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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: Diabetes mellitus is the third leading cause of death and due to limited access to the quality health care, the diabetic patients are more prone to develop complications.
Objective: The aim of the present study was to evaluate α-2-macroglobulin in saliva as marker for glycemic control in type 2 diabetic patients.
Patients and Methods: This cross-sectional observational study included 90 subjects, classified into 3 groups: Group (1) included 40 patients with type 2 DM with HbA1c levels more than or equal to 7% (inadequate glycemic control). Group (2) included 40 patients with type 2 DM with HbA1c levels less than 7% (adequate glycemic control). Group (3) included 10 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%. All patients were subjected to full history taking, complete clinical examination, laboratory investigations and assessment of salivary α-2-macroglobulin. **Results:** The mean level of salivary α-2-macroglobulin (A2MG) in the control group was 173.40 ± 58.76 ng/ml, in the adequate glycemic control group, it was 337.90 ± 86.95 and in the inadequate glycemic control group, the level was 998.81 ± 203.04 ng/ml. There was high statistically significant difference between the three groups.
Conclusion: 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.
Keyword: α-2-macroglobulin, Saliva, Glycemic control, DM, Type 2.

INTRODUCTION

Diabetes mellitus is the third leading cause of death and due to limited access to the quality health care, the diabetic patients are more prone to develop complications. Screening requires measurement of blood sugar levels. The best parameter is glycosylated hemoglobin (HbA1c), which gives an overview of an individual's glycemic control of previous 4 months. However, this procedure is invasive and requires blood sample [(1)].

Glycemic control is essential to manage the disease and to 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 [(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)].

The noninvasive method of estimation of diabetic biomarker in salivary samples is required. Saliva is a source of the alpha-2-macroglobulin (A2MG) other than blood. In type 1 and type 2 diabetes mellitus (DM), the A2MG levels are increased in blood [(4)].

A2MG synthesis is enhanced in diabetic patients. A2MG is produced by liver and acts as a plasma antiproteinase. The high serum A2MG decreases the bioavailability of insulin, leading to impairment of blood sugar control [(5)].

The aim of the present study was to evaluate α-2-macroglobulin in saliva as marker for glycemic control in type 2 diabetic patients.

PATIENTS AND METHODS

This cross-sectional observational study included 90 subjects, who came for follow up at Internal Medicine Department, Benha University Hospitals during the period from January 2021 to December 2021. Patients were classified into 3 groups (using the last criteria of American Diabetes Association):

- **Group 1:** Included 40 patients with type 2 DM with HbA1c levels more than or equal to 7% (inadequate glycemic control).
- **Group 2:** Included 40 patients with type 2 DM with HbA1c levels less than 7% (adequate glycemic control).
- **Group 3:** Included 10 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%.

Inclusion criteria:

- Age between 30 and 60 years.
- T2DM patients according to the last criteria of American Diabetes Association.

Exclusion criteria:

- Associated diabetic complications (diabetic ketoacidosis, hyperosmolar non-ketotic coma).
- Rheumatic diseases.
- Nephrotic syndrome.
- Auto immune disease.
- Associated acute inflammatory conditions in the mouth.
- Pregnant females.
- Association of other neurological disease.

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All patients were subjected to full history taking, complete clinical examination, and laboratory investigations (fasting blood glucose, post-prandial blood glucose, HbA1c, lipid profile, and urinary albumin/creatinine ratio). Assessment of salivary α-2-macroglobulin was performed by using human α2 macroglobulin (α2-MG) ELISA Kit (Shanghai Crystal Day Biotech Co. Limited) (Cat. No: E1097Hu).

