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Research Article

Assessment of Salivary Amylase, IL-6, and Glucose as Non-Invasive Biomarkers for the Early Diagnosis of Type 2 Diabetes Mellitus

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Abstract

Background: Type 2 diabetes mellitus (T2DM) is a chronic, progressive metabolic disorder marked by insulin resistance and progressive β-cell dysfunction. Early diagnosis is critical to preventing long-term complications, including cardiovascular disease and diabetic neuropathy. Conventional diagnostic techniques, such as blood sampling, are invasive and require significant resources. Saliva, a non-invasive and easily accessible biofluid, may contain biomarkers indicative of systemic metabolic changes.

Methods: This case-control study evaluated salivary levels of glucose, interleukin-6 (IL-6), and alpha-amylase in 30 newly diagnosed T2DM patients and 30 age- and gender-matched healthy controls. Unstimulated saliva samples were collected, processed, and analyzed using enzymatic and ELISA-based methods. Data were assessed using independent t-tests and Receiver Operating Characteristic (ROC) curve analysis.

Results: T2DM patients showed significantly higher salivary IL-6 (12.8 ± 3.1 pg/mL and 6.3 ± 2.2 pg/mL, respectively) and glucose (10.2 ± 2.7 mg/dL and 5.1 ± 1.8, respectively) compared to controls. Salivary amylase levels showed no significant difference ($p = 0.28$). ROC curve analysis demonstrated that IL-6 had the highest diagnostic accuracy (AUC = 0.86), followed by glucose (AUC = 0.84). Alpha-amylase exhibited limited diagnostic utility (AUC = 0.63).

Conclusions: Silvery IL-6 and glucose appear to be promising non-invasive biomarkers for the early detection of T2DM. While alpha-amylase alone lacks sufficient diagnostic value, it may have adjunctive utility when combined with other markers in broader panels. These findings support the potential of saliva-based diagnostics in diabetes screening.

Keywords: Type 2 diabetes mellitus, saliva, IL-6, glucose, biomarkers, non-invasive, amylase.

Introduction

Type 2 diabetes mellitus (T2DM) is a long-term and worsening metabolic illness that causes high blood sugar levels because the body doesn't respond to insulin and the beta cells in the pancreas don't work properly. T2DM accounts for over 90% of the diabetics throughout the globe, and it is the source of the increased morbidity and death from the complications, like cardiovascular disease, neuropathy, nephropathy, and retinopathy [1]. T2DM has an increasing incidence, driven by the factors of lifestyle, like poor dietary habits, physical inactivity, obesity, and hereditary disposition [2].

In spite of advances in diagnosis and therapy, most people are not diagnosed until the late stages of the disease because of the insidious and symptom-free presentation of early T2DM [3]. Conventional diagnostic methods, including fasting plasma glucose, oral glucose tolerance tests, and glycated hemoglobin (HbA1c) measurements, are reliable but require venipuncture, specialized equipment, and trained personnel. These limitations hinder large-scale screening, especially in resource-limited settings [4-5].

Consequently, the use of non-invasive, cost-efficient, and readily available diagnostic technologies has become increasingly popular. Saliva has become an attractive substitute biological fluid for the follow-up of diseases and diagnosis [6]. It is easily attainable, can be obtained without discomfort

from the patient, and mirrors the broad physiological and pathological conditions. Modern bioanalytical techniques have made it possible to quantify many biomarkers present in saliva, such as hormones, enzymes, antibodies, and cytokines [7].

From the salivary components, glucose, interleukin-6 (IL-6), and alpha-amylase have emerged with potential applications in diabetic research. Salivary glucose has a correlation with blood glucose levels, providing a reasonable indicator for hyperglycemia [8]. IL-6, an interleukin with pro-inflammatory properties, is central to the action of insulin resistance and chronic inflammation presented by T2DM. Alpha-amylase, the stress-sensitive enzyme, can be an indirect indicator of changes in the autonomic nervous system and the metabolic changes [9].

This study aims to evaluate the diagnostic potential of three salivary biomarkers, glucose, IL-6, and alpha-amylase, for the early detection of T2DM. By comparing their levels in patients with newly diagnosed T2DM and healthy controls, the study seeks to determine their utility as components of a non-invasive screening approach.

Materials and Methods

Study design and participants

This pilot case-control study was conducted in collaboration with three private internal medicine and endocrinology clinics in Kerbala, Iraq, over a four-month period from February to May, 2024. A total of 60 participants were enrolled, comprising 30 newly diagnosed T2DM patients and 30 healthy controls matched by age and gender. We chose 30 individuals per group, following what's common for pilot studies investigating novel biomarkers. Previous work indicated that salivary IL-6 and glucose have obviously different values in diabetics and healthy controls, and thus the sample size would be sufficient to reveal significant trends [10].

