Compare Salivary Alpha-2 Macroglobulin with Glycosylated Hemoglobin as a Marker of Glycemic Control

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Abstract	Original Research Article

Background: Glycemic control is crucial for managing type 2 diabetes mellitus (T2DM), with HbA1c being the gold standard for long-term monitoring. However, HbA1c has limitations, including its inability to reflect short-term glucose fluctuations and susceptibility to conditions like anemia. Salivary alpha-2 macroglobulin (A2MG), a non-invasive biomarker, has emerged as a potential alternative for assessing glycemic control. ***Objective:*** This study aimed to compare the efficacy of salivary A2MG and HbA1c in reflecting glycemic control among patients with T2DM in Bangladesh. ***Methodology:*** A cross-sectional study was conducted among 80 patients diagnosed with T2DM at Bangabandhu Sheikh Mujib Medical University, Dhaka, from March 2022 to February 2023. Participants were divided into adequately controlled (HbA1c <7%) and inadequately controlled (HbA1c ≥7%) groups. Salivary A2MG levels were measured using ELISA, while HbA1c was analyzed using an automated biochemistry analyzer. Statistical analyses included correlation and receiver operating characteristic (ROC) curve analysis to evaluate diagnostic performance. ***Results:*** Mean salivary A2MG levels were significantly higher in inadequately controlled patients (280.6 ± 161.7 ng/ml) compared to adequately controlled patients (83.6 ± 25.3 ng/ml, p<0.001). A strong positive correlation was observed between salivary A2MG and HbA1c (rho=0.738, p<0.001). ROC curve analysis demonstrated an area under the curve (AUC) of 0.915 for salivary A2MG, with a sensitivity of 85.4% and specificity of 81.3% at a cutoff value of 100.1 ng/ml. ***Conclusion:*** Salivary A2MG shows strong potential as a non-invasive biomarker for glycemic control, correlating significantly with HbA1c and offering high diagnostic accuracy. It provides a practical, cost-effective alternative for diabetes management, particularly in resource-limited settings.
Keywords: Salivary alpha-2 macroglobulin, HbA1c, glycemic control, type 2 diabetes mellitus, non-invasive biomarker, Bangladesh.

INTRODUCTION

Glycemic control is a critical aspect of managing diabetes mellitus, a condition that has reached epidemic proportions worldwide, including in Bangladesh. Monitoring blood glucose levels regularly is essential for assessing the effectiveness of diabetes management. Traditional marker like HbA1c (glycosylated hemoglobin) have long been used to evaluate long-term glycemic control [1-3]. However, these markers have certain limitations, such as their inability to accurately reflect fluctuations in blood glucose over short periods or their influence by conditions like anemia. As a result, there is increasing interest in exploring alternative biomarkers that could provide a more accurate and timely assessment of glycemic control [4-5].

Salivary alpha-2 macroglobulin (A2M) has emerged as a promising marker for glycemic control in recent studies. Alpha-2 macroglobulin is a large glycoprotein that plays a role in inflammation and tissue repair [6-7]. Recent findings have suggested that it is present in the saliva of individuals with diabetes in altered concentrations, and may correlate with blood

glucose levels, offering a potential non-invasive biomarker. The use of salivary A2M could provide a convenient and effective method of monitoring diabetes, especially in populations where regular blood sampling may be less feasible [8-9].

On the other hand, glycosylated hemoglobin (HbA1c) remains the gold standard for long-term glycemic control, as it reflects the average blood glucose levels over the past 2-3 months. HbA1c levels are widely used in clinical settings for diabetes diagnosis and management. Even certain medications, which can sometime lead to inaccurate assessments of a patient's true glycemic control [10-13]. Furthermore, its inability to provide real-time data on blood glucose variations limits its effectiveness for short-term adjustments in therapy.

In Bangladesh, where diabetes is a growing public health concern, the need for reliable and accessible markers for glycemic control is more critical than ever. With limited healthcare infrastructure in some regions, the development of non-invasive diagnostic tools like salivary A2M could significantly enhance the management of diabetes, particularly for rural or underserved populations. This research aims to compare the effectiveness of salivary A2M with HbA1c in reflecting glycemic control among diabetic patients in Bangladesh.

