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Evaluation of Oxidative Stress Level and some Antioxidant Enzymes activity Parameters in Patients with Type two Diabetes Mellitus

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ABSTRACT

Diabetes, a group of metabolic disorders characterized by dysregulation of oxidative stress and elevated blood glucose levels. It has been studied with emphasis on malondialdehyde (MDA) and glutathione (GSH) levels as biomarkers of lipid peroxidation and antioxidant activity in the serum of type II Diabetes examines patients. The study involved the analysis of 105 serum samples from 75 type II diabetes patients and 30 healthy individuals. MDA and GSH levels served as measures of oxidative stress and antioxidant activity, respectively. In addition, lipid profiles were examined, which include measurements such as total cholesterol (Total C), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C). The results showed significantly increased MDA, total cholesterol, triglycerides and glucose levels in the diabetic group compared to controls. In contrast, GSH and HDL levels were significantly lower in diabetics. In the type 2 diabetes patient group, a correlation between glucose levels and MDA concentration was observed, while no other significant associations were found between lipid profile parameters, glucose levels and MDA or GSH levels. Studies show the complex connection between diabetes and the increase in free radicals and the corresponding decrease in antioxidant synthesis. This dynamic interaction is an important factor in the development of oxidative stress.

Keywords: Type II diabetes mellitus, MDA, GSH, Lipid profile, Blood sugar.

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a common metabolic disorder marked by insulin resistance and elevated blood glucose levels[1,2]. Diabetes is becoming a growing health problem and affects nations regardless of their level of development[3]. According to the WHO, type II diabetes will be the most common disease in 2030, potentially affecting more than 180 million people worldwide[4].

Normal metabolism produces reactive oxygen radicals in healthy people. It is believed that an imbalance between the antioxidant system, which acts as a defense mechanism, and the antioxidant system, which favors radicals, causes oxidative stress[5]. In pathophysiological situations such as atherosclerosis, diabetes, cancer, chronic inflammatory diseases, and diseases of the central nervous system. One of the main factors in cellular aging and therefore cell destruction, cell damage and cell death is Oxidative stress[6,7]. In addition, it can be crucial in the development of micro- and macro-vascular problems[8]. Uncontrolled hyperglycemia results in an elevation of free radical production, specifically reactive oxygen species (ROS) which occurs via processes of protein glycosylation and autooxidation of glucose[9]. The breakdown of unsaturated lipids, along with increased lipid peroxidation, is a well-known mechanism responsible for cell damage resulting from oxidative stress[10]. There is strong evidence that diabetes is associated with increased oxidative stress, characterized by increased production of reactive oxygen species (ROS) and impaired antioxidant defense mechanisms[11]. The toxicity of the fatty acid peroxides produced and the induction of membrane lipid peroxidation by ROS have been proposed to significantly contribute to cellular dysfunction[12]. Due to its simplicity, measurement of malondialdehyde (MDA) is the most commonly used test for lipid peroxidation. Therefore, assessment of lipid peroxide levels in the bloodstream provides valuable information about the prognosis of diabetes, complications of which often lead to death[13]. Antioxidants, like glutathione (GSH), act as potent electron donors by providing hydrogen atoms to pair with the unpaired electrons of free radicals[14]. This converts reactive free radicals into inactive substances[15]. In the present study, the complex balance of oxidative stress and antioxidant markers with particular emphasis on MDA and GSH levels was investigated through a detailed assessment of the lipid profile in individuals with type 2 diabetes. This research can provide valuable insights into the dynamic interplay of these biomarkers and reveal differences in the biochemical environment compared to the non-diabetic group.

MATERIALS AND METHODS

Patients design

In this study, blood samples from 75 individuals with type II diabetes (males and females) and 30 individuals in excellent health were analyzed for the presence of a variety of biomarkers. The investigated components included total serum cholesterol, triglycerides, lipoproteins (specifically HDL and LDL), and decreased GSH and MDA levels. This examination was conducted at the General Hospital Taq Taq in Koya-Erbil, Kurdistan Region, Iraq.

Ethical consideration

This research followed the guidelines of the Declaration of Helsinki. Approval for the study (ID: 3/45/109) was obtained from the Ethics Committee of the Chemistry Department, Faculty of Science and Health, Koya University. Before participating in the study, all subjects provided written informed consent.

