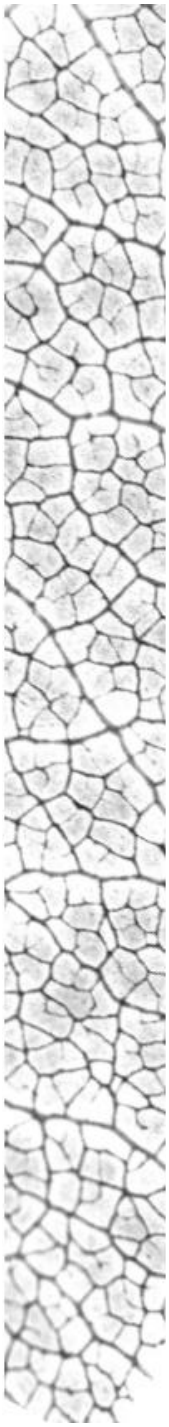

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Uncompensated response to the oxidation stress of the diabetes in both male and female patients with type II diabetes

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Abstract

Background: This study evaluated the level of few antioxidant enzymes through the variation in these enzymatic biomarkers in type II diabetes.
Objectives: The present study aimed to report the variation of some enzymatic antioxidant parameters level as uncompensated response to the oxidation stress in type II diabetes patients.
Materials and Methods: 45 diabetic patients and 44 healthy subjects were recruited (aged 20- 71 years) in Kirkuk province northern Iraq. The levels of (HbA1c) and (FBS), (SOD), (TAS), (GP) and (GPx), were evaluated. The glucose was measured in the patient's serum after 12 hours of fasting following a standard procedure in the kit from (Rondo. United Kingdom Laboratories Ltd), while HbA1c calculated using Stanbio kit (USA). The Antioxidant enzymes, SOD, TAS, GP and GPx were assessed in serum samples using colorimetric Randox kit (Randox, laboratories, Ltd). Body mass index (BMI) was calculated using spscific formula and classifying normal weight as (BMI 18.5- 24.9) Kg/M2, obesity as (BMI 30-39.9) Kg/M2 and morbid obesity as (BMI > 40) Kg/M2).The waist circumference was measured at the narrowest point of the torso width-wise, usually just above the belly button, which is ≤ 102 cm in male and≤88 cm in female.
Results: Plasma (HbA1c) concentrations and (FBS) are higher in patients than in controls (P = 0.04* and 0.001*, respectively). Surprisingly, the amount of (GPx) and (SOD) are lower in patients group comparing to control group (P < 0.002* and P < 0.05*, respectively). Concentrations of (TAS), in contrast, is significantly lower in patients than controls group (P = 0.002*), while no change is noticed in the level of (GR) in patients groups (P = 0.73). There are significant differences (P≤ 0.05) in a mean of age, BMI, waist circumference, (FBS), (GPx), (TAS) and (HbA1c) among female and male of two groups. There are no other statistically significant differences (P ≥ 0.05) between gender in health and patient groups in relation to the serum levels of (GR) and (SOD) enzymes. In addition, data showed there is no significant correlation (P ≥ 0.05) (HbA1c*) and antioxidant enzymes concentration in type II diabetes patients.*
Conclusions: Enzymatic biomarkers can be used to monitor the uncompensated response to the oxidation stress as shown by lower antioxidant level in type II diabetes patients in this study.

Keywords: antioxidants markers, total antioxidant status, GPx, type II diabetes complications

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INTRODUCTION

Diabetes is a global epidemic with 285 million adults suffering from this disease. In the absence of better control or cure, the reported number is more likely to increase, especially in less developed countries and specifically in the poor population areas (Bandeira, et al. 2012; Salari, et al, 2015). The most known syndrome for type II diabetes is a metabolic disorder, the sugar will accumulate in the blood stream rather than acting as source of energy after taking by the cells (Lee, et al. 2015). The cells become resistance to insulin, they ignore it messages to assimilate glucose, this known as Insulin Resistance (Wilkin, 2009). Moreover, Pancreas is unable to produce more Insulin that needed to trigger that resistance to taking glucose from blood stream.

