Salivary Oxidative Stress Markers in Relation to Vascular Disease Risk of Type Two Diabetes Mellitus

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ABSTRACT

Background: Cardiovascular disease (CVD) is an important complication of type 2 diabetes mellitus (T2DM). Oxidative stress plays a major role in the development of CVD. Saliva has a diagnostic properties aiding in the detection of systemic diseases. This study aimed to assess the association between salivary oxidative stress markers and the risk of vascular disease (VD) in T2DM patients.

Materials and Methods: One hundred T2DM patients and fifty apparently healthy males were enrolled in this study. Saliva sample was collected for assessment of oxidative stress markers including: lipid peroxidation plasma thiobarbituric acid-reactive substances (TBARS), uric acid (UA) and total antioxidant capacity (TAC) levels. Arterial stiffness index (ASI) was used for the assessment of VD risk.

Results: According to ASI, T2DM patients were categorized into two groups: Group A: T2DM patients without VD risk. Group B: T2DM patients with VD risk. The mean values of TBARS and UA of group B showed a statistically highly significant elevation compared to group A and controls (P<0.01). The mean value of TAC of group B showed a statistically highly significant decrease when compared to group A and controls (P<0.01).

Conclusion: The increase in salivary TBARS and UA levels and the decrease in the TAC level can be used as an indicator for the increase of risk for VD in T2DM patients.

Key words: type 2 diabetes mellitus, vascular disease risk, salivary oxidative stress.

INTRODUCTION

Type 2 diabetic patients have higher risk of CVD compared with those without diabetes (1). A non-invasive and effective method for early detection of VD used to indicate the stiffness of the arteries is ASI (2, 3). Oxidative stress plays a major role in the development of CVD (4). Reactive oxygen species (ROS) can stimulate oxidation of LDL, cholesterol, cholesterol derived species, protein modifications which can lead to foam cell formation and atherosclerotic plaques and vascular thrombosis (Heart attack and Stroke) (5).

Saliva is like blood, a complex fluid containing a variety of enzymes, hormones, antibodies, antimicrobial constituents. Therefore, most compounds found in blood are also present in saliva (6). Saliva has a dynamic diagnostic properties aiding in the detection of oral and systemic disease by using the salivary biomarkers (7,8).

The relationship of salivary oxidative stress markers including: TBARS, UA and TAC were studied in relation to T2DM in previous studies (9,10). In this study the aim is to investigate the risk of VD in T2DM patients through studying salivary TBARS, UA and TAC all together as an indicator of risk for VD.

MATERIALS AND METHODS

One hundred T2DM patients and fifty apparently healthy males were enrolled in this study recruited from National diabetes center, University of Al-Mustnasiriya, from January 2014 to February 2015. T2DM patients were on oral hypoglycemic drugs. Their age range was 45–55 years and duration of diabetes mellitus was 2-15 years.

The exclusion criteria for the T2DM patients were smokers, patients treated with insulin, patients with a concurrent acute illness or with a major liver, thyroid or other endocrine diseases, patients suffered from endpoints of VD: angina pectoris, myocardial infarction, transient ischemic attack and stroke.

The ASI was measured after training and supervision by a specialist physician in the National diabetes center, University of Al-Mustnasiriya by using an automated digital oscillometric device that is called commercially as (Vital vision) and which provides an indicator, the H-Value (Arterial Hardness Indicator) (figure 1), that quantifies the degree of arterial hardness depending on the variations in pulse wave amplitude obtained while measuring blood pressure (11).

The saliva sample collection was performed without any stimulus in the morning (9 to 11 AM), it was collected on ice, patients and healthy were asked to rinse their mouth with normal saline. All subjects refrained from eating, drinking for a minimum of one hour before saliva collection. Subjects were comfortably seated and,
after a few minutes of relaxation, they were trained to avoid swallowing saliva and asked to lean forward and spit all the saliva they produced for 10 minutes.

The collected saliva was centrifuged at 3000 rpm for 10 minutes; the clear supernatants were separated and stored frozen at (-20°C) until assayed. Oxidative stress markers assessment including TBARS was conducted according to Shah and Walker (12), estimation of UA was conducted according to Fossati et al. (13) and TAC was conducted according to Prieto et al. (14).

ANOVA test for more than two independent means and LSD was used to measure the precision of a variety of means between two mean values. To estimate the diagnostic efficiency for vascular disease risk in T2DM patients of each single parameter of the present study; receiver operating characteristic (ROC), sensitivity and specificity was measured. A probability value (P<0.05) was considered to be statistically significant and (P<0.01) was considered to be statistically highly significant.

