

Salivary α 2-macroglobulin as a marker for glycemic control in patients with type 2 diabetes mellitus

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Introduction

Panic from needle and the cost can discourage some diabetic patients from monitoring their blood glucose levels in a regular manner, so there is a critical need to find a new cost-effective, painless, and sensitive glucose-detection method. Many studies have found that α 2-macroglobulin (A2MG) concentrations are increased in the blood of both type 1 and type 2 diabetic patients.

Aim

The aim of the article is to study the salivary A2MG value as a marker for glycemic control in patients with type 2 diabetes mellitus (DM).

Patients and methods

A total of 60 patients were included and divided into three groups. Group 1 included patients with type 2 DM with glycosylated hemoglobin (HbA1c) levels more than or equal to 7% (inadequate glycemic control). Group 2 included patients with type 2 DM with HbA1c levels less than 7% (adequate glycemic control). Group 3 included healthy persons (control group). All patients were subjected to the following: thorough history taking, full physical examination, and laboratory investigations, including fasting blood glucose, cholesterol, triglycerides, HbA1c, and salivary A2MG.

Results

There were statistical significant relations between salivary A2MG and both BMI and duration of diabetes ($P < 0.05$) in type 2 uncontrolled diabetes group but not in controlled group ($P > 0.05$). There were statistically significant positive correlations between levels of salivary A2MG and HbA1c, cholesterol, triglycerides, fasting blood sugar, duration of DM, BMI, and age. The best cutoff value of salivary A2MG as a predictor of bad glycemic control, in relation to HbA1c, was more than or equal to 645 ng/ml, with area under the receiver operating characteristic curve of 0.92, sensitivity of 91.7%, specificity of 90%, and P value of less than 0.001.

Conclusion and recommendation

With the advantages of rapid, accessible, sensitive, cost-effective, and noninvasive method, salivary A2MG is a promising biological marker for glycemic control in patients with type 2 DM.

Keywords:

α 2-macroglobulin, salivary α 2-macroglobulin, type 2 diabetes mellitus

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Introduction

Diabetes mellitus (DM) is a metabolic disease with chronic hyperglycemia owing to defect in insulin action and/or insulin secretion [1].

Glycemic control is essential to manage the disease and avoid its complications. Fasting, 2 h postprandial, and glycosylated hemoglobin (HbA1c) are considered the methods for evaluation if there is good glycemic control or not. HbA1c is a nonenzymatic glycation between the hemoglobin and glucose particles. When using HbA1c for diabetes diagnosis, it is important to know that HbA1c is an indirect measure of average blood glucose values and to take other factors that may affect hemoglobin glycation into mind independently of glycemia, including race/ethnicity, age, hemoglobinopathies, and other factors

[2]. Moreover, panic from sharp objects (needle) can discourage some patients from monitoring their blood glucose levels in a regular manner. It has been documented that 20.5% of patients who had needle anxiety avoid all medical treatment [3]. Therefore, there is a critical need to find painless and satisfactory glucose-detection methods. In this concern, there is a need to define alternate screening methods and/or other types of biological samples to evaluate if there is good glycemic control or not in patients with type 2 DM [4].

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Saliva may be an important method of diagnosis of many diseases and could be valuable over existing methods. At present, saliva biomarkers indicate the possibility of developing a disease or its occurrence, as well as the response of the disease to the drug therapy. Saliva is useful in both old and young patients and has many diagnostic benefits. It is also useful in epidemiologic studies and screening for many diseases [5].

α 2-macroglobulin (A2MG) is a glycoprotein and considered as an important protease inhibitor. It is present in the circulation of both vertebrates and multiple invertebrates. In humans, it is found at significant plasma concentration and can be measured by many different methods [6].

Many studies have found that A2MG concentrations are increased in the blood of both type 1 and type 2 diabetic patients. This could be owing to enhanced clearance of tetrameric A2MG protease that may be compensated by increased synthesis of A2MG molecules. Moreover, increased values of A2MG may be owing to upregulation of acute-phase proteins in uncontrolled diabetic patients [7].

The aim of the present work is to study the salivary A2MG value as a marker for glycemic control in patients with type 2 DM.

