Upregulation of salivary α2 macroglobulin in patients with type 2 diabetes mellitus

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ABSTRACT. We investigated the expression of salivary α2-macroglobulin (α2-MG) in patients with type 2 diabetes mellitus (T2DM) to investigate its value for predicting damage to the salivary glands. A total of 116 patients with T2DM and 60 patients with impaired fasting glucose (IFG) were included in this study. Sixty health volunteers were enrolled as a control group. Unstimulated saliva was collected at 8 a.m. prior to breakfast. Expression of α2-MG was determined using an enzyme-linked immunosorbent assay. The correlation between salivary α2-MG, serum α2-MG, and concentration of fasting glucose was analyzed using Pearson correlation analysis. No significant difference was observed in the expression of serum α2-MG in the T2DM group, IFG group, and control group (P > 0.05). Compared with the control group and IFG group, a statistical difference was observed in the salivary α2-MG in the T2DM group (P < 0.01). No statistical difference was observed in the salivary α2-MG in the IFG group compared with the control group (P > 0.05). In the patients with T2DM, a close correlation was identified in the expression of serum α2-MG and salivary α2-MG (r = 0.52, P < 0.01). A poor correlation was
identified between salivary α2-MG and blood sugar level ($r = -0.12$, $P = 0.199$). The expression of salivary α2-MG showed a remarkable increase in T2DM patients, which may be associated with functional disorders of the salivary gland.

Key words: α2-MG; Saliva; Salivary gland; Type 2 diabetes mellitus

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a chronic non-communicable disease caused by the decline of human islet function. The main clinical manifestation in patients with T2DM is high blood sugar. In general, a series of complications, including retinopathy, nephropathy, neuropathy, cardiovascular disease, peripheral vascular disease, and periodontitis have been reported in these patients (Agarwal et al., 2012). Currently, oral infection such as periodontitis and tooth loss has also been reported to be associated with the occurrence of T2DM (Border et al., 2012; Chan et al., 2012). In addition, salivary flow and composition in diabetic patients showed a significant difference compared to that in non-diabetic subjects (Bajaj et al., 2012; Lasisi and Fasanmade, 2012). In particular, significant differences were noted in the expression of salivary proteins in diabetic patients compared with non-diabetic groups (Rao et al., 2009).

Adequate flow of saliva into the oral cavity is very important for maintaining oral health. Reduced saliva output may have a negative effect on dental quality and oral infections. For example, xerostoma has been frequently cited as a symptom of diabetes. Therefore, changes of saliva output and the expression of salivary proteins may indicate altered salivary gland function. In this study, the expression of human α2 macroglobulin (α2-MG) was determined in T2DM patients. In addition, the correlation between serum α2-MG, salivary α2-MG, and fasting blood sugar was analyzed to investigate the predictive effects of salivary α2-MG for diagnosing salivary gland disorder.

MATERIAL AND METHODS

Patients

A total of 116 T2DM patients (male: 54, female: 62, average age of 57 ± 12.3 years) were treated in the Endocrinology Department and Outpatient Department of Yantai Yuhuangding Hospital from February 2011 to March 2012 and were included in this study. In addition, 60 cases with impaired fasting glucose (IFG, male: 27, female: 33) with an average age of 55 ± 14.3 years were enrolled in the IFG group. The diagnosis of T2DM was based on the standards established by the American Diabetes Association in 2010 (American Diabetes Association, 2010). Patients with IFG and a fasting blood glucose ≥ 7.0 mM were considered to be in a prediabetic state. In addition, 60 healthy volunteers (male: 22, female: 38, average age of 51 ± 11.3 years) were enrolled as the control group. Fasting blood glucose ranged from 5.6-6.9 mM according to the standards established by the American Diabetes Association in 2003 (International Diabetes Federation, 2003). Those with acute and chronic nephrosis, liver disease, and autoimmune diseases were excluded from this study. All patients signed informed consent. This study was approved by the Ethics Committee of Yuhuangding Hospital.
Prior to enrolling patients, laboratory testing results and clinical symptoms were used as references for grouping. The onset time of T2DM was more than 4 years. However, because of medical ethics considerations, no limitations were imposed for the clinical treatment during sample collection. Patient information is summarized in Table 1.