Ethical consent:

Written informed consent was obtained from every participants before inclusion in the study. The whole study design was approved by Ethical Scientific Committee of Faculty of Medicine, Benha University. Confidentiality and personal privacy was respected in all levels of the study. Patients were free to withdraw from the study at any time without any consequences. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Statistical analysis

Results were statistically analyzed using statistical package of social sciences (SPSS 22.0, IBM/SPSS Inc., Chicago, IL). Two types of statistical analyses were conducted: Descriptive statistics, which included estimates for summarizing the continuous data as mean (X) and standard deviation (SD) for normally distributed data or median (Med) and interquartile range (IQR) for skewed data. Frequency with percentage (%) was used for presenting qualitative data. Pearson Chi-square (χ^2) test was used to compare between two or more groups regarding one qualitative variable. Fisher's Exact Test was used instead of Chi-Square (χ^2) test when the assumption that at least 80% of the expected frequencies are greater than five was violated. One-way ANOVA test was used for continuous data to test for significant difference between more than two normally distributed groups. Assumptions of normality in each group and homogeneity of variances were verified using Shapiro-Wilk test and Levine's test, respectively. Kruskall-Wallis test was used when ANOVA assumptions were violated to compare between more than two groups of skewed data. Tukey honestly significant difference (Tukey-HSD) test was used as a post hoc test to adjust for multiple comparisons after significant ANOVA test to indicate which significant difference between pairs of groups whereas Bonferroni post hoc test was used after significant Kruskall-Wallis test. Spearman rank correlation coefficient (r_s) – a non-parametric equivalent to Pearson correlation coefficient – was calculated to indicate strength and direction of association between two numerical variables, both are continuous but not normally distributed or at least one of them is ordinal. Receiver operating characteristic (ROC) analysis, which is graphical plot of sensitivity against one minus the specificity (false positive rate) for different cutoffs. The optimal cutoff value was determined using Youden index J that is the farthest point on ROC curve from the diagonal line of equality [maximum (sensitivity + specificity)-1]. In all applied tests, the P-values associated with test statistics indicated the significance level at which the null-hypothesis (the hypothesis of no difference) was rejected and it was set at 0.05 so that a P-values > 0.05 are statistically non-significant, P-values ≤ 0.05 are significant, and P-values < 0.01 are highly significant.

RESULTS

The mean age of the control group was 42.17 ± 5.91 years, 42.93 ± 5.91 years in the adequate glycemic control T2DM group and 44.93 ± 6.81 years in the inadequate glycemic control T2DM group with no significant difference between the three groups (p=0.285). Regarding gender distribution, there were (60%, 62.5% and 67.5% males in the control, adequate glycemic control T2DM and inadequate glycemic control T2DM groups respectively) while there were (40%, 37.5% and 32.5% females in the control, adequate glycemic control T2DM and inadequate glycemic control T2DM groups respectively) with no significant difference in the gender distribution of the cases included in the study (p=0.545) (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 level of FBG, 2Hr PPBG and HbA1c (%) were statistically significantly higher in the Inadequate glycemic control group as compared to the control and adequate diabetic control group. Also, statistically significantly higher in the adequate glycemic control group as compared to the control group. The level of Urinary Albumin-creatinine ratio

was statistically significantly higher in the inadequate glycemic control group as compared to the control and adequate diabetic control group (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 mean level of salivary α-2-macroglobulin in the control group was 173.40 ± 58.76 ng/ml, in the adequate glycemic control group, the level was 337.90 ± 86.95 ng/ml while in the inadequate glycemic control group, the level was 998.81 ±203.04 ng/ml. There was high statistically significant difference between the three groups (Figure 1).