Patients and methods

The protocol of this study was approved by the local ethics committee in February 2016. All procedures performed were in accordance with the ethical standards of the institutional research committee and with Helsinki declaration and its later amendments. The authors have no conflicts of interest, and they alone are responsible for the content and writing of the paper. This study was performed in Internal Medicine Department (Endocrinology Unit) and Clinical Pathology Department, Zagazig University Hospitals, in the period between April 2016 and June 2017. Sixty patients were included in this study, with age ranging between 23 and 65 years. The patients were divided into three groups according their DM profile (using the last criteria of American Diabetes Association [8]) as follows: group 1 included 20 patients with type 2 DM with HbA1c levels more than or equal to 7% (inadequate glycemic control); group 2 included 20 patients with type 2 DM with HbA1c levels less than 7% (adequate glycemic control); and group 3 included 20 healthy persons as a control group with fasting plasma glucose less than

100 mg/dl, 2 h plasma glucose less than 140 mg/dl, and HbA1c less than 5.7%.

Patients with rheumatic diseases, terminal illnesses, chronic inflammatory processes in the mouth, and neurological disorders that affect saliva (Alzheimer's disease, Huntington's disease, psychiatric disorders, Parkinsonism, amyotrophic lateral sclerosis, autism disorders, and multiple sclerosis [9]) all were excluded from our study.

After being informed on the purpose and procedures of the study, all patients signed an informed consent form and were subjected to the following: thorough history taking, full physical examination, and laboratory investigations, including fasting blood glucose using hexokinase method by spectrophotometry on cobas 8000 by Roche Diagnostics GmbH D-68298 (c702 module; Mannheim, Germany), cholesterol and triglycerides using spectrophotometer reaction on cobas 8000 (c702 module), HbA1c by turbidimetric inhibition immune assay on cobas 8000 (c501 module), and salivary A2MG using enzyme-linked immunosorbent assay (human A2MG kit; Cell Biolabs, San Diego, California, USA).

Sample collection

First, venous blood (4 ml) from the diabetic and control group after 10 h of fasting was collected and divided aseptically into two tubes: (a) serum tube, in which the blood was left for 10 min to be coagulated at room temperature, and then rapidly centrifuged for 20 min at speed of 2000–3000 rpm to take serum for chemical analysis, and (b) EDTA tube, in which prevention of blood coagulation was done by adding anticoagulant like EDTA for HbA1c analysis.

Second, unstimulated saliva (1 ml) from the diabetic and control group was collected. In brief, at 8 am, the patients were asked to rinse their mouths thoroughly with water before breakfast. They were then required to tilt their heads forward, and saliva was accumulated in the floor of the mouth for 2 min and collected into a sterile container. The saliva samples were centrifuged for 10 min at 1000g, immediately frozen and stored at -20°C until further analysis. On the day of the assay, saliva samples were centrifuged at 2000g for 10 min. The supernatants were used for the detection of salivary proteins.

Statistical analysis

Data were entered, checked, and analyzed using Epi-Info, version 6 (Centers for Disease Control and Prevention, Atlanta, Georgia, USA) and SPP for

Windows, version 8 (Microsoft, Redmond, Washington, USA). Baseline characteristics of the study population were presented as frequencies and percentages or mean values and SD. Analysis of variance test was used to analyze repeated measures.

Differences between two quantitative variables were compared by Student's *t* test. Correlation of numeric data was done by Pearson's correlation. Receiver operating characteristic (ROC) curve analysis was used to identify optimal cutoff values of A2MG with maximum sensitivity and specificity in relation to HbA1c at its diagnostic numbers for prediabetes, diabetes, and uncontrolled diabetes according to the last criteria of American Diabetes Association [7]. Area under curve (AUROC) was also calculated. Criteria to qualify for AUC were as follows: 0.90–1=excellent, 0.80–0.90=good, 0.70–0.80=fair, 0.60–0.70=poor, and 0.50–0.6=fail. The optimal cutoff point was established at point of maximum accuracy. Five percent probability is adopted as the level of statistical significance in all statistical tests ($P<0.05$), and $P<0.001$ was considered as highly significant.

Results

Table 1 describes demographic and clinical data of the studied groups. It demonstrates significant increased number of hypertensive cases in uncontrolled diabetic patients (group 1) when compared with controlled diabetic patients (group 2) and healthy persons

(group 3) ($P<0.05$). There were significant differences among the three groups regarding BMI with increase obesity in uncontrolled diabetic patients than controlled diabetic patients and healthy persons, and there were significant differences between two diabetic groups regarding the duration of DM ($P<0.05$) with increase duration of DM in uncontrolled patients comparing with controlled, but there were no significant differences regarding age, sex, smoking, and ischemic heart disease (IHD) ($P>0.05$) among the studied groups.

