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ORIGINAL ARTICLE

ASSESSMENT OF OXIDATIVE STRESS AND ANTIOXIDANT STATUS IN TYPE 1 DIABETES MELLITUS

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Summary

Increased oxidative stress appears to be a risk factor for insulin resistance, dyslipidemia, β-cell dysfunction, impaired glucose tolerance, and, eventually, diabetes mellitus. The majority of research shows that oxidative stress plays a role in the etiology of diabetes through changes in enzymatic systems, lipid peroxidation, decreased levels of vitamin C, and reduced glutathione metabolism. **Objectives:** the study was conducted to evaluate biomarkers of oxidative stress such as serum malondialdehyde (MDA), myeloperoxidase (MPO), catalase (CAT), vitamins (C, E), nitric oxide (NO), reduced glutathione (GSH) levels and a lipid profile in newly diagnosed type I diabetics (IDDM). **Methods:** Patients are selected by simple randomization after professional diagnosis based on clinical examination and laboratory tests in a case-control study. This study included 24 newly diagnosed type I diabetics and 20 as a control group. **Results:** Newly diagnosed IDDM patients showed significantly higher MDA, MPO, TC, LDL, TC, TG, and AIP than the control group. The diabetic group also showed a significant decrease in CAT, GSH, HDL, NO and vitamins (E and C) when compared to healthy subjects. **Conclusion:** This study demonstrated that patients with type 1 diabetes have a disturbance in oxidant/antioxidant status and lipid profile.

Key words: Antioxidants; Type 1 diabetes mellitus; Oxidative stress; Lipid; Vitamins

Introduction

Oxidative stress is excessive production or inadequate scavenging of free radicals such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) (1).

An antioxidant is any agent that prevents or slows down the oxidation of a substrate, even at very low concentrations. Depending on their mode of action, antioxidants are classified as either chain-breaking antioxidants or protective antioxidants (2). Antioxidants include vitamins C and E, glutathione (oxidized or reduced), cysteine, and other biological antioxidants. Superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT) are antioxidant enzymes that may increase the destruction of or promote the scavenging of ROS in diabetes (3).

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So, differences in these enzyme levels make tissues more sensitive to oxidative stress, which may lead to diabetes complications (4). In DM, oxidative stress damages cellular machinery and enzymes, and increases insulin resistance due to the production of free radicals by the oxidation of glucose, the non-enzymatic glycation of proteins and, the promotion of lipid peroxidation (5). Free radicals have a powerful effect on destroying proteins, lipids and DNA. Therefore, it plays an important role in the initiation and progression of late diabetes complications.

In vitro studies show that myeloperoxidase converts l-tyrosine to 3,3-dityrosine, which acts as a link across the polypeptide protein chains, making it a useful marker for protein oxidation Dyslipidemia is a common feature of DM, which makes cells more susceptible to lipid peroxidation (6). Malondialdehyde (MDA) , produced by lipid peroxidation, is used to assess lipid peroxides (7).

NO is a key modifier with a wide range of biological effects, such as vasodilating, preventing sodium reabsorption in the tubules and increased natriuresis. Blocking nitric oxide production leads to increased blood pressure and vascular damage (8).

Vitamins are essential components of the biological system because they are involved in several biochemical activities. Vitamins such as (A, C and E) detoxify free radicals. Any alterations in their levels are considered important indicators of oxidative stress. In some circumstances, these vitamins also enhance toxicity by forming oxidants. Diabetes has been linked to increased or decreased levels of vitamin E in the body. However, contradictory data indicate that vitamin E has negative effects on the vascular changes associated with diabetes (5).

Glutathione enzymes are affected by diabetes and are responsible for converting glutathione disulfide into glutathione and converting peroxide into water (5). Altering enzyme levels will expose cells to oxidative stress and, as a consequence, cell death. The metabolism of hydrogen peroxide (H2O2) is regulated by catalase (CAT), which may cause damage to RNA, DNA and lipids if produced in excessive quantities. CAT catalytically transforms hydrogen peroxide to H2O and O2, neutralizing it. CAT deficiency leads to oxidative stress to the beta cells of the pancreas, which have a large number of mitochondria, which leads to beta cell dysfunction and eventually diabetes (9). Hyperglycemia, insufficient insulin production and/or resistance to the insulin can cause diabetes to develop (10). The current study was carried out to assess the effect of oxidative status in newly diagnosed type 1 diabetecs.

