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Alpha-2-Macroglobulin in Saliva as a Noninvasive Glycemic Control Marker in Type 2 Diabetes Mellitus Patients

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ABSTRACT

Background: Limited access to quality healthcare makes diabetic patients more susceptible to developing complications, contributing to diabetes mellitus being the third leading cause of death.

Objective: The objective of this study was to investigate the potential use of α-2-macroglobulin in saliva as an indicator of blood sugar regulation in patients with type 2 diabetes (T2DM).

Patients and Methods: In this study, which was cross-sectional and observational in design, 90 participants were divided into three groups. Group 1 included 40 patients diagnosed with type 2 diabetes mellitus and had HbA1c levels equal to or greater than 7% (indicating inadequate glycemic control). Group 2 comprised of 40 patients with type 2 diabetes mellitus with HbA1c levels less than 7% (indicating adequate glycemic control). Finally, group 3 included 10 healthy individuals as a control group, who had a fasting plasma glucose level less than 100 mg/dl, 2-hour plasma glucose less than 140 mg/dl, and HbA1c less than 5.7%. All participants were subjected to a thorough clinical examination, laboratory tests, and assessment of salivary α-2-macroglobulin.

Results: The control group had an average salivary α-2-macroglobulin (α-2-MG) level of 173.40 ± 58.76 ng/ml, while the adequate glycemic control group had a mean level of 337.90 ± 86.95 ng/ml. In contrast, the inadequate glycemic control group had a significantly higher mean level of 998.81 ± 203.04 ng/ml. The difference in A2MG levels between the three groups was statistically significant.

Conclusion: The potential of salivary α-2-MG as a biomarker for glycemic control in T2DM patients is significant. It can be a useful tool for screening and monitoring large populations of individuals with T2DM, and can be easily measured using whole saliva, making it accessible to individuals with minimal training.

Keyword: α-2-macroglobulin, Saliva, Glycemic control, DM, Type 2.

INTRODUCTION

Diabetes is the third most common cause of death, and individuals with limited access to quality health care are at higher risk of developing complications. To screen for diabetes, blood glucose levels need to be measured. The ideal indicator for glycemic control is HbA1c, which provides a four-month assessment of an individual's glucose levels. However, this test is invasive and requires a blood sample [(1)].

Managing blood glucose levels is crucial for the prevention of complications associated with diabetes. Fasting and 2-hour postprandial blood glucose levels, as well as HbA1c, are commonly used to assess glycemic control [(2)]. However, some patients may be deterred from regular blood glucose testing due to a fear of needles. Studies show that needle phobia can lead to avoidance of all medical care in up to 20.5% of individuals [(3)].

The diabetes biomarker should be measured using a noninvasive method in saliva samples. Alpha-2-macroglobulin (α-2-MG) can be found in saliva, as well as blood, and its levels are increased in type 1 and type 2 diabetes [(4)].

The production of α-2-MG is increased in diabetic people. A2MG is generated by the liver and functions as an antiproteinase in plasma. The elevated serum A2MG reduces insulin's bioavailability, resulting in impaired glucose regulation [(5)].

The aim of the present study was to evaluate α-2-MG in saliva as marker for glycemic control in T2DM patients. PATIENTS AND METHODS

This study was conducted at the Internal Medicine Department of Benha University Hospitals from January 2021 to December 2021. A total of 90 subjects participated in the study and were classified into three groups according to the American Diabetes Association criteria. **Group 1** included 40 patients with T2DM and HbA1c levels greater than or equal to 7% (indicating inadequate glycemic control), **Group 2** included 40 patients with T2DM and HbA1c levels less than 7% (indicating adequate glycemic control), and **Group 3** included 10 healthy individuals with fasting plasma glucose levels below 100 mg/dL, 2-hour plasma glucose levels below 140 mg/dL, and HbA1c levels below 5.7%. The study was cross-sectional and observational in design.

