The Bioscan
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
20(4): 99-118, 2025 www.thebioscan.com

Assessment of Caries Risk Using Salivary Biomarkers and Clinical Parameters in Pediatric Patients

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DOI: 10.63001/tbs.2025.v20.i04.pp99-118

KEYWORDS:
Caries risk assessment, Salivary biomarkers, Pediatric dentistry, Secretory IgA, Streptococcus mutans.

Received on:

06-09-2025

Accepted on:

03-10-2025

Published on:

07-11-2025

ABSTRACT

Dental caries remains one of the most prevalent chronic conditions among children worldwide, reflecting a complex interplay between host factors, microbial ecology, diet, and salivary composition. Traditional risk assessment methods relying solely on clinical and dietary parameters often fail to predict disease onset accurately in pediatric populations. This study aims to evaluate the diagnostic utility of salivary biomarkers alongside clinical parameters to develop a more comprehensive, objective, and early predictive framework for caries risk assessment in children. A cross-sectional analytical study was conducted among pediatric patients aged 5 to 12 years, categorized into caries-free, moderate-risk, and high-risk groups based on the Decayed, Missing, and Filled Teeth (DMFT/dmft) index. Unstimulated whole saliva samples were collected under standardized conditions, and biochemical analyses were performed to quantify salivary flow rate, pH, buffer capacity, total protein concentration, calcium, phosphate, and levels of specific biomarkers, including lactoferrin, α-amylase, secretory IgA, and Streptococcus mutans count. Clinical parameters such as plaque index, dietary habits, and oral hygiene practices were also evaluated and correlated with biochemical findings using multivariate regression models. The results demonstrated a statistically significant association between elevated bacterial load, reduced salivary flow, and lower buffer capacity with higher caries activity. Among the biomarkers, lactoferrin and secretory IgA exhibited notable variations, indicating their potential as early predictors of caries susceptibility. Salivary calcium and phosphate levels were inversely correlated with caries severity, underscoring their protective role in enamel remineralization. The combined assessment of salivary and clinical parameters enhanced the predictive accuracy of caries risk models by nearly 30% compared to conventional diagnostic indices alone. These findings affirm that salivary biomarkers, when interpreted in conjunction with clinical data, offer a powerful, non-invasive, and reproducible method for assessing caries risk in pediatric patients. The incorporation of biomarker-based screening into routine pediatric dental examinations could enable clinicians to identify at-risk children earlier, individualize preventive care strategies, and monitor the efficacy of intervention programs. This integrative approach aligns with precision dentistry principles, promoting a shift from restorative to preventive and personalized oral health management in pediatric populations.

Introduction:-

Dental caries continues to be recognized as one of the most pervasive chronic diseases in childhood, affecting nearly 60–90% of school-aged children worldwide, according to estimates from the World Health Organization. Despite significant advancements in oral healthcare, fluoridation, and preventive strategies, dental caries remains a major public health concern, particularly in developing and underprivileged regions. Its multifactorial etiology, influenced by biological, behavioral, and environmental determinants,

complicates efforts toward early identification and targeted prevention. Pediatric patients are especially vulnerable due to developing enamel, dietary transitions, and limited manual dexterity, all of which predispose them to a higher caries risk. Therefore, identifying reliable, non-invasive, and reproducible methods to evaluate caries susceptibility in children has emerged as a critical research priority in contemporary dentistry.

Background and Rationale

Caries development is an intricate process resulting from dynamic interactions between host factors (teeth and saliva), diet (fermentable carbohydrates), and microorganisms within the dental biofilm. Traditionally, caries risk assessment has relied heavily on clinical observations, dietary history, and behavioral parameters. While these approaches remain useful, they cannot quantify subclinical changes or predict future disease progression accurately. Caries, in its early stages, is a reversible biochemical process, and timely diagnosis can prevent the need for restorative intervention. The modern preventive paradigm emphasizes early detection and risk-based management rather than reactive treatment. In this context, salivary diagnostics has garnered considerable attention as a promising adjunctive tool for individualized caries risk profiling.

Saliva serves as the body's first line of defense in maintaining oral homeostasis. It contains a complex mixture of inorganic ions, enzymes, proteins, and antimicrobial agents that collectively contribute to enamel remineralization, pH buffering, and microbial control. The fluid's accessibility, non-invasive collection, and direct interaction with the oral cavity make it a highly suitable biological medium for diagnostic applications. In recent years, saliva has been increasingly explored as a diagnostic matrix for systemic diseases, including diabetes mellitus, cardiovascular disorders, and certain malignancies. Within the field of pediatric dentistry, however, its potential as a caries risk predictor remains underutilized, especially in clinical practice.

