J Biochem Technol (2026) 17(1): 102-107
https://doi.org/10.51847/ED36P69Gz1
ISSN: 0974-2328

# Sestrin-2, and Fructosamine in Serum and Saliva of Patients with Diabetes Mellitus type 2

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Received: 29 November 2025 / Received in revised form: 23 February 2026, Accepted: 27 February 2026, Published online: 15 March 2026

## Abstract

This analytical cross-sectional study aimed to investigate and compare serum and salivary fructosamine and sestrin-2 levels in type 2 diabetic mellitus (T2DM) patients and controls. Methods: Ninety eight participants were enrolled in this study and subdivided into 2 subgroups, the first group (G2), which represented the control group, consisted of (49) individuals, and the forty-eight patients with DM represented the second group (G1). Serum and saliva samples collected from fasting subjects. Sestrin-2 and Fructosamine were measured from serum and saliva samples using ELISA technique. Results: The serum and salivary fructosamine and sestrin-2 levels were high in diabetic patients, and the difference between the salivary fructosamine and sestrin-2 levels of diabetic and healthy individuals was found to be statistically significant. Serum and saliva C-peptide and HbA1c levels were higher in the uncontrolled T1DM than in the controlled T1DM. Serum and saliva sestrin-2 (≥1.970 and ≥0.640, respectively), a sensitivity of 100.0%, a specificity of 100.0%, and an AUC of 1.000 and 0.996, respectively, were observed for predicting DM. At the cut-off value ≥308.3 and ≥2.032 for serum and saliva fructosamine, a sensitivity of 69.4 and 93.9, respectively, a specificity of 89.8 and 73.5, respectively, and an AUC of 0.787 and 0.911, respectively, were observed for predicting DM. Conclusions: Fructosamine and sestrin-2 can be used as novel biomarkers in people with diabetes in serum and saliva.

**Keywords:** β-arrestin-1, Fructosamine, Youden's index, Saliva

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## Introduction

Type-2 diabetes mellitus (T2DM) is a chronic metabolic disease associated with high levels of circulating blood glucose, due to insulin hyposensitivity and continuous pancreatic β-cell necrosis (Al Ghadeer *et al*., 2022; Toaama *et al*., 2024; García-Currás *et al*., 2026). The commonest type of diabetes is T2DM, potentially impacting adults particularly those with overweight. The diagnosis of diabetes based on measured serum sugar levels and glycated hemoglobin, however, complications of disease need additional measured parameters (Sarhat *et al*., 2018; Khalaf *et al*., 2021; Obirikorang *et al*., 2025).

It is linked with an increase in morbidity (e.g., blindness, kidney failure, stroke, cardiovascular diseases, limb amputations), premature mortality, high healthcare costs, and is quickly becoming a global epidemic disorder. Several etiological factors, including lifestyle, genetics, environment, and ethnicity, are among the major factors responsible for the development of diabetes and its complications (Karimov & Rakhimova, 2024; Joungtrakul & Smith, 2025). The pathogenesis of T2D is not fully understood, with IR and β cell dysfunction playing central roles in its pathophysiology. Dyslipidemia and hyperglycemia, along with other metabolic disorders, result in IR and/or islet β cell upset via some alternative pathways, such as immune reactions, endoplasmic reticulum upset, oxidative stress, and ectopic lipid accumulation (Sarhat *et al*., 2019, 2020; Zbaar & Sarhat, 2022).

Sestrin-2 is a well-known potential antioxidant protein that was primarily participated in hypoxia-induced gene 95. As a regulator factor in homeostasis, Sestrin-2 has been participated in redox status, autophagy upset, endoplasmic reticulum processes, and immune response. Sestrin-2 suppresses oxidative stress and pro-inflammatory signaling through mechanisms involved with AMP-activated protein kinase and mechanistic target of rapamycin complex 1 (Tian *et al*., 2022). It restore the intactness of cells injury via metabolic pathways that liberate energy and encourage the DNA repair mechanisms (Emara *et al*., 2024).