This comparison will focus on evaluating the sensitivity and specificity of both markers in detecting poor glycemic control, as well as their practical application in a clinical setting. It will also examine the potential advantages of using salivary A2M over HbA1c, particularly in terms of ease of collection, cost-effectiveness, and the ability to offer real-time monitoring. By exploring the correlation between these two biomarkers in the Bangladeshi population, this study seeks to identify a potentially valuable alternative or complement to the conventional HbA1c test, with the goal of improving diabetes management and patient outcomes in the region.

Objective
To assess the compare salivary alpha-2 macroglobulin with glycosylated hemoglobin as a marker of glycemic control.

METHODOLOGY
Study Design and Setting
This research followed a cross-sectional comparative study design. The study was conducted in two departments of Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, specifically the Department of Laboratory Medicine and the Department of Endocrinology. The study was carried out from March 2022 to February 2023, following approval from the Institutional Review Board (IRB).

Study Population
The study enrolled patients diagnosed with type 2 diabetes mellitus (T2 DM) who attended the outpatient (OPD) and inpatient (IPD) departments of Endocrinology at BSMMU. Participants were selected based on the latest American Diabetes Association (ADA) criteria for the diagnosis of diabetes mellitus (2022). Patients with HbA1c levels less than 7% were categorized as having adequately controlled T2 DM, while those with HbA1c levels equal to or greater than 7% were considered as having inadequately controlled T2 DM.

Sample Size Calculation
The sample size was calculated based on sensitivity, using the following formula and parameters:
- **Anticipated sensitivity (SN)**: 91.7% (as per Nsr-Allah *et al.,* 2019)
- **α**: 5%
- **Z1-α/2**: 1.96 (standard normal deviate)
- **L**: 10% (desired precision)
- **Prevalence**: 40% (estimated prevalence of diabetes in the hospital setting)

The sample size calculation resulted in an estimated 73 patients, accounting for a 9% non-response rate, which increased the total sample size to approximately 80 patients.

Inclusion and Exclusion Criteria
The inclusion criteria for this study were: patients diagnosed with T2 DM, aged over 18 years, and both males and females. Exclusion criteria included liver disease, nephrotic syndrome, stage 4 and onward chronic kidney disease, rheumatoid arthritis, hemoglobinopathies, severe anemia, history of blood transfusion, chronic inflammatory processes in the mouth, autoimmune diseases or collagen vascular disorders affecting the oral cavity, and pregnancy.

Data Collection Procedure
Data collection was initiated after obtaining formal ethical approval from the IRB. Participants were approached during their visits to the OPD and IPD, and informed consent was obtained both verbally and in writing. Data were collected through face-to-face interviews using a semi-structured questionnaire in English and Bengali. Physical measurements such as height, weight, and BMI were taken, and participants were asked to provide saliva samples, which were collected under direct supervision. Venous blood was also drawn for HbA1c analysis. Each patient's data collection process took approximately 35-40 minutes.

Laboratory Procedures
For the saliva sample collection, participants were instructed to rinse their mouths with water and then collect saliva over 5 minutes into a sterile container. Blood samples for HbA1c were collected from the

antecubital vein into EDTA tubes. The saliva samples were centrifuged at 3000 rpm for 10 minutes at 4°C to collect the supernatant, which was then stored at -20°C. Salivary A2MG levels were measured using the enzyme-linked immunosorbent assay (ELISA) method, while HbA1c levels were analyzed using the Siemens Dimension EXL automated biochemistry analyzer based on the photometric technique.

Data Analysis and Statistical Methods

The collected data were entered and analyzed using SPSS version 26.0. Quantitative data were presented as means and standard deviations, while categorical data were expressed as frequencies and percentages. Various statistical tests were applied, including the unpaired t-test for comparisons of continuous variables, chi-square test for categorical variables, and Mann-Whitney U test for skewed distributions. Spearman's rank correlation and Pearson's correlation were used to examine relationships between salivary A2MG and HbA1c, as well as other clinical variables. The diagnostic accuracy of salivary A2MG was assessed using sensitivity, specificity, positive predictive value, negative predictive value, and receiver operating characteristic (ROC) curve analysis.

Confidentiality and Ethical Considerations

Patient confidentiality was maintained throughout the study. Each participant was assigned a unique ID number to protect their identity. Informed consent was obtained from all participants, ensuring they understood that their personal data would remain confidential and that they had the right to withdraw from the study at any time without penalty.