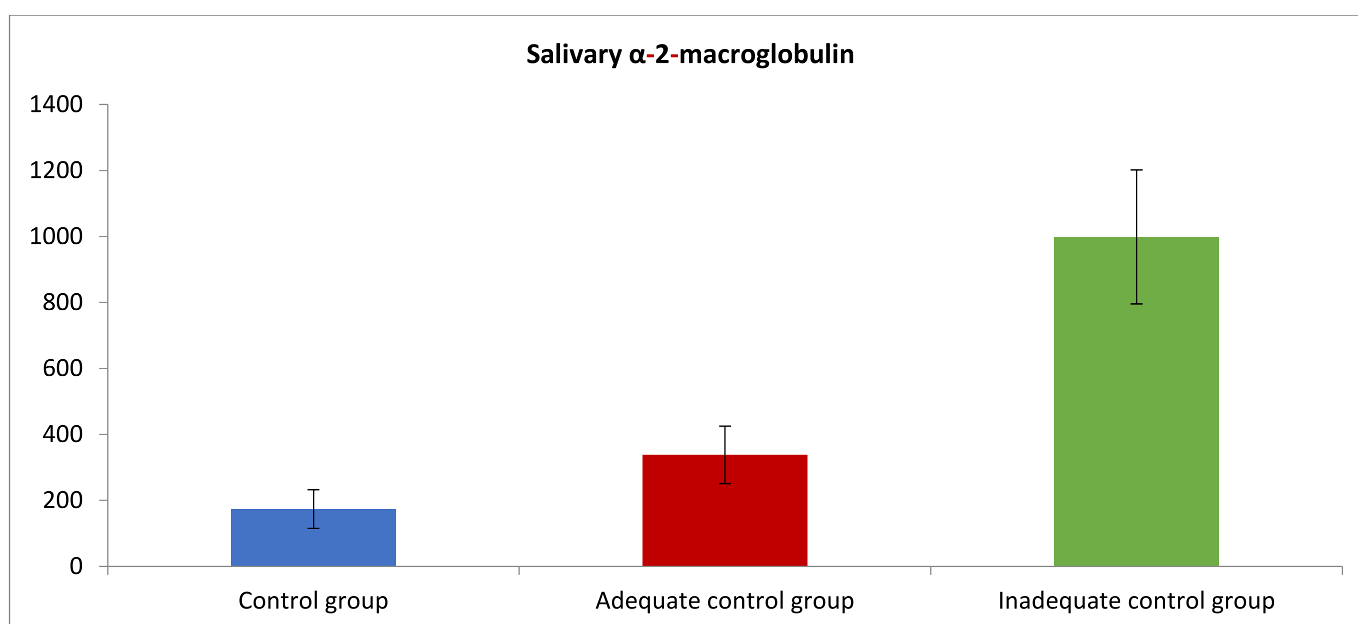

Figure (1): Level of Salivary α2 macroglobulin n the three group

ECG changes were detected in 3 cases (7.5%) with adequate glycemic control and in 5 cases (12.5%) with inadequate glycemic control while no cases revealed ECG changes in the control group. There was no statistically significant difference between the three groups regarding 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.

In both the adequate glycemic control group and the inadequate glycemic control, there was statistically significant moderate positive correlation between *α*-2-macroglobulin with each of FBG, 2Hr PPBG, HBA1c and BMI (Table 4).

Table (4): Correlation between α-2-Macroglobulin 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 best cutoff point of salivary α-2-macroglobulin to differentiate between the control group and adequate glycemic control group was 226.5 ng/ml with 92.5% sensitivity, 80 specificity, 94.5% PPV, 78% NPV and 86.4 % accuracy (Figure 2). The best cutoff point of salivary α-2-macroglobulin to differentiate between the adequate glycemic control group and inadequate glycemic control group was 521.3 ng/ml with 95.5% sensitivity, 98 specificity, 96.4% PPV, 98% NPV and 97.6 % accuracy (Figure 3).

