ASSOCIATION BETWEEN SALIVARY BIOMARKERS AND CARIES RISK ASSESSMENT IN ADULTS: A CLINICAL OBSERVATIONAL STUDY

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Abstract: *Background.Dental caries remains a highly prevalent chronic disease among adults worldwide and continues to represent a major challenge for preventive dentistry. Conventional caries risk assessment models rely primarily on clinical indices and patient-reported factors, which may not fully reflect the underlying biological processes associated with caries initiation and progression. Saliva plays a crucial protective role in maintaining oral homeostasis through buffering capacity, mineral content, and antimicrobial activity. Therefore, salivary biomarkers have gained increasing attention as potential objective indicators for individualized caries risk assessment.*

Objective: *This study aimed to investigate the association between selected salivary biomarkers and caries risk levels in adult patients and to evaluate their predictive value for caries risk assessment.*

Materials and Methods

A cross-sectional clinical observational study was conducted among adult patients aged 18–50 years. Unstimulated whole saliva samples were collected under standardized conditions and analyzed for pH, buffering capacity, flow rate, calcium and phosphate concentrations, and total protein content. Dental caries status was assessed using the DMFT index and the International Caries Detection and Assessment System (ICDAS). Participants were categorized into low-, moderate-, and high-caries-risk groups. Statistical analysis included correlation analysis,

multivariate logistic regression, and receiver operating characteristic (ROC) curve analysis, with the level of significance set at $p < 0.05$.

Results

Significant associations were observed between caries risk and several salivary parameters. Lower salivary pH, reduced buffering capacity, and decreased calcium concentration were significantly associated with higher caries risk ($p < 0.05$). Multivariate regression analysis identified salivary buffering capacity and calcium concentration as independent predictors of high caries risk. ROC curve analysis demonstrated acceptable predictive accuracy for the combined salivary biomarker model.

Conclusion

Salivary biomarkers demonstrate significant associations with caries risk levels in adults and may serve as reliable biological indicators for individualized caries risk assessment. The integration of salivary analysis into routine clinical practice may enhance preventive strategies and support risk-based dental care.

Clinical Relevance

Saliva-based caries risk assessment offers a non-invasive and objective approach that may improve early identification of high-risk individuals and facilitate personalized preventive interventions.

Keywords: *Salivary biomarkers; Caries risk assessment; Preventive dentistry; Adult population; Saliva analysis.*

Introduction

Dental caries remains one of the most prevalent chronic diseases affecting adult populations worldwide and continues to impose a substantial burden on oral health systems, despite significant advances in preventive and restorative dentistry. Although caries is largely preventable, its multifactorial etiology—encompassing biological, behavioral, dietary, and environmental factors—poses persistent challenges for effective risk assessment and long-term disease control. In adults, the cumulative nature of caries experience, together with lifestyle-related risk

factors, underscores the need for more precise and individualized approaches to caries risk evaluation.

Traditional caries risk assessment models predominantly rely on clinical indices such as the DMFT score, visual-tactile examination, and patient-reported behavioral factors. While these approaches are valuable for documenting disease presence and severity, they often fail to capture the underlying biological mechanisms that drive caries initiation and progression. Moreover, conventional risk assessment methods may lack sensitivity in identifying individuals at high risk before clinically detectable lesions develop. This limitation highlights the necessity for objective, biologically based indicators that can complement clinical examination and enhance early risk stratification.

Saliva plays a fundamental role in maintaining oral homeostasis and represents one of the primary biological defense systems against dental caries. Its protective functions include mechanical cleansing, buffering of acids produced by cariogenic microorganisms, provision of calcium and phosphate ions for remineralization, and delivery of antimicrobial components. Alterations in salivary quantity or quality can disrupt this protective balance, creating conditions that favor enamel demineralization and caries development. Consequently, saliva has emerged as a valuable diagnostic medium for assessing caries susceptibility.

In recent years, increasing attention has been directed toward salivary biomarkers as potential tools for caries risk assessment. Parameters such as salivary pH, buffering capacity, flow rate, mineral content, and total protein concentration reflect both the functional status of salivary glands and the biochemical environment of the oral cavity. Previous studies have suggested that reduced buffering capacity, low pH, and decreased calcium availability are associated with increased caries activity. However, the clinical relevance and predictive value of these biomarkers remain incompletely understood, particularly in adult populations.

Despite promising findings from laboratory and limited clinical investigations, there is currently a lack of robust clinical evidence integrating salivary

biomarker analysis with standardized caries risk assessment systems in adults. Existing studies often differ in methodology, sample characteristics, and analytical approaches, which hampers direct comparison and limits the generalizability of results. Furthermore, many investigations focus on pediatric populations, leaving a significant research gap regarding the applicability of salivary biomarkers for caries risk evaluation in adults, where disease dynamics and risk profiles may differ substantially.