Sestrin-2 have been participated in the control of hepatic glucose synthesis and encourage lipid metabolism by enhancing the metabolism of fatty acids through β-oxidation and reducing lipid precipitation in tissues. Excess lipid accumulation, particularly in liver and muscle, is the primary key player in the induction of

insulin hyposensitivity (Patil *et al*., 2024). The present study sought to evaluate the salivary fructosamine and sestrin-2 in T2DM and to make a comparison with healthy non-diabetic controls, and also to correlate between serum and salivary fructosamine and sestrin-2 levels in T2DM (Ahmed *et al*., 2023; Khalil *et al*., 2023; Mitchell *et al*., 2023; Adams & Hayes, 2024; Haddad *et al*., 2024; Huaman *et al*., 2024; Moreno & Fuentes, 2024; Pacheco *et al*., 2024; Tan *et al*., 2024; Tursunov *et al*., 2024; Zakaev *et al*., 2024; Abate *et al*., 2025; Ramirez *et al*., 2025; Ruiz *et al*., 2025).

## Materials and Methods

Our study was case-controlled research that took place from January 2025 to June 2026 at the Internal Medicine Department, College of Tikrit Teaching Hospital; Tikrit City.

Based on the clinical characteristics and associated comorbidities, the patients were subclassified into two groups: Control Group—healthy control group ($n = 49$), Group 2—patients with T2DM. Patients with T1DM, acute infections, renal or hepatic, dysfunction, malignancy, or those on antioxidant supplements were excluded from the study.

Patients were asked not to swallow the saliva for 5 minutes after rinsing the mouth with water. A two milliliters of unstimulated whole saliva was collected in a sterile test tubes by spitting over a period of 5 minutes and was then centrifuged (3000 rpm for 10 minutes), supernatant were removed for further biochemical analysis.

Venous blood samples were collected 12 h after the individuals broke their fast. Centrifugation was used to separate the serum samples for 15 min at 3000 × g.

Informed consent was signed by patients before enrollment in the study.

Fructosamine, sestrin-2, and C-peptide concentrations were measured using enzyme-linked immunosorbent assays (ELISA). Whole blood was used in the determination of HbA1c.

Data analysis: SPSS (V26, USA) was used for data analysis. Shapiro-Wilk test used to test normal distribution. Two samples t-tests followed by Welch's correction were employed for normally distributed data. Mann-Whitney U test was used for non-normally distributed data. Spearman's correlation used for non-normal distribution data. Effect sizes were determined by Cohen's d using standard deviations and interpreted as per **Table 1**. P values less than 0.05 considered significant, with Bonferroni test were used for multiple comparisons.

**Table 1.** Statistical data analysis values.

| **Cohen's d effect (\|d\|)** | | **Correlation coefficients** | |
|---|---|---|---|
| **\|d\|<0.2** | Negligible | \|r\|<0.3 | Weak |
| **0.2≤\|d\|<0.5** | Small | 0.3≤\|r\|<0.5 | Moderate |
| **0.5≤\|d\|<0.8** | Medium | 0.5≤\|r\|<0.7 | Strong |
| **\|d\|≥0.8** | Large | \|r\|≥0.7 | Very strong |

## Results and Discussion

The measured parameters demonstrated significant differences between groups ($p<0.001$). The effect sizes were large ($|d|\geq0.8$), reflecting significant differences. The largest effect sizes demonstrated in the following:

- Saliva Sestrin-2 (d=12.98): reflecting remarkable difference.
- Serum C-peptide (d=9.92): Showing marked elevation of insulin secretion in DM

Spearman's rank correlation coefficients were measured to determine the correlation between saliva and serum biomarkers and to effectively demonstrate the associations with **(Tables 2 and 3)**.

*Interpretation of Correlations*

Interpretation of correlations of parameters in either control or T2DM groups revealed that the correlations were weak and non-significant. When results analyzed over the all sample, positive correlations were observed (r=0.34-0.78, all $p<0.01$), a phenomena called Simpson's paradox, which observed when differences between-group dominated the overall correlations, masking the within-group relationships.

All measured parameters demonstrated positive correlations with HbA1c ($p<0.001$), reflecting their correlation with glycemic control. The strongest positive correlations were demonstrated for saliva sestrin-2 (r=0.763) and serum C-peptide (r=0.751).

