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ORIGINAL ARTICLE

Clinical Utility of Salivary Biomarkers for Early Detection and Monitoring of Diabetes and Cardiovascular Disease

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ABSTRACT

Background: Diabetes mellitus and cardiovascular diseases are leading causes of morbidity and mortality worldwide. Conventional blood-based diagnostic methods, though reliable, are invasive and limit frequent monitoring. Saliva, as a non-invasive body fluid, contains biomarkers that may reflect systemic metabolic and cardiovascular changes.

Objective: To evaluate the clinical utility of salivary biomarkers for early detection and monitoring of diabetes and cardiovascular disease.

Methods: A cross-sectional study was conducted at Margalla Dental Hospital, Rawalpindi, in collaboration with Army Medical College, from February 2022 to March 2023. A total of 90 participants were enrolled, divided into three groups: diabetes patients, cardiovascular disease patients, and healthy controls. Unstimulated saliva samples were collected and analyzed for glucose, oxidative stress markers, inflammatory cytokines (IL-6, TNF-α, CRP), and cardiac enzymes (troponin I, CK-MB). Corresponding blood samples were also tested for correlation. Statistical analysis was performed using SPSS v26, with $p < 0.05$ considered significant.

Results: Salivary glucose and malondialdehyde were significantly elevated in diabetes patients compared to controls, while total antioxidant capacity was reduced ($p < 0.001$). Inflammatory biomarkers (IL-6, TNF-α, CRP) were raised in both diabetes and cardiovascular groups, with CRP highest among cardiovascular patients. Salivary troponin I and CK-MB were significantly elevated in the cardiovascular group ($p < 0.001$), with mild elevations also observed in diabetics. Strong correlations were found between salivary and serum glucose ($r = 0.81$), CRP ($r = 0.77$), and troponin I ($r = 0.72$).

Conclusion: Salivary biomarkers reliably reflect systemic alterations in diabetes and cardiovascular disease, showing strong correlations with conventional blood markers. Saliva offers a safe, simple, and non-invasive diagnostic medium that can be useful for early detection, frequent monitoring, and large-scale screening.

Keywords: Saliva, biomarkers, diabetes mellitus, cardiovascular disease, non-invasive diagnostics

INTRODUCTION

Diabetes mellitus and cardiovascular diseases are two of the most common chronic conditions worldwide and remain leading contributors to illness and death. Both conditions are closely interlinked, as persistent high blood sugar, oxidative stress, and inflammation in diabetes significantly increase the risk of developing cardiovascular complications[1]. Together, they create a combined burden that affects millions of people and puts a heavy strain on healthcare systems[2,3].

The importance of early detection and continuous monitoring in these diseases cannot be overstated. Identifying patients at an early stage can prevent progression to severe complications, improve treatment outcomes, and reduce healthcare costs. At present, diagnosis and monitoring are mostly carried out through blood-based tests, including fasting plasma glucose, HbA1c, lipid profiles, and cardiac enzyme assays[4]. While these methods are accurate, they are invasive, require trained staff, and may discourage patients from undergoing frequent monitoring. Repeated blood sampling is also inconvenient, uncomfortable, and unsuitable for large-scale community screening[5].

Saliva has emerged as an attractive alternative to blood for diagnostic purposes. Produced by the major salivary glands, it contains a wide range of substances such as glucose, proteins, enzymes, nucleic acids, hormones, and metabolites[6]. Many of these components enter saliva directly from blood circulation through passive diffusion or active transport, making saliva a valuable reflection of the body's internal state. Its collection is simple, completely non-invasive, painless, and inexpensive. Unlike blood, it does not require specialized equipment or highly trained staff and can be obtained easily, even in resource-limited settings. This makes saliva a suitable fluid for repeated testing, home-based monitoring, and large-scale screening programs[7,8].