Patients

The prospective study was conducted in the Al-Wafa Center/Ninevah, Iraq. All participants who were diagnosed with IDDM were included in this study; their ages ranged from 32 to 56 years. Before being included in the experiment, all participants gave their informed consent. This study included two groups: the first included 24 newly diagnosed patients with type 1 diabetes (FSG $\geq$ 126 mg/dl, HbA1c $\geq$ 6.5), while the second included 20 as a healthy control group. Pregnant, breastfeeding women, individuals using additional medications, supplements or vitamins, patients with other diseases, those who drink alcohol and smokers were excluded from the study. The BMI was calculated using body parameters such as height and weight.

Blood samples and biochemical analysis

After fasting overnight, venous blood samples from diabetic patients were obtained in simple tubes. Except for the blood glucose level, which was determined immediately after a ten-minutes incubation period (at 37 °C) in a water bath. The serum sample was centrifugated at 4000 rpm for ten minutes, then transferred to an Eppendorf tube and kept (at −20 °C) for later analysis.

Analytical statistics

The Mann Whitney test and Kruskal-Wallis test were used to compare two datasets. All data was reported as mean± standard deviation, and statistical significance was determined at (p-value <0.05). Graphpad Prism software version 8.0 was used for all studies (San Diego, California, USA).

Assessment of blood glucose, insulin and insulin resistance levels

An enzymatic colorimetric method was used to calculate FSG and measure the absorbance (at 505 nm). Serum insulin was measured at 450 nm using an enzyme-linked immunosorbent assay (ELISA) and Homeostatic Model Assessment (HOMA-IR) was used to determine insulin resistance using the equation:

Insulin (μU / mL) × Glucose (mmol/L) / 22.5 = HOMA-IR (10).

MDA

The modified approach (11) was used to assess serum MDA which combines with thiobarbituric acid to produce a colored compound that can be used to determine lipid peroxidation (measurement of absorbance at 532 nm).

NO, MPO and lipid profile

Greiss reagent was used to calculate serum NO levels (12). Equal proportions (200 μl)) of the obtained supernatant and Griess reagent were mixed, and ELISA was used to measure absorbance at 540 nm. The NO content was tested using a standard curve for sodium nitrite. The enzymatic activity of MPO was evaluated by the enzymatic oxidation of 0-dianisidin method (13, 14), (substrate reduction) catalyzed by H2O2 to produce a colored material with a wavelength of 450 nm. A colorimetric approach based on the sulfo-phospho-vanillin reaction was used to determine blood lipid levels (15).

CAT activity

CAT activity was determined by measuring the decrease in hydrogen peroxide absorption using spectroscopy (240 nm) (16).

glutathione (reduced)

A modified standard Ellman technique (17) was used to determine serum GSH levels. The reaction of GSH with Ellman's reagent (DTNB) (5,5'-dithiobis-2-nitrobenzoic acid) and produces a bright yellow product that can be detected (at 412 nm).

vitamin C and vitamin E

The decrease in the uptake of 2,6-dichlorophenolinophenol (absorpance at 520 nM) was used to assess serum vitamin C levels (18). Iron ion absorption from iron reductase was measured at 460 and 520 nm to estimate serum vitamin E levels (19).

Results

Demographic features of newly diagnosed IDDM patients and controls.

The age and BMI of all participants are shown in Table 1. There are no significant differences between them.

Table 1 shows the demographic features of the groups.

Demographic features	Control n=24	Newly diagnosed n=20
Age (year)	19.9 ± 5.2	19.1 ± 4.8
BMI Kg/m^2	21.89 ± 2.79	21.79 ± 1.8

Diagnostic parameters of T1DM (Fasting serum glucose (FSG), insulin and homeostatic model of insulin resistance (HOMA-IR)).