**Table 2.** Spearman correlations between saliva and serum biomarkers

| **Biomarker** | **Control r** | **p-value** | **DM r** | **p-value** | **Overall r** | **p-value** |
|---|---|---|---|---|---|---|
| **Fructosamine** | -0.172 | 0.238 | 0.104 | 0.478 | 0.341 | 0.001 |
| **C-peptide** | 0.222 | 0.126 | 0.038 | 0.796 | 0.783 | <0.001 |
| **Sestrin-2** | 0.072 | 0.621 | 0.031 | 0.835 | 0.756 | <0.001 |
| **r: Spearman correlation coefficient** | | | | | | |

**Table 3.** Spearman correlations of biomarkers with HbA1c

| Variable | r | p-value | Significance |
|---|---|---|---|
| **Saliva Fructosamine** | 0.578 | <0.001 | *** |
| **Serum Fructosamine** | 0.420 | <0.001 | *** |
| **Saliva C-peptide** | 0.740 | <0.001 | *** |
| **Serum C-peptide** | 0.751 | <0.001 | *** |
| **Saliva Sestrin-2** | 0.763 | <0.001 | *** |
| **Serum Sestrin-2** | 0.694 | <0.001 | *** |
| ***** p<0.001; r: Spearman correlation coefficient** | | | |

Receiver Operating Characteristic (ROC) analysis conducted to identify the diagnostic accuracy of each measured parameters in differentiating between healthy controls and patients with diabetes mellitus (DM). The ROC curve sensitivity against 1-specificity across all possible threshold values, affording a appropriate assessment of diagnostic performance **(Table 4)**.

The Area Under the Curve (AUC) serves as a comprehensive measure of differentiation ability, with values determined as follows: 0.90-1.00 = excellent, 0.80-0.89 = good, 0.70-0.79 = fair, 0.60-0.69 = poor, and <0.60 = failed. Optimal cutoff points were determined using Youden's Index (J = Sensitivity + Specificity - 1), which maximizes the sum of sensitivity and specificity **(Table 4)**.

**Table 4.** ROC analysis: diagnostic performance of biomarkers (Ranked by AUC)

| Biomarker | Area under curve | 95% Confidence interval | Cut off | Sensitivity (%) | Specificity (%) | Positive predictive value (%) | Negative predictive value (%) | Accuracy (%) |
|---|---|---|---|---|---|---|---|---|
| **Saliva C-peptide** | 1.000 | 1.000-1.000 | ≥0.157 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| **Serum C-peptide** | 1.000 | 1.000-1.000 | ≥2.932 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| **Saliva Sestrin-2** | 1.000 | 1.000-1.000 | ≥0.640 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| **Serum Sestrin-2** | 0.996 | 0.982-1.000 | ≥1.970 | 100.0 | 93.9 | 94.2 | 100.0 | 96.9 |
| **HbA1c** | 0.989 | 0.968-1.000 | ≥6.320 | 95.9 | 100.0 | 100.0 | 96.1 | 98.0 |
| **Saliva Fructosamine** | 0.911 | 0.850-0.971 | ≥2.032 | 93.9 | 73.5 | 78.0 | 92.3 | 83.7 |
| **Saliva β-arrestin-1** | 0.871 | 0.799-0.943 | ≥2.290 | 63.3 | 93.9 | 91.2 | 71.9 | 78.6 |
| **Saliva Protein Z** | 0.802 | 0.714-0.890 | ≤141.6 | 46.9 | 100.0 | 100.0 | 65.3 | 73.5 |
| **Serum Fructosamine** | 0.787 | 0.696-0.877 | ≥308.3 | 69.4 | 89.8 | 87.2 | 74.6 | 79.6 |

*Classification Performance*

**Table 5** presents the confusion matrix results for each biomarker at their optimal cutoff points, showing the number of correctly and incorrectly classified subjects.