Salivary Biomarkers and Caries Susceptibility

Several salivary components have been identified as potential indicators of caries activity. Among the most studied are salivary flow rate, pH, and buffer capacity factors that directly influence the acid-neutralizing potential and microbial balance within the oral environment. Low salivary flow rate and buffer capacity have consistently been associated with elevated caries incidence, as they impair the mouth's natural ability to counteract demineralization. Additionally, electrolytes such as calcium and phosphate play critical roles in maintaining enamel integrity. A reduction in their concentration compromises remineralization and increases caries susceptibility.

Beyond physicochemical properties, salivary proteins and immune factors offer a deeper insight into host defense mechanisms. Secretory immunoglobulin A (sIgA), the predominant antibody in saliva, acts by agglutinating cariogenic bacteria and inhibiting their adhesion to tooth surfaces. Variations in sIgA concentration have been correlated with differential caries experiences among children, suggesting its role as a potential biomarker of susceptibility. Similarly, lactoferrin, an iron-binding glycoprotein, exhibits antimicrobial activity by depriving pathogens of essential nutrients and disrupting bacterial membranes. Elevated lactoferrin levels have been observed in children with active carious

lesions, reflecting an immune response to microbial challenge. Enzymes such as α-amylase, associated with both salivary defense and stress responses, also demonstrate altered activity in caries-prone individuals. Together, these markers represent a complex interplay of protective and pathogenic influences, offering a biochemical window into oral health status.

Clinical Parameters and Behavioral Determinants

While biochemical analysis provides an objective measure of caries risk, it must be interpreted alongside clinical and behavioral data to achieve a comprehensive assessment. Clinical indices such as the Decayed, Missing, and Filled Teeth (DMFT/dmft) index, plaque index, and gingival status remain essential in evaluating existing disease burden. Moreover, behavioral determinants, including oral hygiene habits, frequency of sugar intake, and parental supervision, play crucial roles in modulating caries risk among pediatric patients. The inclusion of both objective biomarkers and subjective behavioral factors ensures that assessment models capture the full spectrum of influences contributing to disease development.

Recent studies highlight that parental knowledge and attitudes toward oral hygiene significantly impact children's oral health outcomes. Socioeconomic conditions, access to fluoridated water, and cultural dietary practices further compound the risk. Despite this complexity, existing diagnostic frameworks in pediatric dentistry often fail to integrate these dimensions effectively, resulting in inconsistent prevention outcomes. Therefore, the integration of clinical, behavioral, and salivary data presents a more holistic strategy, capable of predicting risk with greater sensitivity and specificity.

Technological Advances and Emerging Approaches

The advent of molecular diagnostic technologies and biosensor platforms has revolutionized the potential of salivary testing. Techniques such as enzyme-linked immunosorbent assays (ELISA), polymerase chain reaction (PCR), and mass spectrometry now enable quantification of salivary proteins, microbial DNA, and metabolic markers with remarkable precision. Microfluidic "lab-on-chip" devices, capable of simultaneous multi-analyte detection, are gradually being incorporated into research and clinical setups. These technologies facilitate rapid chairside evaluation, enabling clinicians to make informed preventive decisions during routine visits. In pediatric dentistry, where patient cooperation and attention span are often limited, such minimally invasive diagnostic modalities provide immense practical value.

The use of salivary diagnostics also aligns with the principles of **precision dentistry**, a concept analogous to precision medicine. This approach emphasizes individualized treatment based on genetic, environmental, and lifestyle variables. Incorporating biomarker-based assessment into preventive dental care supports the transition from population-based to personalized disease management. Such integration allows early identification of high-risk children, customization of fluoride therapy, dietary counseling, and recall intervals tailored to the patient's risk profile.

Challenges and Research Gaps

Despite its promising applications, several limitations continue to hinder the routine

clinical use of salivary biomarkers for caries risk assessment. Salivary composition is highly variable, influenced by factors such as circadian rhythm, hydration status, and dietary intake. Standardization of collection protocols remains a challenge, especially among pediatric subjects, where cooperation can be inconsistent. Additionally, the biological variability across individuals makes it difficult to establish universal threshold values for specific biomarkers. Most existing studies have small sample sizes and heterogeneous methodologies, limiting their generalizability.

Another major research gap pertains to the lack of correlation between biochemical markers and longitudinal caries outcomes. While cross-sectional studies demonstrate significant associations, limited longitudinal data are validating whether these biomarkers can reliably predict future caries development. Furthermore, the combined predictive value of multiple salivary markers, when analyzed alongside established clinical indices, remains underexplored in pediatric cohorts. This gap underscores the need for comprehensive, multi-parameter studies that integrate both biochemical and clinical dimensions of risk assessment.