RESULTS

Table shows the distribution of patients with T2 DM according to their age groups and their glycemic control status. The table reveals that the majority of participants were in the age range of 41-50 years, with 40.6% of them being adequately controlled and 31.3% inadequately controlled. There was no significant difference in the mean age between the adequately controlled group (mean age=43.2±9.72 years) and inadequately controlled group (mean age=44.7±10.2 years) (p=0.513).

Table-1: Age distribution of the participants (N=80)

Age group (years)	Adequately controlled (HbA1c <7%) (n=32)	Inadequately controlled (HbA1c ≥7%) (n=48)	Total (N=80)	p-value
<30	4(12.5%)	5(10.4%)	9(11.3%)	
31-40	9(28.1%)	14(29.2%)	23(28.7%)	
41-50	13(40.6%)	15(31.3%)	28(35.0%)	
51-60	5(15.6%)	11(22.9%)	16(20.0%)	
>60	1(3.1%)	3(6.3%)	4(5.0%)	
Total	32(100.0%)	48(100.0%)	80(100.0%)	
Mean±SD (years)	43.2±9.72	44.7±10.2	44.1±9.97	0.513[ns]

p-value obtained by Unpaired t-test, p <0.05 considered as a level of significance

ns = not significant

Table shows the gender distribution of the participants with T2 DM based on their glycemic control status. Out of the total 80 participants, 58.8% were female and 41.2% were male. In the group of adequately controlled T2 DM patients, there were 16 males (50.0%) and 16 females (50.0%). In contrast, among the inadequately controlled T2 DM patients, there were 17 males (35.4%) and 31 females (64.6%). The difference in glycemic control between males and females was not statistically significant (p=0.194).

Table-2: Gender distribution of the participants (N=80)

Gender	Adequately controlled (HbA1c <7%) (n=32)	Inadequately controlled (HbA1c ≥7%) (n=48)	Total (N=80)	p-value
Male	16(50.0%)	17(35.4%)	33(41.2%)	0.194[ns]
Female	16(50.0%)	31(64.6%)	47(58.8%)	
Total	32(100.0%)	48(100.0%)	80(100.0%)	

p-value obtained by Chi-square test, p <0.05 considered as a level of significance

ns = not significant

Table shows the comparison of salivary A2MG and HbA1c in adequately controlled and inadequately controlled patients with T2 DM.

For salivary A2MG, the mean±SD was 83.6±25.3 ng/ml in adequately controlled patients and 280.6±161.7 ng/ml in inadequately controlled patients. The median values were 74.3 ng/ml and 293.1 ng/ml in adequately controlled patients and inadequately controlled patients respectively. The difference in salivary A2MG levels between the two groups was highly significant (p<0.001†).

For HbA1c, the mean±SD was 6.34±0.36% in adequately controlled patients and 9.68±2.13% in

inadequately controlled patients. The difference in HbA1c levels between the two groups was highly significant (p<0.001*).

Table-3: Comparison of salivary A2MG and HbA1c levels between adequately and inadequately controlled T2 DM patients (N=80)

Variables	Adequately controlled (HbA1c <7%) (n=32) Mean±SD	Inadequately controlled (HbA1c ≥7%) (n=48) Mean±SD	p-value
Salivary A2MG			
Mean±SD	83.6±25.3	280.6±161.7	<0.001ˢ †
Median	74.3	293.1	
HbA1c			
Mean±SD	6.34±0.36	9.68±2.13	<0.001ˢ *

p-value obtained by †Mann-Whitney test and *Unpaired t-test, p <0.05 considered as a level of significance
s = significant

Table shows the correlation analysis between Salivary A2MG and HbA1c (%) among 80 patients. The correlation coefficient (rho) between salivary A2MG and HbA1c (%) was 0.738, indicating a significant strong positive correlation between the two variables (rho=0.738, p<0.001).