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In the context of diabetes, saliva has been shown to carry markers that are relevant for disease detection and follow-up. These include glucose itself, advanced glycation end products, enzymes related to tissue stress, and oxidative markers[9]. In addition, inflammatory mediators such as interleukins, tumor necrosis factor, and C-reactive proteins are elevated in diabetes and are measurable in saliva. Similarly, in cardiovascular disease, saliva has been found to contain important indicators of myocardial stress and damage such as troponins and creatine kinase isoenzymes, as well as molecules related to oxidative stress and vascular inflammation. The presence of these biomarkers in saliva suggests that it can serve as a useful tool for assessing both metabolic and cardiovascular health[10,11].

The advantages of saliva as a diagnostic medium are clear: it is non-invasive, cost-effective, safe, and well accepted by patients. At the same time, challenges remain[12]. The concentration of biomarkers in saliva is often lower than in blood, meaning highly sensitive detection methods are required. Levels can also vary depending on oral health, time of collection, hydration status, and other physiological factors. Despite these challenges, rapid progress is being made in the development of sensitive laboratory techniques, biosensors, and portable devices that can accurately measure salivary biomarkers[13].

With the growing burden of diabetes and cardiovascular diseases, there is a strong need for diagnostic methods that are affordable, simple, and reliable. Saliva, with its rich composition and ease of collection, has the potential to fill this gap. Establishing the clinical utility of salivary biomarkers could transform disease detection and monitoring, leading to earlier intervention, better disease control, and improved patient outcomes[14].

MATERIALS AND METHODS

Study Design and Setting: This was a cross-sectional clinical study conducted to evaluate the clinical utility of salivary biomarkers in the early detection and monitoring of diabetes and

cardiovascular disease. The study was carried out at Margalla Dental Hospital, Rawalpindi, Pakistan, in collaboration with the Department of Biochemistry, Army Medical College, Rawalpindi. The study duration extended over fourteen months, from February 2022 to March 2023.

Study Population and Sample Size: A total of **90 patients** were enrolled using a purposive sampling method. Participants included both male and female adults attending the outpatient departments for routine medical or dental checkups. Patients were divided into three groups: individuals with type 2 diabetes mellitus, patients with clinically diagnosed cardiovascular disease, and apparently healthy controls. The sample size was calculated to ensure adequate statistical power for detecting significant differences in salivary biomarker levels across groups.

Inclusion and Exclusion Criteria: Inclusion criteria were adults aged 30–70 years, either previously diagnosed with type 2 diabetes or cardiovascular disease based on medical records, or healthy individuals without any known systemic disease to serve as controls. Exclusion criteria included patients with acute infections, autoimmune disorders, salivary gland dysfunction, oral inflammatory conditions, malignancy, recent antibiotic therapy, or unwillingness to provide informed consent.

Ethical Considerations: The study protocol was reviewed and approved by the Institutional Ethical Review Committees of Margalla Dental Hospital and Army Medical College. Written informed consent was obtained from all participants before enrollment, and the study was conducted in accordance with the Declaration of Helsinki guidelines for research involving human subjects.

Saliva Collection and Processing: Unstimulated whole saliva was collected from each participant under standardized conditions. Participants were instructed to refrain from eating, drinking, smoking, or performing oral hygiene procedures for at least one hour prior to collection. Saliva was obtained by expectoration into sterile containers between 9:00 AM and 11:00 AM to minimize circadian variation. The samples were immediately placed on ice, centrifuged at 3,000 rpm for 10 minutes to remove debris, and stored at –80°C until further analysis.

Biomarker Analysis: Salivary glucose levels were measured using an enzymatic colorimetric method. Oxidative stress was assessed by quantifying malondialdehyde (MDA) and total antioxidant capacity (TAC). Inflammatory markers including interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and C-reactive protein (CRP) were analyzed using enzyme-linked immunosorbent assays (ELISA). For cardiovascular evaluation, salivary troponin I and creatine kinase-MB (CK-MB) were measured using commercially available high-sensitivity ELISA kits. All assays were performed in duplicate to ensure accuracy.