Serum sampling

After fasting, five milliliters of venous blood are drawn from both patients and healthy individuals. The collected blood was immediately deposited in yellow tubes and centrifuged at 4000 revolutions per minute (rpm) for 10 min at 4 degrees Celsius. The serum was then refrigerated until it was ready to be analyzed.

Laboratory serum analysis

Following the methodology proposed by[16,17], the enzymatic total serum concentration of cholesterol and triglyceride were estimated depending on the enzymatic conversion of cholesterol to quinonimine pigment and the hydrolysis of triglyceride with lipases, then the absorbance spectrophotometrically was measured. The LDL- and HDL-cholesterol levels were also measured according to an outlined method[18,19], and serum lipid profiles were measured through the use of standard kits from BIOLAB GOD-PAP (France).

Serum glucose is determined through the use of standard kits from BIOLAB GOD-PAP (France), which depend upon the enzymatic conversion of glucose by the glucose oxidase (GOD) to red-violet quinonimine as an indicator.

The assessment of reduced GSH levels was carried out following the method studied by Buege and Aust[20]. The concentration of glutathione (GSH) was determined by observing the reaction's final product, which is formed when non-sulfhydrylated (SH) proteins react with the precipitation solution. The addition of sulfhydryl groups to DTNB (5', 5'-) (dithiobis 2-nitrobenzoic acid) produced a solution with a distinct yellow hue. The spectrophotometer measured reduced glutathione in blood samples treated with EDTA anticoagulant at a wavelength of 412 nm after a 24-h period.

The concentration of MDA (malondialdehyde), an indicator of lipid peroxidation, was determined by reacting MDA with thiobarbituric acid (TBA), which produced a mauve substance. Following the procedure outlined in the reference, this product was allowed to come to room temperature before having its absorbance measured at a wavelength of 532 nm using a UV/Vis spectrophotometer[20].

Data analysis

The data was analyzed using a T-test on independent samples using the statistical software Social Science Package (SPSS). The results were presented as the mean plus standard deviation (mean+S.D.). In statistical analysis, findings with a P-value were greater than 0.05 were deemed non-significant (NS), whereas those with a P-value less than 0.05 were deemed significant. In addition, the Person's Correlation coefficient (r) was used to evaluate the relationships between the evaluated parameters.

RESULTS

A total of 75 people with type II diabetes took part in our study. The average age was 56 years and ranged from 20 to 90 years with some other characteristics of patients showed in demographic table (Table 1).

Table 1: Sociodemographic of participants

Variable	Frequency (N)	Percentage %
Age		
20–40 years	12	15.5%
40–60 years	33	44.4%
Above 60 years	30	40%
Gender		
Male	38	50.6%
Female	37	49.4%
Family history with Types II Dm		
With family history Types II Dm	43	57.7%
Without family history Types II DM	32	42.7%
Comorbidity		
Hypertensive	47	62.7%
Non hypertensive	28	33.3%

The results presented in Table 2 and Fig. 1 show a significant (P<0.05) increase in the levels of cholesterol (284.0 mg/dl) and triglyceride (TG) (390.4 mg/dl) in individuals with diabetes compared to their healthy individuals, which is approximately (163.0 mg/dl) and (110.0 mg/dl) respectively. However, this examined increase in the LDL value from (95.18 to 118.1) mg/dl did not reach statistical significance. Furthermore, HDL levels showed a significant decrease in the sera of diabetic subjects (50.54 mg/dL) compared to the sera of healthy subjects (56.80 mg/dL) serving as controls.

Table 2: Values of lipid profile parameters in individuals diagnosed with type 2 diabetes mellitus

Parameter	Patients (N=75) Mean ± S.D	Control (N=30) Mean ± S.D	P-value
Cholesterol	284.0±38.30*	163.0±5.840	0.002
Triglyceride	390.4±70.60*	110.0±5.850	0.0001
HDL	50.54±1.850*	56.80±2.520	0.049
LDL	118.1±14.90	95.18±5.870	0.141

*P<0.05: significant

Analysis of the data in Table 3 and Fig. 2 showed that serum glucose (257.8 mg/dl) and malondialdehyde (MDA) (0.550 µmol/L) levels in diabetic patients are significantly (P<0.05) higher than those observed in healthy controls, while glutathione (GSH) levels (0.004 µM) in the sera of diabetics are significantly (P<0.05) lower than those in healthy individuals (0.030 µM).