Table 2 shows the comparison among the studied groups regarding laboratory findings. It demonstrates that there were significant increases in triglyceride and cholesterol levels in uncontrolled diabetic patients when compared with controlled diabetic and control groups ($P<0.05$), and there were highly statistical significant increases in fasting plasma glucose level in uncontrolled diabetic patients compared with controlled diabetic and control groups ($P<0.001$). There were also high statistically significant increases in HbA1c and salivary A2MG levels in poor glycemic control group than controlled diabetic group and healthy group ($P<0.001$).

Table 3 shows the relation between salivary A2MG and demographic data in controlled (G1) and uncontrolled (G2) DM groups. There were no statistically significant differences regarding sex, age, and smoking and hypertension in both uncontrolled and controlled group ($P>0.05$).

Table 1 Comparison between studied groups regarding demographic and clinical data

Demographic data	Uncontrolled T2DM (G1) (N=20)	Controlled T2DM (G2) (N=20)	Healthy group (G3) (N=20)	Test	<i>P</i> value
Sex	[N(%)]	[N(%)]	[N(%)]		
Male	7 (35)	9 (45)	13 (65)	3.737	0.154
Female	13 (65)	11 (55)	7 (35)		
Age (mean \pm SD)	49.75 \pm 10.74	50.90 \pm 10.54	48.9 \pm 11.47	5.72	0.21
Smoking					
No	14 (70)	16 (80)	17 (85)	1.38	0.50
Yes	6 (30)	4 (20)	3 (15)		
Any comorbidity					
No	9 (45)	14 (70)	17 (85)	7.35	<0.05
Yes	11 (55)	6 (30)	3 (15)		
Hypertension					
No	9 (45)	15 (75)	17 (85)	8.01	<0.05
Yes	11 (55)	5 (25)	3 (15)		
IHD					
No	18 (90)	19 (95)	20 (100)	2.11	0.35
Yes	2 (10)	1 (5)	0 (0)		
BMI (mean \pm SD)	32.40 \pm 5.77	30.35 \pm 6.60	24 \pm 4.07	20.90	<0.001
DM duration (mean \pm SD)	13.85 \pm 7.23	8.20 \pm 3.59	0 \pm 0	-2.794	<0.05

IHD, ischemic heart disease; T2DM, type 2 diabetes mellitus.

Table 2 Comparison between studied groups regarding laboratory findings

Laboratory findings	Uncontrolled T2DM (G1) (N=20)	Controlled T2DM (G2) (N=20)	Healthy group (G3) (N=20)	P value
Fasting plasma glucose (mg/dl)				
Mean±SD	172.20±26.52	100.65±21.30	90.95±8.66	<0.001
Median (range)	178.50 (111–205)	95.50 (68–141)	90.50 (71–98)	
Triglycerides (mg/dl)				
Mean±SD	290.80±87.65	193.10±115.47	184.40±106.60	<0.05
Median (range)	289.50 (188–483)	141 (91–436)	140 (76–419)	
Total cholesterol (mg/dl)				
Mean±SD	274.50±93.22	192.05±69.81	195.70±88.10	<0.05
Median (range)	284.50 (144–585)	197.50 (97–389)	161.50 (103–397)	
HbA1c (%)				
Mean±SD	9.02±1.38	6.20±0.61	5.35±0.44	<0.001
Median (range)	8.90 (7.20–12.20)	6.30 (4.50–6.99)	5.55 (4.60–5.65)	
Salivary α2 macroglobulin (ng/ml)				
Mean±SD	820.65±190.17	331±98.01	146.90±42.01	<0.001
Median (range)	785 (580–1323)	329.50 (200–501)	135 (100–250)	

HbA1c, glycosylated hemoglobin; T2DM, type 2 diabetes mellitus.