<table>
<thead>
<tr>
<th>Item</th>
<th>T2DM group</th>
<th>IFG group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number (M/F)</td>
<td>116 (54/62)</td>
<td>60 (27/33)</td>
<td>60 (22/38)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>57 ± 12.3</td>
<td>55 ± 14.3</td>
<td>51 ± 11.3</td>
</tr>
<tr>
<td>Time of onset (years)</td>
<td>&gt;4</td>
<td>First diagnosis or recheck</td>
<td>NA</td>
</tr>
<tr>
<td>Tooth loss (N)</td>
<td>2</td>
<td>1</td>
<td>NA</td>
</tr>
<tr>
<td>Diabetic nephropathy (N)</td>
<td>9</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Diabetic foot (N)</td>
<td>8</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Diabetic eye disease (N)</td>
<td>3</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Fasting blood sugar (mM)</td>
<td>10.08 ± 2.44</td>
<td>6.58 ± 0.24</td>
<td>5.01 ± 0.41</td>
</tr>
<tr>
<td>Serum α2-MG (g/L)</td>
<td>1.70 ± 0.55</td>
<td>1.57 ± 0.36</td>
<td>1.54 ± 0.38</td>
</tr>
<tr>
<td>Salivary α2-MG (ng/mL)</td>
<td>192.6 ± 65.3</td>
<td>158.1 ± 60.1</td>
<td>134.8 ± 63.2</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>8.7 ± 1.7</td>
<td>5.8 ± 1.1</td>
<td>5.7 ± 0.7</td>
</tr>
</tbody>
</table>

**Sample collection and processing**

Unstimulated saliva (1 mL) from the diabetic and control group was collected. Briefly, at 8 a.m., the subjects were asked to rinse their mouths thoroughly with water prior to break- fast. They were then required to tilt their heads forward, and saliva was collected into a sterile container. The saliva samples were immediately frozen and stored at -20°C until further analysis. Saliva samples were centrifuged at 2000 g for 10 min at 4°C. The supernatants were used for the detection of salivary proteins using an enzyme-linked immunosorbent assay kit (RapiBio, Inco, CA, USA) following the manufacturer instructions.

For serum samples, 3 mL venous blood was collected from the ulnar vein. Next, the blood was transferred to a vacuum blood tube. After centrifugation at 2000 g for 5 min at 4°C, the samples were immediately frozen and stored at -20°C. The concentration of α2-MG was determined using a BNII automatic protein analyzer (Siemens, Munich, Germany). The level of blood glucose was detected using a DXC800 automatic biochemistry analyzer (Beckman Coulter, Brea, CA, USA) according to manufacturer instructions.

**Statistical analysis**

SPSS16.0 was used for statistical analysis (SPSS, Inc., Chicago, IL, USA). All data are reported as means ± standard deviation. One-factor analysis of variance was performed for inter-group comparison. The correlation between salivary α2-MG, serum α2-MG, and concentration of fasting glucose was analyzed by Pearson correlation analysis. P < 0.05 was considered to be statistically significant.

**RESULTS**

**Expression of serum α2-MG and salivary α2-MG**

Table 2 summarizes blood glucose concentration, serum α2-MG, and saliva α2-MG in
Upregulation of α2-MG in T2DM patients, the IFG group, and the control group. No statistical difference was observed in the blood glucose and serum α2-MG among groups (P > 0.05). Compared with the control group, a statistical difference was observed in salivary α2-MG in the T2DM group and IFG group (P < 0.01). However, no statistical difference was observed in the T2DM and IFG group (P > 0.05).

Table 2. Comparison between serum α2-MG and salivary α2-MG.

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Blood glucose (mM)</th>
<th>Serum α2-MG (g/L)</th>
<th>Salivary α2-MG (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T2DM group</td>
<td>116</td>
<td>10.08 ± 2.44</td>
<td>1.70 ± 0.55</td>
<td>192.6 ± 65.3*</td>
</tr>
<tr>
<td>IFG group</td>
<td>60</td>
<td>6.58 ± 0.24</td>
<td>1.57 ± 0.36</td>
<td>158.1 ± 60.1*</td>
</tr>
<tr>
<td>Control group</td>
<td>60</td>
<td>5.01 ± 0.41</td>
<td>1.54 ± 0.38</td>
<td>134.8 ± 63.2</td>
</tr>
</tbody>
</table>

*P < 0.05, compared with control group.

Correlation between blood sugar and salivary α2-MG in T2DM patients

Correlation analysis was performed by analyzing the blood sugar level in 116 T2DM patients as an independent variable and salivary α2-MG level as a dependent variable. A poor correlation was observed between blood sugar and salivary α2-MG in T2DM patients (r = 0.12, P = 0.199, Figure 1).

Figure 1. Correlation analysis between blood sugar and salivary α2-MG in T2DM.