Table 3: Serum glucose, malondialdehyde (MDA), and glutathione (GSH) levels in patients with type 2 diabetes mellitus.

Parameter	Patients (N=75) Mean ± S.D	Control (N=30) Mean ± S.D	P-value
Glucose	257.8±9.370*	91.45±1.520	0.0001
MDA	0.550±0.070*	0.210±0.001	0.004
GSH	0.004±0.0007*	0.030±0.001	0.0001

*P<0.05: significant

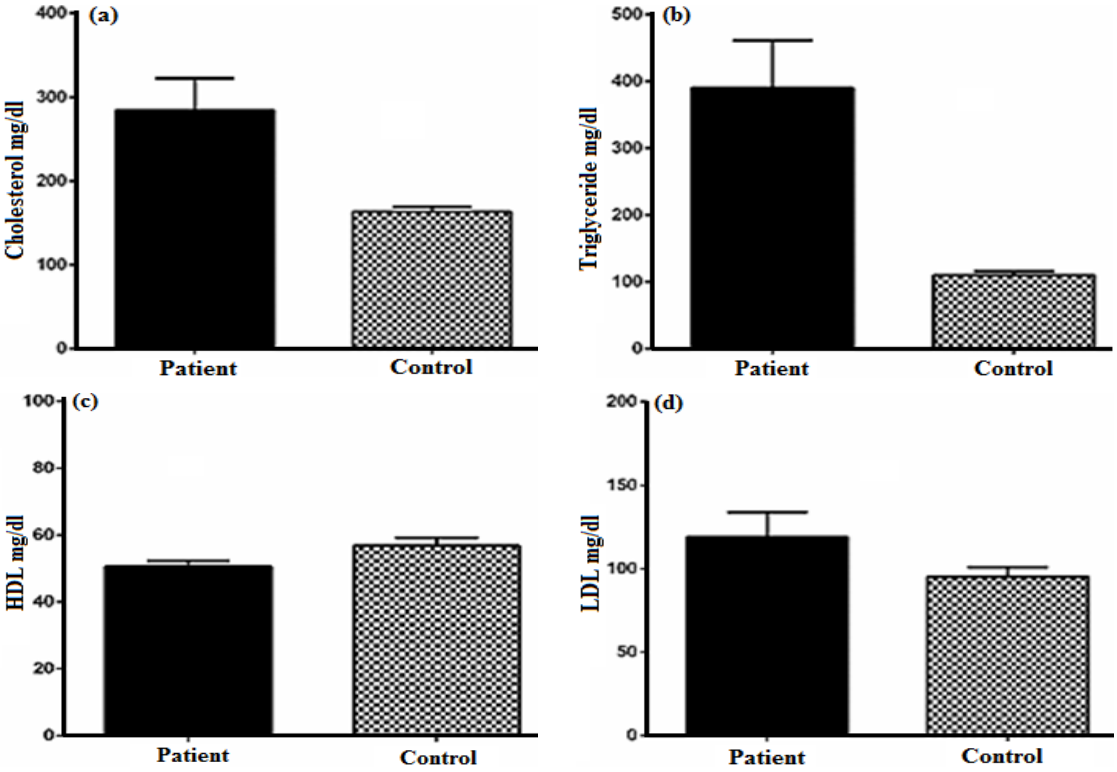

Fig. 1. Comparison of (A) total cholesterol, (B) triglyceride, (C) HDL-C, and (D) LDL-C levels between control and diabetic subjects