Practical considerations such as finite patient availability, time, and assay expense also played a role in our choice. Although the sample size is not sufficient for generalization on a wide scale, it is a firm foundation for the evaluation of these markers and paves the way for larger, future studies. The mean age was 51.8 ± 8.9 years for the diabetic group and 52.9 ± 8.5 years for the control group. The male-to-female ratio was 1.1:1 among diabetics (16 males and 14 females) and 0.9:1 among controls (14 males and 16 females), with no statistically significant difference in demographic distribution between the groups.

Inclusion and exclusion criteria

Inclusion criteria for the T2DM group were adults aged 30–65 years, newly diagnosed with diabetes within the past 6 months according to American Diabetes Association (ADA) criteria (fasting plasma glucose ≥126 mg/dL, HbA1c ≥6.5%, or 2-hour oral glucose tolerance test (OGTT) ≥200 mg/dL) [11], not currently on insulin or anti-inflammatory therapy, and able to provide informed consent.

Exclusion criteria for diabetic participants included current smoking or tobacco use; presence of systemic infections, autoimmune conditions, or acute illness; diagnosed oral or periodontal inflammatory disease; salivary gland disorders; use of medications affecting salivary flow or composition (e.g., anticholinergics or corticosteroids); and pregnancy or lactation.

Control participants were included if they were healthy adults aged 30–65 years, normoglycemic (fasting glucose <100 mg/dL and HbA1c <5.7%) [11], with no personal history of diabetes or prediabetes, and matched to diabetic participants by age (±5 years) and gender.

Exclusion criteria for controls mirrored those of the diabetic group, with additional exclusion for individuals with a family history of diabetes in first-degree relatives or any known systemic or chronic inflammatory condition. Other excluded participants were those who smoked, had systemic infections or oral inflammatory diseases, or were taking medications known to affect salivary flow or composition [12].

Sample collection

Saliva samples without stimulation were collected from all participants in the period from 8:00 am to 10:00 am. Participants were asked to avoid eating, drinking, or brushing teeth for at least an hour before collecting. The samples were cooled using dry ice and then stored at -80 ° C until the analysis [4, 12].

Biochemical analysis

Upon thawing, saliva samples were centrifuged at 3,000 rpm for 10 minutes at 4 °C to remove cellular debris, and the clear supernatant was used for all biochemical assays. The clear supernatant was used for all biochemical analyses [13]. All analyses were performed in duplicate to ensure consistency and reproducibility.

Salivary IL-6 levels were measured using a high-sensitivity enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems, USA). The assay was performed according to the manufacturer's instructions. Standards, samples, and controls were added to pre-coated microplates, followed by the addition

of a biotin-conjugated detection antibody. After incubation and washing, streptavidin-HRP was added, and a colorimetric substrate (TMB) was used to develop the signal. The reaction was stopped with sulfuric acid, and optical density was read at 450 nm using a BioTek ELx800 microplate reader. The lower limit of detection for the kit was 0.5 pg/mL, and the intra-assay and inter-assay coefficients of variation were <10% and <12%, respectively, indicating good precision. The reference value for the salivary IL-6 in this study was < 1 pg/mL for healthy adults [14].

Salivary glucose was measured using the glucose oxidase-peroxidase (GOD-POD) enzymatic method with a commercially available diagnostic kit (Biolabo, France). This method is widely validated for saliva and is based on the enzymatic oxidation of glucose to gluconic acid and hydrogen peroxide, with subsequent color development via a peroxidase reaction, measured at 505 nm [15]. The reference value for the salivary IL-6 in this study was up to 2.51 mg/mL for healthy adults [16].

Salivary alpha-amylase was assessed using a kinetic enzymatic colorimetric assay (Spinreact, Spain), based on the hydrolysis of a chromogenic starch substrate. The increase in absorbance at 405 nm was proportional to enzyme activity and was measured kinetically over one minute. All reagents were equilibrated to room temperature prior to testing, and standard curves were constructed using known concentrations for each assay. In this study, normal unstimulated salivary α-amylase levels were set with values from 1 to 371 U/mL, reflecting broad physiological variation [16].

Quality control procedures included the use of internal controls, calibration curves, and validation against known reference ranges. All samples were analyzed under the same conditions and within the same analytical run to minimize inter-assay variability.

Ethical approval

This study was conducted in accordance with the ethical standards of the Declaration of Helsinki. Ethical approval was obtained from the College of Medicine at the University of Kerbala, Scientific Affairs Committee, with reference number 3028 issued on the 14th of November 2023. Informed consent was obtained from all participants prior to sample collection and data analysis. To protect their identity, all personal details were removed from the data and replaced with anonymous codes. Only the research team had secure access to this information, and all files were stored safely.