Table-4: Correlation between salivary A2MG (ng/ml) and HbA1c (%) among the patients (N=80)

	Correlation coefficient (rho)	p-value
Salivary A2MG vs HbA1c(%)	0.738	<0.001ˢ

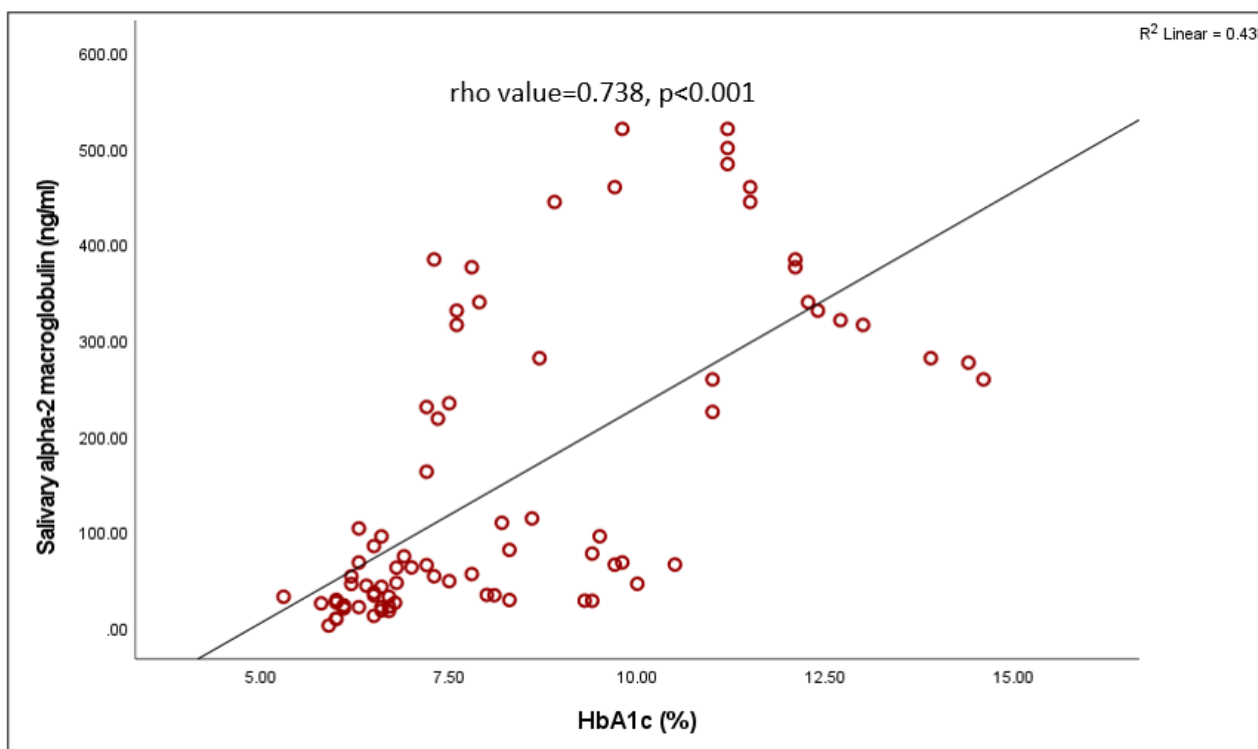p-value was determined by Spearman's correlation test.
s = significant

Figure-1: Scatter diagram showing correlation between salivary A2MG and HbA1c (%) among the patients (N=80)
Positive correlation was found between salivary A2MG and HbA1c (%) (rho=0.738, p<0.001.

Figure shows the ROC curve analysis of salivary A2MG for predicting inadequate glycemic control in a sample of 80 individuals. The cut-off value used was ≥100.1ng/ml. The area under the curve (AUC) was calculated to be 0.915 with a 95% confidence interval of 0.858 to 0.973 (p<0.001). The sensitivity of the test was found to be 85.4%, indicating its ability to correctly identify individuals with inadequate glycemic

control, while the specificity was 81.3%, indicating its ability to correctly identify individuals with adequate glycemic control. These results suggest that salivary