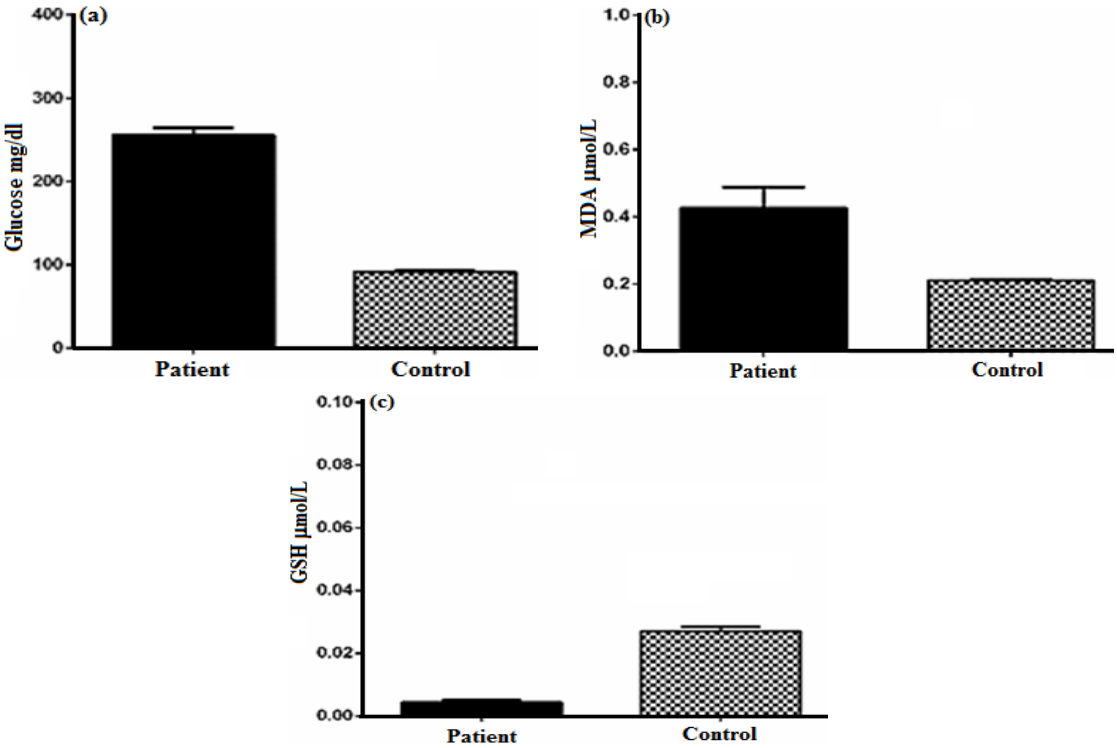

Fig. 2. Comparison of (A) glucose levels, (B) malondialdehyde (MDA) levels, and (C) reduced glutathione peroxidase levels between the control and diabetic groups

The correlation coefficients between oxidative stress (MDA) and antioxidants (GSH) and other evaluated parameters in patients with type 2 diabetes are shown in Table 4. Consequently, there is a non-significant positive correlation between lipid profile parameters and MDA concentration in diabetic patients. Notably, glucose concentration is the only parameter that has a significant positive correlation with MDA. Conversely, GSH levels in diabetics show a negative correlation that, although not statistically significant, is observed in parameters other than LDL such as cholesterol, HDL, triglycerides and glucose levels.

Table 4: Correlations among various biochemical parameters, antioxidants, and oxidative stress indicators in patients with type 2 diabetes.

Diabetic type II	GSH		MDA	
	r	P	R	P
Cholesterol	-0.06205	0.5635	0.2145	0.0745
HDL	-0.04733	0.6596	0.1647	0.1729
LDL	0.02100	0.8452	0.05267	0.6650
Triglyceride	-0.1291	0.2335	0.1480	0.2284
Glucose	-0.06439	0.5488	0.2245	0.0345*