Statistical analysis

Data analysis was conducted using SPSS version 25. Descriptive statistics were calculated, and independent t-tests were used to compare the groups. Diagnostic performance was evaluated through Receiver Operating Characteristic (ROC) curve analysis. A p-value less than 0.05 was considered statistically significant.

Results

Demographic and clinical features

Table 1 summarizes the demographic and clinical characteristics of the study groups. Age and sex distribution were comparable between the T2DM and control groups, indicating appropriate matching. However, the T2DM group had significantly higher levels of fasting blood glucose.

A visual comparison of the main biochemical markers between the T2DM and control groups is illustrated in Figure 1. Individuals in the T2DM group showed significantly elevated mean values for fasting glucose (162.4 ± 22.6 mg/dL), salivary IL-6 (12.8 ± 3.1 pg/mL), and salivary glucose (10.2 ± 2.7 mg/dL) compared to controls (91.3 ± 9.4 mg/dL, 6.3 ± 2.2 pg/mL, and 5.1 ± 1.8 mg/dL, respectively), all with p-values < 0.01. On the other hand, there was no significant difference in salivary amylase levels between the groups (108.7 ± 24.6 U/L for T2DM and 102.4 ± 21.5 U/L for controls; p = 0.28). These results reflect alterations in glucose metabolism and inflammatory responses in T2DM, as detected in both blood and saliva samples.

Table 1: Comparison of demographic and biochemical parameters between study groups

Parameter	T2DM group (n=30)	Control group (n=30)	p-value
Age (years)	51.8 ± 8.9	52.9 ± 8.5	0.63
Gender (M/F)	16 / 14	14 / 16	0.59
Fasting glucose (mg/dL)	162.4 ± 22.6	91.3 ± 9.4	< 0.001 *
Salivary IL-6 (pg/mL)	12.8 ± 3.1	6.3 ± 2.2	< 0.01 *
Salivary glucose (mg/dL)	10.2 ± 2.7	5.1 ± 1.8	< 0.01 *
Salivary amylase (U/L)	108.7 ± 24.6	102.4 ± 21.5	0.28

* Highly significant statistical difference

ROC curve analysis

Receiver Operating Characteristic (ROC) curve analysis revealed the following diagnostic performance: IL-6 had an Area Under the Curve (AUC) of 0.86 with 82% sensitivity and 80% specificity; glucose followed with an AUC of 0.84, 78% sensitivity, and 83% specificity. In contrast, amylase had a lower diagnostic value with an AUC of 0.63.

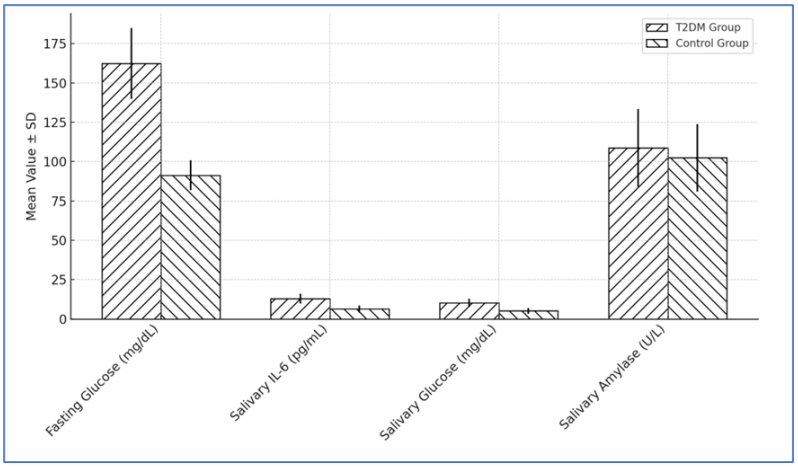

Figure 1: Comparing mean values (± SD) of fasting glucose, salivary IL-6, salivary glucose, and salivary amylase between the T2DM and control groups. Bars with stripes indicate T2DM (//) and control (\\) groups, respectively. Significant differences ($p < 0.01$) were noted for fasting glucose, salivary IL-6, and salivary glucose. No significant difference was found for salivary amylase ($p = 0.28$).