Inclusion criteria were age between 30 and 60 years, T2DM patients according to the last criteria of American Diabetes Association.

Exclusion criteria were associated diabetic complications (hyperosmolar non-ketotic coma, diabetic ketoacidosis), Nephrotic syndrome, rheumatic diseases, Auto-immune disease, Associated acute inflammatory conditions in the mouth, Pregnant females and association of other neurological disease.

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All patients underwent a comprehensive assessment that included taking their full medical history, conducting a thorough clinical examination, and performing laboratory tests to determine fasting blood glucose, post-prandial blood glucose, HbA1c, lipid profile, and urinary albumin/creatinine ratio. The levels of salivary α2-MG were evaluated using a human α2-MG ELISA Kit (Shanghai Crystal Day Biotech Co. Limited) (Cat. No: E1097Hu).

Ethical consent:

Before being included in the study, all participants provided written informed consent. The study design was approved by the Ethical Scientific Committee of the Faculty of Medicine at Benha University, and the principles of confidentiality and personal privacy were upheld throughout the study. Participants were free to withdraw from the study at any time without any negative consequences. This study was conducted in accordance with the World Medical Association's Code of Ethics (Declaration of Helsinki) for research involving human subjects.

Statistical analysis

Data was analyzed using SPSS 22.0. Descriptive statistics were used for continuous data and frequency with percentage for qualitative data. Different tests were used based on data distribution, including Pearson Chi-square, Fisher's Exact Test, One-way ANOVA, Kruskall-Wallis, Tukey-HSD, and Spearman rank correlation coefficient. ROC analysis was used to determine optimal cutoff values. The significance level was set at 0.05, and P-values > 0.05 were non-significant, P-values ≤ 0.05 were significant, and P-values < 0.01 were highly significant.

RESULTS

Demographic characteristics (Age and gender) were insignificantly different among the studied groups (P-value > 0.05) (Table 1).

Table (1): Demographic characteristics in the study groups

Parameters	Group I (n = 10)	Group II (n = 40)	Group III (n = 40)	P-value
Age (years)				
Mean ± SD	42.17 ± 5.91	42.93 ± 5.91	44.93 ± 6.81	0.285
Gender				
Males n (%)	6 (60)	25 (62.5)	27 (67.5)	0.545
Females n (%)	4 (40)	15 (37.5)	13 (32.5)	

One-way ANOVA test was used for age. Chi-square test was used for gender

The group with poor glycemic control had significantly higher levels of FBG, 2Hr PPBG, and HbA1c compared to the control group and the group with good diabetic control. Moreover, the group with good glycemic control had significantly higher levels of these parameters compared to the control group. The urinary albumin-creatinine ratio was significantly higher in the poor glycemic control group compared to the control group and the group with satisfactory diabetic control (Table 2).

Table (2): Laboratory investigations in the studied groups

Parameters	Group I (n = 10)	Group II (n = 40)	Group III (n = 40)	P-value
FBG (mg/dL)	94.00 ± 20.15	122.00 ± 25.66	157.50 ± 33.98	**< 0.001**
2Hr PPBG (mg/dL)	107.00 ± 21.25	181.50 ± 44.51	224.50 ± 49.87	**< 0.001**
HbA1c (%)	5.40 ± 1.11	6.45 ± 1.43	8.4 ± 1.77	**< 0.001**
Total Cholesterol (mg/dL)	182.50 ± 37.58	202.00 ± 42.15	186.50 ±38.55	0.415
Triglycerides (mg/dL)	103.00 ± 19.55	147.50 ± 23.12	163.00 ± 28.66	**<0.001**
HDL (mg/dL)	51.50 ± 11.12	44.00 ± 8.55	41.00 ± 6.54	**0.004**
LDL (mg/dL)	104.50 ± 18.51	117.00 ± 22.14	120.00 ± 24.68	0.223
Hb (g/dL)	12.79 ± 2.15	12.35 ± 1.81	12.60 ± 2.24	0.420
Platelets (10^3/L)	264.00 ± 48.69	297.00 ± 51.21	282.00 ± 57.36	0.457
WBCs (10^3/L)	6.10 ± 1.02	6.22 ± 1.10	6.86 ± 1.31	0.329
Urinary Albumin-creatinine ratio	19.00 ±4.51	19.00 ± 4.31	140.00 ± 32.25	**< 0.001**

