

# Association of salivary alpha 2-macroglobulin levels and clinical characteristics in type 2 diabetes

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## Keywords

Alpha 2-macroglobulin, Diabetes, Saliva

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## ABSTRACT

**Aims/Introduction:** Studies suggest that salivary proteins can be used as potential non-invasive markers for clinical diagnosis and screening for diabetes. Previous reports showed that plasma alpha 2-macroglobulin (A2MG) levels were higher in diabetic patients, especially with diabetic complications. We investigated the relationship between salivary A2MG values and clinical characteristics in patients with type 2 diabetes.

**Materials and Methods:** A total of 91 adults were recruited from our outpatient clinics. The study the patients' collected general and biochemical data, and blood glucose (fasting glucose, glycated hemoglobin [HbA1c]) data. Plasma and salivary A2MG levels were examined by enzyme-linked immunosorbent assay.

**Results:** The salivary A2MG levels were significantly positively correlated with plasma A2MG levels, fasting glucose HbA1c and periodontitis status. After 3 months of follow up, the net change of salivary A2MG values positively correlated with the net change of fasting glucose, HbA1c and triglyceride levels, but negatively correlated with high-density lipoprotein cholesterol changes. Furthermore, the correlations between salivary A2MG and fasting glucose HbA1c were better than plasma A2MG, respectively.

**Conclusions:** Our data show that salivary A2MG levels have better correlation with fasting glucose HbA1c and periodontitis status than plasma A2MG in diabetic patients. Salivary A2MG concentration might serve as a non-invasive marker for clinical diabetic control.

## INTRODUCTION

Currently, diabetes detection and tracking methods are invasive, which decreases the patients' likelihood to monitor. If a non-invasive and convenient method to monitor diabetes is found, it could improve the willingness of patients to monitor diabetes or lead to an earlier diagnosis, thus reducing the complications and mortality caused by diabetes. Saliva is a complex fluid containing an entire library of hormones, proteins, enzymes, antibodies, antimicrobial constituents and cytokines. Most analyses detected in blood serum are also found in saliva<sup>1</sup>. Therefore, saliva might serve as an effective and non-invasive indicator of both local and systemic disorders<sup>2</sup>. In recent studies, markers in saliva could be related to many diseases including

autoimmune disease, such as Sjögren's syndrome<sup>3</sup>; malignancies, such as breast cancer<sup>4</sup>, pancreatic cancer and oral cancer<sup>5</sup>; end-stage renal disease<sup>6</sup>; and infectious diseases<sup>7</sup>, and could be used in the assessment of therapeutic levels of drugs<sup>8,9</sup>.

Regarding diabetes, analysis of saliva might also be a potential cost-effective and a non-invasive alternative to blood for screening, diagnosis and monitoring of diabetes. An earlier report showed a unique proteomic signature in saliva obtained from type 2 diabetics compared with saliva obtained from control subjects; 65 proteins showed a more than twofold change<sup>10</sup>. In addition, a recent study with few participants showed that salivary pH, flow rate and salivary amylase, calcium were significantly lower in diabetic patients<sup>11</sup>. Likewise, other salivary markers, such as alpha 2-macroglobulin (A2MG), alpha-1-antitrypsin and transthyretin, showed a positive correlation with

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blood sugar, and could be potential biomarkers for screening and diagnosis of prediabetes and diabetes<sup>10</sup>.

A2MG is part of the alpha macroglobulin family, which consists of alpha 1-macroglobulin, A2MG, complement components and pregnancy zone protein. Macroglobulins have been isolated from the hemolymph of invertebrates, plasma of vertebrates, and egg whites of birds and reptiles<sup>12</sup>. The primary function of A2MG is to entrap proteinase and deliver proteinase to an endocytotic clearance pathway<sup>13,14</sup>. In past studies, A2MG could be used as a marker for the diagnosis and prognosis of a number of diseases, and to predict the stage of liver fibrosis without the need for liver biopsy<sup>15</sup>. In addition, the concentration of A2MG could be used as a prognostic marker of the severity of acute pancreatitis<sup>16</sup>, and is elevated in inflammatory bowel disease<sup>17</sup>. A2MG has been found to be associated with diabetes in past studies. For example, the cardiac isoform of A2MG is an early marker in cardiac hypertrophy and left ventricular mass in humans, and could also be used as a marker for the diagnosis of myocardial infarcted diabetic patients and differentiates them from diabetic patients without myocardial infarction<sup>18</sup>. Furthermore, the plasma A2MG level was found to be elevated in the blood of patients with diabetes, especially in those with diabetic complications<sup>19</sup>.

