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Original Article

Investigating the Relationship Between Salivary Sialic Acid and HbA1c and Fasting Blood Sugar in Prediabetic and Type 2 Diabetic Individuals

Running Title: Salivary Sialic Acid and Glycemic Control Markers (HbA1c and FBS)

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Abstract

Background and Objectives: Diabetes monitoring requires repeated invasive methods. Saliva is a rich source of biomarkers without the need for an invasive method for its collection. Sialic acid is proposed as an acute-phase protein in chronic inflammation of diabetes. This study aimed to determine the association between total salivary sialic acid (TSA) and HbA1c and fasting blood sugar (FBS) in prediabetic and type 2 diabetic individuals, and to evaluate its potential diagnostic utility as a non-invasive biomarker for differentiating these two groups.

Methods: This cross-sectional study was carried out on 70 participants (34 with prediabetes, 36 with type 2 diabetes) who were referred to Shahid Moffateh Clinic, Yasuj, Iran. Convenience sampling was employed. Unstimulated saliva (spitting method) and fasting venous blood samples were obtained. TSA was assessed by spectrophotometry, whereas HbA1c and FBS were measured using standard biochemical methods. Data were analyzed with SPSS 23 using Spearman/Pearson correlation tests.

Results: Mean age of participants was 50.38±10.14 and 51.50±9.13 years in prediabetic and diabetic groups, respectively. Mean FBS (mg/dL), HbA1c (%), and TSA (mg/dL) levels in prediabetics were 108.94±9.04, 5.63±0.246, and 1764.7±742.02, and in diabetics were 167.05±55.83, 7.02±1.67, and 1672.51±576.59, respectively. No significant correlation was found between TSA and FBS (P=0.53 for prediabetic, P=0.19 for diabetic) and TSA and HbA1c (P=0.90 for prediabetic, P=0.62 for diabetic) in either group. The ROC curve area was 0.44 (P=0.38), indicating poor diagnostic power.

Conclusion: Based on our findings, total salivary sialic acid is not a suitable biomarker to replace HbA1c for monitoring prediabetic and type 2 diabetic individuals. Further studies investigating other salivary biomarkers or free sialic acid are recommended.

Keywords: Total Salivary Sialic Acid, HbA1c, Type 2 Diabetes, Prediabetes, Fasting Blood Sugar.

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Introduction

Diabetes mellitus is a common chronic metabolic disorder characterized by chronic hyperglycemia due to defects in insulin secretion or action (1). This disease is known as one of the leading causes of death and disability worldwide, and its prevalence is increasing (2). Type 2 diabetes is the most common type of diabetes and is associated with numerous microvascular and macrovascular complications (3). Continuous blood glucose monitoring through measurement of HbA1c and fasting blood glucose (FBS) is essential to prevent these complications (4). However, these methods are invasive, painful, and sometimes difficult to sample, especially in children and older people (5). Human saliva, as a non-invasive and accessible biological fluid, mirrors the body's physiological status, and many analytes present in plasma are found in it (6). The thin epithelium in the salivary ducts facilitates the easy exchange of substances between plasma and saliva (7). Low-grade chronic inflammation is a feature of the pathogenesis of type 2 diabetes (8). During this inflammation, cytokines such as TNF-α and IL-6 are released, which stimulate the synthesis of acute-phase proteins (such as CRP, haptoglobin, and α1-antitrypsin) in the liver. Many of these acute-phase glycoproteins have terminal sialic acid chains (9, 10). Sialic acid is a terminal monosaccharide present on glycoproteins and glycolipids and plays an essential role in cell–cell recognition, immune responses, microbial interactions, and inflammatory processes (11). Therefore, increased total serum sialic acid levels have been associated with diabetes and its complications as an inflammatory biomarker (12). Some studies have reported a positive relationship between serum sialic acid and HbA1c (13, 14), but regarding salivary sialic acid and its relationship with glycemic control, studies are limited and contradictory.