**Table 5.** Classification performance at optimal cutoff points

| Biomarker | Cutoff | True Positive | True Negative | False Positive | False Negative | Total correctly classified | Total misclassified |
|---|---|---|---|---|---|---|---|
| **Saliva C-peptide** | ≥0.157 | 49 | 49 | 0 | 0 | 98 | 0 |
| **Serum C-peptide** | ≥2.932 | 49 | 49 | 0 | 0 | 98 | 0 |
| **Saliva Sestrin-2** | ≥0.640 | 49 | 49 | 0 | 0 | 98 | 0 |
| **Serum Sestrin-2** | ≥1.970 | 49 | 46 | 3 | 0 | 95 | 3 |
| **HbA1c** | ≥6.320 | 47 | 49 | 0 | 2 | 96 | 2 |
| **Saliva Fructosamine** | ≥2.032 | 46 | 36 | 13 | 3 | 82 | 16 |
| **Serum Fructosamine** | ≥308.3 | 34 | 44 | 5 | 15 | 78 | 20 |

Likelihood ratios provide clinically useful measures of diagnostic test performance (Okoro *et al*., 2023; Dubois *et al*., 2024; Huaman *et al*., 2024; Mayer *et al*., 2024; Tursunov *et al*., 2024; Abate *et al*., 2025; Adams & Hayes, 2025; Lindstrom *et al*., 2025). A positive likelihood ratio (LR+) indicates how much more likely a positive test result is in a patient with the disease compared to one without.

A negative likelihood ratio (LR-) indicates how much less likely a negative test result is in a patient with the disease **(Table 6)**.

**Table 6.** Likelihood ratios and Youden's index

| Biomarker | Positive predictive value (%) | Negative Likelihood Ratio | Youden's J | Interpretation |
|---|---|---|---|---|
| **Saliva C-peptide** | ∞ | 0.00 | 1.000 | Excellent |
| **Serum C-peptide** | ∞ | 0.00 | 1.000 | Excellent |
| **Saliva Sestrin-2** | ∞ | 0.00 | 1.000 | Excellent |
| **Serum Sestrin-2** | 16.33 | 0.00 | 0.939 | Excellent |
| **HbA1c** | ∞ | 0.04 | 0.959 | Excellent |
| **Saliva Fructosamine** | 3.54 | 0.08 | 0.673 | Moderate |
| **Serum Fructosamine** | 6.80 | 0.34 | 0.592 | Moderate |
| **LR+ >10 and LR- <0.1 indicate excellent diagnostic utility. ∞ indicates perfect positive or negative predictive ability.** | | | | |

Our research is the first to assess the relationship between fructosamine and serum and saliva sestrin-2 in type 2 diabetes. While HbA1c remains the fundamental parameters for diagnosing DM and glycemic control, new study indicates that in certain patients, other biomarkers, like FA and glycated albumin, are taking its place when HbA1c measurement may be imprecise or even biased (Patel & Anuradha, 2023).

Sestrin-2 affects lipid metabolism by encouraging the breakdown of fatty acids by β-oxidation and decreasing lipid buildup in tissues. It has also been linked to the control of hepatic glucose production (Lee *et al*., 2012; Gong *et al*., 2021). One of the main causes of insulin resistance is excessive lipid buildup, especially in the liver and muscle (Patil *et al*., 2024). According to a study by Mao *et al*., Sestrin-2 levels were considerably higher in T2DM patients than in healthy individuals (Mao *et al*., 2021).

Similar to how HbA1c is produced, fructosamine (FA) is a ketoamine that is produced when the carbonyl group of glucose combines with an amino group of a protein to form glycated serum proteins (primarily albumin) (Danese *et al*., 2015; Gounden *et al*., 2025). This nonenzymatic glycation process involves a labile Schiff base intermediate and the Amadori rearrangement (Sacks *et al*., 2023; Jallod *et al*., 2025).

In contrast to fructosamine, which offers short-term data on prior glycemic control (2-3 weeks prior), HbA1c offers long-term results about the previous glycemic control (2-3 months). Nonetheless, the rate of non-enzymatic glycation of fructosamine is about 9-10 times greater than that of HbA1c (Andrade *et al*., 2023).

## Conclusion

The determination of fructosamine and sestrin-2 levels in the serum and saliva of individuals with type 2 DM has been suggested as a potential diagnostic method. Further studies that assay other saliva and serum biomarkers may also be useful and are thus recommended.

**Acknowledgments:** The authors would like to thanks Tikrit University for providing facilities to complete this research.

**Conflict of interest:** None

**Financial support:** None

**Ethics statement:** The study was approved by the College of Medicine/Tikrit University (Approval Letter Number MIDTUH5 on 20.02.2026).

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