Significance of the Present Study

Given these gaps, the present research aims to systematically evaluate the role of salivary biomarkers in conjunction with clinical parameters to establish an evidence-based caries risk assessment model for pediatric patients. By analyzing multiple salivary constituents such as pH, buffer capacity, calcium, phosphate, total protein, lactoferrin, secretory IgA, α-amylase, and bacterial load alongside clinical indices like DMFT/dmft, plaque score, and oral hygiene habits, this study seeks to identify the most reliable predictors of caries susceptibility. The investigation extends beyond mere correlation by employing statistical modeling to determine the relative weight of each parameter in predicting caries risk.

This study also contributes to the broader understanding of how physiological defense mechanisms in saliva respond to microbial and environmental challenges in the oral cavity. The incorporation of both biochemical and clinical data allows for the development of a comprehensive diagnostic framework that can guide personalized preventive care strategies. Such an approach is particularly valuable in pediatric dentistry, where behavioral interventions alone may be insufficient to mitigate biological predispositions to disease.

Implications for Pediatric Dental Practice

The successful integration of salivary biomarker analysis into clinical practice could redefine preventive dentistry for children. Routine screening of salivary parameters during dental visits could enable early identification of at-risk individuals, allowing for timely implementation of interventions such as fluoride varnish applications, dietary counseling, and microbial control. Moreover, longitudinal monitoring of salivary biomarkers could help evaluate the effectiveness of preventive programs and guide modifications in patient management. The availability of portable diagnostic tools and biosensors may further enhance the feasibility of such monitoring in community and school-based dental health initiatives.

Beyond individual patient care, this approach has implications for public health policy. Large-scale surveillance of salivary biomarkers could assist in identifying population-level trends in caries risk and guide targeted resource allocation. In the long

term, integrating salivary diagnostics with electronic health records and predictive analytics could contribute to a data-driven model of oral healthcare delivery, one that prioritizes prevention, personalization, and early intervention.

In summary, the assessment of caries risk in pediatric patients requires a paradigm shift from conventional, observation-based methods to a more scientific, biomarker-driven approach. Saliva, with its multifaceted diagnostic potential, offers a unique and underutilized avenue for understanding the biological mechanisms underlying caries susceptibility. When combined with clinical parameters and behavioral insights, salivary analysis provides a robust, non-invasive, and objective means to evaluate risk and guide preventive care. The current research endeavors to bridge existing knowledge gaps by developing and validating a comprehensive caries risk model that integrates biochemical, microbial, and clinical dimensions. Through this integration, pediatric dentistry can move closer to the ideals of precision prevention, ensuring that every child receives care tailored to their unique physiological and environmental context.

Methodology:-

The present investigation was designed to evaluate the relationship between salivary biomarkers and clinical parameters associated with caries risk among pediatric patients. The methodological framework was carefully structured to ensure precision, reproducibility, and ethical compliance. This study employed a **cross-sectional analytical design**, integrating clinical examination, biochemical salivary assessment, and statistical modeling to establish potential correlations and predictive indicators for dental caries risk in children.

1. Study Design and Ethical Approval

This study followed a **cross-sectional observational design**, aimed at comparing salivary biomarker profiles and clinical indices between caries-active and caries-free pediatric populations. Ethical clearance was obtained from the Institutional Ethics Committee (IEC Ref. No: DENT/RES/2025/031), ensuring compliance with the **Declaration of Helsinki (2013)** on research involving human participants. Written informed consent was obtained from parents or legal guardians, and verbal assent was taken from the children before their inclusion in the study.

2. Study Population

The study included **children aged 6 to 12 years**, selected from outpatient pediatric dental clinics and school-based dental health programs in the region. This age range was chosen as it represents the mixed dentition period, a critical developmental phase with increased susceptibility to caries due to the coexistence of primary and permanent teeth.

2.1 Inclusion Criteria

- Healthy children with no systemic illness or long-term medication use.
- Children within the age group of 6–12 years.
- Participants with fully erupted molars suitable for clinical examination.
- Parents are willing to provide informed consent.

2.2 Exclusion Criteria

- Children on antibiotic therapy within the past month.
- Patients with salivary gland disorders, xerostomia, or chronic illnesses.

- Children wearing orthodontic appliances.
- Uncooperative children are unable to comply with saliva collection protocols.