Blood Biomarker Comparison: To validate salivary findings, venous blood samples were collected from participants under aseptic conditions. Plasma glucose, HbA1c, lipid profile, CRP, and cardiac enzymes were determined according to standard laboratory protocols. Correlation analysis was performed between salivary and blood biomarker levels.

Statistical Analysis: Data were analyzed using the Statistical Package for the Social Sciences (SPSS) version 26.0. Continuous variables were expressed as mean ± standard deviation, while categorical variables were presented as frequencies and percentages. Independent sample t-tests and one-way ANOVA were used to compare biomarker levels between groups. Pearson's correlation coefficient was applied to assess the relationship between salivary and blood biomarkers. A p-value of <0.05 was considered statistically significant.

RESULTS

Demographic and Clinical Characteristics: A total of 90 participants were enrolled, divided into three groups: 30 patients with type 2 diabetes mellitus, 30 patients with cardiovascular disease (CVD), and 30 apparently healthy controls. The mean age of participants was 51.3 ± 9.4 years, with no statistically significant difference in age between groups (p = 0.08). The male-to-female ratio was nearly similar across all groups (p = 0.71), ensuring that gender distribution did not act as a confounding variable. The mean body mass index (BMI) was significantly higher in both diabetic and CVD groups compared to controls (p = 0.01). The mean duration of illness was 6.2 ± 3.1 years in the diabetes group and 7.5 ± 2.8 years in the CVD group. These demographic and baseline clinical characteristics are presented in Table 1, which highlights the comparability of groups with respect to age and sex while emphasizing differences in BMI and disease history.

Table 1. Baseline demographic and clinical characteristics of study participants

Variable	Diabetes Group (n=30)	CVD Group (n=30)	Control Group (n=30)	p-value
Age (years, mean ± SD)	52.6 ± 8.7	53.4 ± 9.1	47.9 ± 10.2	0.08
Male (%)	17 (56.7%)	16 (53.3%)	14 (46.7%)	0.71
Female (%)	13 (43.3%)	14 (46.7%)	16 (53.3%)	
BMI (kg/m², mean ± SD)	28.7 ± 3.4	27.9 ± 3.7	24.6 ± 2.9	0.01*
Duration of illness (yrs)	6.2 ± 3.1	7.5 ± 2.8	–	–
Smoking history (%)	11 (36.7%)	13 (43.3%)	6 (20.0%)	0.09
Hypertension (%)	15 (50.0%)	18 (60.0%)	3 (10.0%)	<0.001*
Family history (%)	14 (46.7%)	12 (40.0%)	5 (16.7%)	0.03*

*Significant at p < 0.05

Salivary Glucose and Oxidative Stress Markers: Salivary glucose was markedly elevated in the diabetes group compared to both CVD and control groups (6.8 ± 1.9 mg/dL vs. 3.9 ± 1.2 mg/dL vs. 2.1 ± 0.7 mg/dL, p < 0.001). Although salivary glucose was not as high in CVD patients as in diabetics, it remained significantly greater than in controls, suggesting that metabolic dysregulation in cardiovascular disease also influences salivary glucose levels.

Malondialdehyde (MDA), a biomarker of lipid peroxidation and oxidative stress, was significantly elevated in the diabetic group, reflecting chronic oxidative damage associated with hyperglycemia. CVD patients also demonstrated elevated MDA levels compared to controls, highlighting the role of oxidative stress in cardiovascular pathology. Total antioxidant capacity (TAC), however, was markedly reduced in both disease groups compared to controls, emphasizing that antioxidant defense is compromised in systemic disease states. These findings are summarized in Table 2.

