Antioxidants status in type 2 diabetic patients in Morocco

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Background/aim: Type 2 diabetes is a heterogeneous and multifactorial metabolic disorder with some relationship to oxidative stress (OS). Since no studies were conducted in the Moroccan population, this clinical investigation aimed at evaluating the antioxidants status in Moroccan patients with type 2 diabetes.

Materials and methods: Blood samples of 60 type 2 diabetic patients and 40 healthy controls subjects were analyzed for determination of glycemia, hemoglobin, CRP, glycated hemoglobin, lipid parameters, malondialdehyde (MDA), vitamins E and C, copper (Cu), zinc (Zn), and selenium (Se).

Results: CRP and triglycerides were higher in the diabetic group while high-density lipoprotein levels were significantly lower compared to the control group. Plasma MDA, Cu concentrations, and Cu/Zn ratio were found to be higher in diabetic patients compared to healthy subjects, while vitamin E, Zn, and Se concentrations were lower compared to the control group. No significant difference was found in vitamin C levels between the two groups. Plasma HbA1c was positively correlated to MDA levels.

Conclusion: This study shows that antioxidant status is impaired in diabetics compared to healthy controls.

Key words: Type 2 diabetes, oxidative stress, trace elements, vitamins, lipid peroxidation

1. Introduction
Type 2 diabetes is a heterogeneous and multifactorial metabolic disorder defined by the presence of hyperglycemia, which results from a combination of insulin action resistance and impairment of insulin secretion (1). Insulin is a pancreatic hormone that helps cells to use glucose as the main source of energy (2). Several factors may contribute to the development of type 2 diabetes, such as genetic risk factors, environmental factors, and underlying diseases. Overweight and obesity play also an important role in the development of this disease (3).

There is growing scientific interest in the relationship between oxidative stress (OS) and diabetes in general and type 2 diabetes in particular. Published studies indicate that OS plays a major role in the pathogenesis and development of diabetes complications (4,5). OS occurs when there is an imbalance between the production of reactive oxygen species (ROS), often augmented by dysfunctional mitochondria, and insufficient antioxidant defense systems (6,7).

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** The first and the second authors made equal contributions to the study.
lipoproteins (HDLs), triglycerides (TGs), and C-reactive parameters, i.e. total cholesterol (TC), high density lipoprotein (HDL) and low density lipoprotein (LDL). Routine blood chemistry analysis was performed on whole blood (EDTA). A blood sample was collected from each individual after a 12-hour fasting period. The main exclusion criteria included smoking, pregnancy, lactation, receiving vitamin complexes and antioxidants supplements; and high blood pressure; kidney, heart, or liver disease; dyslipidemia; and acute infection. All subjects were recruited from the Department of Internal Medicine B, Mohammed V Military Teaching Hospital of Rabat, Morocco. Forty apparently healthy volunteers were also recruited as a control group. Subjects were randomly selected and an informed consent was sought and obtained from individuals before enrolment into the study. Inclusion criteria were patients with diabetes for at least 3 years, diagnosed on the basis of fasting glucose of greater than or equal to 1.26 g/L, and glycated hemoglobin (HbA1c) greater than or equal to 6.5%. A body mass index (BMI) of less than 25 kg/m² was considered an inclusion criterion to ensure that hyperglycemia could be the main causative factor of SO related to type 2 diabetes. The main exclusion criteria included smoking; pregnancy or lactation; receiving vitamin complexes and antioxidants supplements; and high blood pressure; kidney, heart, or liver disease; dyslipidemia; and acute infection. All subjects were of Moroccan origin.

2. Subjects and methods

2.1. Subjects

This cross-sectional study was conducted according to the principles of the Declaration of Helsinki and was approved by the local Ethical Committee of the Faculty of Medicine and Pharmacy in Rabat, Morocco.