3. Sample Size Determination

A pilot study was conducted on 20 children to estimate mean differences in salivary pH and buffer capacity between caries-active and caries-free groups. Based on the preliminary data, a **sample size of 120 participants** was calculated using G*Power software (v3.1), maintaining a 95% confidence interval and 80% statistical power. Participants were divided equally into two groups:

Group	Description	Sample Size (n)
Group I	Caries-Free (DMFT/dmft = 0)	60
Group II	Caries-Active (DMFT/dmft ≥ 3)	60

4. Data Collection Procedures

Data collection was conducted in two phases: **clinical examination** and **saliva sample analysis**, both performed on the same day to eliminate diurnal variations. All assessments were carried out between **9:00 a.m. and 11:00 a.m.** to ensure consistency in salivary flow and composition.

5. Clinical Examination

Clinical examination was performed by two calibrated pediatric dentists. Inter-examiner reliability was assessed using Cohen's Kappa statistics ($\kappa = 0.89$), confirming substantial agreement. Clinical data were recorded using a standardized proforma including demographic details, oral hygiene practices, and dietary habits.

5.1 Caries Experience

Caries experience was recorded using the **DMFT/dmft index** according to the **World Health Organization (WHO) Oral Health Survey Methods (2013)**. Teeth were examined under adequate illumination using a mouth mirror and a WHO periodontal probe.

5.2 Plaque and Gingival Indices

The **Silness and Löe Plaque Index (1964)** and **Löe and Silness Gingival Index (1963)** were used to assess oral hygiene status and gingival health, respectively.

5.3 Dietary and Behavioral Assessment

Parents were interviewed to gather information regarding children's frequency of sugar intake, snacking habits, and toothbrushing routines using a validated

dietary frequency questionnaire adapted from **Moynihan et al. (2015)**.

6. Saliva Collection Procedure

Unstimulated whole saliva samples were collected using the **passive drool technique**. Each participant was instructed to refrain from eating, drinking, or brushing teeth for at least **90 minutes** before sample collection. Participants were seated upright, head slightly tilted forward, and asked to allow saliva to pool in the mouth and then drool into a sterile polypropylene tube over a **five-minute period**.

6.1 Handling and Storage

Immediately after collection, samples were placed on ice and transported to the Biochemistry Laboratory within 30 minutes. The samples were centrifuged at **3,000 rpm for 15 minutes** to remove cellular debris, and the supernatant was stored at **–20°C** until biochemical analysis.

7. Biochemical Analysis of Salivary Parameters

A comprehensive panel of salivary biomarkers was selected based on their known association with caries development and host defense mechanisms. Each parameter was analyzed using standard laboratory techniques under controlled conditions.

Table 1. Overview of Salivary Biomarkers and Analytical Methods

Parameter	Analytical Method	Instrument/Kit Used	Unit of Measurement
Salivary pH	Digital pH meter	Eutech pH 700	–
Buffer Capacity	Ericsson's Titration Method	Manual titration	meq/L
Flow Rate	Gravimetric method	Digital balance	mL/min
Calcium	Arsenazo III colorimetric method	Spectrophotometer	mg/dL
Phosphate	Fiske-Subbarow method	Spectrophotometer	mg/dL
Total Protein	Lowry method	UV-Vis Spectrophotometer	mg/mL
α-Amylase	CNPG3 substrate method	Enzyme kinetic analyzer	U/mL

Parameter	Analytical Method	Instrument/Kit Used	Unit of Measurement
Secretory IgA	ELISA (Human sIgA Kit, Sigma-Aldrich)	Microplate reader	µg/mL
Lactoferrin	Sandwich ELISA	Microplate reader	ng/mL

Each sample was analyzed in triplicate to minimize analytical error, and the mean value was used for statistical evaluation.

8. Microbial Analysis

For assessing the microbial load, a selective culture of **Streptococcus mutans** and **Lactobacillus spp.** was performed. A 1 mL aliquot of saliva was serially diluted and plated on **mitis salivarius bacitracin agar** for *S. mutans* and **Rogosa agar** for *Lactobacillus spp.* Plates were incubated at **37°C for 48 hours** under microaerophilic conditions. Colony-forming units (CFUs) were counted and expressed as **CFU/mL of saliva**.

9. Quality Control and Calibration

All instruments were calibrated daily. Reagents were prepared fresh, and control assays were performed with every batch of tests. The intra-assay coefficient of variation was maintained below **5%** for biochemical assays. Data entry was verified by the dual entry method to avoid transcriptional errors.

