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ORIGINAL ARTICLE

Study of Salivary Alpha-2 Macroglobulin in Type 2 Diabetic Egyptian Patients

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Abstract

Background: **Evaluating glycemic control in saliva samples in a noninvasive method is necessary. Alpha-2 macroglobulin (A2MG) might be present in saliva; therefore, it has the potential to be employed as a noninvasive approach for tracking type 2 diabetes mellitus individuals' glycemic control.**

Aim and objectives: **Evaluating the efficacy of salivary A2MG as a biomarker for glycemic control in Egyptians with type 2 diabetes mellitus.**

Patients and methods: **The Internal Medicine Outpatient Clinic at Al-Azhar University Hospital in Cairo enrolled 100 participants for a cross-sectional observational research throughout September 2021 to March 2022.**

Results: **Age, family history, hypertension, morbidities, post-hoc test for multiple contrasts among examined groups with respect to fasting blood sugar, postprandial blood sugar, and glycated hemoglobin, post-hoc test for multiple contrasts among examined groups with respect to salivary A2MG and diagnostic performance of salivary A2MG in discrimination were all highly statistically significant.**

Conclusion: **As a result of the fact that the current study discovered an adequate correlation among salivary A2MG levels and the percentage of glycated hemoglobin, the measurement of A2MG in saliva provides a noninvasive, effective alternative way for assessing glycemic index, hence avoiding the comorbidities that have been related to diabetes.**

Keywords: Glycemic control, Salivary alpha-2 macroglobulin, Type 2 diabetic Egyptian patients

1. Introduction

High rates of incidence and prevalence define diabetes mellitus (DM), a condition marked by persistently high blood glucose that may result either from insufficient insulin production by the pancreas or from improper insulin use by the body. Different types of DM exist. Damaged beta cells in the pancreas cause type 1 diabetes mellitus (T1DM) by preventing the pancreas from releasing insulin, which keeps blood glucose levels from dropping quickly enough. Insulin resistance and insufficient insulin production have a part in the pathogenesis of type 2 diabetes mellitus (T2DM).[1]

Approximately 370 million people throughout the world are living with T2DM right now. T2DM develops at various rates in persons of diverse racial and/or ethnic backgrounds, likely due to genetics and lifestyle factors (such as a high-sugar diet and inactivity).[2,3]

Noninvasive strategies for assessment of diabetic biomarker are required. Clinical diagnosis and screening for diabetes may benefit from the use of salivary proteins as noninvasive biomarkers, according to a recent study. Higher levels of plasma alpha 2-macroglobulin (A2MG) have previously been reported in diabetic individuals, particularly those who also suffer from diabetes complications.[4,5]

A2MG is a glycoprotein secreted by the liver that functions as an anti-proteinase in the plasma. Proteolytic enzymes involved in inflammation and homeostasis are captured and inhibited. Diabetic

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individuals have increased production of A2MG. Insulin bioavailability is decreased and blood sugar management is compromised by elevated serum A2MG.[6]

Major and minor salivary gland secretions are found in saliva, but there are also numerous proteins originating from the blood in the saliva. When plasma levels of A2MG are high, the protein may be exocytotically transported to the saliva; therefore, it could become a useful noninvasive source in determining A2MG as a biomarker for glycemic control in cases with T2DM.[4,7]

This work aimed to evaluate salivary A2MG as a biomarker for glycemic control in Egyptian cases with T2DM.

2. Patients and methods

This research was a cross-sectional observational study performed at the Outpatient Clinic of the Internal Medicine Department, Al-Azhar University Hospital in Cairo during the course of 6 months starting from September 2021 till March 2022 on 100 patients.

Inclusion criteria: age between 30 and 60 years old and according to the most recent ADA guidelines, people with T2DM.

Exclusion criteria: participants suffering from T1DM, people who have additional diabetes complications, such as diabetic ketoacidosis or hyperosmolar nonketotic unconsciousness. Individuals who suffer from rheumatic diseases, any type of cancer, pregnant women, or end-stage illnesses; people who have a history of both an inflammatory and an infectious illness within the last 3 months; individuals who are in their latter stages of life. Those who have chronic inflammatory processes in the mouth, as well as those who have neurological illnesses that influence saliva (such as Alzheimer's disease, Huntington's disease, mental disorders, Parkinsonism, amyotrophic lateral sclerosis, autistic disorders, or multiple sclerosis), are not the candidates for this treatment.

