Evaluation of Antioxidant Levels in Blood Sera of Iraqi Patients with T2DM

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Abstract

Background: oxidative stress is one of the problems that can encounter of diabetic patients. Reactive oxygen species formed as a result of oxidative stress. Antioxidants system serves to protection of cells from damage that may be occurred due the action of these species. In this work, we attempt to investigate the biochemical parameters including enzymatic and non-enzymatic antioxidants in patients with type 2 diabetic mellitus. Subjects and Methods: Blood samples were collected from 58 subjects include 29 males and 29 females, in age range of 40-72 years. They were classified into two groups, the first group represents patients group that include 30 patients. Second group consist of 28 healthy persons as a control group. BMI was calculated, fasting blood glucose, uric acid, urea and creatinine levels were measured by enzymatic methods. Glutathione peroxidase (GP x) and glutathione reductase (GR) levels were measured by enzyme linked immunosorbent assay (ELISA) method. Trace elements levels (zinc, magnesium and selenium) were estimated by flame atomic absorption spectrophotometer. Results: Results showed a significant increase in levels of FBG, UA, creatinine and significant decrease in levels of GP x, GR, Zn, Mn, and Se in patients group related to the control group. There is no significant difference in level of urea between patients and control groups. Conclusion: Free radical generation increase in diabetic case, that lead to decrease in antioxidants levels of GPx1, GSR, Zinc, Selenium and Manganese. The levels of GPx1, GSR, Se, Zn, and Mn are the better indicators to evaluate the oxidative stress, and can be used as markers for early detection of diabetes.

Keywords: T2DM, glutathione peroxidase, glutathione reductase, zinc, manganese, selenium.

Introduction

Diabetes considers the second problem in world after cancer, simply, it describes in terms of higher glucose level than normal in blood [1-3]. Diabetes disease has more than one type, but all its types have the general abnormality "a high level of glucose in blood" [4, 5]. Insulin is promoting the absorption of glucose in to the cells. The defect of this hormone causes hyperglycemia, in another hand, insulin may not works as should be when it gets to the cell, this state called "insulin resistance".

This may be treating by drugs, proper diet, and exercise or by insulin [8,9]. Free radicals are associated with oxidative stress. Cells protected from the damage that can be occurred by oxidants. Antioxidants are molecules present in low concentrations that functions to neutralize oxidants through certain mechanisms.

Glutathione system is one of the antioxidants mechanisms to scavenger free radicals [10-12]. Antioxidants can be classified into two major groups. Enzymatic antioxidants, which include different enzymes such as catalase, superoxide dismutate, glutathione reductase, glutathione peroxidase and non-enzymatic antioxidants that consisting of glutathione, lipio acid, selenium, zinc and the dietary supplements [13].

Diabetes may be lead to diabetic complications such as osteoporosis [14,15], retinopathy, neuropathy, and nephropathy [16,17]. The aim of this work is to study some enzymatic and non-enzymatic antioxidants in blood sera of Iraqi patients with diabetes as biochemical markers that can be used in early detection of diabetes.
Subjects and Methods

Blood samples were obtained from thirty patients with T2DM whom attended to National Diabetes Center, AL-Mustansiriya University and 28 healthy persons during the period from January 2017 to May 2017. Age with range (40-72) years for all patients and control groups. Venous blood samples (7ml) from each patient and healthy persons were collected in serum separator tube without anticoagulant.

Samples were allowed to clot for 30 minutes at room temperature. Serum was separated under centrifugation at 3000 rpm for 10 min. then; serum was removed and divided into four parts that kept in eppendorf tube. The first part was used to determine of (fasting blood glucose, urea, uric acid and creatinine) in serum immediately, by Auto Analyzer Kenza 240 TX; a clinical chemistry analyzer performs diagnostic tests.

The second, third and fourth parts were stored at -20°C to determine each of GR and GPx that were measured by enzyme–linked immunosorbent assay (ELISA), using kit produced by Aviva Systems Biology, USA, and the trace elements (Zn, Mn and Se) in serum immediately, by Auto Analyzer Kenza 240 TX; a clinical chemistry analyzer performs diagnostic tests.

The results revealed no significance differences in BMI and age between the studied groups (p>0.05) but significant differences increasing in patients group in comparison with control group of FBG and creatinine levels (p<0.05), also the result showed no significant differences in urea level between the studied groups (p>0.05).