10. Data Processing and Statistical Analysis

Data were compiled using **Microsoft Excel 2021** and analyzed with **SPSS (Version 26.0, IBM Corp.)**. The following statistical tests were applied:

1. **Descriptive Statistics:** Mean, standard deviation (SD), and range for each parameter.
2. **Inferential Statistics:**
    - **Independent t-test**: to compare mean values between caries-active and caries-free groups.
    - **Pearson's correlation coefficient (r):** to determine relationships between salivary biomarkers and DMFT/dmft index.
    - **Multiple linear regression analysis:** to identify independent predictors of caries risk.
    - **Receiver Operating Characteristic (ROC) curve analysis:** to assess the diagnostic accuracy of key biomarkers.

A *p*-value of **<0.05** was considered statistically significant.

Table 2. Statistical Analysis Framework

Objective	Statistical Test Applied	Variables Involved
Compare biomarker levels between groups	Independent t-test	Group I vs. Group II
Correlation between biomarkers and caries score	Pearson's correlation	DMFT/dmft vs. biochemical parameters
Identify predictors of caries risk	Multiple regression	sIgA, lactoferrin, buffer capacity, calcium
Evaluate diagnostic efficiency	ROC curve analysis	Biomarkers with an AUC > 0.70 are considered effective

11. Ethical and Biosafety Considerations

All procedures were performed using standard infection control protocols. Disposable gloves, masks, and sterilized instruments were used throughout the study. Biohazard waste was handled according to institutional biosafety guidelines and disposed of through approved medical waste management systems.

Confidentiality of participants was maintained by assigning unique identification codes. Personal identifiers were removed from datasets before statistical analysis.

12. Data Validation and Reliability

To ensure methodological rigor, **intra-observer reliability** for clinical examinations was tested by re-examining 10% of the participants after one week. The mean intra-observer kappa score was 0.91, confirming high reliability. Analytical reproducibility for biochemical tests was evaluated by duplicate testing of 10% of randomly selected samples, yielding a **mean deviation of less than 3%**.

13. Integration of Clinical and Biochemical Data

A **Caries Risk Assessment Model (CRAM)** was designed to integrate clinical and salivary parameters. Each variable was assigned a weighted score based on its relative contribution to caries susceptibility derived from regression coefficients.

Table 3. Caries Risk Assessment Model (CRAM) – Parameter Scoring Scheme

Parameter	Range/Value	Risk Score Assigned
DMFT/dmft	0 / ≥3	0 / 3
Plaque Index	<1.0 / ≥2.0	1 / 3
Salivary Flow Rate	>1.0 / <0.5 mL/min	0 / 3
Buffer Capacity	>8 / <4 meq/L	0 / 3
Calcium	>5.0 / <3.0 mg/dL	0 / 2
Phosphate	>4.0 / <2.0 mg/dL	0 / 2
sIgA	>100 / <50 µg/mL	0 / 3
Lactoferrin	<20 / >50 ng/mL	0 / 3
S. mutans Count	$<10^4$ / $>10^6$ CFU/mL	0 / 3

The cumulative CRAM score (ranging from 0 to 25) was used to categorize participants into **low, moderate,** and **high caries risk** groups for comparative analysis.

14. Validation of Predictive Model

To test the robustness of the CRAM framework, **cross-validation** was performed by randomly dividing the dataset into training (70%) and testing (30%) subsets. Regression coefficients derived from the training set were applied to the testing subset to predict caries status. Predictive performance was assessed through accuracy, sensitivity, specificity, and AUC values.

Table 4. Validation Metrics of Predictive Model

Metric	Training Set (%)	Testing Set (%)
Accuracy	88.4	84.7
Sensitivity	90.2	86.1

Metric	Training Set (%)	Testing Set (%)
Specificity	86.7	82.9
AUC Value	0.91	0.89

The high predictive accuracy confirmed the validity of combining salivary biomarkers and clinical indices as reliable tools for caries risk estimation in children.

Although the methodology incorporated strict standardization, certain limitations were acknowledged. The cross-sectional design limited causal inferences. Diurnal variation and transient physiological factors could influence salivary composition despite efforts to control collection time. Furthermore, socioeconomic and dietary influences, though documented, were not included in the regression analysis due to data variability.

In summary, this study adopted a rigorous multi-parameter approach combining **clinical, biochemical, and microbiological assessments** to determine caries risk among pediatric patients. The methodology ensured scientific integrity through standardized saliva collection, validated analytical techniques, robust statistical modeling, and ethical compliance. The integration of salivary biomarkers such as pH, buffer capacity, calcium, phosphate, sIgA, and lactoferrin with traditional clinical indices represents a novel and comprehensive strategy for early caries risk assessment in pediatric dentistry.

Results and Discussion:-

The present study assessed the relationship between salivary biomarkers, clinical parameters, and dental caries risk among pediatric patients aged 6–12 years. Data were analyzed for 120 participants divided equally into caries-free (Group I) and caries-active (Group II) groups. Statistical analyses were performed to compare biochemical and clinical variables and determine their predictive value in caries risk estimation.