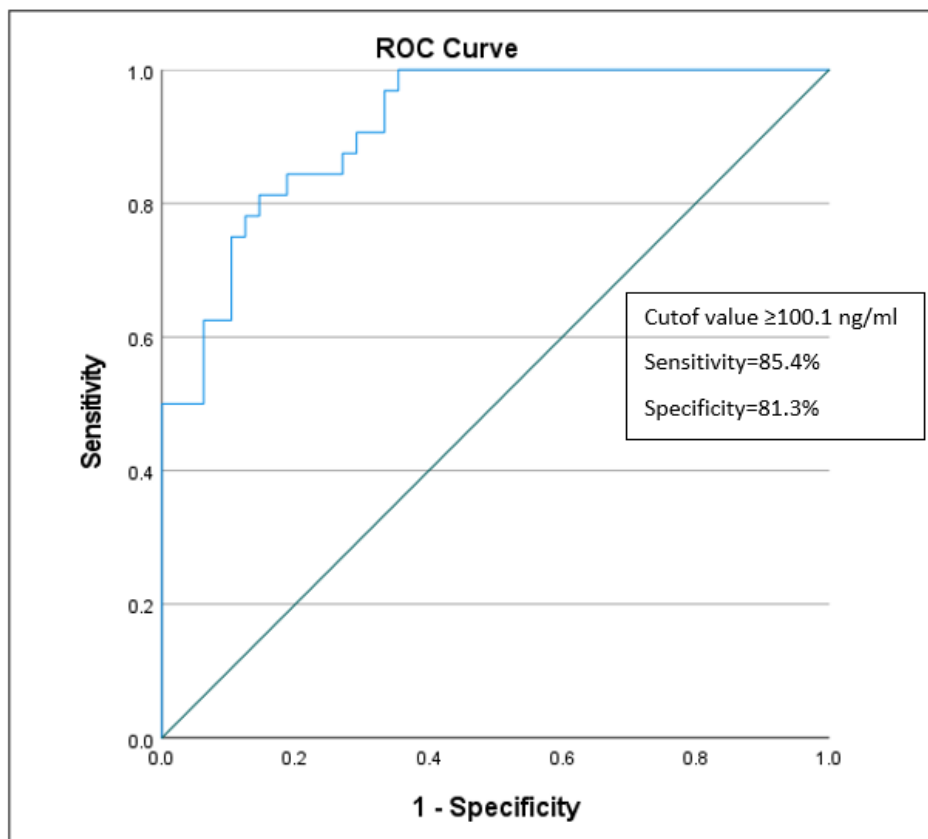A2MG has a strong discriminatory power in predicting inadequate glycemic control.

Figure-2: ROC curve analysis of salivary A2MG in the prediction of inadequate glycemic control (N=80)

Table shows the diagnostic accuracy of salivary A2MG in predicting inadequate glycemic control in the 80 individuals. Among the 48 participants with inadequate glycemic control, 41 were correctly identified as positive (true positives), while 7 were misclassified as negative (false negatives). Among the 32 participants with adequate glycemic control, 6 were incorrectly identified as positive (false positives), and 26 were correctly identified as negative (true negatives).

Table-5: Diagnostic accuracy test of salivary A2MG in the prediction of inadequate glycemic control (N=80)

Salivary A2MG	Adequately controlled (HbA1c <7%) (n=48)	Inadequately controlled (HbA1c ≥7%) (n=32)	Total N=80
>100.1 (ng/ml)	41 (TP)	6 (FP)	44
<100.1 (ng/ml)	7 (FN)	26 (TN)	36
Total	48	32	80

Table shows that salivary A2MG demonstrated a sensitivity of 85.4%, meaning it correctly identified 85.4% of individuals with inadequate glycemic control. It also exhibited a specificity of 81.3%, indicating it correctly identified 81.3% of individuals with adequate glycemic control. The positive predictive value was 87.2%, meaning that among those identified as positive by the test, 87.2% actually had inadequate glycemic control. The negative predictive value was 78.8%, indicating that among those identified as negative by the test, 78.8% actually had adequate glycemic control. The overall accuracy of the test was 83.8%, which represents the proportion of correctly classified individuals (both true positives and true negatives) out of the total sample.

