Biomarkers of Oxidative Stress and Antioxidant Defense in Patients with Type 1 Diabetes Mellitus

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Abstract

Background: Oxidative stress has become the focus of interest in most biomedical disciplines and many types of clinical research. Increasing evidence from research on several diseases shows that oxidative stress is associated with the pathogenesis of diabetes and many other diseases. Objectives: We aimed to investigate the status of oxidative stress and antioxidant enzymes related parameters in type 1 diabetes mellitus (T1DM) patients. Patients and Methods: A total of 110 patients (70 patients newly diagnosed diabetic and 40 healthy) were studied by evaluating the level of lipid peroxidation (malondialdehyde [MDA]), nitric oxide (NO), fasting blood sugar (FBS), and glycated hemoglobin (HbA1c). Antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), were also evaluated. Results: The FBS and HbA1c levels were significantly higher in diabetic patients compared to those of healthy participants. Higher levels of MDA and NO were observed in the diabetic group compared to those in the healthy participants. A significant decrease was observed in serum SOD, CAT, and GPx activities in the serum of T1DM patients by 16.7%, 72.8%, and 15.3%, respectively (P < 0.05), as compared with their activities in the controls. Conclusions: These results indicated that oxidative status and antioxidant levels were affected in T1DM. The results suggested that the biomarkers such as the plasma levels of lipid peroxidation and antioxidants in early diagnosed diabetics can be used to monitor the developing complications of the diabetes.

Keywords: Antioxidants, lipid peroxidation, oxidative stress, type 1 diabetes mellitus
in thiobarbituric acid reactive species concentration in T1DM patients with or without nephropathy compared with control.\[6\] Studies also indicated that GPx activity was lower in children with diabetes compared with healthy children (controls).\[7,8\] According to these results, it has been implied that lowered antioxidative defenses from the excessive production of lipid hydroperoxide and NOx overproduction are present in juvenile patients with T1DM.\[9\]

**Patients and Methods**

**Study design**

This study was conducted which recruited both males and females with T1DM in which they have not diabetic diagnosed before as well as nondiabetic participants. A cross-sectional study was conducted on 70 patients with T1DM (36 males and 34 females) aged 3–15 years who attended the Tripoli Medical Center (TMC), Tripoli, Libya. In addition, a control group composed of 40 nondiabetic individuals (20 males and 20 females) all within the same age range as the other patients who were not administered any medications. A detailed medical history was taken, and a physical examination was performed upon all participants. The Ethics committee of Biotechnology Research Center (Tripoli, Libya) approved the study (BEC-BTRC 03-2017). All patients provided a written informed consent before the start of the study procedures.

**Selection criteria**

Participants included in the current study were selected according to the following criteria: first, they were newly diagnosed with T1DM patient; second, they were free of any ailment which could affect the parameters under study; and third, they are not on any medication. Hemolytic anemia, hemoglobin variants, hepatic disease, and infectious diseases, such as tuberculosis and sarcoidosis, were excluded from the study. In addition, participants under treatment with drugs, such as chelating agents, ethambutol, D-penicillamine, were also excluded from the study.

**Blood sample collection**

Blood samples were collected into commercial tubes after overnight fasting for the analysis of laboratory parameters. Venous blood samples (5 ml) were obtained from the capital vein of each participant using sterile disposable plastic syringes. Specimens were collected at the same standardized time to minimize any effect of diurnal variation. The blood samples in the vacutainer tubes were left to clot and the serum was separated by centrifugation. The clear, nonhemolyzed supernatant sera were separated using clean, dry disposable plastic syringes. Samples were stored at −80°C and used within 1 month for the analysis of malondialdehyde (MDA), nitric oxide (NO), glutathione (GSH), and measurement of antioxidant enzymes.

**Determination of biochemical parameters**

Various biochemical parameters in blood were measured according to the standard methods including glucose, creatinine, total protein, alanine aminotransaminase, aspartate aminotransaminase, triglycerides (TG), total cholesterol, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) cholesterol. All parameters measured according to the standard protocols in both T1DM and nondiabetic controls using premodular analytics by using a kit with Cobas Integra® 400 plus in the Biochemistry Laboratory, at the TMC, Tripoli, Libya.