1. Demographic and Clinical Characteristics

The mean age of participants in Group I was **8.9 ± 1.7 years**, and in Group II, **9.1 ± 1.5 years**, with no statistically significant difference ($p = 0.45$). The gender distribution was nearly equal in both groups (52% males, 48% females). Table 1 summarizes the baseline demographic and clinical parameters.

Table 1. Demographic and Clinical Characteristics of Participants

Parameter	Group I (Caries-Free)	Group II (Caries-Active)	p-value
Age (years, Mean ± SD)	8.9 ± 1.7	9.1 ± 1.5	0.45 (NS)
Gender (M/F)	31/29	32/28	
DMFT/dmft	0.0 ± 0.0	4.2 ± 1.8	<0.001*
Plaque Index	0.92 ± 0.38	1.87 ± 0.52	<0.001*
Gingival Index	0.64 ± 0.28	1.12 ± 0.41	<0.001*
Frequency of sugar intake/day	2.1 ± 0.9	4.8 ± 1.4	<0.001*

(*p < 0.05 significant; NS = not significant*)

The results indicated that while demographic characteristics were comparable, caries-active children exhibited significantly higher plaque accumulation, gingival inflammation, and frequency of sugar intake, factors that can influence both oral microbial ecology and salivary composition.

2. Salivary Flow Rate, pH, and Buffer Capacity

Salivary flow rate was significantly reduced in the caries-active group (**0.46 ± 0.15 mL/min**) compared to the caries-free group (**0.78 ± 0.18 mL/min**, $p < 0.001$). The mean salivary pH in Group I was **7.12 ± 0.28**, whereas in Group II, it dropped to **6.48 ± 0.33** ($p < 0.001$). Buffer capacity followed a similar pattern, being lower in caries-active subjects (**4.23 ± 1.16 meq/L**) compared to caries-free subjects (**7.96 ± 1.43 meq/L**).

Table 2. Comparison of Salivary Physical-Chemical Properties

Parameter	Group I (Mean ± SD)	Group II (Mean ± SD)	p-value
Flow Rate (mL/min)	0.78 ± 0.18	0.46 ± 0.15	<0.001*
pH	7.12 ± 0.28	6.48 ± 0.33	<0.001*
Buffer Capacity (meq/L)	7.96 ± 1.43	4.23 ± 1.16	<0.001*

Lower flow rate and buffering potential in caries-active subjects are consistent with the notion that reduced saliva volume diminishes its capacity to neutralize acids and remineralize enamel. Similar findings were reported by **Fejerskov et al. (2019)** and **Almeida et al. (2021)**, emphasizing that even modest reductions in salivary buffering can significantly elevate caries susceptibility.

3. Biochemical Profile of Saliva

Salivary concentrations of calcium, phosphate, total protein, α-amylase, secretory immunoglobulin A (sIgA), and lactoferrin were quantified for both groups.

Table 3. Comparative Analysis of Salivary Biomarkers

Biomarker	Group I (Mean ± SD)	Group II (Mean ± SD)	p-value
Calcium (mg/dL)	5.42 ± 0.73	3.68 ± 0.84	<0.001*
Phosphate (mg/dL)	4.12 ± 0.65	2.73 ± 0.79	<0.001*
Total Protein (mg/mL)	0.89 ± 0.22	1.46 ± 0.37	<0.001*
α-Amylase (U/mL)	126.4 ± 21.3	158.9 ± 25.4	<0.001*
Secretory IgA (µg/mL)	105.6 ± 21.7	62.3 ± 19.6	<0.001*
Lactoferrin (ng/mL)	18.4 ± 5.3	46.2 ± 7.1	<0.001*

(*$p < 0.05$ significant*)

The data revealed that calcium and phosphate concentrations were significantly lower in the caries-active group, whereas total protein, α-amylase, and lactoferrin levels were markedly elevated. Reduced mineral content reflects a diminished capacity for enamel remineralization, while elevated total protein and α-amylase may indicate heightened bacterial activity and host response to infection.

In contrast, salivary sIgA, an important immunoglobulin contributing to oral defense, was significantly higher in caries-free children, suggesting enhanced mucosal immunity. This aligns with reports by **Miletic et al. (2018)** and **Zhang et al. (2022)**, which demonstrated inverse associations between sIgA levels and caries prevalence in pediatric populations.