Kruskall Wallis test was used. Post hoc analysis was done using Bonferroni method. Different letters indicate significant pair

The average value of salivary α2-MG was 173.40 ± 58.76 ng/ml in the control group, 337.90 ± 86.95 ng/ml

in the group with acceptable glycemic control, and 998.81 ± 203.04 ng/ml in the group with inadequate glycemic control. There was a significant difference between the three groups (Figure 1).

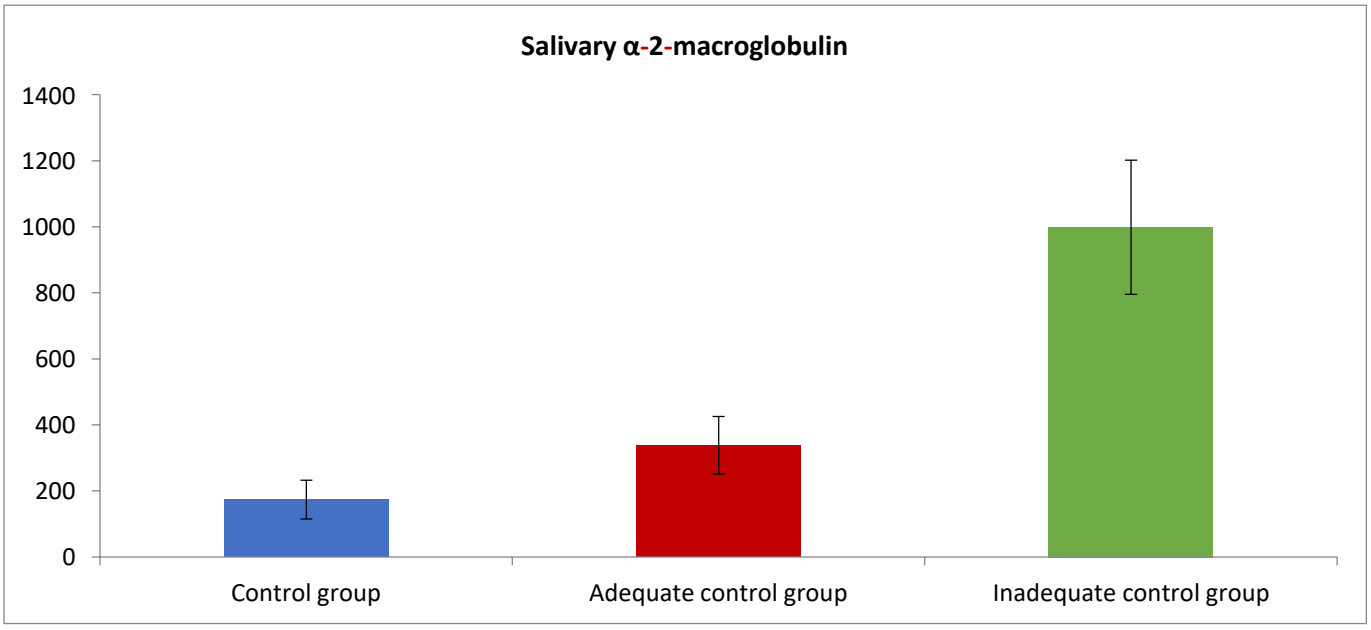

Figure (1): Level of Salivary α2 macroglobulin n the three group

ECG changes were found in 3 individuals (7.5%) with proper glycemic control and 5 individuals (12.5%) with insufficient glycemic control, while none of the individuals in the control group showed ECG changes. There was no significant difference among the three groups with respect to ECG changes (Table 3).