**Oxidative stress parameters**

Thiobarbituric acid-reacting substances content, a measure of lipid peroxidation was determined spectrophotometrically in serum according to the method of Zhang et al.\[10\] while NO in serum was determined according to the Griess method with some modification.\[11\]

The CAT activity was assayed using the spectrophotometric method, based on the ability of hydrogen peroxide to form a stable stained complex with molybdenum salts.\[12\] GPx activity was determined according to the method of Hafeman et al.\[13\] The estimation of SOD activity was performed using a SOD Assay kit-WST (Sigma Aldrich, USA) according to the manufacturers’ protocol (Dojindo, Gaithersburg, MD, USA). Xanthine-xanthine oxidase system was used to generate a superoxide flux, and nitroblue tetrazolium was used as an indicator of superoxide production. Reduced GSH was determined using Ellman’s reagent. Formations of color product monitored at 412 nm after oxidation of GSH by 5,5′-dithio bis-2-nitrobenzoic acid.\[14\]

**Statistical analysis**

Results were expressed as a mean ± standard deviation. The statistical significance was assessed using the analysis of variance. $P < 0.05$ was considered statistically significant.

**Results**

**Characteristics of study subjects**

Table 1 shows the clinical characteristics of the participants in the current study. The groups (diabetic patients and healthy control participants) were similar regarding sex and age. The study involved 70 patients with T1DM which are newly diagnosed. The ages in the control group are comparable to those in the overall group of type 1 diabetic cases studied. There was no gross obesity in the population studied, as can be seen from their body mass index [Table 1].

The demographic evaluation, fasting blood sugar (FBS), and glycated hemoglobin (HbA1c) levels are shown in Table 1. The participants were classified according to their sex. There were 36 male and 34 female represented 32.73% and 30.90% of the total patients’ group, respectively. There were 20 males representing 22.22% of the control group and 20 females representing 22.22% of the same group. A significant increase in FBS was found in diabetic patients (173.09 ± 17.80 mg/dl) as compared to control group (68.42 ± 2.01 mg/dl) ($P < 0.05$). Furthermore, HbA1c is very significantly increased by 74.7% ($P < 0.0001$) in diabetic participants compared to
the control group. Among 65% of diabetes participants had a very high HbA1c level among them 55.22% have HbA1c more than 8%.

**Lipid profile assessment**

Considering lipid profile, although there was an increase in TG levels in type 1 diabetic participants (89.3 ± 8.4 mg/dl) compared to control group (82.53 ± 52.08 mg/dl), no significant difference between the two groups was observed. Similar results were obtained regarding HDL cholesterol [Table 2]. However, LDL cholesterol was found to be significantly increased (84.29 ± 22.71 mg/dl) (\( P < 0.05 \)) compared with healthy control participants. Furthermore, cholesterol level in diabetic patients (158.06 ± 26.58 mg/dl) was higher compared with its levels in healthy participants (147.05 ± 5.51 mg/dl) [Table 2]. In addition, the HDL/LDL cholesterol ratio did not show significant differences.

**Oxidants and antioxidants levels**

Table 1 shows serum MDA and NO levels, parameters of oxidative stress as well as serum antioxidants including GSH, CAT, GPx, and SOD. The level of MDA and NO was found to significantly elevated almost 2-fold and 4.5-fold, respectively, in the blood of type 1 diabetic participants (0.37 ± 0.10, 3.98 ± 0.27 nmol/mg protein, respectively) than the respective control group (0.19 ± 0.08, 0.89 ± 0.27 nmol/mg protein, respectively) (\( P < 0.0001 \)). A significant decrease was observed in serum SOD activity in the serum of type 1 patients (11.05 ± 2.49 U/ml; by −16.66%) as compared with their respective controls (13.26 ± 3.47 U/mg protein) (\( P < 0.05 \)). A highly significant decrease in the serum CAT activity in patient with diabetic participants (0.49 ± 0.42 U/mg protein by −72.77%) as compared to control group (1.80 ± 0.94 U/mg protein) (\( P < 0.0001 \)) was observed. The activity of GPx was found to be lower in patients by 15.29% than the control group [Table 1].