Hence, the purpose of the present study was to evaluate the correlation among salivary A2MG, plasma A2MG, and blood glucose level in type 2 diabetic patients before and after 3 months of stable follow up to identify the possibility of salivary A2MG as a non-invasive screening and monitoring method for diabetes.

## MATERIALS AND METHODS

### Participants

This study enrolled outpatients at the Department of Endocrinology and Metabolism of Tri-Service General Hospital, Taipei, Taiwan, with ages ranging from 35 to 80 years who had been first diagnosed with type 2 diabetes after 30 years-of-age. Inclusion criteria were diabetic patients with stable medication during the preceding 3 months who regularly received diabetes education. Patients meeting any of the following criteria were excluded from the study: type 1 diabetes mellitus, history of rheumatoid disease, malignancy of any kind, history of inflammatory and an infectious disease within the past 3 months. A total of 91 patients were selected to enter the study and received regular outpatient tracking for 3 months without change of medications. The diagnosis of chronic periodontitis was made according to the principles defined by Eke *et al.*<sup>20</sup> Patients were classified as having moderate periodontitis if they had two or more interproximal sites with a clinical attachment level (CAL)  $\geq 3$  mm, or if they had two or more interproximal sites with a probing depth (PD)  $\geq 4$  mm (not at the same tooth). The patients' general characteristics of anthropometric variables and blood pressure were recorded, and changes in anthropometric variables and parameters were measured. Finally, the net change in plasma and salivary A2MG

levels was compared with these parameters. The institutional review board of the Tri-Service General Hospital approved the protocol, and all participants gave written informed consent.

### Laboratory Measurements

Blood samples were collected after 10 h of fasting. Glucose and lipid levels were measured in the samples. Serum total cholesterol, triglyceride, and low-density lipoprotein cholesterol were measured using the dry, multilayer analytical slide method in the Fuji Dry-Chem 3000 analyzer (Fuji Photo Film Corporation, Tokyo, Japan). Serum levels of high-density lipoprotein cholesterol were determined by an enzymatic cholesterol assay method after dextran sulfate precipitation. The levels of glycated hemoglobin (HbA1c) were estimated using ion-exchange high-pressure liquid chromatography (HPLC; BIO-RAD VARIANT II, Los Angeles, CA, USA). Plasma glucose concentrations were determined using the glucose oxidase method on a Beckman Glucose Analyzer II (Beckman Instruments, Fullerton, CA, USA). Plasma insulin was measured with a commercial immunoradiometric kit (BioSource Europe S.A., Nivelles, Belgium). The concentrations of the aforementioned biochemical variables were determined in duplicate, and the values were averaged. Plasma high-sensitivity C-reactive protein levels were measured using the Tina-quant (Latex) high-sensitivity assay (Roche, Mannheim, Germany). The patients were seated quietly and were instructed to rinse their mouth with water, then 2 min after, 5–6 mL of unstimulated whole saliva was collected. The accumulated saliva in the floor of the mouth was drawn by a plastic disposable pipette and collected into a plastic polyethylene tube of 10-mL capacity. The collection period was 20 min, and sampling time was always between 10.00 and 13.00 h. Saliva was sterile filtered to remove bacteria and mucosal cell contamination using Millex-GV 0.22- $\mu$ m pore size filters (Millipore, Billerica, MA, USA). The collected saliva was centrifuged at 1006 g for 10 min; this was carried out within 1 h of collection to eliminate debris and samples were stored frozen at  $-20^{\circ}\text{C}$  in polyethylene tubes until assayed. On the day of assay, saliva and plasma samples were thawed and centrifuged before analysis of A2MG concentrations using a commercially available high-sensitivity sandwich enzyme-linked immunosorbent assay (anti-alpha-2-MACROGLOBULIN Antibody-200-101-207S; Rockland Immunochemicals Inc., Limerick, PA, USA). Measurements were repeated three times.

### Statistical Analysis

Descriptive results of continuous variables were expressed as mean  $\pm$  standard deviation, and  $P < 0.05$  was considered statistically significant. We used the paired *t*-test for comparisons of quantitative variables. Relationships between A2MG and other variables (both anthropometric and biochemical) were analyzed by Spearman's rank-order correlations. The partial correlation coefficient was used to control for the effects of periodontitis, age and diabetes duration. All the statistical analyses were carried out using the program SPSS 18 (SPSS, Chicago, IL, USA).