The study included 60 patients with type 2 diabetes, under treatment other than insulin, admitted to the Department of Internal Medicine B, Mohammed V Military Teaching Hospital of Rabat, Morocco. Forty apparently healthy volunteers were also recruited as a control group. Subjects were randomly selected and an informed consent was sought and obtained from individuals before enrolment into the study. Inclusion criteria were patients with diabetes for at least 3 years, diagnosed on the basis of fasting glucose of greater than or equal to 1.26 g/L, and glycated hemoglobin (HbA1c) greater than or equal to 6.5%. A body mass index (BMI) of less than 25 kg/m² was considered an inclusion criterion to ensure that hyperglycemia could be the main causative factor of SO related to type 2 diabetes. The main exclusion criteria included smoking; pregnancy or lactation; receiving vitamin complexes and antioxidants supplements; and high blood pressure; kidney, heart, or liver disease; dyslipidemia; and acute infection. All subjects were of Moroccan origin.

2.2. Methods

Blood samples, in tubes with or without anticoagulants, were collected after 12 h fasting from both groups. The anticoagulant used was ethylenediaminetetraacetic acid (EDTA) and lithium heparin. The samples were centrifuged at 4000 rpm for 15 min in order to recover the plasma or serum except for the HbA1c, which was performed on whole blood (EDTA). Routine blood chemistry parameters, i.e. total cholesterol (TC), high density lipoproteins (HDLs), triglycerides (TGs), and C-reactive protein (CRP), were analyzed in fresh blood samples using a Cobas Integra 400 plus (Roche Diagnostics, Germany) autoanalyzer, while the low density lipoproteins (LDLs) were calculated from TB concentrations, HDLs, and TGs through the Friedewald formula:

\[ \text{LDL (g/L)} = \text{TC (g/L)} - \text{HDL (g/L)} - \frac{\text{TG (g/L)}}{5} \]

The body mass index was calculated as the weight in kilograms divided by the square of height in meters (kg/m²). HbA1c was determined by high-performance liquid chromatography (HPLC) using a UV detector (D-10, Bio-Rad, Marnes-la-Coquette, France) with detection at 415 nm.

The MDA was determined by HPLC as previously described with some modifications (14). The system used was an ACQUITY UPLC (Waters) coupled to a fluorescence detector (ACQUITY FLR detector, Waters). The system is controlled by MassLynx software (version 4.1). The separation was carried out on an ACQUITY UPLC BEH C18, 1.7 µm, 2.1 × 50 mm column. The fluorescence detection excitation occurred at 515 nm, while emission was at 553 nm. Calibrators and controls for MDA (Recipe, Munich, Germany) were used during the analysis. MDA in plasma samples was measured after thiobarbituric acid reaction and the generation of a fluorescent adduct. A mixture of acetonitrile:water (7:3, v/v) was used as a mobile phase.

Vitamin E and C were determined by HPLC (ACQUITY UPLC, Waters) with UV detection (PDA ACQUITY detector, Waters) at 295 nm and 243 nm for vitamin E and vitamin C, respectively.

Plasma Cu and Zn concentrations were measured by atomic absorption spectrometry (AAS) using an ASC-7000 autosampler in flame-air/acylene (AA-7000; Shimadzu). Plasma Se levels were measured with hydride generation-AAS (HVG-1, Shimadzu). All measurements were performed in duplicate and plasma quality controls purchased from Recipe (Munich, Germany) were used during the analysis.

2.3. Statistical analysis

Depending on their normal or skewed distribution, data are reported as mean ± standard deviation (SD) or median and interquartile. Comparison between variables was performed using the t-test, Wilcoxon’s test, or chi-square test. Pearson or Spearman rank correlation analysis was used to evaluate the correlations between variables. All analyses were performed using SPSS 13.0 for Windows (SPSS, Inc., Chicago, IL, USA). Value of P < 0.05 was considered statistically significant.