Table 3 Relation between salivary α2 macroglobulin (ng/ml) and demographic data in uncontrolled (G1) and controlled (G2) diabetes mellitus groups

Groups	Parameters	N	Salivary α2 macroglobulin (ng/ml)		Test	P value
			Mean±SD	Median (range)		
G1	Sex				0.630	0.540
	Male	7	347.333±125.22	385 (200–501)		
	Female	13	317.63±72.61	320 (200–411)		
	Age				0.535	0.599
	≤50 years	7	347.28±117.05	390 (200–481)		
	>50 years	13	322.23±90.12	320 (200–501)		
	Smoking				–0.250	0.817
	No	14	326.75±81.26	329.50 (200–470)		
	Yes	6	348.00±165.37	345.50 (200–501)		
G2	HTN				–0.04	0.595
	No	9	806.66±193.84	750 (600–1250)		
	Yes	11	832.09±195.79	800 (580–1323)		
	Sex				0.909	0.393
	Male	9	886.85±284.35	780 (600–1323)		
	Female	11	785.00±113.57	790 (580–970)		
	Age				–0.04	0.543
	≤50 years	9	773.88±119.94	750 (600–970)		
	>50 years	11	858.90±231.57	800 (580–1323)		
G2	Smoking				–1.098	0.317
	No	16	779.28±111.19	770 (580–970)		
	Yes	4	917.16±298.85	815 (600–1323)		
	HTN				–0.087	0.931
	No	15	329.86±99.16	339 (200–501)		
Yes	5	334.40±105.82	305 (200–470)			

HTN, hypertension.

Table 4 shows the relation between salivary A2MG and clinical data in uncontrolled (G1) and controlled (G2) DM groups. There were statistically significant relations between salivary A2MG and both BMI and duration of DM ($P<0.05$) in type 2 DM uncontrolled group; otherwise, the controlled group did not show any significant relation ($P>0.05$).

In Table 5, salivary A2MG (ng/ml) level and study parameters were statistically analyzed and correlated with each other in all our 60 patients. We found that there were highly statistically significant positive correlations regarding HbA1c ($r=0.927$, $P<0.001$), total cholesterol ($r=0.496$, $P<0.001$), triglycerides ($r=0.540$, $P<0.001$), fasting blood sugar ($r=0.788$, $P<0.001$), duration of DM ($r=0.564$, $P<0.001$), and

Table 4 Relation between salivary α 2 macroglobulin (ng/ml) and clinical data in uncontrolled (G1) and controlled (G2) diabetes mellitus groups

Groups	Parameters	N	Salivary α 2 macroglobulin (ng/ml)		Test	P value
			Mean \pm SD	Median (range)		
G1	BMI				-1.753	<0.05
	Average	2	785.00 \pm 96.04	780 (690–950)		
	Overweight	7	800.45 \pm 191.08	750 (580–1250)		
	Obese	11	1056.50 \pm 376.88	1056 (790–1323)		
	Duration of DM				-2.270	<0.05
	5–15 years	14	763.21 \pm 157.15	735 (580–1250)		
	>15 years	6	954.66 \pm 205.97	942 (700–1323)		
G2	BMI				0.186	0.832
	Average	5	354.00 \pm 103.63	390 (210–470)		
	Overweight	8	318.62 \pm 112.78	327.50 (200–501)		
	Obese	7	328.71 \pm 88.15	320 (210–481)		
	Duration of DM				0.444	0.662
	<5 years	3	354.66 \pm 56.92	385 (289–390)		
	5–15 years	17	326.82 \pm 104.30	320 (200–501)		

DM, diabetes mellitus.

Table 5 Correlation between salivary α 2 macroglobulin (ng/ml) and study parameters

Parameters	Salivary α 2 macroglobulin (ng/ml)							
	Group 1 (N=20)		Group 2 (N=20)		Healthy group (N=20)		All Subjects (N=60)	
	r	P value	r	P value	r	P value	r	P value
Age	+0.208	0.380	+0.103	0.665	+0.155	0.514	+0.341	<0.05
BMI	-0.101	0.673	-0.248	0.292	-0.256	0.277	+0.467	<0.001
DM Duration	+0.533	<0.05	+0.244	0.300	–	–	+0.564	<0.001
Fasting sugar	+0.586	<0.05	+0.146	0.539	+0.650	<0.05	+0.788	<0.001
Triglycerides	+0.316	0.174	+0.235	0.319	+0.621	<0.05	+0.540	<0.001
Total cholesterol	+0.284	0.224	+0.329	0.157	+0.298	0.202	+0.496	<0.001
HbA1c	+0.778	<0.001	+0.666	<0.05	+0.474	<0.05	+0.927	<0.001

DM, diabetes mellitus; HbA1c, glycosylated hemoglobin.