Patients who participated in the research were separated into three groups on the basis of the inclusion and exclusion criteria, as well as the DM profiles of the participants (using the most recent criteria established by the American Diabetes Association). Group A composed of 35 cases with T2DM who had glycated hemoglobin (HbA1C) levels of more than 7 %, group B composed of 35 cases with T2DM who had HbA1C levels of less than or equal to 7 %, and group C (the control group) consisted of 30 healthy participants whose fasting plasma glucose was less than 100 mg/dl, their 2-h plasma glucose was less than 140 mg/dl, and their HbA1C was less than 5.7 %.

Ethics committee approval and quality control: the requirements of the AL-Azhar University ethics committee were adhered to throughout all of the processes, written consents were obtained from individuals before enrolling them in the study, the aims of the study as well as any potential risks were addressed with individuals, and the patients' confidentiality was protected with regard to the data that was gathered.

3. Methods

Patients were exposed to a thorough history and physical examination as well as laboratory measurements of fasting blood sugar (FBS), postprandial blood sugar (PPBS), and cholesterol and triglycerides.

Biomarkers measurements: saliva samples were tested for human-2-macroglobulin concentrations with an ELISA kit (Human Alpha 2-Macroglobulin DuoSet; R&D Systems, Minneapolis, USA) and for HbA1C concentrations with a kit from the Bio-Rad Variant II brand team (Bio-Rad Inc., Hercules, California, USA).

The three groups were compared regarding different criteria including: demographic characteristics and BMI, risk factors and DM duration. The laboratory parameters including plasma levels of cholesterol and triglycerides and levels of FBS, PPBS, and HbA1C, levels of salivary A2MG, diagnostic performance of salivary A2MG in discrimination of studied groups and correlations between salivary A2MG and each of demographic and laboratory data.

3.1. Statistical analysis

The data was examined employing SPSS, version 24 (Statistical Package for the Social Sciences). Utilizing the Statistical Package for Social Science (SPSS) (Released 2015), the data were gathered, edited, and input into a personal computer (PC). Armonk, New York: IBM Corp., IBM SPSS Statistics for Windows, Version 23.0. The numerical information was summarized as a mean ± SD. Frequency and percentages were utilized to represent qualitative information. If you take the sum of a collection of numbers and divide it by the total number of numbers in the set, you get the mean of the set. The SD can be used to evaluate how widespread a set of numbers is. When the SD is small, the values cluster near to the set's mean, but when it is large, the values are more dispersed.

Table 1. Comparison among examined groups regarding age and sex.

	Groups [*n* (%)]						Statistical test	*P* value
	Group A ($N = 35$)		Group B ($N = 35$)		Group C ($N = 30$)			
Sex								
Male	19	54.3	21	60	18	60	$\chi^2 = 0.3$	0.859 NS
Female	16	45.7	14	40	12	40		
Age (years)	Mean ± SD	53.7 ± 6.3	45.5 ± 8.2	39.3 ± 5.1	KW = 42.9	<0.001 HS		

KW, Kruskal–Willis test.
HS, *P* value less than 0.001 is highly significant; NS, *P* value more than 0.05 is nonsignificant.

4. Results

Table 1 shows that there was not a significant distinction in sex among the groups tested ($P = 0.859$). There is a statistically substantial age gap ($P < 0.001$) among the two groups.

Table 2 demonstrates that there was not significant distinction ($P = 0.073$) in relation to smoking among any of the groups that were investigated. A statistically significant rise in the number of persons in groups A (26 patients, 74.3 %) and B (21 participants, 60 %) who had a positive family history in comparison to group C (0 patients, 0 %). Increase in the prevalence of hypertension that is statistically significant in groups A (19 individuals, 54.3 %) and B (10 people, 28.9 %) contrasted to group C (three people, 10 %). There was a significant rise in the proportion of individuals in groups A (18 individuals, 51.4 %) and B (12 individuals, 34.3 %) who had comorbidities in comparison to group C (which had no individuals, 0 %).

The data presented in Table 3 indicates that the duration of DM symptoms in group A has risen significantly ($P = 0.001$; (7.2 ± 4.6) versus group B (3.7 ± 2.2; Fig. 1, Table 4).

The variance was statistically significant and the results showed that both group A (198.8 ± 53.2) and group B (129.4 ± 19.9) experienced an increase in their FBS levels. It was shown that there was a highly significant variance among the FBS of group A (198.8 53.2) and the FBS of group C (87.1 ± 9.6). In comparison, the FBS levels found in group B were significantly higher than those obtained in group C (123.4 ± 8.4).