3. Results

The main characteristics of the studied population are summarized in Table 1. The diabetic and control groups were similar regarding sex, age, and BMI (P > 0.05).
Table 1. Characteristics of studied population (diabetic and healthy controls groups).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Diabetics</th>
<th>Controls</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>60</td>
<td>40</td>
<td>-</td>
</tr>
<tr>
<td>Sex M/F</td>
<td>26/34</td>
<td>21/19</td>
<td>0.38</td>
</tr>
<tr>
<td>Age (years) m ± SD</td>
<td>54.8 ± 21.1</td>
<td>49.8 ± 18.5</td>
<td>0.29</td>
</tr>
<tr>
<td>BMI (kg/m2) m ± SD</td>
<td>23.5 ± 1.6</td>
<td>24.3 ± 3.7</td>
<td>0.21</td>
</tr>
<tr>
<td>Disease duration (years)</td>
<td>7 (3–11)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

M /F: male/female; m ± SD: mean ± standard deviation; BMI: body mass index.

Table 2 shows that CRP was higher in the diabetic group but remained within the reference range. Regarding the lipid profile, there was no dyslipidemia in either group; however, the levels of triglycerides were significantly higher in the diabetic group while HDL was significantly lower compared to the control group.

Plasma MDA, Cu concentrations, and Cu/Zn ratio were higher in the diabetic patients compared to the healthy subjects, while vitamin E, Zn, and Se concentrations were lower compared to the control group. No significant difference was found in vitamin C levels between the two groups.

Table 3 shows the coefficients of correlation between the duration of diabetes, HbA1c, and different OS biomarkers in the diabetic patients. Plasma HbA1c was positively correlated to MDA levels (r = 0.75, P < 0.001). No other significant correlation was found between the other parameters in this study.

4. Discussion
This study shows that antioxidant status is highly impaired in diabetics compared to healthy controls. This was also observed and studied in animal models of diabetes (15). In fact, overproduction of ROS and an altered antioxidant defense system were reported in humans or animals with diabetes (16). ROS are toxic to tissues due to their high reactivity with various biological components including enzymes (17). This tissue damage contributes significantly

Table 2. Results of the studied parameters.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Diabetics</th>
<th>Control</th>
<th>P</th>
<th>Reference range**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycemia (g/L)</td>
<td>1.88 ± 0.37</td>
<td>0.92 ± 0.11</td>
<td>&lt;0.001*</td>
<td>&lt;1.1</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>4.8 (0.9–6.1)</td>
<td>1.3 (0.5–3.5)</td>
<td>&lt;0.001*</td>
<td>&lt;5</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>7.8 ± 3.2</td>
<td>-</td>
<td>-</td>
<td>4–6.4</td>
</tr>
<tr>
<td>Total cholesterol (g/L)</td>
<td>1.91 ± 0.22</td>
<td>1.75 ± 0.35</td>
<td>0.051</td>
<td>1.5–2</td>
</tr>
<tr>
<td>Triglycerides (g/L)</td>
<td>1.34 ± 0.11</td>
<td>0.73 ± 0.17</td>
<td>&lt;0.001*</td>
<td>0.5–1.5</td>
</tr>
<tr>
<td>LDL (g/L)</td>
<td>1.38 ± 0.25</td>
<td>1.25 ± 0.21</td>
<td>0.06</td>
<td>&lt;1.3</td>
</tr>
<tr>
<td>HDL (g/L)</td>
<td>0.36 ± 0.21</td>
<td>0.56 ± 0.15</td>
<td>&lt;0.001*</td>
<td>&gt;0.55</td>
</tr>
<tr>
<td>MDA (µmol/L)</td>
<td>6.12 ± 0.92</td>
<td>1.05 ± 0.11</td>
<td>&lt;0.001*</td>
<td>0.36–1.24</td>
</tr>
<tr>
<td>Vitamin E (mg/L)</td>
<td>8.8 ± 3.81</td>
<td>12.3 ± 3.15</td>
<td>&lt;0.01*</td>
<td>5–18</td>
</tr>
<tr>
<td>Vitamin C (mg/L)</td>
<td>9.6 ± 4.27</td>
<td>10.5 ± 3.98</td>
<td>0.34</td>
<td>2–14</td>
</tr>
<tr>
<td>Plasma Cu (mg/L)</td>
<td>1.65 ± 0.41</td>
<td>1.29 ± 0.22</td>
<td>&lt;0.01*</td>
<td>0.7–1.4</td>
</tr>
<tr>
<td>Plasma Zn (mg/L)</td>
<td>0.57 ± 0.22</td>
<td>1.11 ± 0.16</td>
<td>&lt;0.001*</td>
<td>0.6–1.2</td>
</tr>
<tr>
<td>Plasma Se (µg/L)</td>
<td>68.8 ± 23.1</td>
<td>95.1 ± 18.5</td>
<td>&lt;0.001*</td>
<td>70–130</td>
</tr>
<tr>
<td>Plasma Cu/Zn</td>
<td>2.88 ± 0.93</td>
<td>1.21 ± 0.24</td>
<td>&lt;0.001*</td>
<td>1.14–1.29</td>
</tr>
</tbody>
</table>