Studies have shown that inflammatory gum diseases such as gingivitis and periodontitis are associated with a significant increase in salivary sialic acid, and the concentration of this marker is correlated with the severity of inflammation and clinical periodontal indices (15, 16). Furthermore, some recent systematic reviews have shown that although saliva has high potential for non-invasive monitoring, the heterogeneity of sampling methods, differences in saliva composition, and the diversity of analytical techniques have limited the reproducibility of many salivary biomarkers. Variability in saliva composition, periodontal status, and biomarker transfer mechanisms may influence the correlation between salivary biomarkers and systemic glycemic indices (17). Considering the high prevalence of diabetes in Iran (18) and the need for non-invasive methods for screening and monitoring patients, this study aimed to determine the relationship between total salivary sialic acid levels and glycemic control indices (FBS and HbA1c) in prediabetic and type 2 diabetic individuals referred to Shahid Mofatteh Clinic in Yasuj. As far as we know, limited studies have simultaneously evaluated the relationship between total salivary sialic acid and glycemic markers in prediabetic and type 2 diabetic individuals. Furthermore, the diagnostic performance of salivary TSA using ROC analysis has not been adequately investigated in the Iranian population.

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Materials and Methods

Study type and population: This was a cross-sectional (descriptive-analytical) study conducted in 2025 on prediabetic and type 2 diabetic individuals referred to Shahid Mofatteh Clinic in Yasuj. After obtaining ethics approval from the Ethics Committee of Yasuj University of Medical Sciences (IR.YUMS.REC.1404.166) and written informed consent, eligible individuals were included in the study.

Sample size and sampling method: The sample size was calculated based on the study by Olaru et al. (13), considering a correlation coefficient of 0.61 (R^2 = 0.38) between serum sialic acid and serum HbA1c, α = 0.01, power (1-ß) = 90%, and using the following formula for each group (diabetic and prediabetic patients), resulting in 37 individuals. Because no published study reporting the correlation between salivary sialic acid and HbA1c in prediabetic and type 2 diabetic individuals was available at the time of study design, the serum-based effect size was used as the closest available estimate for sample size calculation. Finally, 36 people with diabetes and 34 prediabetic individuals were selected using a non-probability convenience sampling method.

Inclusion and exclusion criteria: Inclusion criteria included willingness to participate in the study, a definitive diagnosis of type 2 diabetes (based on ADA criteria) with at least three years of disease duration for the diabetic group, and a diagnosis of prediabetes (based on ADA criteria) for the prediabetic group. Exclusion criteria included psychiatric disorders, liver disease, kidney disease, immunodeficiency, hemoglobinopathies (such as thalassemia and sickle cell), malnutrition, and use of diuretics, immunosuppressants, and psychoactive drugs. Schematic 1 shows a visual abstract of the research.

Sample collection: Saliva sample: Unstimulated saliva samples were collected using the "spitting" method between 9:00 and 11:00 AM. Individuals refrained from eating, drinking, and brushing their teeth for at least 90 minutes before sampling. The collected saliva was placed in a sterile tube, sealed with parafilm, and centrifuged at 2000 rpm for 10 minutes. The samples were then stored at -20°C until analysis (19). After 12-14 hours of fasting, 4 ml of venous blood was drawn from each participant. 2 ml was used for FBS measurement (after centrifugation at 3000-3300 rpm for 7 minutes), and 2 ml was used for HbA1c measurement (stored in a -20°C freezer) (16).

Biochemical measurements:

- Total salivary sialic acid (TSA): Total salivary sialic acid was measured using the colorimetric method of Skoza and Mohos (20). Briefly, the saliva sample (100µl) was hydrolyzed with 0.1 N sulfuric acid (H_2SO_4) at 80°C for 1 hour. Then, 70 µl of periodate reagent (25 mM periodic acid (HIO_4) in 0.125 N sulfuric acid) was added to the sample and incubated at 37°C for 30 minutes. The reaction was stopped by adding 70 µl of 2% sodium arsenite ($NaAsO_2$). After removing the iodine color, 140 µl of 0.1 M thiobarbituric acid ($C_4H_4N_2O_2S$) (pH = 9) was added to the solution and heated in a boiling water bath for 7.5 minutes. After cooling, 560µl of dimethyl sulfoxide was added to the solution, and then the absorbance was measured at a wavelength of 532 nm using a spectrophotometer (Biowave 2, UK). The optical density (OD) was read, and using the Beer-Lambert law and a molar coefficient of 57,000

for total salivary sialic acid, the results were reported as mg/dl (16, 21).