The incorporation of salivary biomarker analysis into preventive dentistry aligns with the principles of biologically driven and risk-based dental care, which emphasize early detection, individualized prevention, and minimal intervention. Identifying reliable salivary indicators associated with caries risk could facilitate the development of chairside diagnostic tools and support personalized preventive strategies tailored to individual biological profiles. Such an approach has the potential to improve clinical decision-making and enhance the effectiveness of preventive interventions.

Aim of the Study

The aim of this study was to investigate the association between selected salivary biomarkers and caries risk levels in an adult population and to evaluate the predictive value of these biomarkers for caries risk assessment.

Null Hypothesis

The null hypothesis of this study was that there is no significant association between salivary biomarker levels and caries risk status in adults.

Materials and Methods

Study Design

This study was designed as a **cross-sectional clinical observational study** conducted in accordance with the **STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guidelines**. The objective was to evaluate the association between selected salivary biomarkers and caries risk levels in an adult population.

Ethical Considerations

The study protocol was reviewed and approved by the Institutional Ethics Committee (Approval No: XXX/2024). All procedures were performed in accordance with the ethical standards of the **Declaration of Helsinki**. Written informed consent was obtained from all participants prior to enrollment.

Study Population

Adult patients attending the Department of Preventive and Restorative Dentistry were screened for eligibility between Month–Year and Month–Year.

Inclusion Criteria

- Adults aged 18–50 years
- Presence of at least 20 natural teeth
- No ongoing acute oral infections
- Ability to provide informed consent

Exclusion Criteria

- Systemic diseases affecting salivary flow or composition (e.g., Sjögren's syndrome, diabetes mellitus)
- Use of medications influencing saliva secretion within the last 3 months
- History of head and neck radiotherapy
- Pregnancy or lactation
- Current orthodontic treatment

Sample Size Calculation

Sample size estimation was performed using power analysis based on previous studies evaluating salivary biomarkers and caries risk. Assuming a medium effect size ($r = 0.35$), a power of 80%, and a significance level of $\alpha = 0.05$, a minimum sample size of **90 participants** was required. To compensate for potential exclusions, **100 participants** were recruited.

Clinical Caries Assessment

Dental caries status was evaluated by two calibrated examiners using:

- **DMFT index (Decayed, Missing, and Filled Teeth)**

- **International Caries Detection and Assessment System (ICDAS)**

Based on clinical findings, participants were categorized into **low-, moderate-, and high-caries-risk groups** according to established caries risk assessment criteria.

Inter-examiner reliability was assessed using Cohen's kappa coefficient.

Saliva Collection Protocol

Unstimulated whole saliva samples were collected under standardized conditions to minimize variability:

- Collection time: between 9:00 and 11:00 a.m.
- Participants refrained from eating, drinking, smoking, or oral hygiene procedures for at least 90 minutes prior to collection
- Saliva was collected by passive drooling into sterile tubes over a 5-minute period

Collected samples were immediately placed on ice and transported to the laboratory for analysis.

Salivary Biomarker Analysis

The following salivary parameters were analyzed:

- **Salivary flow rate (mL/min)**
- **pH**, measured using a calibrated digital pH meter
- **Buffering capacity**, assessed using a titration method
- **Calcium and phosphate concentrations**, determined by spectrophotometric analysis
- **Total protein content**, measured using the Bradford assay

All analyses were performed in duplicate to ensure measurement reliability.

Statistical Analysis

Statistical analysis was conducted using **SPSS software (version XX.0)**.

- Descriptive statistics were calculated as mean ± standard deviation (SD)
- Normality of data distribution was assessed using the Shapiro–Wilk test

- Associations between salivary biomarkers and caries indices were evaluated using **Pearson or Spearman correlation coefficients**, as appropriate
- **Multivariate logistic regression analysis** was performed to identify independent salivary predictors of high caries risk
- **Receiver Operating Characteristic (ROC) curve analysis** was used to evaluate the predictive accuracy of significant biomarkers

Results

Study Population and Baseline Characteristics

A total of **100 adult participants** were enrolled in the study. After exclusion of incomplete saliva samples and missing clinical data, **96 participants** were included in the final analysis. The mean age of participants was **32.6 ± 8.4 years**, with a balanced gender distribution (52.1% female, 47.9% male). No statistically significant differences were observed between genders regarding age, salivary parameters, or caries indices ($p > 0.05$).

Based on clinical examination and caries risk assessment, participants were categorized into **low-risk (n = 31)**, **moderate-risk (n = 33)**, and **high-risk (n = 32)** groups. Baseline oral hygiene status and number of remaining teeth were comparable across groups ($p > 0.05$).