## RESULTS

A comparison of the anthropometric variables and the biomarker data between baseline and after 3 months are shown in Table 1. Only the HbA1c value was significantly lower after 3 months of follow up. Although plasma fasting glucose concentrations were lower after 3 months of follow up, the difference was not statistically significant.

As shown in Table 2 and Figure 1, variants, plasma A2MG and salivary A2MG were analyzed. The plasma A2MG levels showed a significant positive correlation with salivary A2MG, diastolic blood pressure, HbA1c, fasting glucose, low-density lipoprotein cholesterol and periodontitis status (Figure 1). The salivary A2MG protein levels were significantly positively correlated with plasma A2MG levels, HbA1c, fasting glucose and periodontitis status (Figure 1). We also observed a significant negative correlation between salivary A2MG levels, age and

diabetes duration. Interestingly, salivary A2MG levels correlated more with HbA1c ( $r = 0.443$ ,  $P < 0.001$ ), fasting glucose ( $r = 0.295$ ,  $P = 0.005$ ) and periodontitis status ( $r = 0.312$ ,  $P = 0.002$ ) than plasma A2MG levels correlate with HbA1c ( $r = 0.342$ ,  $P = 0.001$ ), fasting glucose ( $r = 0.246$ ,  $P = 0.020$ ) and periodontitis status ( $r = 0.286$ ,  $P = 0.005$ ), respectively.

As documented in Table 3, the net changes of plasma A2MG level showed a significant positive correlation with the net changes of low-density lipoprotein cholesterol, and a negative correlation with the net changes of body mass index and high-density lipoprotein cholesterol. However, there were no significant correlations between the net changes of plasma A2MG level and salivary A2MG level ( $r = 0.157$ ,  $P = 0.167$ ), HbA1c ( $r = -0.074$ ,  $P = 0.325$ ) and fasting glucose ( $r = -0.118$ ,  $P = 0.235$ ). Notably, the net changes of salivary A2MG level still showed a significant positive correlation with the net changes of HbA1c ( $r = 0.421$ ,  $P = 0.003$ ), fasting glucose ( $r = 0.381$ ,  $P = 0.008$ ) and triglyceride ( $r = 0.374$ ,  $P = 0.009$ ), and were significantly negatively correlated with the net changes of high-density lipoprotein cholesterol ( $r = -0.403$ ,  $P = 0.005$ ).

Table 4 showed that after correcting for the effects of periodontitis, age and diabetes duration, the only variable that showed significant partial correlation with salivary A2MG was HbA1c, suggesting that it was the only variable whose associa-