4. Microbial Profile

Microbial load, represented by *Streptococcus mutans* and *Lactobacillus spp.* Counts were significantly elevated in caries-active children. The mean *S. mutans* count was **6.8×10^6 CFU/mL** compared to **9.4×10^4**

CFU/mL in caries-free children ($p < 0.001$). Similarly, *Lactobacillus spp.* counts were **5.2 × 10^5 CFU/mL** vs **7.8 × 10^3 CFU/mL** respectively.

These findings support the role of these acidogenic bacteria in demineralization processes, corroborating evidence from **Marsh (2020)** and **Kidd & Beighton (2021)** that identify *S. mutans* as a critical initiator of cariogenic biofilm formation.

5. Correlation Between Biomarkers and Caries Index

Pearson's correlation analysis was performed to determine relationships between salivary biomarkers and DMFT/dmft scores.

Table 4. Correlation Coefficients (r) Between Biomarkers and Caries Experience

Parameter	Correlation with DMFT/dmft	p-value
Salivary Flow Rate	–0.63	<0.001*
Buffer Capacity	–0.58	<0.001*
Calcium	–0.61	<0.001*
Phosphate	–0.55	<0.001*
sIgA	–0.59	<0.001*
Lactoferrin	+0.64	<0.001*
Total Protein	+0.52	<0.001*
α-Amylase	+0.48	0.002*

(*$p < 0.05$ significant*)

The results revealed a **negative correlation** between caries index and protective factors such as flow rate, buffering capacity, calcium, phosphate, and sIgA. Conversely, lactoferrin, total protein, and α-amylase showed **positive correlations**, indicating their elevation in response to increased bacterial and inflammatory activity.

These trends underscore the multifactorial interplay between host defense, salivary composition, and microbial colonization in determining caries susceptibility.

6. Regression and Predictive Modeling

A **multiple linear regression model** was developed using significant biomarkers as independent variables and DMFT/dmft as the

dependent variable. The final model explained **78.6% of variance (R^2 = 0.786, p < 0.001)**, with sIgA, calcium, and lactoferrin emerging as the most significant predictors of caries activity.

Table 5. Regression Coefficients of Predictive Biomarkers

Variable	β Coefficient	Standard Error	p-value
sIgA	–0.41	0.07	<0.001*
Calcium	–0.34	0.06	<0.001*
Lactoferrin	+0.39	0.08	<0.001*
Buffer Capacity	–0.27	0.09	0.004*

(*p < 0.05 significant*)

This predictive strength demonstrates that an integrated assessment of biochemical and clinical parameters can effectively stratify caries risk in pediatric populations. The regression findings corroborate previous studies by **Lenander-Lumikari & Loimaranta (2020)** and **Shetty et al. (2021)**, who emphasized sIgA and calcium as major determinants in pediatric caries defense mechanisms.

7. ROC Curve Analysis and Diagnostic Efficacy

Receiver Operating Characteristic (ROC) curves were plotted to evaluate the diagnostic accuracy of the most significant biomarkers. The **Area Under Curve (AUC)** values were as follows:

- sIgA: **0.92**
- Lactoferrin: **0.88**
- Calcium: **0.86**
- Buffer Capacity: **0.83**

These values suggest excellent discriminatory power of these biomarkers in differentiating between high- and low-risk groups. The sensitivity and specificity of sIgA were **89.6%** and **87.3%**, respectively, highlighting its potential as a noninvasive screening tool.

8. Comparative Discussion with Literature

The results align with the established view that saliva serves as a dynamic diagnostic fluid reflecting both systemic and oral health. The observed reduction in pH, buffer capacity, and flow rate among caries-active children reinforces the findings of **Humphrey and Williamson (2020)**, who linked acidogenic shifts in salivary chemistry with early enamel dissolution.

Low calcium and phosphate concentrations among the caries-active participants confirm the crucial role of mineral homeostasis in maintaining enamel integrity. The results are consistent with **Fadel et al. (2018)**, who reported that salivary ionic imbalances

increase enamel demineralization rates and alter the hydroxyapatite equilibrium.

The inverse relationship between sIgA and caries incidence found in this study mirrors findings by **Zhang et al. (2022)** and **de Farias et al. (2020)**, where higher sIgA levels enhanced bacterial aggregation and clearance, thereby reducing cariogenic biofilm adhesion. Elevated lactoferrin levels in the caries-active group may reflect compensatory antimicrobial responses, a phenomenon discussed by **Marcotte and Lavoie (2021)**.

Furthermore, the integration of biochemical and microbial parameters into a composite model (CRAM) demonstrates enhanced predictive precision, echoing trends in recent biomarker-based diagnostic research, such as **Patil et al. (2023)**, which advocated for multifactorial saliva-based diagnostics.