Table 2. Salivary glucose and oxidative stress markers in study groups

Parameter	Diabetes Group (n=30)	CVD Group (n=30)	Control Group (n=30)	p-value
Salivary glucose (mg/dL)	6.8 ± 1.9	3.9 ± 1.2	2.1 ± 0.7	<0.001*
MDA (nmol/mL)	4.7 ± 1.1	3.9 ± 0.9	2.3 ± 0.8	<0.001*
TAC (U/mL)	0.72 ± 0.19	0.81 ± 0.21	1.25 ± 0.22	<0.001*
Salivary urea (mg/dL)	10.2 ± 2.8	9.7 ± 2.5	6.8 ± 2.1	<0.001*
Salivary creatinine (mg/dL)	0.31 ± 0.09	0.28 ± 0.08	0.21 ± 0.07	0.02*

*Significant at p < 0.05

Salivary Inflammatory Biomarkers: Inflammatory biomarkers were significantly elevated in both diabetic and CVD patients compared to healthy controls. IL-6 levels in diabetic patients were almost threefold higher than in controls, consistent with chronic low-grade inflammation. Similarly, TNF-α and CRP were significantly raised in both disease groups, with CRP particularly

higher in cardiovascular patients. This suggests that salivary inflammatory markers can serve as indicators not only of systemic inflammation but also of cardiovascular risk. These findings are presented in Table 3.

Table 3. Salivary inflammatory biomarkers among study groups

Parameter	Diabetes Group (n=30)	CVD Group (n=30)	Control Group (n=30)	p-value
IL-6 (pg/mL)	18.6 ± 4.5	15.4 ± 3.9	6.3 ± 2.1	<0.001*
TNF-α (pg/mL)	22.1 ± 5.2	20.8 ± 4.7	9.2 ± 2.8	<0.001*
CRP (mg/L)	3.7 ± 1.1	5.1 ± 1.6	1.2 ± 0.6	<0.001*
IL-1β (pg/mL)	14.2 ± 3.8	12.9 ± 3.1	5.7 ± 1.9	<0.001*
IFN-γ (pg/mL)	11.5 ± 2.9	10.8 ± 2.5	4.9 ± 1.4	<0.001*

Salivary Cardiac Biomarkers: Salivary cardiac biomarkers showed significant elevations in CVD patients compared to both diabetics and controls. Troponin I was highest in the CVD group, but interestingly, diabetics also demonstrated mild elevations compared to controls, suggesting the possibility of subclinical myocardial stress in long-standing diabetes. CK-MB levels followed a similar trend, with CVD patients showing nearly threefold higher levels compared to controls. These findings are displayed in Table 4.

Table 4. Salivary cardiac biomarkers in study groups

Parameter	Diabetes Group (n=30)	CVD Group (n=30)	Control Group (n=30)	p-value
Troponin I (ng/mL)	0.019 ± 0.007	0.051 ± 0.014	0.009 ± 0.004	<0.001*
CK-MB (U/L)	4.2 ± 1.5	7.9 ± 2.1	2.6 ± 1.1	<0.001*
Myoglobin (ng/mL)	34.5 ± 10.2	56.8 ± 13.4	21.7 ± 8.6	<0.001*
BNP (pg/mL)	92.1 ± 24.6	145.2 ± 31.7	68.9 ± 19.8	<0.001*

Correlation analysis confirmed strong positive associations between salivary and serum biomarkers. Salivary glucose showed an excellent correlation with serum glucose ($r = 0.81$, $p < 0.001$). Similarly, salivary CRP strongly correlated with serum CRP ($r = 0.77$, $p < 0.001$), and salivary troponin I correlated with serum troponin I ($r = 0.72$, $p < 0.001$). These findings validate the reliability of saliva as a diagnostic fluid that reflects systemic metabolic and cardiovascular alterations.

DISCUSSION

The present study evaluated the clinical utility of salivary biomarkers for early detection and monitoring of diabetes and cardiovascular disease[12]. Our findings demonstrate that saliva, a non-invasive and easily obtainable fluid, contains measurable concentrations of glucose, oxidative stress markers, inflammatory mediators, and cardiac proteins that closely reflect systemic disease status. These results strongly support the use of salivary diagnostics as a reliable adjunct or alternative to conventional blood-based investigations[13,14].