**Table 1** | Participant characteristics

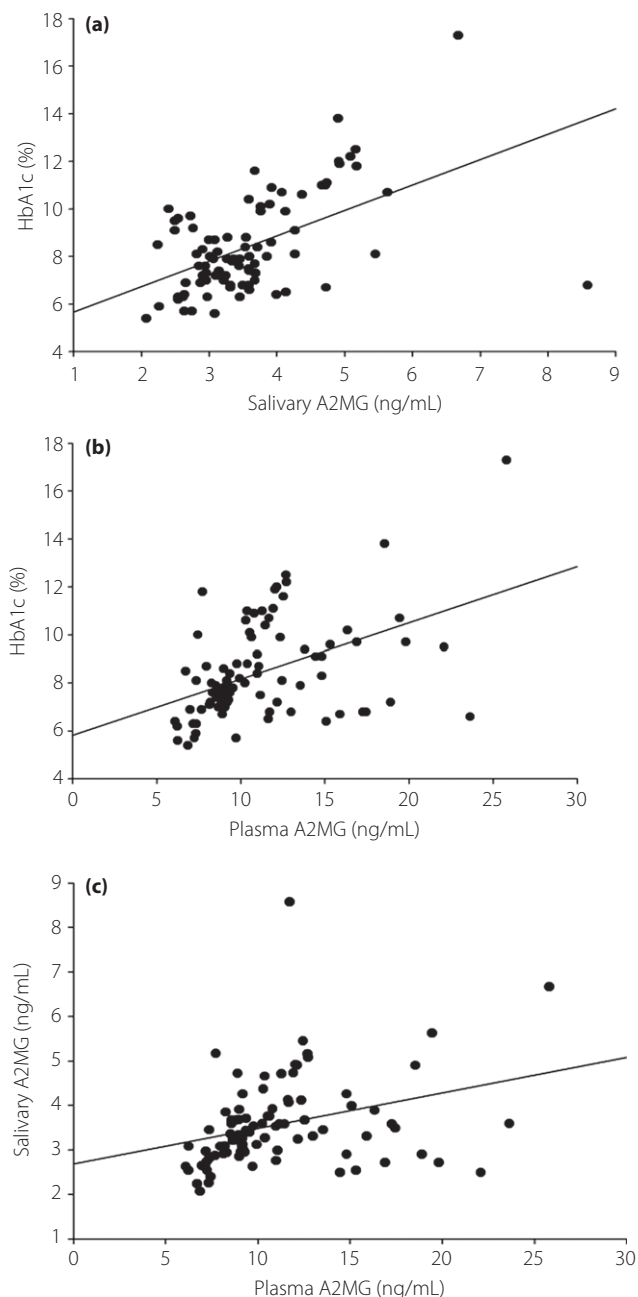
<i>n</i> = 91	Baseline	After 3 months	<i>P</i>
Sex, male (%)	57.58	57.58	NS
Age (years)	55.89 ± 12.21	55.94 ± 11.42	NS
DM years	8.67 ± 7.31	8.68 ± 6.34	NS
BMI (kg/m <sup>2</sup> )	25.93 ± 3.66	25.87 ± 3.65	NS
W/H (cm/cm)	0.90 ± 0.007	0.91 ± 0.008	NS
SBP (mmHg)	130.86 ± 11.93	129.52 ± 8.54	NS
DBP (mmHg)	79.15 ± 9.36	77.74 ± 8.54	NS
HbA1c (%)	7.93 ± 1.79	7.54 ± 1.2	<0.001
FPG (mmol/L)	9.01 ± 3.12	8.18 ± 2.62	0.063
Fasting insulin (pmol/L)	82.3 ± 10.22	80.2 ± 7.48	NS
TC (mmol/L)	4.80 ± 0.84	4.71 ± 1.22	NS
TG (mmol/L)	1.70 ± 0.87	1.68 ± 0.88	NS
HDL-C (mmol/L)	1.08 ± 0.36	1.08 ± 0.37	NS
LDL-C (mmol/L)	2.88 ± 0.70	2.84 ± 0.77	NS
GPT (U/L)	24.61 ± 12.94	26.41 ± 14.18	NS
Cr (mg/dL)	0.89 ± 0.27	0.89 ± 0.22	NS
hsCRP (mg/L)	1.88 ± 1.43	1.82 ± 1.36	NS
Periodontitis (%)	76.92	73.62	NS
Smoking (%)	18.18	18.18	NS
Drinking (%)	10.61	10.61	NS
Betel nut (%)	3.03	3.03	NS
Diabetes therapy			
Sulfonylureas (%)	76.92	76.92	NS
Glinides (%)	13.18	13.18	NS
Biguanides (%)	86.81	86.81	NS
TZDs (%)	9.89	9.89	NS
α-GIs (%)	15.38	15.38	NS
Insulins (%)	9.89	9.89	NS

Data presented as mean ± standard deviation. α-GIs, α-glucosidase inhibitors; BMI, body mass index; Cr, creatinine; W/H, waist to hip ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting blood sugar; GPT, glutamate pyruvate transaminase; HbA1c, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; hsCRP, high sensitive C-reactive protein; LDL-C, low-density lipoprotein cholesterol; NS, not significant; TC, total cholesterol; TG, triglycerides; TZDs, thiazolidinediones.

**Table 2** | Correlations of plasma and salivary alpha 2-macroglobulin and clinical variables

	Plasma A2MG		Saliva A2MG	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
PA2MG (ng/mL)	–	–	0.318	0.002
SA2MG (ng/mL)	0.318	0.002	–	–
Sex	–0.057	0.594	0.140	0.189
Age (years)	–0.047	0.661	–0.239	0.023
DM years	0.010	0.923	–0.232	0.028
BMI (kg/m <sup>2</sup> )	–0.050	0.635	0.072	0.503
W/H (cm/cm)	0.013	0.908	0.023	0.833
SBP (mmHg)	0.197	0.061	0.025	0.812
DBP (mmHg)	0.297	0.004	0.010	0.923
HbA1c (%)	0.342	0.001	0.443	<0.001
FPG (mmol/L)	0.246	0.020	0.295	0.005
Fasting insulin (pmol/L)	0.122	0.208	0.133	0.198
TC (mmol/L)	0.143	0.176	0.156	0.142
TG (mmol/L)	0.154	0.148	0.182	0.089
HDL-C (mmol/L)	–0.112	0.300	–0.202	0.062
LDL-C (mmol/L)	0.018	0.023	0.065	0.555
Periodontitis (%)	0.286	0.005	0.312	0.002

$P < 0.05$ . BMI, body mass index; DBP, diastolic blood pressure; DM years, diabetes duration; FPG, fasting plasma glucose; HbA1c, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; PA2MG, plasma alpha 2-macroglobulin; SA2MG, salivary alpha 2-macroglobulin; SBP, systolic blood pressure; TC, total cholesterol; TG, triglycerides; W/H, waist-to-hip ratio.