9. Interpretation of Findings

The data collectively suggest that caries risk in pediatric patients is strongly associated with alterations in salivary composition. Reduced salivary flow and mineral content, combined with lowered sIgA and increased inflammatory proteins, form a distinct biochemical signature of caries susceptibility.

This highlights the **potential of saliva as a diagnostic biofluid**, capable of reflecting host-pathogen interactions, immune status, and environmental influences in a noninvasive manner.

Moreover, the high predictive accuracy of the combined biomarkers (AUC > 0.85 for all major markers) suggests clinical utility for early detection and personalized prevention strategies.

10. Implications and Future Perspectives

The integration of salivary biomarkers into routine pediatric screening could revolutionize preventive dentistry by allowing early, chairside risk assessment without radiation or invasive sampling. Coupling biochemical assays with digital data analytics may facilitate individualized caries management protocols.

However, inter-individual variability and environmental influences necessitate longitudinal studies to confirm causality and develop standardized biomarker thresholds. Future work should incorporate molecular techniques such as **proteomics** and **metabolomics** to expand biomarker panels beyond classical biochemical indicators.

This study demonstrates that caries-active pediatric patients exhibit distinctive alterations in salivary pH, buffer capacity, calcium, phosphate, and immune proteins, particularly sIgA and lactoferrin. These variables collectively explain nearly 80% of caries risk variance, confirming the diagnostic power of saliva-based assays.

The strong agreement with existing literature and the robust statistical performance of predictive models underscore the reliability of salivary biomarkers as noninvasive, efficient, and clinically meaningful tools for pediatric caries risk assessment.

Conclusion:-

The findings of this study underscore the vital role of salivary biomarkers as powerful, noninvasive indicators in the assessment of caries risk among pediatric patients. Dental caries, though multifactorial in nature, is ultimately the result of an imbalance between demineralization and remineralization processes at the tooth surface, influenced by

microbial activity, host factors, diet, and time. This investigation demonstrated that the biochemical composition of saliva reflects these underlying interactions, thereby providing a reliable diagnostic window into a child's oral health status. The significant differences observed in salivary flow rate, pH, and buffer capacity between caries-free and caries-active groups highlight the protective function of saliva in maintaining oral equilibrium. A reduction in flow rate and buffering potential in caries-active children compromises the neutralization of acids, facilitating an environment favorable for cariogenic bacterial growth and enamel dissolution. This physiologic alteration, combined with lowered pH, enhances the cariogenic potential of the oral microenvironment. These clinical parameters, when analyzed alongside biochemical indices, provide a comprehensive understanding of individual caries susceptibility. Among the biochemical markers analyzed, calcium and phosphate demonstrated strong negative correlations with caries activity, confirming their essential roles in enamel remineralization. Their depletion in saliva among caries-active subjects suggests an impaired mineral exchange mechanism. Conversely, elevated total protein, α-amylase, and lactoferrin levels in the same group indicate a compensatory host response to microbial challenge and inflammation. The inverse relationship between salivary secretory immunoglobulin A (sIgA) and caries prevalence further supports its immunoprotective role in mucosal defense. Collectively, these parameters not only distinguish high-risk children from low-risk counterparts but also reflect the dynamic nature of salivary biochemistry in response to microbial load and dietary influences.

The correlation and regression analyses validated the predictive capacity of these biomarkers, explaining nearly 80% of caries risk variability when considered collectively. The strong diagnostic performance of sIgA, calcium, and lactoferrin, demonstrated through ROC curve analysis, emphasizes their potential for integration into clinical risk assessment models. Such an evidence-based biomarker approach could complement conventional methods like DMFT scoring, enabling early detection of susceptibility before irreversible tooth damage occurs. Beyond the biochemical domain, this study also reinforces the importance of comprehensive pediatric oral health management. Routine evaluation of salivary parameters can be incorporated into preventive programs in schools and dental clinics, particularly in populations with high caries prevalence. The approach is simple, child-friendly, cost-effective, and easily repeatable, aligning with the broader shift toward minimally invasive and preventive dentistry. In summary, the assessment of caries risk through salivary biomarkers and clinical indicators presents a promising frontier in pediatric dentistry. The integration of biochemical, immunologic, and microbial analyses within routine dental examinations offers the potential to transition from reactive treatment toward proactive prevention. Future studies with larger cohorts, multiethnic samples, and longitudinal follow-ups are warranted to establish standardized biomarker thresholds and validate their clinical utility. As research advances, saliva-based diagnostics may evolve into a cornerstone of personalized oral healthcare, allowing for early intervention, improved patient education, and sustainable reduction in caries incidence among children.

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