Newly diagnosed patients had significantly higher FSG levels than the control group. The diabetic group also showed significantly lower insulin levels compared to healthy individuals.

Table 2 shows the diagnostic parameters of the groups.

Diagnostic parameters	Control n=24	Newly diagnosed n=20
FSG (mmol/l)	4.621 ± 0.5767	12.98 ± 0.9856 ****
Insulin (µu/L)	11.29 ± 0.9314	3.685 ± 0.9227 ****
HOMA-IR	2.122 ± 0.3892	2.212 ± 0.3836

The data is presented as mean ± SD. *Statistically significant variances were assessed using the Mann Whitney test and Kruskal-Wallis test (****$p < 0.0001$).

Effect of oxidation variables

MDA, MPO, AIP, LDL, TG, and TC levels were found to be significantly higher in newly diagnosed patients than in control individuals. Also, newly diagnosed patients had significantly lower levels of CAT, GSH, NO, vitamins (C and E) and HDL compared to the healthy subjects.

Table 3 shows the oxidant and antioxidant parameters between groups.

Parameters	Control n=24	Newly diagnosed n=20
MDA (µmol/L)	2.334 ± 0.5537	4.113 ± 0.6668 ****
MPO (U/ml)	14.79 ± 1.39	25.78 ± 1.598 ****
CAT (U/ml)	0.1576 ± 0.03526	0.07684 ± 0.07286 ****
GSH (µmol/L)	14.89 ± 0.976	7.134 ± 0.6324 ****
NO (µmol/L)	14.49 ± 0.9797	12.29 ± 0.6232 ****
Vitamin C (µmol/L)	39.21 ± 3.989	28.89 ± 3.215 ****
Vitamin E (µmol/L)	21.32 ± 3.421	12.87 ± 1.126 ****
AIP	-0.05801 ± 0.08012	0.2798 ± 0.09305 ****
HDL (mmol/L)	1.3412 ± 0.1120	1.112 ± 0.1213 ****
LDL (mmol/L)	2.622 ± 0.5213	4.112 ± 0.3121 ****
VLDL (mmol/L)	0.5488 ± 0.07998	0.9612 ± 0.0897 ****
TG (mmol/L)	1.197 ± 0.1901	2.088 ± 0.1976 ****
TC (mmol/L)	4.387 ± 0.7124	5.732 ± 0.1867 ****

The data is presented as mean ± SD. *Statistically significant variances were assessed using the Mann Whitney test and Kruskal-Wallis test (****$p < 0.0001$).

Discussion

The current study confirmed that type 1 diabetics have an imbalance in oxidant and antioxidant levels. We found that diabetic group had significantly high levels of MDA, MPO, AIP, LDL, TG, and TC and significantly low levels of GSH, NO, HDL, and vitamins (C and E) compared to control group. Diabetes mellitus depletes

antioxidants and promotes lipid peroxidation which involves an interaction of ROS with lipids that causes damage to lipid membrane of the cell. MDA, a byproduct of lipid oxidation, is a biomarker of oxidative stress (20). In this study, newly diagnosed IDDM patients showed significantly increased MDA levels compared to healthy individuals (Table 3), supporting the theory that persistent hyperglycemia in diabetes has been linked with an an increasing perioxidation of lipid markers such as MDA. Firouzray *et al*. (2007) found that MDA levels, TC and LDL were significantly raised in diabetic patients compared to the control group. They studied the effect of antidiabetic drugs on the lipid profile and oxidative stress in diabetics. In a later study, the authors hypothesized that patients with type 1 diabetes are at increased risk of atherosclerosis; they also found that dyslipidemia was correlated with oxidative stress (21). The reduction in insulin sensitivity could be caused by oxidative stress, since ROS can pass through cell membranes and damage beta cells in the pancreas (22).