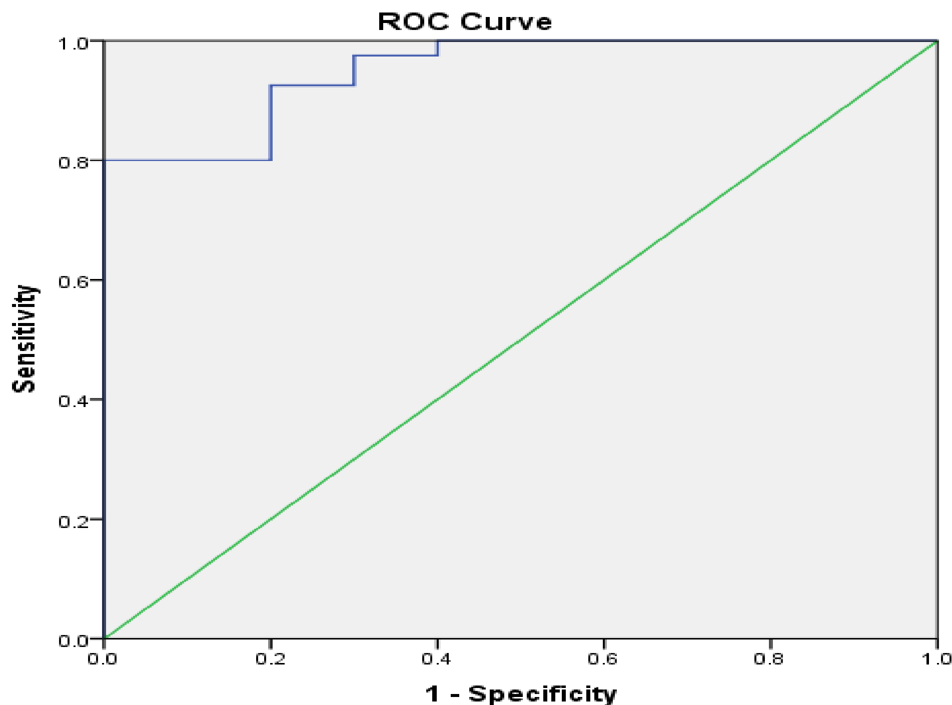

Figure (2): Roc curve of salivary α2 macroglobulin to differentiate between control and adequate

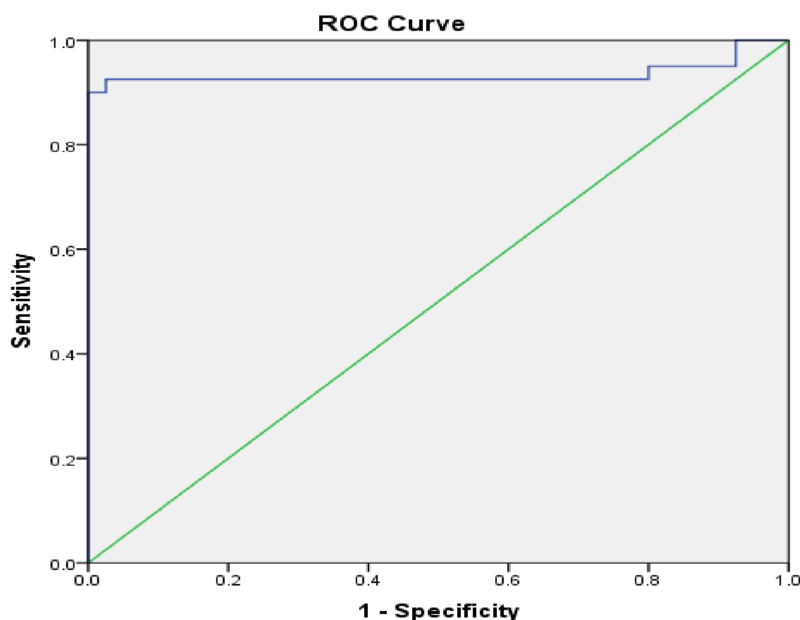

Figure (3): Roc curve of salivary α2 macroglobulin to differentiate between adequate glycemic control and inadequate glycemic control groups.