BMI ($r=0.467$, $P<0.001$) and significant positive correlation with age ($r=0.341$, $P<0.05$), and we can notice that this positive correlation was more evident in uncontrolled type 2 DM group (G1), especially regarding the DM duration and DM control parameters (fasting sugar and HbA1c).

The regression equation for prediction of HbA1c using the level of A2MG is as follows: HbA1c (%) = 3.13 + [0.006 \times the level of salivary A2MG (ng/ml)]. Considering HbA1c (%) as a dependent variable, 3.13 as a constant, 0.006 is the beta coefficient, and salivary A2MG (ng/ml) as the independent variable.

Table 6 shows ROC curve to evaluate validity of salivary A2MG as a predictor for glycemic control, in relation to HbA1c. Our results revealed that the best cutoff value of salivary A2MG as a predictor for HbA1c = 7% (as alarming number for bad glycemic control in type 2 DM) is 645 ng/ml [AUROC 0.92, sensitivity 91.7%, specificity 90%, positive predictive value (PPV) 96.5%, negative predictive value (NPV)

78.3%, accuracy 91.2%; $P<0.001$], the cutoff value of salivary A2MG as a predictor for HbA1c = 5.7 (as a diagnostic number for prediabetes) is 425 ng/ml (AUROC 0.83, sensitivity 83.3%, specificity 80%, PPV 92.6%, NPV 61.5%, accuracy 82.5%; $P<0.001$), but the cutoff value of salivary A2MG as a predictor for HbA1c = 6.5 (as a diagnostic number for diabetes) is 565 ng/ml (AUROC 0.92, sensitivity 91.7%, specificity 85%, PPV 94.8%, NPV 77.3%, accuracy 90%; $P<0.001$).

Discussion

During past three decade, HbA1c has been used as a biomarker for evaluating glycemic control in type 1 and type 2 diabetes [10]. It is well recognized that HbA1c predicts the microvascular and macrovascular diabetic complications [11]. The obtaining of a blood sample for HbA1c is considered as an invasive procedure and is associated with psychological and physical insult to the patients, making them worried about monitoring their diabetic state. As there are many factors which can

Table 6 Receiver operating characteristic curve to evaluate validity of cutoff value for salivary α 2 macroglobulin as a predictor for glycemic control, in relation to glycosylated hemoglobin

Salivary A2MG (ng/ml)	HbA1c (%)	AUROC	Sens (%)	Spec (%)	PPV (%)	NPV (%)	Accuracy (%)	P
425	5.7	0.83	83.3	80	92.6	61.5	82.5	<0.001
565	6.5	0.92	91.7	85	94.8	77.3	90	<0.001
645	7	0.92	91.7	90	96.5	78.3	91.2	<0.001

A2MG, α 2 macroglobulin; AUROC, area under receiver operating characteristic curve; HbA1c, glycosylated hemoglobin; NPV, negative predictive value; PPV, positive predictive value; Sens, sensitivity; Spec, specificity.

affect HBA1c levels including race/ethnicity, age, hemoglobinopathies, uraemia and other factors [2], that give a critical need to evaluate painless, simple and satisfactory new alternate screening methods for DM detection and mandate usage of other types of biological samples to measure glycemic control in type 2 DM [4].

A2MG is a glycoprotein generated by the liver and induced by other factors including cytokines in an acute-phase condition. A2MG synthesis may be enhanced in diabetic patients with upregulation of the acute-phase proteins. Therefore, increased clearance of tetrameric A2MG protease complexes will be compensated by enhanced formation of entire A2MGs, resulting in an increase in the circulating form of A2MG molecules [12].

A2MG is considered as the main plasma anti-proteinase protein, and many previous studies defined and applied its plasma levels as a marker for the screening, diagnosis, and prognosis of many diseases, including diabetes. Many low-molecular-weight plasma proteins may be lost in urine and affected in diabetic nephropathy such as albumin (65 kDa), cystatine (13 kDa), transferrin, and α 1 microglobulin; however, A2MG is a large molecule and has a molecular weight of 725 000 kDa, so is not affected by nephropathy [13].

As saliva contains many serum proteins secreted from the salivary glands, if A2MG is present at high levels in plasma, it is possible that A2MG molecules could be exocytosed from the blood to the saliva [14].