**Figure 1** | Simple linear regression for the association of (a) salivary alpha 2-macroglobulin (A2MG) with plasma glycated hemoglobin (HbA1c;  $r = 0.443$ ,  $P < 0.001$ ), (b) plasma A2MG with plasma HbA1c ( $r = 0.342$ ,  $P = 0.001$ ) and (c) salivary A2MG with plasma A2MG ( $r = 0.318$ ,  $P = 0.002$ ).

tion with salivary A2MG was independent of periodontitis, age and diabetes duration.

## DISCUSSION

A2MG, which is produced by the liver, is the major plasma anti-proteinase protein present in the plasma of vertebrates<sup>21</sup>.

**Table 3** | Correlations among the changes of plasma alpha 2-macroglobulin, salivary alpha 2-macroglobulin values and variation factors after glucose control

	$\Delta$ Plasma A2MG		$\Delta$ Saliva A2MG	
	$r$	$P$	$r$	$P$
$\Delta$ PA2MG (ng/mL)	–	–	0.157	0.167
$\Delta$ SA2MG (ng/mL)	0.157	0.167	–	–
$\Delta$ BMI (kg/m <sup>2</sup> )	–0.277	0.042	0.151	0.176
$\Delta$ W/H (cm/cm)	–0.054	0.371	0.187	0.125
$\Delta$ SBP (mmHg)	0.062	0.704	0.039	0.812
$\Delta$ DBP (mmHg)	0.24	0.136	–0.145	0.371
$\Delta$ HbA1c (%)	–0.074	0.325	0.421	0.003
$\Delta$ FPG (mmol/L)	–0.118	0.235	0.381	0.008
$\Delta$ TC (mmol/L)	0.038	0.408	0.033	0.420
$\Delta$ TG (mmol/L)	0.104	0.262	0.374	0.009
$\Delta$ HDL-C (mmol/L)	–0.317	0.023	–0.403	0.005
$\Delta$ LDL-C (mmol/L)	0.278	0.041	0.073	0.326

$P < 0.05$ . BMI, body mass index; DBP, diastolic blood pressure; DM years, diabetes duration; FPG, fasting plasma glucose; HbA1c, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; PA2MG, plasma alpha 2-macroglobulin; SA2MG, salivary alpha 2-macroglobulin; SBP, systolic blood pressure; TC, total cholesterol; TG, triglycerides; W/H, waist-to-hip ratio.

**Table 4** | Significant partial correlations of baseline salivary alpha 2-macroglobulin after corrections

	Correction for periodontitis		Correction for periodontitis and age		Correction for periodontitis, age and DM years	
	$r$	$P$	$r$	$P$	$r$	$P$
Periodontitis (%)						
Age (years)	–0.214	0.038				
DM years	–0.202	0.040	–0.184	0.094		
FPG (mmol/L)	0.254	0.008	0.214	0.036	0.175	0.099
HbA1c (%)	0.402	0.001	0.326	0.004	0.267	0.006

$P < 0.05$ . DM years, diabetes duration; FPG, fasting plasma glucose; HbA1c, glycated hemoglobin.

From previous studies, serum or plasma A2MG could be used as a marker for the diagnosis and prognosis of a number of diseases, including patients with diabetes. Annapoorani *et al.*<sup>18</sup> found that levels of serum cardiac isoform A2MG significantly elevate in diabetic patients suffering myocardial infarction. Furthermore, in diabetes patients with proliferative retinopathy, serum A2MG plays a key role in the control and regulation of the ocular neovascularization<sup>22</sup>. In addition, Ahmad *et al.*<sup>23</sup> 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.*<sup>23</sup> observed no significant relationship between