Correlation between serum α2-MG and salivary α2-MG in T2DM patients

For the correlation analysis between serum α2-MG and salivary α2-MG in T2DM patients, serum α2-MG was considered the independent variable, while salivary α2-MG level was considered the dependent variable. A significant correlation was observed between serum α2-MG and salivary α2-MG in T2DM patients (r = 0.52, P < 0.01, Figure 2).
Correlation between serum α2-MG and salivary α2-MG in the control group

In our study, the correlation between serum α2-MG and salivary α2-MG in the control group was also investigated using serum α2-MG as the independent variable and salivary α2-MG level as the dependent variable. Our results showed a poor correlation between serum α2-MG and salivary α2-MG in the control group (r = 0.182, P = 0.163, Figure 3).

Figure 2. Correlation analysis between serum and salivary α2-MG in T2DM.

Figure 3. Correlation analysis between serum and salivary α2-MG in the normal control group.
DISCUSSION

The incidence of T2DM continues to increase each year. Great progress has been achieved in treatment methods, and the therapeutic schedule is rigorous and has been standardized. To date, effective treatment plans for diabetes have improved the quality of life and delayed the incidence of a variety of complications. However, a large number of patients develop chronic complications in elderly years (Agarwal et al., 2012). A recent study indicated that the levels of some salivary proteins such as α2-MG, α1-antitrypsin, and chalone C in patients with T2DM was 2-fold higher than these values in non-diabetic subjects (Rao et al., 2009). These proteins are considered to be associated with glucose metabolism and immunoregulation pathways.

We tested serum and salivary α2-MG levels in T2DM patients and IFG patients. The results showed that the serum α2-MG in T2DM patients was higher than those in the control group and IFG group. However, no statistically significant difference was noted among serum α2-MG in these patients. A previous study (James et al., 1980) reported a remarkable increase in serum α2-MG in patients with T2DM, but this was largely influenced by gender and age. In our study, salivary α2-MG level was significantly higher than in the normal control group and IFG group, which was consistent with a previous report (Rao et al., 2009). In contrast, Yin et al. (2012) reported that in edentulous patients with T2DM, downregulation of salivary α2-MG was observed. We speculated that tooth loss, gingival tissue repair, and diet may affect the secretion of salivary proteins. In a recent study investigating putative local etiologic factors on implant bone loss in relation to T2DM, salivary osteoprotegerin was higher in the diabetes group than in the control group at baseline levels, while the levels of IL-4 and IL-10 in the diabetes group showed a remarkable decrease compared to that in the control group (Tatarakis et al., 2013).

As a poor correlation between blood sugar and salivary α2-MG was identified in patients with T2DM, we speculated that the significant increase in salivary α2-MG in diabetes patients was not induced by the increased blood glucose concentration. The level of serum α2-MG was positively related to salivary α2-MG in T2DM patients, but a poor correlation was observed between serum α2-MG and salivary α2-MG in the normal control group. This indicated that some alternations may occur when α2-MG is secreted from the blood into the saliva in T2DM patients. However, the mechanism of how α2-MG enters the saliva has not been well defined. Based on our results, the significant increase in salivary α2-MG in T2DM patients may be associated with a higher level of serum α2-MG and the secretion function of the salivary gland. In addition, alterations in the secretion function of salivary glands may indicate damage to the salivary gland.

Further histopathological examinations are needed to confirm the salivary gland lesion. However, biopsy is more invasive than other methods. In this study, we only collected unstimulated saliva prior to breakfast. Further studies are needed to investigate the effects of stimulated whole saliva and salivary flow on the altered function of the salivary gland.

Most biological markers in the blood can be detected in the saliva. However, their concentrations are low (Denny et al., 2008). The level of certain biomarkers in the saliva changes during the disease course (Hu et al., 2007). Wong (2008) paved the way for the saliva research in 2008, and saliva diagnostics have been extensively applied in molecular diagnostics since.

In clinical experiments, it is easy to collect and store saliva. In addition, the procedure is non-invasive and rapid. Along with the clinical application of sensitive experimental tech-
nology and equipment, saliva can be used for disease diagnosis. Saliva is important for disease diagnosis and progression monitoring (Greenberg et al., 2010).

In conclusion, the expression of salivary α2-MG showed a remarkable increase in T2DM patients compared with the control group. For patients with T2DM, a close correlation was identified in the expression of serum α2-MG and salivary α2-MG. Our study may provide some evidence regarding the association between expression of salivary α2-MG and functional disorders of the salivary gland.

Conflicts of interest

The authors declare no conflict of interest.

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REFERENCES