*P<0.05: significant

DISCUSSION

Diabetes mellitus, a common metabolic disease, is closely linked to oxidative stress- a condition that results from an imbalance between the production of reactive oxygen species (ROS) and the body's ability to neutralize their harmful effects[30]. The present study aimed to evaluate oxidative stress and related antioxidants (GSH) and some biochemical parameters in a control group and patients with type 2 diabetes. This relationship plays a crucial role in the physiological disorders of diabetes and influences the progression of the disease and the occurrence of its complications. In type 2 diabetes, insulin resistance and a sustained increase in blood glucose levels combine to produce changes in lipid metabolism, resulting in an atypical lipid profile[31]. As characterized by this disorder, this study showed significant differences by increased total cholesterol, triglycerides and low-density lipoprotein cholesterol (LDL-C) in association with decreased high-density lipoprotein cholesterol (HDL-C) in diabetics compared to healthy people. The majority of diabetics suffer from insulin resistance, and several studies have shown that insulin affects the production of apo-lipoproteins in the liver as well as the regulation of lipoprotein lipase activity and cholesterol transport protein, leading to dyslipidemia in diabetes mellitus[21]. Similar to our results, recently also showed an increase in lipid profile parameters in Iranian patients with diabetes[22]. Cardiovascular complications of diabetes, as well as diseases such as hypertension, dyslipidemia, and insulin resistance, are thought to result from reactive oxygen species (ROS) production, oxidative stress, and endothelial damage[23]. Recently, Hyperglycemia can activate many signaling pathways, making cells sensitive to necrosis, apoptosis, and/or necrosis[31]. In individuals with poorly controlled diabetes, elevated blood sugar levels result in the release of free radicals, most notably reactive oxygen species, produced by the autoxidation of glucose and peroxidation of lipids[5]. Studies have shown that oxidative stress not only worsens but is also influenced by dyslipidemia in diabetes[32]. The process of lipid peroxidation induced by oxidative stress produces reactive byproducts such as malondialdehyde (MDA), which can act as a biomarker indicating oxidative damage to lipids[33]. The current study found increased MDA levels in people diagnosed with diabetes compared to non-diabetics who are in optimal health. Mallick *et al.,* discovered a comparable increase in lipid peroxidation of erythrocyte membranes in both male and female diabetics in India[24]. In the past, it was believed that oxidative stress plays a significant role in the pathogenesis of problems associated with non-insulin dependent diabetes mellitus (NIDDM), especially cardiovascular diseases[25]. In addition, the highest MDA value was recently measured in patients with diabetic retinopathy in the city of Salah-Addin, Iraq[26].

The redox couple that has the greatest importance in antioxidant defense is GSH/GSSG, which also has a central function in metabolic processes and the regulation of the body's homeostasis pathways. Oxidative stress occurs as a result of reduced glutathione (GSH) levels and is associated with aging processes and the development of various ailments and diseases[27]. When glucose levels are elevated, particularly in hyperglycemia, the use of glucose in the polyol pathway is preferred. This pathway consumes NADPH, a crucial cofactor essential for glutathione (GSH) regeneration facilitated by the enzyme GR[34]. As a result, hyperglycemia indirectly contributes to the breakdown of GSH. The increased activity in the polyol pathway may deplete

NADPH because the first step involves the reduction of glucose to sorbitol, a process that is dependent on NADPH. This breakdown of NADPH in turn keeps GSH levels in a reduced state[35]. This study showed a significant decrease in mean GSH levels in diabetic patients compared to a healthy control group, consistent with a previous study that found GSH deficiency in type 2 diabetes patients[28]. Sekhar, R.V. *et al.,* studied that diabetes patients had GSH deficiency and low GSH levels, which were associated with increased oxidative stress and increased markers of oxidative damage in plasma[29].

CONCLUSION

In summary, our study highlights the complicated relationship between diabetes mellitus, oxidative stress and associated complications. Increased oxidative stress in type 2 diabetes is closely associated with altered lipid metabolism, reflected in an abnormal lipid profile with increased total cholesterol, triglycerides and LDL-C, and decreased HDL-C- a pattern consistent with the existing literature and the Prevalence highlights of dyslipidemia in diabetes. Furthermore, our study investigates the complex dynamics of the redox couple GSH/GSSG and highlights its crucial role in antioxidant defense and metabolic regulation. The study establishes a connection between GSH deficiency, oxidative stress and the development of diabetes-related symptoms and highlights the importance of maintaining redox homeostasis. Regarding lipid peroxidation induced by oxidative stress, malondialdehyde (MDA) emerges as a biomarker indicating lipid damage in diabetes. Elevated MDA levels in diabetics' further support the connection between oxidative stress and lipid metabolism. In conclusion, this study provides crucial insights into the complex relationship between diabetes, oxidative stress and lipid metabolism and lays the foundation for understanding the molecular mechanisms behind diabetes-related complications.

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Conflict of interest

The author declare that we have no conflict of interest.

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