A2MG level with either FPG or HbA1c. In the present study, we found that the level of plasma A2MG was significantly positively correlated to FPG and HbA1c in type 2 diabetic patients with stable follow up. The findings were similar to a previous study, which found that the serum or plasma A2MG level was significantly higher in diabetes patients than in normal populations<sup>24</sup>. Furthermore, a high A2MG level was strongly associated with poor blood sugar control and complications<sup>24</sup>. The correlation between serum or plasma A2MG and diabetes control and complications has been associated with serum A2MG either binding to insulin in the serum or influencing the internalization of insulin by the target cell<sup>25</sup>. Therefore, it is conceivable that high serum of A2MG might thereby decrease the bioavailability of insulin, leading to impairment of blood sugar control. Furthermore, the A2MG gene was induced by a variety of factors, including cytokines, in the acute and chronic inflammatory conditions<sup>26</sup>; hence, A2MG synthesis might be enhanced in diabetic patients with upregulated acute and chronic inflammatory cytokines.

A2MG is not only present in blood, but also predominant in human saliva. As early as 1988, Grimoud<sup>27</sup> showed that both sex and age have a statistically significant influence on either serum or salivary A2MG. However, to date, the correlation between salivary and serum A2MG has not been well studied. In the present study, we first showed that salivary A2MG has a significant positive correlation with plasma A2MG in type 2 diabetic patients. The present study suggested that A2MG expression in type 2 diabetic patients was consistent in saliva and plasma, and that detection of salivary A2MG values could reflect systemic A2MG expression profile in type 2 diabetic patients. Interestingly, our data showed that salivary A2MG levels have better correlation with HbA1c, fasting glucose and periodontitis status than plasma A2MG levels, respectively. From previous reports, salivary A2MG was not only an essential component of the innate salivary immunity, acting as a natural inhibitor against swine influenza A virus, but also was reported as a pro-inflammatory factor<sup>28,29</sup>. Meanwhile, A2MG levels in whole saliva were found directly positively related to an individual's periodontitis severity<sup>30</sup>. Recently, a study 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<sup>10</sup>. In the present study, positive and significant correlations with HbA1c, fasting glucose and periodontitis status were found in both plasma and salivary A2MG values. Consistent with the bidirectional model between diabetes and periodontitis, the diabetes-related pro-inflammatory status might suggest an increased risk for the development of periodontitis. Hence, it can explain that pro-inflammatory A2MG levels have a significant association with diabetes and periodontitis status in the present study.

Furthermore, we observed that the net changes of salivary A2MG levels still showed a significant positive correlation with

the net changes of HbA1c and fasting glucose after 3 months of stable follow up. However, there were no significant correlations between the net changes of plasma A2MG level with HbA1c and fasting glucose. Caseiro *et al.*<sup>31</sup> 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<sup>31</sup>. However, in the present study, we also evaluated the association between plasma and salivary A2MG and diabetic complications, such as neuropathy and retinopathy. Unfortunately, there were no significant correlations at baseline and net change after 3 months in type 2 diabetes. To our knowledge, this correlation between the net changes of salivary A2MG levels and the net changes of HbA1c, fasting glucose, and lipid profiles in type 2 diabetes has not been reported in the past. This finding strengthens the hypothesis that detecting salivary A2MG might be a more effective and non-invasive method to monitor diabetes control than plasma A2MG. Meanwhile, we found the correlation between salivary A2MG and HbA1c was stronger than fasting glucose in both baseline characteristics and net change after 3 months. It also showed that after correcting for the effects of periodontitis, age and diabetes duration, the only variable that showed significant partial correlation with salivary A2MG was HbA1c. Therefore, the salivary A2MG level might reflect as a better marker for long-term blood sugar control than single-point blood sugar control.

There were many limitations to the present study. First, the study had a small sample size and short duration. Larger samples and longer study duration are required to determine the value of salivary A2MG necessary for evaluating diabetes control and complications. Second, the present study could not completely exclude the possible influence of oral antidiabetes drugs in salivary A2MG levels.

In summary, we first explored the association among salivary A2MG, plasma A2MG and blood glucose variants in type 2 diabetic patients. We found that salivary A2MG statistically correlates with plasma A2MG. Furthermore, salivary A2MG more significantly correlates with fasting glucose and HbA1c than plasma A2MG. In addition, the net changes of fasting glucose and HbA1c after 3 months of follow up also statistically correlate with the net changes of salivary A2MG, but not with the net changes of plasma A2MG. With the advantages of easy access and non-invasive collection by individuals with modest training, whole saliva provides a cost-effective approach for the continued disease monitoring and screening of large populations in studies of systemic diseases. Therefore, we consider salivary A2MG as a useful non-invasive and biological indicator of diabetes control.

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## DISCLOSURE

The authors declare no conflict of interest.

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