Our results revealed a low significant level of NO compared to the healthy subjects. Various studies have shown changes in nitrogen oxide levels in patients with diabetes, although the results are mixed. Some studies have indicated low serum NO levels in diabetic patients (23). Nitric oxide is an example of ROS. It is produced in the body during normal physiological processes and is flushed out by endogenous antioxidant systems (enzymatic and non-enzymatic) such as glutathione peroxidase, catalase, and vitamins (E and C) (24). ROS cause tissue damage; therefore, in diabetic patients, they reduce the presence of endothelial-derived nitric oxide (25, 26).

In IDDM patients, serum levels of MPO were significantly higher than the control group, according to our results. In a study by (27), it was found that the blood MPO level in children with type 1diabetes was significantly higher than in healthy controls, which may explain the increased risk of cardiovascular disease in these patients. Although the exact mechanism is unknown, the role of MPO is clearly related to one mechanism proposed as a mediator of vascular injury (28). NO has recently been proposed as a regulator and substrate for MPO act. As a consequence, increased MPO activity is causing overuse of NO and endothelial dysfunction. The reactive nitrating oxidant species of NO is produced by MPO. Therefore, the MPO pathway have a significant role in the inflammatory process as well as diabetic vascular impairment (29).

T1DM and dyslipidemia have been shown to be closely related (30). To reduce CVD, it appears that a focus on dyslipidemia in patients with type 1 diabetes is critical. Several studies have found that individuals with type 1 diabetes have higher circulating levels of CVD risk markers, such as AIP, TC, TG, LDL, and VDLA, as well as lower levels of HDL (31). These studies, interestingly, support our results. The cause of the adiposity is unknown, but it is thought to be correlated with insulin insufficiency and hyperglycemia (32). Insulin has an important role in regulating lipid metabolism by inhibiting lipase in adipose tissue. Insulin also affects HDL metabolism by inhibiting lipase activity in the liver (33). Therefore, our study revealed that a significant decrease in the insulin level causes dyslipidemia.

Glutathione and catalase and act as protective mechanisms against damage caused by oxidative stress. In the initial stages of type 1 diabetes mellitus, the level of glutathione was found to be significantly lower in diabetics (34). Decreased glutathione concentration under severe oxidative stress could explain the reduced activity of glutathione peroxidase (35). However, several researchers have noted no differences in glutathione peroxidase activity between newly diagnosed IDDM patients and healthy subjects (36). In patients with type 1 diabetes, Dave *et al*. (2007) found significant reductions in catalase and glutathione when compared with healthy individuals (37). This suggests that the depletion of endogenous antioxidants is caused by increased lipid peroxidation during the disease's early stages. The decreasing in antioxidant levels (enzymatic and non-enzymatic) was observed in newly diagnosed diabetics are due to their overuse of ROS waste, as evidenced by increased lipid peroxidation, in response to hyperglycemia (38). This result is in agreement with (39). In a study of twenty IDDM patients, it was found that MDA levels improved while vitamin E and glutathione levels decreased (40). After three months of treatment with vitamin E (600 mg/day), malondialdehyde levels were significantly reduced, while vitamin E and glutathione increased significantly. Data show that vitamin E strengthens the antioxidant defense mechanism and reduces oxidative stress in IDDM patients. To combat various types of free radicals, all antioxidants collaborate in a synergistic mechanism. Vitamin C prevents the development of hydroperoxide when combined with vitamin E and vitamin E slows down the spread of lipid peroxidation (Laight *et al*., 2000), Further investigations are needed to find an association between reactive oxygen species and diabetes complications. This will be useful for the type 1 diabetic in terms of prevention, monitoring, and treatment options.

Conclusion

This study revealed an increase in lipid peroxidation, lipid profile and MPO in newly diagnosed IDDM patients, while a decrease in antioxidants (enzymatic and non-enzymatic) and NO. Low levels of vitamins (C, E) in IDDM patients indicate the need for their supplementation in addition to the main therapy.

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Conflict of Interest

The authors declare no conflicts of interest.

Adherence to Ethical Standards

The study was approved by the ethical committee in the Nineveh Health/ Iraqi Ministry of Health, the approval number and date (33009 on 27/2/ 2022).

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