DISCUSSION

In this study, the mean level of salivary α-2-macroglobulin in the control group was 173.40 ± 58.76 ng/ml, in the adequate glycemic control group, the level was 337.90 ± 86.95, while in the inadequate glycemic control group, the level was 998.81 ± 203.04 ng/ml. There was high statistically significant difference between the three groups. This came in accordance with **Nsr-Allah and his colleagues** [(6)]**,** who reported that there were also high statistically significant increases in salivary A2MG levels in poor glycemic control group than in controlled diabetic group and healthy group (P<0.001). Our results are in agreement with the results of **Chung *et al.*** [(7)] 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. Moreover, our data are in alignment with a study of **Takada *et al.*** [(8)] where they 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. Another study **Rao *et al.*** [(9)] enrolled 20 participants with either type 2 diabetes or prediabetes, and found that salivary proteins including A2MG 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 shown that the salivary A2MG profile highlights the importance of the innate immune system in the pathogenesis of type 1 diabetes and related complications. A higher salivary A2MG level was only found in the group of type 1 diabetes patients complicated with retinopathy and nephropathy rather than in non- complicated type 1 diabetes patients.

In this study, in both the adequate glycemic control group and the inadequate glycemic control group, there was statistically significant moderate positive correlation between α-2-macroglobulin with each of FBG, 2Hr PPBG and HBA1C. This comes in accordance with **Nsr-Allah *et al.*** [(6)] who reported that salivary A2MG was strongly positively correlated with fasting blood glucose and HbA1c levels in patients with type 2 DM. In addition, this comes in agreement with the study of **Chung *et al.*** [(7)] which declared a strong positive correlation between HbA1c and both blood and salivary A2MG in patients with type 2 DM. Moreover, **Rastogi *et al.*** [(11)] found correlation between saliva levels of A2MG and HbA1c (r = 0.994 and P =0.001) in DM2. HbA1c groups highly correlated with A2MG. Similarly, Pearson correlation coefficient was calculated for HbA1c and A2MG. It showed that there was good linear correlation between HbA1c and A2MG (r = 0.977, P < 0.001). Our results are in line with those of **Rao *et al.*** [(9)] which detected higher concentrations of salivary and blood A2MG in prediabetic patients compared to healthy control group, indicating that there is a strong association between glycemic control and salivary levels of A2MG. Additionally, our results come in agreement with the results of **Aitken *et al.*** [(5)] 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). Our results also agree with **Nsr-Allah *et al.*** [6] who reported that salivary A2MG shows a strong positive correlation with BMI and duration of diabetes in type 2 DM. **Ahmad *et al.*** [12] found that in patients with type 2 diabetes, the plasma A2MG level shows a direct positive correlation with the duration of diabetes and different levels of microalbuminuria. However, **Ahmad *et al.*** [12] observed no significant relationship between A2MG level with either FPG or HbA1c.

In this study, the best cutoff point of salivary α-2-macroglobulin to differentiate between the control group and adequate glycemic control group was 226.5 ng/ml with 92.5% sensitivity, 80 specificity, 94.5% PPV, 78% NPV and 86.4 % accuracy. The best cutoff point of salivary α-2-macroglobulin to differentiate between the adequate glycemic control group and the inadequate glycemic control group was 521.3 ng/ml with 95.5% sensitivity, 98 specificity, 96.4% PPV, 98% NPV and 97.6 % accuracy. **Nsr-Allah *et al.*** [6] showed that according to 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, with sensitivity of 91.7%, specificity 90% and P<0.001) which was optimal to predict uncontrolled DM2 patients (HBA1c ≥ 7%) indicating that A2MG could be used as a diagnostic method for detection of inadequate glycemic control. **Aitken *et al.*** [5] showed that 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.

The different cutoff points reported in different studies could be explained to be due to different criteria of the included cases and different kits used that could reveal differences in their sensitivity. 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 patients with aggressive periodontitis compared to those with chronic periodontitis [13]. For this strong association, further studies are required for careful assessment of periodontal status by an oral clinical and complementary investigations such as radiography.

CONCLUSION

With the advantages of rapid, accessible, cost-effective, and noninvasive method, 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.

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Conflicts of interest: No conflicts of interest.

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