Table (3): ECG changes in the studied groups

Parameters	Group I (n = 10)	Group II (n = 40)	Group III (n = 40)	P-value
ECG changes Present n (%)	0 (0%)	3 (7.5)	5 (12.5)	0.247

Chi-square test was used.

There was a moderate positive correlation that was statistically significant between α α2-MG and FBG, 2Hr PPBG, HBA1c, and BMI in both the group with adequate glycemic control and the group with inadequate glycemic control (Table 4).

Table (4): Correlation between α α2-MG and various parameters in groups II & III

Parameters correlated	α-2-Macroglobulin			
	Group II		Group III	
	r_s	P-value	r_s	P-value
Age (years)	0.11	0.560	-0.17	0.358
FBG (mg/dL)	**0.47**	**0.009**	**0.45**	**0.013**
2Hr PPBG (mg/dL)	**0.46**	**0.011**	**0.41**	**0.024**
HbA1c (%)	**0.45**	**0.010**	**0.46**	**0.011**
Total cholesterol (mg/dL)	0.04	0.833	0.06	0.755
Triglycerides (mg/dL)	0.00	0.985	0.21	0.262
HDL (mg/dL)	0.07	0.701	0.12	0.525
LDL (mg/dL)	0.28	0.135	-0.09	0.654
BMI (kg/m^2)	**0.51**	**0.015**	**0.47**	**0.021**
SBP (mmHg)	0.11	0.550	0.21	0.269

DBP (mmHg)	0.01	0.969	0.20	0.282
Hb (g/dL)	-0.06	0.748	-0.16	0.403
Platelets (10^3/L)	0.19	0.321	-0.07	0.726
WBCs (10^3/L)	-0.34	0.066	-0.01	0.966
Urinary Albumin-creatinine ratio	0.218	0.118	0.194	0.146
r_s : Spearman correlation coefficient				

The optimal threshold value for salivary α2-MG to distinguish between the control group and the group with good glycemic control was determined to be 226.5 ng/ml, with a sensitivity of 92.5%, a specificity of 80%, a PPV of 94.5%, an NPV of 78%, and an overall accuracy of 86.4% (Figure 2).

The optimal threshold value of salivary α2-MG to distinguish between the adequate glycemic control group and the inadequate glycemic control group was found to be 521.3 ng/ml with a sensitivity of 95.5%, specificity of 98%, PPV of 96.4%, NPV of 98%, and accuracy of 97.6% (Figure 3).