It was found a significant decrease in GPx1 and GSR levels in diabetic group compare with control group (p<0.05). The results revealed significance difference in UA levels in first group compare to control group (p<0.05) and showed a significant decrease in trace elements levels in first group compare to control group (p<0.05) as shown in Table (2).

The results in table (3) showed that FBG level was inversely correlated (negative correlation) with GPx1,GSR, Se, Zn and Mn levels in diabetic group, but FBG level was positive correlation with UA, Urea, Creatinine, BMI, age and duration in the same group as shown in Table (4).

Table 1: Values of Age, BMI, FBG, Urea and Creatinine in both diabetic patients and control groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>AGE (year) Mean±SE</th>
<th>BMI(Kg/m²) Mean±SE</th>
<th>FBG (mg/dl) Mean±SE</th>
<th>Urea (mg/dl) Mean±SE</th>
<th>Creatinine (mg/dl) Mean±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Diabetic group</td>
<td>56.83±0.91a</td>
<td>33.01±0.85a</td>
<td>174.36±12.59a</td>
<td>29.23±1.53a</td>
<td>1.06±0.04a</td>
</tr>
<tr>
<td></td>
<td>Control group</td>
<td>53.96±1.39a</td>
<td>30.90±0.77a</td>
<td>137.60±45.58b</td>
<td>31.28±0.76a</td>
<td>0.94±0.02b</td>
</tr>
<tr>
<td>LSD</td>
<td>3.8276</td>
<td>2.6499</td>
<td>29.747</td>
<td>3.7559</td>
<td>0.1118</td>
<td></td>
</tr>
</tbody>
</table>

The means with same letters in the same column are no significant difference (P> 0.05). The means with different letters in the same column are significant differences (P<0.05).

Table 2: levels of GPx1, GR, UA, Zn, Mn, Se in diabetic patient and control groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>GPx1 (U/ml) Mean±SE</th>
<th>GSR (ng/ml) Mean±SE</th>
<th>UA (mg/dl) Mean±SE</th>
<th>Zn (µg/dl) Mean±SE</th>
<th>Mn (µg/L) Mean±SE</th>
<th>Se (µg/L) Mean±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Diabetic group</td>
<td>147.66±38.60b</td>
<td>137.69±45.58b</td>
<td>5.68±0.25a</td>
<td>60.37±3.71b</td>
<td>22.37±1.31b</td>
<td>48.72±6.31b</td>
</tr>
<tr>
<td></td>
<td>Control group</td>
<td>392.30±32.43a</td>
<td>594.31±31.59a</td>
<td>4.01±0.14b</td>
<td>89.23±1.26a</td>
<td>38.51±2.08a</td>
<td>119.51±3.78a</td>
</tr>
<tr>
<td>LSD</td>
<td>85.559</td>
<td>91.87</td>
<td>0.6115</td>
<td>9.0691</td>
<td>4.3684</td>
<td>14.932</td>
<td></td>
</tr>
</tbody>
</table>

The means with different letters in the same column are significant differences (P<0.05).
Table 3: Correlation coefficient of FBG level with GPx, GR, Se, Mn and Zn levels in diabetic patients and control groups

<table>
<thead>
<tr>
<th>correlation coefficient</th>
<th>FBG (mg/dl) in diabetic group</th>
<th>GPx</th>
<th>GR</th>
<th>Se</th>
<th>Mn</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>-0.285</td>
<td>-0.118</td>
<td>-0.188</td>
<td>-0.046</td>
<td>-0.122</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>0.127</td>
<td>0.536</td>
<td>0.320</td>
<td>0.808</td>
<td>0.521</td>
</tr>
</tbody>
</table>

Table 4: Correlation coefficient of FBG level with urea, creatinine, UA, age and duration in diabetic patients and control groups

<table>
<thead>
<tr>
<th>Correlation Coefficient</th>
<th>Urea</th>
<th>Creatinine</th>
<th>UA</th>
<th>BMI</th>
<th>Age</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>FBG (mg/dl) in diabetic group</td>
<td>r</td>
<td>0.158</td>
<td>0.078</td>
<td>0.143</td>
<td>0.124</td>
<td>0.123</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>0.406</td>
<td>0.681</td>
<td>0.451</td>
<td>0.514</td>
<td>0.516</td>
</tr>
</tbody>
</table>

Discussion

The results that listed in (Table 1) showed no significant difference (p>0.05) in age and BMI of the studied groups (patients and control groups). In the present study, patients and the control groups were matched regarding for age and BMI. This matching is a useful result to prevent any effects of differences in age and BMI of the study. This result was agreed with Marwan M. Merkhan et al. [18], and Kameran Hassan et al. [19] whom showed that there was no significant difference between age of controls and patients with T2DM.