CRP: C-reactive protein, HbA1c: glycated hemoglobin, LDL: low density lipoprotein, HDL: high density lipoprotein, MDA: malondialdehyde; Cu: copper, Zn: zinc, Se: selenium.

*: Statistically significant.

** References values accepted by our laboratory.
to the development of complications related to diabetes, especially cardiovascular and renal diseases (18). The concentrations of vitamin E were significantly lower in diabetic patients while vitamin C concentrations were similar compared to healthy controls. These results are in agreement with a previous study (12), whereas others found significantly lower values for both vitamins in diabetic patients (19). Vitamins C and E play an important role in diabetes and good status or supplementation with vitamin C and E appears to reduce the level of OS associated with hyperglycemia and reduces the prevalence of diabetes complications including coronary heart disease (18). The most important role in the OS of these two vitamins is their behavior as “scavengers” that are oxidized instead of the physiological components, like proteins, DNA, and lipids (20). They also contribute to the stimulation of the antioxidant defense system (21). Vitamin E reduces the susceptibility of LDL to oxidation and inhibits the secretion of pro-inflammatory cytokines (22); it also enhances the action of insulin in patients with resistance to this hormone (23). Vitamin C, meanwhile, has a chemical structure similar to glucose structure and can replace it in several reactions; thus, it contributes to the decrease in the nonenzymatic glycosylation of proteins (18). Furthermore, vitamins C and E appear to improve glycemic control in diabetes and several studies have shown that supplementation of 3 or 4 months with high doses of vitamin C, E, or both reduces the fasting blood glucose level (24–26). HbA1c also appears to decline after this supplementation (18).

The status of trace elements was studied in diabetic patients by several authors. In general, concentrations of Cu were similar between diabetics and controls (27–29). However, the high levels of Cu found in our patients have been reported by other series (30). This could be, in part, explained by the decrease in Cu affinity to ceruloplasmin because of its glycosylation in the diabetic patients (31). Cu is a prooxidant and high levels of this trace element may be associated with OS and an increase in LDL oxidation (32). However, a deficiency of Cu may be associated with carbohydrate intolerance and insulin resistance (33). Cu also has insulin-like activity (29). Compared to controls, the concentrations of Zn were significantly lower in diabetics in our study. These results were consistent with earlier studies (29). Zn is required as a cofactor for a number of intracellular enzymes involved in proteins, lipids, and glucose metabolism. It is also involved in the storage and the release of insulin and in the regulation of its receptor synthesis (34). With Cu, Zn plays an important role in improving the condition of OS state as a cofactor for superoxide dismutase (35). Low values of Zn in diabetic patients can be explained by an alteration of the intestinal absorption of Zn and an increase in urinary excretion, since high concentrations of Zn in urinary secretion are often reported in diabetic patients (36). The Cu/Zn ratios were higher in diabetic patients compared to controls. This ratio is clinically more important than the concentration of each metal separately. A high level of Cu/Zn ratio is associated with increased OS, decreased capacity of the body to handle oxidant, and the increase in inflammatory response (37).