Table-6: Diagnostic performance test of salivary A2MG (N=80)

Results	Values	95% CI
Sensitivity	85.4%	72.24% to 93.9%
Specificity	81.3%	63.6% to 92.8%
Positive Predictive Value	87.2%	76.7% to 93.4%
Negative Predictive Value	78.8%	64.7% to 88.3%
Accuracy	83.8%	73.8% to 91.1%

DISCUSSION

In the present study, it was observed that mean salivary A2MG was 83.6±25.3 ng/ml in adequately controlled T2 DM group and was 280.6±161.7 ng/ml in inadequately controlled group. The difference in salivary A2MG levels between the two groups was highly significant (p<0.001). Salivary A2MG was significantly higher among the patients with inadequate glycemic control which was similar to the previous studies, where they found salivary A2MG significantly higher in patients with inadequate glycemic control compared to those with adequate glycemic control [1-3]. (Caixeta *et al.,* 2022; Aitken *et al.,* 2015; Rastogi *et al.,* 2019). For HbA1c, the mean value was 6.34±0.36% in adequately controlled patients and 9.68±2.13 was in inadequately controlled patients. The difference in HbA1c levels between the two groups was highly significant (p<0.001).

It was observed that the correlation coefficient (rho) between salivary A2MG and HbA1c was 0.738, indicating that a significant strong positive correlation between the two variables (rho=0.738, p<0.001). A previous study by Caixeta *et al.,* also revealed that the correlation between A2MG and HbA1c was strong (r = 0.838) (Caixeta *et al.,* 2022) [1]. Aitken *et al.,* found a correlation between saliva levels of A2MG and HbA1c (%) (r=0.7748 and p< 0.0001) in patients with T_2 DM (Aitken *et al.,* 2015) [2]. The study by Rastogi *et al.,* observed a strong positive correlation between saliva levels of A2MG and HbA1c (r = 0.994 and P =0.001) in T_2 DM (Rastogi *et al.,* 2019). Chung *et al.,* observed that the net change of salivary A2MG levels showed a significant positive correlation with the net change of HbA1c after 3 months of stable follow up. Therefore, the salivary A2MG level might reflect a better marker for long term blood sugar control [3].

For HbA1c, Pearson's correlation analysis showed a positive correlation with age (r = +0.257, p = 0.021), duration of diabetes (r = +0.350, p = 0.001), and BMI (r = +0.467, p = <0.001). That means, higher HbA1c levels were associated with older age, longer duration of diabetes and higher BMI in patients with T2 DM. On the other hand, for salivary A2MG, Spearman's rho correlation analysis did not show significant correlation with age (rho = +0.038, p = 0.740) and duration of T2 DM (rho = +0.061, p = 0.589). However, it showed a positive correlation with BMI (rho = +0.227, p = 0.013), indicating that higher levels of salivary A2MG are associated with higher BMI among the patients with T2 DM. In a previous study by Nsr-Allah *et al.,* salivary A2MG showed strong positive correlation with BMI, duration of diabetes and age of the patients with T2 DM [4].

ROC curve analysis of A2MG in the prediction of inadequate glycemic control, a cut-off value ≥100.1 ng/ml showed sensitivity and specificity 85.4% and 81.3% respectively. For individuals with insufficient glycemic control, the area under the ROC curves showed a positive discrimination threshold of A2MG (AUC = 0.915, CI 95%: 0.858-0.973, p< 0.001). According to this study, the PPV, NPV and diagnostic accuracy were 87.2%, 78.8% and 83.8% respectively. In agreement with our result, Aitken *et al.,* established similar results, where the best cutoff point for A2MG levels in saliva to identify insufficient glycemic control was 840 ng/ml with a sensitivity was 81.9% and specificity was 89.6% (Aitken *et al.,* 2015). [2] The results of Nsr-Allah *et al.,* revealed that the best cutoff value of salivary A2MG as a predictor for HbA1c=7% was 645 ng/ml with 91.7% sensitivity, 90% specificity, 96.5% PPV, 78.3% NPV and 91.2% accuracy (Nsr-allah *et al.,* 2019). [4] El-Alfy and Khalil in their study showed that the best cutoff point of salivary A2MG to differentiate between adequate 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 (El-Alfy and Khalil, 2022). [5]

CONCLUSION

The study highlights that the majority of participants with T2DM were aged 41-50 years, with no significant difference in age or gender distribution between adequately and inadequately controlled groups. Salivary A2MG levels were significantly elevated in inadequately controlled patients, showing a strong positive correlation with HbA1c levels (rho=0.738, p<0.001). ROC analysis demonstrated the high diagnostic accuracy of salivary A2MG for predicting inadequate glycemic control (AUC=0.915, sensitivity=85.4%, specificity=81.3%). These findings suggest that salivary A2MG is a promising non-invasive biomarker for assessing glycemic control in T2DM patients.

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