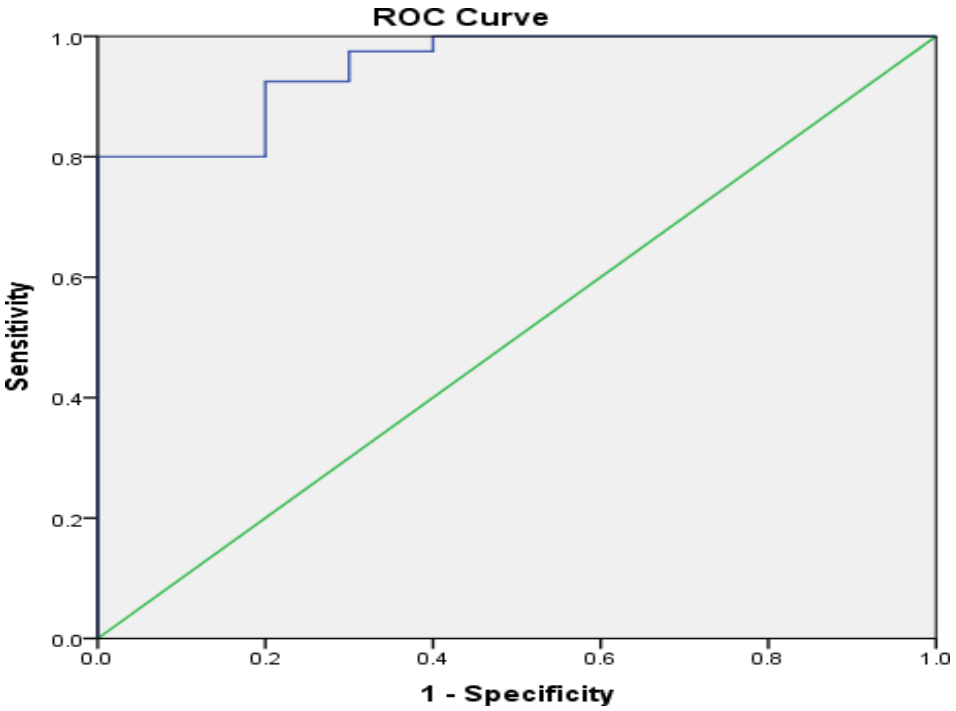

Figure (2): Roc curve of salivary α2-MG to differentiate between control and adequate

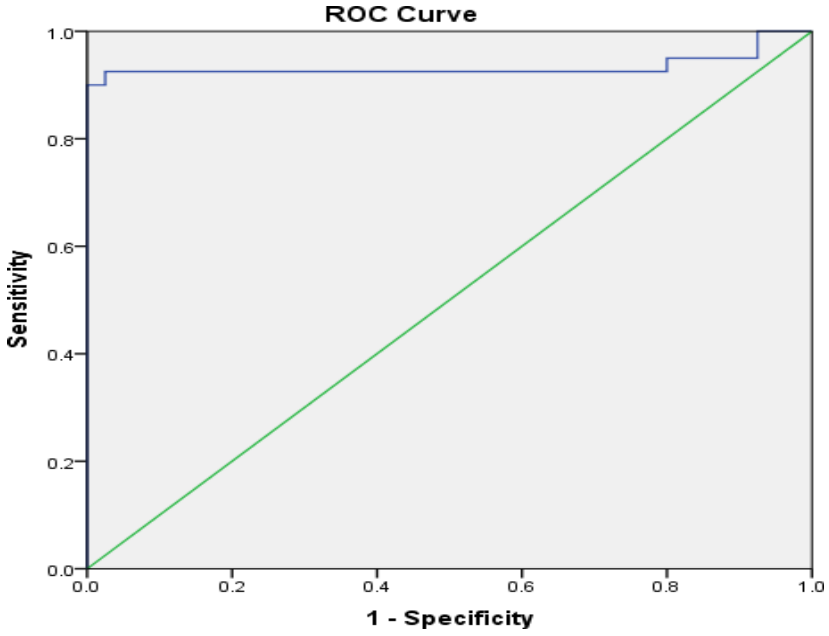

Figure (3): Roc curve of salivary α2-MG to differentiate between adequate glycemic control and inadequateglycemic control groups.

DISCUSSION

In this study, the levels of salivary α2-MG were found to be significantly higher in the inadequate glycemic control group compared to the control group and the adequate glycemic control group. These findings are consistent with previous studies conducted by Nsr-Allah et al. (6) and Chung et al. (7) that reported significant increases in salivary α2-MG levels in poorly controlled diabetic patients. Takada et al. (8) also found a relationship between α2-MG and glycemic control in diabetic patients without anemia or nephropathy, further supporting the potential use of salivary α2-MG as a monitoring method for diabetes. The authors of this study suggest that detecting salivary α2-MG levels could be an effective method for monitoring diabetes control. Another study **Rao *et al.*** [9] enrolled 20 participants with either T2DM or prediabetes, and found that salivary proteins including α2-MG showed a relative increase in abundance with disease progression of prediabetes to the diabetic state and could be potential biomarkers for prediabetes screening.