Pattern 1:
1 to 3 bars appear when the cardiovascular system is normal

Pattern 2:
4 to 6 bars suggest a condition of progressing arterial hardness

Figure 1: H-Value measurement result on the LCD Display of ASI measuring device

RESULTS
According to ASI, T2DM patients were categorized into two groups:
- Group A: T2DM patients with normal ASI (without VD risk)
- Group B: T2DM patients with abnormal ASI (with VD risk)

Table (1) shows a comparison of salivary oxidative stress markers among group A, group B and controls. The mean values of TBARS and UA of group B showed a statistically highly significant elevation compared to group A and controls (P<0.01). Concerning the comparison between group A vs. controls for the mean values of TBARS and UA, the results revealed significant (P<0.05), highly significant (P<0.01) difference, respectively (Table 1).

The mean value of TAC of group B showed a statistically highly significant decrease when compared to group A and controls (P<0.01). Concerning the comparison between group A vs. controls for the mean values of TAC the results revealed no significant difference (P>0.05) (Table 1).

The area under the curve was highest for salivary TAC (ROC area = 0.842, P<0.01), followed by salivary UA (ROC area = 0.805, P<0.01) and salivary TBARS (ROC area = 0.752, P<0.01) (Table 2 and figure 1).

DISCUSSION
The identification of T2DM patients with a higher risk to VD is a priority need since such patients suffer from risk of CVD more than double in comparison with those age-matched individuals (1).

In this study, TBARS level was significantly higher in T2DM patients than in controls. This result is in accordance with a study conducted by Al-Rawi (9), who found that salivary TBARS level was elevated in T2DM patients. Furthermore, the results of the current study showed that salivary TBARS level was significantly higher in T2DM patients with VD risk than in those without risk, such results are consistent with an Iraqi study conducted by Zaidan (15) who found that serum and saliva TBARS were significantly higher in patients with myocardial infarction than in controls.

The finding in the current study that UA level was higher in T2DM patients compared with...
controls and in T2DM patients with VD risk compared with those without risk is in accordance with the results of many studies that investigated the role of UA as an antioxidant in T2DM and VD. It was noted that T2DM patients have higher levels of serum UA (16), and so they might represent an additional VD risk factor in these patients (17, 18).

In an epidemiological study conducted by Zoppini et al. (19) assessing the association of serum UA levels with all-cause and CVD mortality in T2DM patients and after adjusting for several baseline confounding factors, the major finding of this study suggested that higher serum UA levels are associated with increased risk of VD mortality in T2DM patients, independent of conventional risk factors.

In this study salivary TAC level was lower in T2DM patients with VD risk compared to T2DM patients without VD risk. In a recent study conducted by Mussavira et al. (20), TAC was decreased in the saliva of T2DM patients in comparison with healthy controls. In regard to TAC level in patients with CVD, several studies had found a significantly lower serum TAC level in patients with CVD (21, 22).

In conclusion, the increase in salivary TBARS and UA levels and the decrease in the TAC level in T2DM patients is an indicator of risk for VD and this may make salivary oxidative stress markers another non-invasive method to detect VD risk in T2DM patients.

Table 1: Salivary oxidative stress markers of T2DM patients according to arterial stiffness index (ASI) in comparison with controls

<table>
<thead>
<tr>
<th>Variables</th>
<th>Study groups</th>
<th>LSD-test</th>
<th>ANOVA P-value</th>
<th>Group A vs. Group B</th>
<th>Control vs. Group A</th>
<th>Control vs. Group B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group A Mean ±SD N=50</td>
<td>Group B Mean ±SD N=50</td>
<td>Control Mean ±SD N=50</td>
<td>Group A vs. Group B</td>
<td>Control vs. Group A</td>
<td>Control vs. Group B</td>
</tr>
<tr>
<td>TBARS (µmol/L)</td>
<td>1.28±0.38</td>
<td>1.60±0.37</td>
<td>1.10±0.27</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Uric acid (mg/dl)</td>
<td>3.02±1.51</td>
<td>4.43±1.31</td>
<td>2.03±1.02</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TAC (µmol/L)</td>
<td>543.51±239.93</td>
<td>270.74±171.31</td>
<td>580.16±240.87</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&gt;0.05</td>
</tr>
</tbody>
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Table 2: Area under receiver operator characteristic (ROC) curve for prediction of vascular disease risk in T2DM patients depending on the levels of salivary oxidative stress markers

<table>
<thead>
<tr>
<th>Variables</th>
<th>ROC Area under curve</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBARS (µmol/L)</td>
<td>0.752</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Uric acid (mg/dl)</td>
<td>0.805</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TAC (µmol/L)</td>
<td>0.842</td>
<td>&lt;0.001</td>
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Figure 1: ROC diagram showing the trade-off between sensitivity (rate of true positive results) and 1-specificity (rate of false positive test results) for A. TBARS, B. uric acid and C. total antioxidant measurements when used as a test to predict VD risk in T2DM patients, differentiating them from T2DM patients without VD risk.
REFERENCES