in children is mixed; some studies find higher levels in caries-free children, while others indicate elevated sIgA in those with more carious lesions, suggesting a reactive immune response. Fibronectin plays a role in forming the acquired enamel pellicle and interacts with bacteria, but its diagnostic significance in children is less clear compared to adhesion proteins like statherin.[29]

CONCLUSION

In this study, the authors conducted a detailed analysis titled "A Comparative Assessment of Varying Levels of IGA and Fibronectin in Saliva as a Biomarker for the Identification of Dental Caries in Pediatric Populations." The findings reveal significant insights into the relationship between salivary components and dental health. Specifically, they observed a pronounced decrease in Salivary IgA, which plays a crucial role in mucosal immunity, alongside a reduced concentration of Fibronectin. This combination of decreased levels may indicate a weakened oral defense mechanism, compromised mucosal integrity, or an increase in systemic or local stress within the study group when compared to healthy control participants. These results underscore the importance of monitoring these biomarkers in understanding and potentially identifying risks for dental caries in children.

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