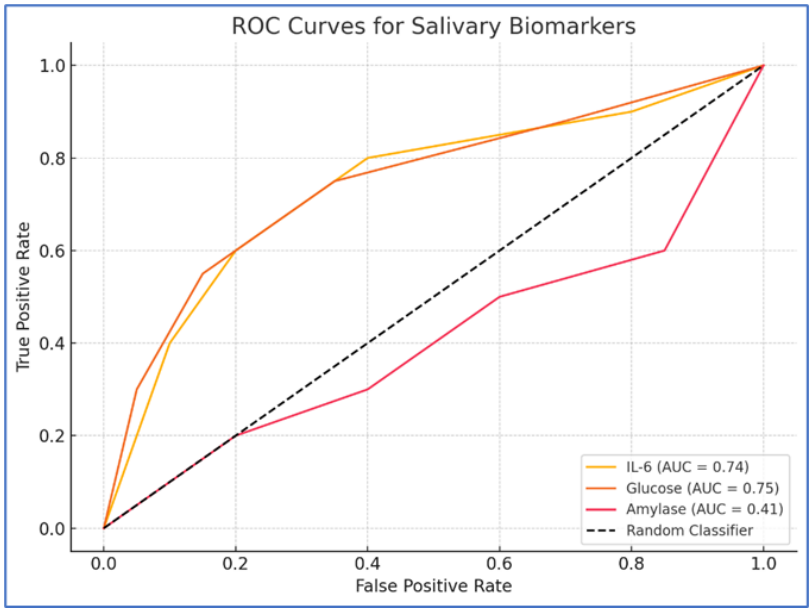

Figure 2: Receiver Operating Characteristic (ROC) curves for salivary IL-6, glucose, and amylase as diagnostic biomarkers for Type 2 Diabetes Mellitus. The IL-6 biomarker demonstrated the highest Area Under the Curve (AUC = 0.86), followed by glucose (AUC = 0.84), indicating strong diagnostic performance. Salivary amylase showed limited diagnostic accuracy (AUC = 0.63). The diagonal dashed line represents the reference line for a random classifier (AUC = 0.5).

These findings, visualized in figure 2, showed that IL-6 was the most effective marker, followed closely by glucose, while amylase appeared less useful for diagnostic purposes.

Discussion

The results support the hypothesis that salivary IL-6 and glucose can serve as reliable, noninvasive biomarkers for early T2DM detection. Elevated IL-6 levels reflect chronic low-grade inflammation, a hallmark of T2DM pathophysiology. Similarly, increased salivary glucose mirrors plasma hyperglycemia. Alpha-amylase showed inconsistent results, possibly influenced by stress or individual metabolic variation [17-18].

The diagnostic accuracy of IL-6 and glucose was further supported by ROC curve analysis, where both biomarkers demonstrated high AUC values (>0.80), indicating strong sensitivity and specificity. These findings are consistent with previous studies highlighting the role of inflammatory cytokines and metabolic byproducts in diabetes development and progression. The elevated IL-6 levels in diabetic patients align with the inflammatory model of insulin resistance, where cytokine imbalance contributes to impaired insulin signaling [19-20].

The observation that salivary glucose levels correlate with systemic glucose concentrations provides a practical advantage for saliva-based testing, par-

ticularly in populations averse to invasive procedures or in settings lacking medical infrastructure. This strengthens the argument for integrating salivary diagnostics in community-based screening programs [20-21].

Alpha-amylase, although not statistically significant in this study, may still hold indirect diagnostic potential. Its secretion is modulated by the autonomic nervous system and can reflect psychosocial or metabolic stress, which often coexists with or exacerbates diabetic pathology. Its role may become clearer when interpreted alongside cortisol or other stress markers [22].

Additionally, the use of private clinic settings in this study reflects real-world conditions, enhancing the generalizability of the findings. The sample size, though modest, was sufficient to reveal significant differences and patterns. Future research should focus on expanding the sample size, incorporating diverse populations, and assessing biomarker fluctuations over time [23-24].

Ultimately, this study contributes to the growing body of evidence advocating the use of saliva as a diagnostic fluid. Its accessibility, ease of handling, and rich composition make it ideal for early screening, particularly in asymptomatic or high-risk individuals. Multimarker panels incorporating IL-6 and glucose may offer a cost-effective, patient-friendly alternative to traditional blood tests.

Study limitations

While the findings are promising, several limitations should be acknowledged. The first is the sample size was relatively small, which may affect the statistical power and generalizability of the results. The second is all participants were recruited from private clinics in a single geographic area, which may introduce selection bias and limit the applicability of the findings to broader populations. The third is that this study employed a cross-sectional design, which limits the ability to assess biomarker changes over time or establish causality. Lastly, potential confounding factors such as diet, oral hygiene, and stress levels were not strictly controlled and could influence salivary composition. Future research should address these limitations through multicenter studies, larger sample sizes, and longitudinal designs.

Conclusions

This study demonstrates that salivary IL-6 and glucose are promising candidates for noninvasive screening of T2DM. While alpha-amylase was less reliable, combining these biomarkers may improve diagnostic accuracy. Further longitudinal studies with larger sample sizes are needed to validate these preliminary findings and integrate salivary diagnostics into routine clinical practice.

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