Therefore, the aim of our work was to study the salivary A2MG as a biological indicator of diabetes with the advantages of easy noninvasive collection of saliva by individuals with limited training, and no special equipment needed for its collection. Diagnosis of a disease by doing the analysis of saliva is potentially valuable for children and older adults, as collection of the fluid is associated with fewer compliance problems in comparison with the collection of blood, like no risk of infections transmitted through needle injection. Furthermore, the analysis of saliva seems to be a

simple and cost-effective approach in the screening of large populations; therefore, we hope to consider salivary A2MG as a useful biological indicator of diabetes control.

Our study was conducted on 60 cases divided into three groups. Group 1 included 20 patients with HbA1c levels more than or equal to 7% (inadequate glycemic control), group 2 included 20 patients with HbA1c levels less than 7% (adequate glycemic control), and group 3 included 20 nondiabetic persons as a control group.

As shown in Table 1, 29 (48%) cases were males, whereas females represented 31 (52%), with age ranged from 25 to 73 years. Thirteen (22%) cases were smokers. There were no significant differences among the three groups regarding age, sex, and smoking. Regarding comorbidities, namely, hypertension and IHD, which are the most common comorbidities occurring in DM, 16 patients from diabetic groups were hypertensive (40% of diabetic patients). This result was near to the results of Kowall *et al.* [15], where 48.3% of diabetic cases were hypertensives; Animaw and Seyoum [16], where 48.8% of diabetic patients had hypertension; and Robinson *et al.* [17], which had 42.2% of hypertensive diabetic cases in their diabetic groups. However, the results were contrary to the results of Perotto *et al.* [18], which had 76.6% of diabetic cases with hypertension. This difference may be explained by the limited number of cases in our study and all of studied diabetic patients were obese.

Similarly, in Table 1, there were three patients only from the two diabetic groups who had IHD (7.5% of diabetic patients). This matches the results of Piniés *et al.* [19], which had 8.8% prevalence of coronary heart disease in type 2 diabetic patients in their study. Our results were dissimilar to the results of Perotto *et al.* [18], which showed that 21.8% of the diabetic patients had ischemic heart disease, and Robinson *et al.* [17], which showed that 19.34% of the diabetic patients had coronary heart disease. This difference may be explained owing to only one-half of our diabetic patients (20 patients) had poorly controlled diabetes

and that the incidence of coronary heart disease increases with poor glycemic control.

Our results revealed significant increased BMI in uncontrolled diabetic group in comparison with controlled diabetic group and healthy group. These results agree with the results of Piniés *et al.* [19], who expected that individuals with BMI greater than 40 kg/m² are seven times more likely to develop diabetes than those with normal BMI. The association between obesity and type 2 diabetes is so strong that a new term called diabetes has appeared [20].

Moreover, in Table 1, the duration of diabetes was significant longer in uncontrolled diabetic group owing to the progressive nature of the disease [1].

Regarding laboratory parameters of the studied group listed in Table 2, there were statistical significant differences among the three groups in total cholesterol, triglyceride, and fasting blood glucose levels. These results agree with the results of Tsujimoto and Kajio [21] and Elley *et al.* [22], which showed high prevalence of dyslipidemia in their diabetic patients.

As described in the same Table 2 regarding the indicators of glycemic control, there were highly statistically significant increases in HbA1c and salivary A2MG levels in poor glycemic control group than controlled diabetic group and healthy group. Our results are in agreement with the results of Chung *et al.* [23], who used enzyme-linked immunosorbent assay for detection of salivary A2MG and found that there were significant increases in HbA1c and salivary A2MG levels in diabetic patients before and after 3 months of stable follow-up to prove that salivary A2MG might be used as a screening and monitoring method of diabetes.

Our results matched the results of a study which used quantitative radial immunodiffusion for detection of serum A2MG and found that A2MG was significantly higher in diabetic patients than in normal populations and found that there was an association between high level of this protein and diabetic retinopathy, which may provide additional information about relation between high level of A2MG and retinal complication [24].

Moreover, our data are in alignment with another study which used mass spectrometer for detecting A2MG and found that A2MG levels were associated with glycemic control and also found a relation between

A2MG and HbA1c in 43 diabetic patients without anemia or nephropathy, because anemia might cause lower HbA1c and proteinuria might cause massive loss of low-molecular-weight proteins, resulting in elevation of high-molecular-weight proteins such as A2MG. These findings strengthen the hypothesis that detecting salivary A2MG level may be an effective method for monitoring diabetes control [7].