Caseiro et al. (10) have demonstrated that the salivary α2-MG profile underscores the significance of the innate immune system in the etiology of T1DM and its related complications. They found that elevated levels of salivary α2-MG were only observed in type 1 diabetes patients complicated with retinopathy and nephropathy, but not in non-complicated type 1 diabetes patients. In the present study, there was a statistically significant moderate positive correlation between α2-MG and each of FBG, 2Hr PPBG, and HBA1C in both the adequate glycemic control group and the inadequate glycemic control group. These findings are in line with those reported by Nsr-Allah et al. (6), who demonstrated a strong positive correlation between salivary α2-MG and fasting blood glucose and HbA1c levels in patients with type 2 diabetes mellitus. Additionally, the results of Chung et al.'s study (7) support our findings as they found a strong positive correlation between HbA1c and both blood and salivary α2-MG in patients with type 2 DM. Furthermore, Rastogi et al. (11) reported a significant correlation between saliva levels of A2MG and HbA1c ($r = 0.994$ and $P = 0.001$) in patients with type 2 DM. Their study also found that α2-MG and HbA1c levels were highly correlated. Similarly, Pearson correlation coefficient was calculated for HbA1c and α2-MG, which demonstrated good linear correlation between HbA1c and α2-MG ($r = 0.977$, $P < 0.001$). These findings suggest that salivary A2MG may be a useful marker for monitoring diabetes control and its related complications.

The results of our study align with the findings of previous research. Rao et al. (9) found higher levels of salivary and blood α2-MG in prediabetic patients compared to healthy controls, indicating a strong link between glycemic control and salivary α2-MG levels. Our results also support the positive correlation between salivary α2-MG and HbA1c percentage in T2DM patients, as reported by Aitken et al. (5) and Nsr-Allah et al. (6). In addition, our study agrees with Nsr-Allah et al. (6) regarding the strong positive correlation between salivary α2-MG and BMI and duration of diabetes in T2DM patients. However, Ahmad et al. (12) found no significant relationship between α2-MG level and FPG or HbA1c in patients with T2DM, despite observing a direct positive correlation between plasma α2-MG and the duration of diabetes and various levels of microalbuminuria.

The study determined the optimal cutoff point of salivary α2-MG to differentiate between the control group and the adequate glycemic control group at 226.5 ng/ml with a sensitivity of 92.5%, specificity of 80%, PPV of 94.5%, NPV of 78%, and accuracy of 86.4%. Meanwhile, the optimal cutoff point of salivary α2-MG to differentiate between the adequate and inadequate glycemic control groups was 521.3 ng/ml with a sensitivity of 95.5%, specificity of 98%, PPV of 96.4%, NPV of 98%, and accuracy of 97.6%. Similarly, Nsr-Allah et al. (6) identified the most discriminating cutoff value of salivary α2-MG using HBA1c as the gold standard for diagnosis of glycemic control, which was 645 ng/ml with an area under curve of 0.92, sensitivity of 91.7%, specificity of 90%, and $P<0.001$. The study concluded that salivary α2-MG could be a diagnostic method for detecting inadequate glycemic control in patients with T2DM. Aitken et al. (5) also found a positive discrimination threshold of α2-MG with an optimal cutoff value of 840 ng/ml, a sensitivity of 81.9%, and a specificity of 89.6% for predicting poor glycemic control in patients with uncontrolled T2DM.

The variations in cutoff points found in different studies may be due to differences in the characteristics of the participants and the sensitivity of the assay kits used. Moreover, there is evidence to suggest that α2-MG levels in saliva are associated with periodontal status. One study found that α2-MG levels in crevicular fluid were significantly higher in patients with aggressive periodontitis compared to those with chronic periodontitis (13). Thus, future studies should consider carefully assessing the periodontal status of participants through oral clinical examinations and complementary investigations such as radiography in order to better understand the association between α2-MG levels and glycemic control.

CONCLUSION

Salivary α2-MG has the potential to be a valuable biological marker for glycemic control in patients with T2DM, thanks to its quick, convenient, affordable, and non-invasive nature. With minimal training, whole saliva could be used as an effective tool for T2DM screening and monitoring in large populations.

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Conflicts of interest: None.

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