The results of BMI of the patients groups were higher than control group, and according to the BMI categories the mean of BMI in diabetic group (33.01±0.85Kg/m²) was referred to the diabetic condition. Fasting Blood glucose (FBG) level was found to be increase significantly (p<0.05) in the group of patients (174.36±12.59) in comparison with the control group (90.78±1.22). The results of the present study agrees with others who found that the increasing of oxidative stress in diabetes lead to over generation of reactive oxygen species (ROS) which considered a direct consequence of hyperglycaemia and production of advanced glycation end products [20].

The result of Urea level of diabetes and control group was revealed that there was no significant difference (p>0.05) in both studied groups. The level value of patients group (29.23±1.53) and control group (31.28±0.76) are within the normal range (15-45 mg/dl). This result in agreement with Jay Prakash Sah et al. [21]. Also, the results showed a significant increasing in creatinine level in patients group rather than control group in this study. This result is in agreement with Arindam Sur [22] and Priti Singh et al. [23], whom noticed an increasing in serum creatinine in diabetic patients group than healthy group. In hyperglycemia, the increasing of blood sugar level can be induced damage in podocytes and basement which leads to increase of the permeability of albumin [24]. Also, the thickening of the basement membrane leads to decrease filtration function of kidney which intern causes an increasing of serum creatinine level [25]. A significant decrease (P< 0.05) in both GP x and GR levels was noticed in patients group than control group.

The mean value of the GPx1 in the control group (392.30±32.43 U/ml) was significantly higher than the mean values of the diabetic group (147.66±38.60 U/ml), also the results of GR show significant differences, the level of the GSR of the control group (594.31±31.59 ng/ ml) was significantly higher than the levels of the diabetic group (137.60±45.58ng/ml). Endogens antioxidants responses to the effect result by free radicals due to increase of oxidative stress. Detoxification of reactive oxygen species, or to convert them from high reactive state to less reactive one, requires consumption of the antioxidants to conduct this process [25-27].

Glutathione neutralizes hydrogen peroxide in presence of GPX, at the same time, the oxidized form of GSSG converts to glutathione in presence of GR and NADPH [28-30]. The results revealed a significant increasing in uric acid level in patients group than healthy subjects. This result may be attributed to increase of xanthine oxidase activity or to increase of lipid peroxidation level that needs to a free radical scavengers which are product by xanthine oxidase [31,32]. The levels of Zn, Mn and Se were significant decrease in patients group in comparison with control group. Decreasing of Zn level in sera of patients may be interpreted on the basis of oxidative stress, especially Zn acts at specific sites by bind with SH groups to protect of proteins from oxidants [33-39].
Due to increase oxidative stress, manganese level decreases which in return lead to reduce insulin production and impair glucose metabolism [53]. Our results indicated that Se level in patients groups less than control, this may attributed to the presence of Se in GP x as cofactor which acts as antioxidants for protection against cellular oxidation [38, 39]. The results of this study showed that the level of FBG was inversely correlated (negative correlation) with GP x, GR, Se, Zn and Mn levels in first group as shown in (Table 3), but FBG level was positively correlated with UA, Urea, Creatinine, BMI, age and duration in first groups as shown in (Table 4). This result attributed to the oxidative stress that occurred in disease case. Consumption of endogenous antioxidants like GP x, GR Zn, Mn, increase with increasing of antioxidants [10-12], consequently the oxidative stress leads to decrease their levels and forms imbalance between oxidants-antioxidants. In contrast, other antioxidants like uric acid level increases in patients than control [35].

**Conclusion**

Free radicals generation increase in diabetic case, that lead to decrease in antioxidants levels of GPx1, GSR, Zinc, Selenium and Manganese. The levels of GPx1, GSR, Se, Zn, and Mn are the better indicators to evaluate the oxidative stress, and can be used as markers for early detection of diabetes. Consequently, low level in endogenous antioxidants may cause acceleration in diabetic complications such as cardiovascular disease, nerve damage, blindness, and nephropathy.

**References**


31. Ihatana et al (2014) The Uric Acid Transporter SLC2A9 is a Direct Target Gene of the Tumor Suppressor p53 Contributing to Antioxidant Defense. Source: Duke-NUS Graduate Medical School; Photo: lunar caustic/Flickr/CC.