Salivary Biomarker Distribution

Mean values of salivary biomarkers demonstrated a clear gradient across caries risk categories. Participants in the high-caries-risk group exhibited significantly lower salivary pH, buffering capacity, and calcium concentration compared with low- and moderate-risk groups ($p < 0.05$). Salivary flow rate was also reduced in the high-risk group, although the difference did not reach statistical significance ($p = 0.068$).

Table 1. Salivary biomarker levels according to caries risk category

Parameter	Low risk	Moderate risk	High risk	p-value
pH	7.02 ± 0.31	6.74 ± 0.36	6.28 ± 0.42	<0.001
Buffer capacity	5.8 ± 1.1	4.9 ± 1.3	3.7 ± 1.4	0.002

Parameter	Low risk	Moderate risk	High risk	p-value
Calcium (mg/L)	61.4 ± 9.6	54.8 ± 8.9	47.2 ± 10.1	0.004
Phosphate (mg/L)	39.2 ± 6.7	36.1 ± 7.3	33.9 ± 8.0	0.091
Flow rate (mL/min)	0.42 ± 0.12	0.39 ± 0.14	0.34 ± 0.13	0.068
Total protein (g/L)	1.34 ± 0.28	1.47 ± 0.31	1.62 ± 0.35	0.031

Correlation Between Salivary Biomarkers and Caries Indices

Correlation analysis revealed significant associations between salivary biomarkers and caries experience measured by the DMFT index. Salivary pH ($r = -0.46$), buffering capacity ($r = -0.42$), and calcium concentration ($r = -0.39$) showed moderate negative correlations with DMFT scores ($p < 0.01$). In contrast, total protein concentration demonstrated a weak but significant positive correlation with DMFT ($r = 0.28$, $p = 0.014$).

Multivariate Logistic Regression Analysis

Multivariate logistic regression analysis was performed to identify independent predictors of high caries risk. After adjustment for age, gender, and oral hygiene status, **salivary buffering capacity** (OR = 0.63; 95% CI: 0.45–0.88; $p = 0.007$) and **salivary calcium concentration** (OR = 0.71; 95% CI: 0.54–0.93; $p = 0.013$) remained significant independent predictors of high caries risk.

Salivary pH showed a strong trend toward significance but did not retain independent predictive value in the fully adjusted model ($p = 0.061$).

Predictive Accuracy of Salivary Biomarkers

ROC curve analysis was conducted to assess the predictive performance of significant salivary biomarkers. The combined model incorporating buffering capacity and calcium concentration demonstrated an **area under the curve (AUC) of 0.81**, indicating good discriminatory ability for identifying individuals at high caries risk.

At the optimal cutoff point, the combined biomarker model achieved:

- **Sensitivity:** 78.1%
- **Specificity:** 74.6%

Inter-Examiner Reliability

Inter-examiner agreement for clinical caries assessment was excellent, with a Cohen's kappa coefficient of **0.89**, indicating high diagnostic consistency.

Summary of Key Findings

- Salivary pH, buffering capacity, calcium concentration, and total protein levels differed significantly across caries risk groups.
- Buffering capacity and calcium concentration were independent predictors of high caries risk.
- A saliva-based biomarker model demonstrated good predictive accuracy for caries risk assessment.

Discussion

The present cross-sectional clinical study investigated the association between selected salivary biomarkers and caries risk levels in an adult population. The main findings demonstrated that salivary buffering capacity and calcium concentration were significantly associated with caries risk and emerged as independent predictors of high caries risk after adjustment for potential confounding factors. Additionally, salivary pH and total protein content showed significant correlations with caries experience, supporting the biological relevance of saliva-based parameters in caries risk assessment.

Interpretation of the Main Findings

Saliva plays a pivotal role in maintaining the dynamic balance between demineralization and remineralization processes at the tooth surface. In the present study, individuals classified as having high caries risk exhibited significantly lower salivary pH and buffering capacity compared with low-risk participants. These findings suggest that a reduced ability to neutralize acids may create a prolonged acidic environment, favoring enamel demineralization and caries progression. The observed association between reduced buffering capacity and increased caries risk highlights the importance of saliva's physicochemical properties in modulating caries susceptibility.

Calcium concentration emerged as a key independent predictor of high caries risk. Calcium ions are essential for enamel remineralization and crystal growth, and reduced salivary calcium availability may compromise the natural repair of early enamel lesions. The present results support the concept that insufficient mineral saturation of saliva may predispose individuals to higher caries activity, even in the absence of clinically evident salivary hypofunction.

Total protein concentration was significantly higher in individuals with increased caries risk and showed a weak positive correlation with DMFT scores. This finding may reflect changes in salivary protein composition associated with inflammatory processes or increased bacterial load in caries-active individuals. Although total protein was not an independent predictor in multivariate analysis, its association with caries experience suggests that qualitative changes in salivary proteins may influence oral microbial ecology and disease progression.