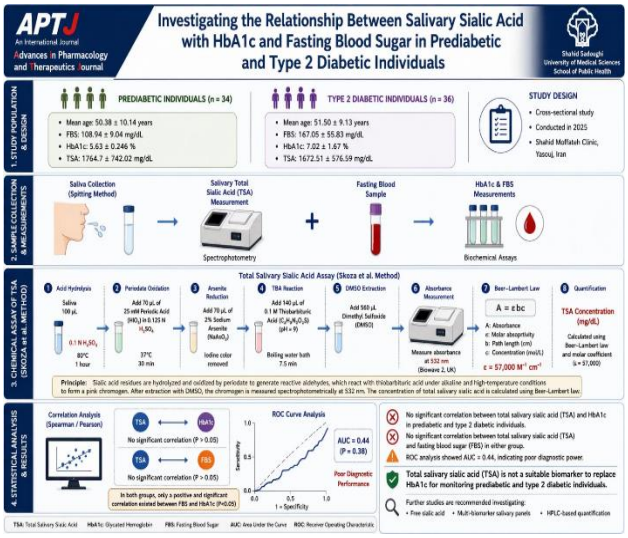

Figure 1. Relationship between salivary sialic acid and HbA1c and fasting blood sugar in prediabetic and type 2 diabetic individuals.

➢ HbA1c and FBS: HbA1c was measured by an enzymatic method using a Diazyme kit (Iran), and FBS was measured by the glucose oxidase (GOD-PAP) method. Statistical analysis: Data were analyzed using SPSS software version 23. Normality of data was assessed using the Kolmogorov-Smirnov test. Due to the non-normal distribution of most variables **(Table 1)**, Spearman's correlation test was used to examine the correlation between variables. The significance level was set at less than 0.05. A ROC curve was plotted to determine the diagnostic power of salivary sialic acid in differentiating the two groups.

The Mann–Whitney U test was used to compare continuous variables between the prediabetic and diabetic groups.

Results

In this study, 70 individuals, including 34 prediabetic [38.2% male (13 individuals) and 61.8% female (22 individuals)] and 36 type 2 diabetic individuals [38.9% male (14 individuals) and 61.1% female (22 individuals)], were examined. The mean age in the prediabetic group was 50.38 ± 10.14 years, and in the diabetic group was 51.50 ± 9.13 years. The two groups did not differ significantly in age or sex. Status of biochemical variables: The mean and standard deviation of FBS, HbA1c, and salivary sialic acid in the two groups are summarized in **Table 1**. As expected, the mean FBS and HbA1c were significantly higher in the diabetic group than in the prediabetic group.

Table 1. Comparison of biochemical variables in the two study groups.

Variable	Prediabetic group (n=34): Mean ± SD	Diabetic group (n=36): Mean ± SD	P-value
FBS (mg/dl)	108.94 ± 9.04	167.05 ± 55.83	**P < 0.001**
HbA1c (%)	5.63 ± 0.246	7.02 ± 1.67	**P < 0.001**
TSA (mg/dl)	1764.7 ± 742.02	1672.51 ± 576.59	**0.05 < P**

Table 2. Correlation between variables in prediabetic and diabetic groups

Group	Variables	Correlation	P-value
Prediabetic	FBS with HbA1	0.49	0.003
Prediabetic	Sialic acid with FBS	0.11	0.53
Prediabetic	Sialic acid with	0.02	0.90
Diabetic	FBS with HbA1	0.80	<0.001
Diabetic	Sialic acid with FBS	0.22	0.19
Diabetic	Sialic acid with	0.08	0.62

Correlation between variables:

The results of Spearman's correlation test are shown in **Table 2**. In both groups, only a positive and

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significant correlation existed between FBS and HbA1c (P<0.05). No significant relationship was observed between salivary sialic acid and FBS and HbA1c in any of the groups (P>0.05). Diagnostic power of salivary sialic acid: The area under the ROC curve for salivary sialic acid in distinguishing diabetic from prediabetic individuals was 0.44 (P>0.05), standard error 0.069, significance 0.38, 95% confidence interval 0.3-0.57, which was not statistically significant, indicating a lack of adequate diagnostic power for this biomarker **(Figure 1).**

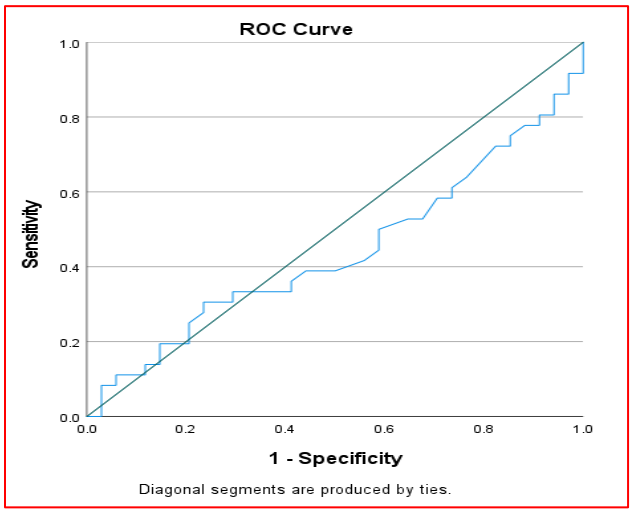

Figure 1:ROC curve for determining the cut-off point of salivary sialic acid.