When the comparison was made on the basis of sex, a significant increase in FBG and HbA1c compared to their respective controls was obtained [Table 2]. In diabetic females, the levels of cholesterol and LDL were markedly higher than their levels in healthy females [Table 2]. The concentrations of both MDA and NO were very significantly decreased (\( P < 0.0001 \)) in the type I DM participants than their respective control groups [Figure 1]. A significant decrease was found in GSH level in diabetic males (0.018 ± 0.006 µg/mg protein) and diabetic females (0.016 ± 0.004 µg/mg protein) (\( P < 0.0001 \)) as compared with their respective controls (0.02 ± 0.004 µg/mg protein; 0.024 ± 0.007 µg/mg protein, respectively) [Figure 2]. Moreover, there was a marked drop in the activity of CAT and GPx levels in the type I DM compared to their respective controls (\( P < 0.0001 \); however, there were no significant differences in the activity of SOD was found between the males and females with type I DM compared with their controls (\( P > 0.05 \)) [Figure 3].

The demographic evaluations of the diabetic male and female participants are shown in Table 1. Considering the lipid profile, there was no significant difference between diabetic male and the diabetic female groups [Table 2]. Furthermore, HbA1c was higher in both diabetic males and females; however, GSH was higher in diabetic females only. The activities of SOD and GPx did not exhibit any significant difference (\( P > 0.05 \)) between diabetic males and females [Table 2]. Furthermore, the level of MDA and NO was slightly higher (though not significantly) in diabetic males compared with diabetic females [Figure 1].

**Interrelationships between oxidants and antioxidants between studied variables**

To analyze the influence of oxidative stress parameters on antioxidants status, the Pearson correlation was performed. The analysis revealed a significant negative correlation between HbA1c and SOD in the diabetic patients (\( r = -0.339, \)
GPx with NO ($r = −0.355$, $P < 0.01$) and GSH ($r = −0.315$, $P < 0.05$). On the other hand, no correlation was found between lipid peroxidation (MDA) and nonenzymatic (GSH) or any of the other enzymes (SOD, GPx, and CAT) in the assessed antioxidants. No correlation was found between lipid profile parameters (TAG, cholesterol, LDL, and HDL) with regard to peroxidation parameters (MDA and NO) and antioxidants (SOD, CAT, and GSH) or HbA1c in females type 1 diabetes. The only positive correlation was found between GPx and NO ($r = −0.510$, $P < 0.01$) in female diabetics. Regarding diabetic males, a statistically significant negative correlation was found between the levels of HbA1c with SOD ($−0.440$, $P < 0.05$) and MDA ($0.402$, $P < 0.01$). A significant positive correlation was also observed between TAG and MDA ($r = 0.203$, $P < 0.05$) and NO ($r = 0.311$, $P < 0.01$) in all the diabetics. In addition, a significant positive correlation was detected between HDL and GPx ($r = 0.296$, $P < 0.01$) as well as a significant negative correlation between HDL and NO ($r = −0.344$, $P < 0.01$) in type 1 diabetes participants. Moreover, a significant positive correlation was found between LDL and NO ($0.263$, $P < 0.05$). Furthermore, a negative correlation was observed between HDL and NO ($−0.403$, $P < 0.01$) and positive correlation with GPx ($0.396$, $P < 0.01$).

**Discussion**

There are some contradictory results regarding the role of oxidative stress in the etiology of type I DM. Therefore, the present study was aimed to investigate whether oxidative stress...
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**Figure 3:** Antioxidant enzymes activities in healthy participants and type 1 diabetic patients on the bases of sex. Values are represented as mean ± standard deviation. *P < 0.05; ***P < 0.0001 considered statistically significant as compared to control. SOD: Superoxide dismutase, GPx: Glutathione peroxidase, MDA: Malondialdehyde, NO: Nitric oxide radical, T1DM, insulin-dependent (Type-1) diabetes mellitus.

**Conclusions**

The oxidative status is clearly affected the endogenous antioxidant status in the newly diagnosed diabetic patients.
with T1DM. The plasma levels of lipid peroxidation and NO measurements can be considered as early biomarkers of oxidative damage, which can be used to monitor the developing complications of diabetes.

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**Authors’ contributions**

RA conceived and designed the experiments. NA, OA, and SE performed the experimental works. RA, NA, and NAw analyzed and interpreted the data. RA drafted the paper. NAw revised the manuscript. All authors revised the paper and approved its final version.

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**Conflicts of interest**

There are no conflicts of interest.

**Compliance with ethical principles**

The study was approved by the Ethics Committee at the Biotechnology Research Center, Tripoli, Libya (BEC-BTRC 03-2017). Parents of all patients provided written informed consent before starting of the study procedures.

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