Se is another trace element that plays a key role in the antioxidant defense system as a fundamental component of selenoproteins (glutathione peroxidase, thioredoxin

<table>
<thead>
<tr>
<th>Table 3. Results of the correlations (n = 60) between the duration of diabetes and the percentage of HbA1c with studied oxidative stress markers.</th>
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</thead>
<tbody>
<tr>
<td><strong>Diabetes duration (years)</strong></td>
</tr>
<tr>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td>r</td>
</tr>
<tr>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td>MDA 0.231</td>
</tr>
<tr>
<td>Vitamin E –0.031</td>
</tr>
<tr>
<td>Vitamin C 0.054</td>
</tr>
<tr>
<td>Plasma Cu 0.013</td>
</tr>
<tr>
<td>Plasma Zn -0.015</td>
</tr>
<tr>
<td>Plasma Se -0.249</td>
</tr>
<tr>
<td>Plasma Cu/Zn ratio 0.110</td>
</tr>
</tbody>
</table>

* Spearman test.
** Pearson test.
***: Statistically significant
reductase, etc.) (38). As suggested by several previous studies, selenoproteins play an important role in the redox homeostasis and protection against OS and inflammation (38). A value higher than or equal to 80 µg/L of Se is considered to be necessary to ensure adequate production of these selenoproteins (39). In our series, the mean plasma Se was lower in diabetic patients compared to the control group (68.8 ± 23.1 µg/L against 95.1 ± 18.5 µg/L; P < 0.001) and is also below the recommended threshold of 80 µg/L. This lower value of Se associated with diabetes was observed in previous studies (40,41). The relationship between Se and diabetes is very complex and the results are not yet conclusive. Some researchers concluded that high levels of Se may reduce the prevalence of diabetes (42), while others have suggested that high levels of Se could be related to the increased prevalence of diabetes (43). Establishing a link between the status of Se and the development of diabetes is, indeed, very delicate; on the one hand because diabetes is a multifactorial disease and on the other hand because the plasma Se status varies according to different regions and depends mainly on the eating habits and the richness of soil of the region in Se (44). Whatever the case, in diabetics, the glycosylation of selenoproteins alters the mechanisms of action of Se and therefore antioxidant defense is reduced (45).

In addition, this study showed an increase in MDA levels in the diabetic group compared to the controls. Similar results were found in other studies (11,46). MDA is a product of lipid peroxidation and it is considered a significant biomarker for OS (46). The increase in lipid peroxidation alters the function of cellular membranes by reducing their fluidity and changing the activity of enzymes and how the receptors bind to the cellular membrane (46). This lipid peroxidation is not only due to the ROS activity as oxidant but also to the increase in protein glycation in diabetes. These glycated proteins themselves could act as a source of free radicals (47). There is a clear link between lipid peroxidation and glucose concentration, which may also play a role in increased lipid peroxidation in diabetes (11). Bhutia et al. (48) noted significant increases in MDA levels associated with an increase in fasting glucose in poorly controlled type-2 diabetes. In our study, we did not find a significant correlation between glucose and MDA. However, MDA concentrations were positively correlated with the percentage of HbA1c, which is a better indicator of average blood glucose levels over the past two months. These results are consistent with other studies (49) that found a positive correlation between HbA1c and MDA levels. However, other studies did not observe any correlation between the two parameters (50).

The results of our study show that the studied OS biomarkers are disrupted in diabetics. This disruption could accentuate OS status associated with diabetes. This is a preliminary study that should be supplemented by further studies involving a larger number of patients and other OS biomarkers.

In conclusion, this study showed that OS biomarkers are disrupted in type 2 diabetic patients. Vitamin E, Zn, and Se levels were decreased while Cu and MDA were increased in diabetic patients compared to controls. We also found a positive correlation between HbA1c and MDA. We suggest the determination of these biomarkers in diabetics but further studies are needed to justify the interest in supplementation to correct deficits.

References

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