Discussion

This study aimed to investigate the relationship between salivary sialic acid and glycemic control indices (FBS and HbA1c) in prediabetic and type 2 diabetic individuals, and whether it could replace blood biomarkers. The main finding of this research was the absence of a significant relationship between total salivary sialic acid and FBS and HbA1c in both groups. Furthermore, the ROC curve also showed poor diagnostic power for this marker. These results are consistent with some previous studies on saliva but differ from others on serum. Salivary biomarkers are strongly influenced by pre-analytical variables such as sample collection method, circadian rhythms, and oral environmental factors, which may reduce their reproducibility and diagnostic reliability. Some recent studies have also reported that many salivary biomarkers in diabetic patients, despite systemic inflammatory changes, show a weak or non-significant correlation with standard glycemic control indices (22). Several studies have reported a positive relationship between serum sialic acid and diabetes and HbA1c. For example, Sabzwari et al. (2006) found higher serum sialic acid levels in type 2 diabetic patients than in the control group (23). Patel et al. (2024), in a large study (843 individuals), showed a significant correlation between serum sialic acid and FBS and HbA1c (14). Also, Olaru et al. (2021) reported a strong relationship between serum sialic acid and HbA1c in diabetic postmenopausal women (13). This fundamental difference between the results of the present study and serum studies is likely due to the difference in the biological matrix (saliva vs. serum). The concentration of proteins and analytes in saliva is typically much lower than in serum, and the transfer of these molecules from blood to saliva may be influenced by several factors, such as salivary flow rate, pH, and ductal membrane integrity (11). Recent studies have also shown that physiological changes in saliva and local oral cavity conditions can significantly affect the concentration of salivary biomarkers and reduce their diagnostic accuracy. Previous reviews have reported significant heterogeneity and inconsistencies in salivary

biomarker studies, mainly due to the lack of standardized protocols for sample collection and analysis (24). Regarding salivary biomarkers of diabetes, studies have shown different results. Some studies have found a positive relationship between salivary inflammatory biomarkers and diabetes. For example, Agho et al. (2021) showed that salivary CRP levels positively correlate with HbA1c in diabetic patients (17). Pakfetrat et al. (2023) also reported a significant relationship between salivary malondialdehyde (MDA) and FBS and HbA1c (25). However, no similar study was found regarding salivary sialic acid itself. Interestingly, although sialic acid is generally considered an inflammatory biomarker, the mean salivary total sialic acid (TSA) level was slightly lower in the diabetic group than in the prediabetic group. However, this difference was not statistically significant (Table 1) and therefore should be interpreted with caution. The relatively small difference in mean TSA levels between the two groups, together with the large standard deviations, indicates substantial inter-individual variability, which may have masked any potential association between salivary TSA and glycemic status.This unexpected finding may reflect the substantial biological variability of salivary biomarkers, the relatively large inter-individual variation observed in both groups, differences in salivary secretion and composition, medication use, oral inflammatory conditions, and other uncontrolled confounding factors. Furthermore, salivary biomarker concentrations do not necessarily parallel systemic inflammatory changes because multiple physiological mechanisms influence the transfer of biomolecules from blood to saliva. In addition, chronic hyperglycemia may alter salivary gland function and salivary protein secretion, which may also contribute to changes in salivary sialic acid concentrations independently of systemic inflammation. Another possible explanation is the potential effect of antidiabetic treatment. Patients with type 2 diabetes are generally more likely to receive pharmacological treatment, with metformin being the recommended first-line therapy for most patients with type 2 diabetes. Previous studies have shown that metformin therapy is associated with lower serum sialic acid concentrations compared with rosiglitazone (26). In addition, metformin has well-documented anti-inflammatory effects and has been shown to reduce circulating inflammatory markers through improved glycemic control and modulation of inflammatory signaling pathways (27, 28). Therefore, antidiabetic treatment may have contributed to the relatively lower salivary TSA levels observed in the diabetic group. However, because medication use and treatment duration were not recorded in the present study, and the available evidence is limited to serum rather than saliva, this explanation remains speculative and requires further investigation. On the other hand, some studies have not confirmed the use of saliva for blood glucose monitoring. Several recent studies have also concluded that salivary biomarkers cannot yet replace reliable indicators like HbA1c and are more considered as auxiliary tools or for initial screening. The correlation between salivary biomarkers and glycemic control indices appears weaker in individuals with lower glycemic burden or in early

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stages of glucose dysregulation (29). Gupta et al. (2015) found no significant correlation between salivary and blood glucose (30). Vaziri et al. (2010) also observed no difference in salivary glucose between diabetic and non-diabetic individuals (31).