Concerning the PPBS, there was a very statistically significant ($P = 0.001$) enhanced PPBS in group A (261.7 ± 48.7) as compared with group B (183.4 17.7). When compared with group C, which had a PPBS that was 123.4 ± 8.4, the PPBS in group A, which was 261.7 ± 48.7, rose by a highly significant amount statistically. In comparison to group C, which had a PPBS that was 123.4 ± 8.4, the PPBS for group B was significantly higher at 183.4 ± 17.7. This variation had a *P* value of less than 0.001. In terms of HbA1C, there was a highly statistically significant rise in HbA1C in group A (8.7 ± 1.4), in comparison to group B (6.8 ± 0.2). When compared with group C, which had a HbA1C level that was 4.9 ± 0.4, the HbA1C level in group A was 8.7 ± 1.4, which showed a statistically significant rise.

When contrast to group C, which had a HbA1C level that was 4.9 ± 0.4, the HbA1C level in group B was significantly higher (6.8 ± 0.2) (Table 5).

Concerning the A2MG levels in the saliva, there were when compared with group B, which had lower levels of salivary A2MG (590.2 ± 28.5), group A had significantly higher levels of salivary A2MG (751.6 ± 146.6). This distinction had a *P* value of less than 0.001. When compared with group C

Table 2. Comparisons of risk variables among several groups that were evaluated.

	Groups [*n* (%)]						Statistical test	*P* value
	Group A ($N = 35$)		Group B ($N = 35$)		Group C ($N = 30$)			
Family history								
Negative	9	25.7	14	40	30	100	$\chi^2 = 39.4$	<0.001 HS
Positive	26	74.3	21	60	0	0		
Hypertension								
No	16	45.7	25	71.4	27	90	$\chi^2 = 14.8$	0.001 S
Yes	19	54.3	10	28.9	3	10		
Smoking								
No	23	65.7	27	77.1	15	50	$\chi^2 = 5.2$	0.073 NS
Yes	12	34.3	8	22.9	15	50		
Comorbidities								
No	17	48.6	23	65.7	30	100	$\chi^2 = 20.8$	<0.001 HS
Yes	18	51.4	12	34.3	0	0		

Table 3. Correlation of the several groups examined with regard to the length of diabetes mellitus.

	Group A ($N = 35$)	Group B ($N = 35$)	Statistical test	P value
DM duration (years)				
Mean ± SD	7.2 ± 4.6	3.7 ± 2.2	Mann–Whitney = 324.5	0.001 S

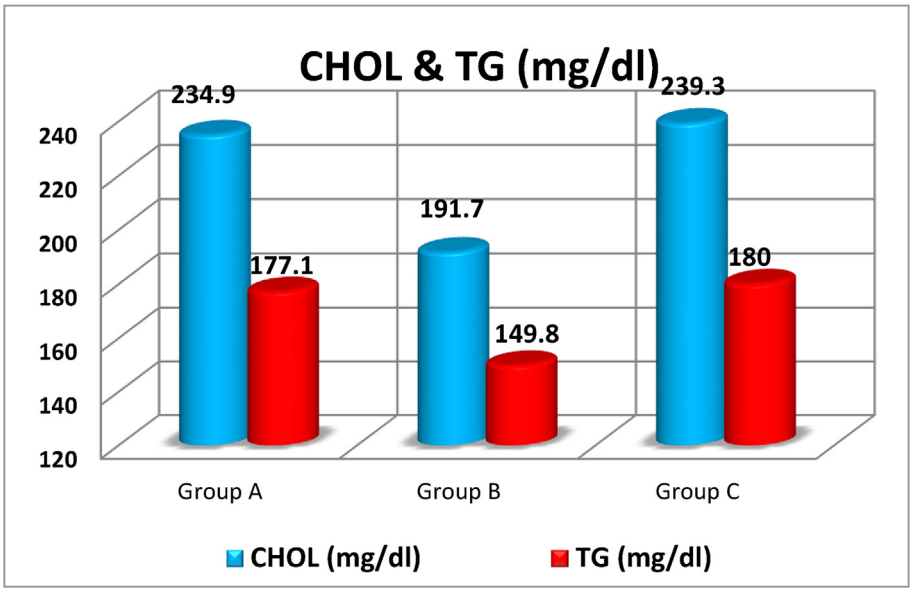

Fig. 1. Comparison among examined groups as concern cholesterol and triglycerides.