As listed in Table 3, salivary A2MG was strongly positively correlated with fasting blood glucose and HbA1c levels in patients with type 2 DM. This comes in agreement with the study of Chung *et al.* [23] which declared a strong positive correlation between HbA1c and both blood and salivary A2MG in patients with type 2 DM. Our results are in line with those of Rao *et al.* [25], which detected higher concentrations of salivary and blood A2MG in prediabetic patients compared with healthy control group, indicating that there is a strong association between glycemic control and salivary levels of A2MG.

Moreover, in Table 3, salivary A2MG shows a strong positive correlation with BMI and duration of diabetes in type 2 DM. These results match with that of Ahmad *et al.* [26], which explored that in patients with type 2 diabetes, the serum A2MG level showed a direct positive correlation with the duration of diabetes, but the same study observed no significant relationship between serum A2MG level and either fasting blood glucose or HbA1c.

Moreover, our results revealed a significant positive correlation between salivary A2MG and both total cholesterol and triglycerides, which comes in alignment with Chung *et al.* [23], which showed high salivary A2MG level associated with high total cholesterol and triglycerides in diabetic patients, but it was statistically nonsignificant, which may be owing to higher BMI in our study than that of Chung *et al.* [23]. Regarding HbA1c, our results came in agreement with the results of Aitken *et al.* [27], who found a positive correlation between salivary A2MG level and HbA1c percentage ($r=0.7748$ and $P<0.001$) in patients with type 2 DM, which is approximately the same as our results ($r=0.778$ and $P<0.001$).

Salivary A2MG was also higher in older age than younger ages, especially with long diabetic course. It may be attributed to higher incidence of gingival and dental complications in older ages. Periodontal status is also related with saliva levels of A2MG; in fact, one study has described that A2MG levels in crevicular fluid are significantly higher in diabetic patients with

acute periodontitis compared with those with chronic periodontitis [28].

In our study, we examined the periodontal status by an oral clinical examination only, but this diagnosis may require other complementary examination such as radiography. In this regard, we may assume that patients with inadequate glycemic control included in our study may also present with some sort of periodontitis that may contribute to the high levels of A2MG. It was previously described that edentulous patients with type 2 DM had high concentrations of salivary biomarkers associated with inflammatory processes, including A2MG [29].

Table 6 demonstrated the use of salivary A2MG level for prediction of poor glycemic control in DM2. The results obtained from ROC curve, in relation to HbA1c as the gold standard for diagnosis of glycemic control, ROC curve was constructed at the most discriminating cutoff value (645 ng/ml) with significant area under curve (AUC=0.92, sensitivity of 91.7%, specificity 90% and $P<0.001$) which was optimal to predict uncontrolled DM2 patients (HbA1c $\geq 7\%$) indicating that A2MG could be used as a diagnostic method for detection of inadequate glycemic control.

In agreement with our results, Aitken *et al.* [27] showed similar results. The area under ROC curve indicated a positive discrimination threshold of A2MG (AUC=0.903, 95% confidence interval, $P<0.001$), and also the optimal cutoff value for prediction of poor glycemic control was 840 ng/ml (sensitivity of 81.9% and specificity of 89.6%) for patients of uncontrolled type 2 DM. According to our results, the HbA1c can be predicted by using the level of salivary A2MG by the following regression equation: $HbA1c (\%) = 3.13 + [0.006 \times \text{the level of salivary A2MG (ng/ml)}]$. However, more future studies are needed to conduct on a larger scale to evaluate this equation.

Conclusion

With the advantages of rapid, accessible, cost-effective, and noninvasive method [30], salivary A2MG is a promising biological marker for glycemic control in patients with type 2 DM. For individuals with modest training, whole saliva provides a good method for type 2 DM screening and/or monitoring of large populations. Our conclusion was supported by previous studies [27,31,32] which have considered saliva in diagnosis and monitoring of type 2 DM, as a simple alternative to blood.

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Authors contribution: Ayman Abd-Elrahman Mohamed Nsr-Allah designed the research and collected the materials and clinical data. Saad El-Osh supervised the laboratory methods. Azza M. Ahmed performed statistical analysis. Sara Hazem wrote the paper. All authors provided a critical revision of the article and approved the final version.

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Conflicts of interest

There are no conflicts of interest.

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