Several reasons could be proposed for the lack of association in our study

1. Difference in the type of sialic acid measured. The free form of sialic acid may show a better relationship with glycemic control (32). It has also been suggested that using a multi-biomarker panel rather than relying on a single marker could increase the diagnostic accuracy of saliva in metabolic diseases (33).

2. Characteristics of the study population: The mean HbA1c in our diabetic group was 7.02 ± 1.67, indicating relatively good glycemic control in these patients. It is possible that in patients with uncontrolled or longer-standing diabetes, systemic inflammation is greater, and consequently, salivary sialic acid levels might also change. In addition, the relatively small number of participants may have limited the statistical power to detect weak associations between salivary sialic acid and glycemic indices. Larger studies are warranted to validate the present findings.

3- Lack of control for oral health status and periodontal diseases: Studies have shown that inflammatory gum diseases such as gingivitis and periodontitis are associated with a significant increase in salivary and serum sialic acid, and the concentration of this marker is correlated with the severity of inflammation and clinical periodontal indices (15, 16).

4- Limitations of saliva collection method: Although unstimulated saliva, which is a standard method for measuring salivary biomarkers, was used in this study, saliva composition can be affected by local oral factors such as periodontal disease, salivary flow rate, oral hygiene status, and other oral conditions (15, 16).

5- Influence of salivary-related biological factors on study results: In addition to oral health status, natural variation in salivary composition, differences in periodontal status of individuals, and mechanisms of biomarker transfer from blood to saliva may affect the degree of correlation between salivary biomarkers and systemic indicators of glycemic control such as HbA1c and FBS (17). It should also be noted that the ROC analysis in the present study was designed to evaluate the ability of salivary total sialic acid to discriminate between prediabetic and type 2 diabetic individuals rather than between diabetic patients and normoglycemic healthy controls. Therefore, the present findings reflect the performance of this biomarker across different stages of dysglycemia rather than its diagnostic accuracy for diabetes itself. Future studies including healthy control subjects are warranted further to evaluate the diagnostic performance of salivary sialic acid.

Conclusion

The results of this study showed that there is no statistically significant relationship between total salivary sialic acid and HbA1c levels and fasting blood sugar in prediabetic and type 2 diabetic individuals, and the area under its ROC curve is also less than 0.5. Then, total salivary sialic acid cannot be recommended as a suitable alternative biomarker for

HbA1c for the non-invasive monitoring of diabetic and prediabetic patients in the studied population. Consequently, larger studies with standardized saliva collection protocols and comprehensive periodontal assessment are recommended to further clarify the clinical value of salivary sialic acid as a non-invasive biomarker for diabetes monitoring.

Declarations

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Conflict of Interest: All listed authors have disclosed any financial and personal relationships with other individuals or organizations that could potentially influence or bias the objectivity of the submitted work and/or its consideration for publication in this journal. The authors declare that they have no conflicts of interest.

Financial support or sponsorship: No

Protection of Human: This study was approved by the Ethics Committee of Yasuj University of Medical Sciences (IR.YUMS.REC.1404.166). All ethical principles of research involving human participants were observed throughout the study. Participation was voluntary, informed consent was obtained from all participants, and the confidentiality of personal information was maintained.

Authors' contribution: Conceptualization: Hojatollah Hossainian, Mojgan Ferdosifar, Eshagh Moradi Sheybani, Amir Hossein Doustimotlagh, Leila Manzouri; Data Curation and Investigation: Mojgan Ferdosifar; Formal Analysis and Methodology: Leila Manzouri; Project Administration: Amir Hossein Doustimotlagh, Leila Manzouri, Mojgan Ferdosifar; Validation: Eshagh Moradi Sheybani, Amir Hossein Doustimotlagh, Leila Manzouri; Visualization: Hojatollah Hossainian, Mojgan Ferdosifar; Writing – Original Draft: Hojatollah Hossainian, Mojgan Ferdosifar; Writing – Review & Editing: Hojatollah Hossainian. All authors read and approved the final version of the manuscript.

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