Table 4. Post-hoc test for multiple comparisons among the groups that were looked at with relation to fasting blood sugar, postprandial blood sugar, and glycated hemoglobin.

	A vs. B	A vs. C	B vs. C
FBS			
LSD	69.3	111.7	42.3
P value	<0.001 HS	<0.001 HS	<0.001 HS
PPBS			
LSD	78.3	138.3	60.02
P value	<0.001 HS	<0.001 HS	<0.001 HS
HbA1C			
LSD	1.95	3.8	1.9
P value	<0.001 HS	<0.001 HS	<0.001 HS

FBS, fasting blood sugar; HbA1C, glycated hemoglobin; LSD, least significant difference; PPBS, postprandial blood sugar.

(382.1 ± 30.2), group A had significantly higher salivary A2MG levels (751.6 ± 146.6), and this difference was statistically significant. When contrasted with group C, which had lower levels of salivary A2MG (382.1 ± 30.2), group B had significantly higher levels of salivary A2MG (590.2 ± 28.5). This difference had a P value of less than 0.001 (Table 6).

Table 5. Post-hoc test for making multiple comparisons among the groups that were evaluated with relation to salivary alpha-2 macroglobulin.

	A vs. B	A vs. C	B vs. C
Salivary A2MG			
LSD	161.4	369.5	208.1
P value	<0.001 HS	<0.001 HS	<0.001 HS

A2MG, alpha-2 macroglobulin; LSD, least significant difference.

At a threshold level of more than 662.5, the A2MG in saliva has the potential to differentiate between group A and group B with a sensitivity of 97.1 %, a specificity of 100 %, a positive predictive value (PPV) of 100 %, and an negative predictive value (NPV) of 97.2 % [area under the curve (AUC) = 0.97, $P = 0.001$]. A receiver operating characteristic (ROC) curve was utilized to unearth this information. Salivary A2MG can be utilized to distinguish between group A and group C when the threshold level is greater than 550, and it does so with a sensitivity of 97.1 %, a specificity of 100 %, a PPV of 100 %, and an NPV of 97.2 % (AUC = 0.97). Additionally, it has a PPV of 100 % and an NPV of 97.2 %. Salivary A2MG has the ability to distinguish among group B and group C when the cutoff level is more than 492.5, and it does so with a sensitivity of 100 %, a specificity of 100 %, a PPV of 100 % and a NPV of 100 % (AUC = 1.0).

5. Discussion

Chronic illness that involves insulin secretion failure, DM causes progressive damage to vital organs and tissues. The high incidence and

Table 6. The diagnostic performance of salivary alpha-2 macroglobulin in differentiating among the groups that were investigated.

	Cut off	AUC	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	*P* value
A vs. B	>662.5	0.97	97.1	100	100	97.2	<0.001
A vs. C	>550	0.97	97.1	100	100	97.2	<0.001
B vs. C	>492.5	1.0	100	100	100	100	<0.001

AUC, area under the curve; NPV, negative predictive value; PPV, positive predictive value.

consequential disability and death make this a major global health problem.[8]

The immediate metabolic consequence of diabetes is hyperglycemia. Uncontrolled DM is related to a multitude of complications, involving diabetic neuropathy, retinopathy, nephropathy, and cardiovascular disease.[9]

Regarding the DM duration of the examined cases, the present research indicated a statistically significant increase in mean DM duration among group A in contrast with group B (7.2 ± 4.6 vs. 3.7 ± 2.2 years, respectively) ($P < 0.001$). This study demonstrated the presence of a significant rise in cases of hypertension among group A in comparison with group B and control group (54.3 vs. 28.9 vs. 10 %, respectively) ($P = 0.001$).

Such findings are in agreement with Nsr-Allah et al.[4] that indicated a significant increase in duration of DM among the uncontrolled patients in in contrast to controlled (13.85 ± 7.23 vs. 8.20 ± 3.59, respectively) and uncontrolled diabetics have a statistically significant higher rate of hypertension than managed diabetics and healthy people.

Similarly, Aktas et al.[10] revealed a statistically significant increase in both systolic and diastolic blood pressures among cases with poorly controlled T2DM in contrast with well-controlled T2DM and control groups.

The comparison among the examined groups showed a highly significant increase in FBS (198.8 ± 53.2 vs. 129.4 ± 19.9 vs. 87.1 ± 9.6 mg/dl, respectively), PPBS (261.7 ± 48.7 vs. 183.4 ± 17.7 vs. 123.4 ± 8.4 mg/dl, respectively), and HbA1C (8.7 ± 1.4 vs. 6.8 ± 0.2 vs. 4.9 ± 0.4 %, respectively) among group A in contrast with group B and the control group ($P > 0.001$).