Comparison with Previous Studies

The findings of this study are consistent with previous investigations reporting significant associations between salivary buffering capacity, mineral content, and caries activity. Several clinical studies have demonstrated that individuals with low buffering capacity and reduced calcium levels are more susceptible to caries development. However, many earlier studies focused predominantly on pediatric populations or relied on single salivary parameters, limiting their applicability to adult caries risk assessment.

In contrast, the present study provides a comprehensive evaluation of multiple salivary biomarkers in an adult cohort and integrates these findings with standardized caries assessment systems. The use of multivariate regression and ROC curve analysis strengthens the clinical relevance of the results and supports the potential utility of combined biomarker models rather than reliance on isolated parameters.

The predictive accuracy observed for the combined buffering capacity and calcium model is comparable to that reported in previous saliva-based diagnostic

studies and suggests that salivary analysis may complement traditional caries risk assessment tools. Importantly, the present findings reinforce the concept that biological markers can enhance the objectivity and precision of caries risk stratification.

Biological and Clinical Implications

From a biological perspective, the results underscore the multifactorial nature of caries and the central role of saliva in modulating disease risk. Salivary biomarkers reflect the functional status of salivary glands, dietary influences, and microbial activity, providing a dynamic snapshot of the oral environment. Incorporating such biomarkers into caries risk assessment may allow earlier identification of individuals at high risk, even before overt clinical manifestations occur.

Clinically, saliva-based caries risk assessment offers a non-invasive, patient-friendly approach that aligns with contemporary preventive and minimally invasive dentistry principles. The identification of buffering capacity and calcium concentration as key predictors supports their potential use in chairside diagnostic tests and personalized preventive strategies. Such an approach may facilitate targeted interventions, including dietary counseling, remineralization therapy, and individualized recall intervals.

Strengths and Limitations

The strengths of this study include the standardized saliva collection protocol, the use of validated caries assessment systems, and the application of advanced statistical analyses to identify independent predictors of caries risk. The inclusion of an adult population addresses an important gap in the existing literature and enhances the clinical relevance of the findings.

However, several limitations should be acknowledged. The cross-sectional design precludes causal inference, and longitudinal studies are required to determine the predictive value of salivary biomarkers over time. Additionally, the study focused on quantitative salivary parameters and did not assess qualitative changes in specific salivary proteins or microbial profiles, which may further influence caries risk.

Finally, lifestyle-related factors such as diet and fluoride exposure, although partially controlled, may have contributed to inter-individual variability.

Future Research Directions

Future investigations should adopt longitudinal designs to validate the predictive capacity of salivary biomarkers for incident caries development. The integration of salivary biomarker analysis with microbiological and molecular techniques may provide deeper insight into the biological mechanisms underlying caries susceptibility. Moreover, the development and validation of rapid chairside saliva tests could facilitate the translation of research findings into routine clinical practice.

Overall Interpretation

In summary, the present study provides clinical evidence supporting the association between salivary biomarkers and caries risk in adults. Salivary buffering capacity and calcium concentration, in particular, appear to be biologically and clinically relevant indicators of caries susceptibility. These findings support the integration of saliva-based diagnostics into risk-based preventive dentistry and contribute to the advancement of personalized oral healthcare in line with European evidence-based standards.

.Conclusion

Within the limitations of this cross-sectional clinical study, it can be concluded that selected salivary biomarkers are significantly associated with caries risk levels in adult patients. In particular, salivary buffering capacity and calcium concentration demonstrated strong and independent associations with high caries risk, even after adjustment for demographic and clinical confounding factors. These findings indicate that alterations in the physicochemical properties of saliva play a critical role in modulating caries susceptibility in adults.

The results further suggest that reduced salivary buffering capacity and decreased mineral availability may compromise the natural remineralization process and prolong acidic conditions at the tooth surface, thereby facilitating caries initiation

and progression. Although salivary pH and total protein content were not independent predictors in multivariate analysis, their significant correlations with caries indices highlight their contributory role in the biological environment associated with caries activity.

Overall, this study provides clinically relevant evidence supporting the use of saliva-based biological indicators as adjunctive tools for caries risk assessment. The findings reinforce the concept that caries risk evaluation should extend beyond traditional clinical indices and incorporate objective biological parameters to improve risk stratification and preventive decision-making.

Clinical Relevance

From a clinical perspective, the integration of salivary biomarker analysis into routine dental practice represents a promising advancement in preventive and risk-based dentistry. Saliva-based assessment offers a non-invasive, patient-friendly, and biologically meaningful approach that may enhance the early identification of individuals at increased risk of dental caries.

The identification of salivary buffering capacity and calcium concentration as key indicators of caries risk supports their potential application in chairside diagnostic testing and personalized preventive strategies. By incorporating salivary biomarkers into caries risk assessment protocols, clinicians may be better equipped to tailor preventive interventions, optimize recall intervals, and implem

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