Salivary glucose levels were significantly elevated in diabetic patients compared to healthy controls, with values showing a strong correlation with serum glucose. This finding is consistent with previous reports highlighting that salivary glucose mirrors glycemic fluctuations and can serve as a marker for glycemic control[15]. Importantly, salivary oxidative stress markers such as malondialdehyde were also increased, while total antioxidant capacity was reduced, reflecting the imbalance between oxidants and antioxidants that is central to diabetic pathophysiology. These results reinforce the concept that saliva can provide insights into both metabolic and oxidative disturbances in diabetes[16,17].

Inflammatory cytokines, including IL-6, TNF-α, and CRP, were found to be elevated in both diabetic and cardiovascular disease groups. This reflects the shared mechanism of chronic low-grade inflammation that underlies both disorders[18]. The higher salivary CRP observed in the cardiovascular group highlights its potential role in detecting vascular inflammation and atherosclerotic risk. These findings align with evidence that systemic inflammation is a critical driver of vascular dysfunction, endothelial injury, and long-term cardiovascular complications[19].

Cardiac biomarkers, particularly troponin I and CK-MB, were significantly elevated in patients with cardiovascular disease. Notably, diabetic patients also demonstrated modest increases in salivary troponin I compared to controls, suggesting that saliva may capture subclinical myocardial stress even before overt cardiovascular disease is diagnosed. This is a key finding, as it highlights saliva's potential not only in detecting established disease but also in risk stratification and early identification of high-risk individuals[20,21].

The correlation analysis confirmed strong associations between salivary and serum biomarkers, including glucose, CRP, and troponin I. This further validates the clinical relevance of saliva as a diagnostic fluid. The non-invasive nature of saliva collection makes it particularly attractive for repeated monitoring, community-based screening, and use in patients with needle phobia or bleeding disorders[22].

Our study adds to the growing body of evidence supporting saliva as a diagnostic medium. However, several challenges must be addressed before routine clinical adoption. The concentration of biomarkers in saliva is generally lower than in blood, necessitating highly sensitive detection technologies[23]. Inter-individual variability due to oral health status, diet, hydration, and circadian rhythm can affect results. Standardization of collection and processing methods is therefore essential. Despite these limitations, advancements in biosensor technology and salivaomics are rapidly bridging these gaps, making salivary diagnostics a realistic clinical tool in the near future.

A particular strength of this study is its dual focus on diabetes and cardiovascular disease, both of which represent overlapping epidemics in Pakistan. By demonstrating the clinical utility of salivary biomarkers in both conditions, our findings highlight the potential of saliva as a low-cost, patient-friendly diagnostic approach suitable for large-scale screening and longitudinal monitoring in resource-limited healthcare systems[24,25].

CONCLUSION

This study demonstrates that salivary biomarkers including glucose, oxidative stress indicators, inflammatory cytokines, and cardiac enzymes are significantly altered in patients with diabetes and cardiovascular disease compared to healthy individuals. Strong correlations between salivary and serum levels of key biomarkers confirm saliva's potential as a non-invasive and reliable diagnostic medium. Saliva offers unique advantages of simplicity, safety, cost-effectiveness, and patient acceptability, making it highly suitable for early detection, continuous monitoring, and community-based screening of chronic diseases. While further large-scale validation and standardization are required, our findings support the integration of salivary diagnostics into clinical practice as a complementary tool alongside traditional blood-based method. By providing a non-invasive approach to monitor systemic health, salivary biomarkers may play a transformative role in the early identification and management of diabetes and cardiovascular diseases, ultimately contributing to improved patient outcomes and reduced healthcare burden.

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Conflicts of Interest: The authors declare no conflicts of interest related to this research.

Ethical Approval: The study protocol was reviewed and approved by the Institutional Ethical Review Committees of Margalla Dental Hospital and Army Medical College. Written informed consent was obtained from all participants prior to sample collection.

Authors' Contributions

- AY designed the study and supervised data collection.
- AAM contributed to methodology development and laboratory analysis.

- MA performed data interpretation and statistical analysis.
- MAli assisted in patient recruitment, sample collection, and data acquisition.
- MAs contributed to manuscript drafting, critical revision, and final approval.

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