Rastogi et al.[5] that demonstrated that the FBS and PPBS were highly statistically significantly increased among the uncontrolled diabetic patients in comparison with patients with controlled diabetes (FBS: 235.44 ± 77.160 vs. 132.37 ± 25.583 mg/dl, respectively and PPBS: 290.58 ± 96.126 vs. 172.83 ± 39.955 mg/dl, respectively).

Similarly, Nsr-Allah et al.[4] determined that uncontrolled diabetes patients' fasting plasma glucose levels were substantially higher than those of managed diabetic patients and controls (172.20 ± 26.52 vs. 100.65 ± 21.30 vs. 90.95 ± 8.66 mg/dl, respectively) and there was a statistically significant increase in HbA1C among (9.02 ± 1.38 vs. 6.20 ± 0.61 vs. 5.35 ± 0.44 %, respectively).

A previous study by Babic et al.[11] demonstrated that cases in the T2DM HbA1C more than or equal to 7 % group had significantly higher values of FBG, 2 h PPBG, and HbA1C (%) contrasted with T2DM individual in T2DM HbA1C less than 7 % group.

Regarding the salivary A2MG, the current study indicated a highly statistically significant rise of salivary A2MG among group A in comparison with group B and control (751.6 ± 146.6 vs. 590.2 ± 28.5 vs. 382.1 ± 30.2 ng/ml, respectively) ($P < 0.001$).

Such findings are in agreement with Rastogi and colleagues that studied the connection of salivary A2MG with glycosylated HbA1C on a total of 87 patients of T2DM who were divided according to HbA1C levels in to two groups (<7 and ^{3}7) and found a statistically significant increase in A2MG among diabetic patients with HbA1C more than or equal to 7 contrasted with diabetic cases with HbA1C less than 7 (2017.42 ± 575.13 vs. 772.54 ± 118.32, respectively).[5]

Similarly, El-Alfy and Khalil[12] demonstrated that the control group had a mean salivary A2MG level of 173.40 ± 58.76 ng/ml, the adequate group 337.90 ± 86.95, and the deficient group 998.81 ± 203.04, with statistically significant variations among the three groups.

The current research shown that the optimal cutoff point for salivary A2MG to discriminate among group A and group B was more than 662.5 ng/ml. This cutoff point had a sensitivity of 97.3 %, a specificity of 95.4 %, a PPV of 98.4 %, and an NPV of 97.2 % (AUC = 0.97 and $P < 0.001$). In addition, the optimal cutoff point for salivary A2MG to discriminate between group A and the control group was more than 550 ng/ml, which had a sensitivity of 97.1 %, a specificity of 94.7 %, a PPV of 97.6 %, and an NPV of 96.2 % (with an AUC = 0.97 and a $P < 0.001$). The optimal cutoff limit for salivary A2MG to discriminate among group B and the control group was more than 492.5 ng/ml, with 91.8 % sensitivity, 90.7 % specificity, 90 % PPV, and 88 % NPV (AUC = 0.92 and $P < 0.001$).

Nsr-Allah and colleagues that indicated that the best cutoff value of salivary A2MG as a predictor of

bad glycemic control (HbA1C > 7) was more than or equal to 645 ng/ml, with AUC of 0.92, sensitivity of 91.7 %, specificity of 90 %, moreover, the cut off value of salivary A2MG as a predictor for HbA1C of 5.7 (as a diagnostic number for prediabetes) was 425 ng/ml (ROC = 0.83, sensitivity 83.3 %, specificity 80 %, PPV 92.6 %, NPV 61.5 %, accuracy 82.5 %). On the other hand, the cutoff value for salivary A2MG as a predictor for HbA1C of 6.5 (as a diagnostic number for diabetes) was 565 ng/ml (ROC = 0.92, sensitivity = 91.7 %, specificity = 85 %, PPV = 94.8 %, NPV = 77.3 %, accuracy = 90 %).[4]

5.1. Conclusion

Since the present study discovered a strong correlation among levels of salivary A2MG and percentage of HbA1c, the measurement of A2MG in saliva provides a noninvasive and effective alternative approach to estimate glycemic index. This method has the potential to prevent the comorbidities of DM, which is a major benefit of the method.

Conflicts of interest

There are no conflicts of interest.

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