Several noticeable symptoms are associated with type II diabetes, including weakness, drowsiness, and blurred vision. About one in four people are unaware

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they have type II diabetes (Van der Does, et al. 1996). Early diagnosis of diabetes is important because by time high level of glucose in the blood stream will cause damages to small blood vessels and therefor can damage the organs leading to a health complications, such as, vision problem, nervous damage and kidney disease (Högestätt, Andersson, & Edvinsson, 1983). More health complication can appear such as heart disease, stroke, and poor blood circulation by time due to destruction to large blood vessels. Major causes of developing type II diabetes are overweight, sedentary life style, lake of exercise, family history of diabetes, and ethnic origins (Astrup, 2001. Mohamed, 2015). Oxidative stress configuring a crucial mechanism in the pathophysiology of diabetes. The evidence showed that there are considerable biochemical pathways specifically combined with hyperglycemia, thus boost a generation of reactive oxygen species (ROS) which in turn, enormous impact in diabetic complications (Son, et al. 2004). There are many enzymatic antioxidant in the serum can be considered as biomarkers for developing type II diabetes thus can be used clinically as indicators for early diagnosis including, superoxide dismutase (SOD), Total Antioxidant Status (TAS), Glutathione Reductase (GP) and glutathione peroxidase (GPx) (Asmat, Abad, & Ismail, 2016). Superoxide dismutase enzyme SOD is a metal containing antioxidant enzyme that reduces harmful free radicals of oxygen formed during normal metabolic cell processes to oxygen and hydrogen peroxide. It is an enzyme that alternately catalyses' the dis-mutation of the superoxide radical into either ordinary molecular oxygen or hydrogen peroxide H_2O_2. This enzyme produces as by-product of oxygen metabolism and if not regulated, causes many type of cell damage (Johnson, & Giulivi, 2005). SOD has been used for the treatment of soft tissue inflammation (human inflammatory disease) (Shingu, et al. 1994). Another important antioxidant that produced by human own body is glutathione peroxidase (GPx). It specialized as large complex protein that designed specifically to remove oxidants and bring the body in balance in oxidative stress. What makes it unique is that it acts much faster than any other antioxidant. It also can remove multiple oxidants before it loses it activity and it can be easily recycled back to its active form by biding to co-factors (Arthur, 2001). This study aims to evaluate the variation in the anti-oxidation enzymatic levels as biomarkers in the diagnosis of type II diabetes patients in a specific population of Kirkuk city northern Iraq.

MATERIAL AND METHODS

Patients and Control

Case-control study was conducted between September 2019- January 2020. The work was invoved 89 individuals, 45 patients with type II diabetes (21 males and 24 females) were selected in Kirkuk city. Patient's age ranged was between 20-71 years. While healthy objectives included 44(19 males and 25 females) as control group.

Anthropometric Measurements

Information taken from all patients and control groups sheets included: age, sex, weight, height, and waist circumference. We excluded patients with hypertension, liver disease, disorder of some hormones, (thyroid, testosterone, LH and FSH), heart failure, renal failure, malignant disease, smoking and alcohol intake with complications of diabetes like diabetic neuropathy, nephropathy, retinopathy. Body mass index (BMI) was calculated using the formula BMI= weight (kg)/ height2 (m) ^{2}. The normal weight (BMI 18.5- 24.9)Kg/M2, obesity (BMI 30-39.9) Kg/M^2 and morbid obesity (BMI > 40) Kg/M^2) were classified.The waist circumference was measured at the narrowest point of the torso width-wise, usually just above the belly button while the subject standing up, which classified as ≤ 102 cm in male and≤88 cm in female.

Preparing Blood Samples

Approximately five milliliters of venous blood were collected from each individual after 8-12 hours fasting then placed into EDTA containing tubes. About one milliliters used for HbA1c assay using a kit supplied by (Stanbio marker made in USA) and nearly three milliliters of whole blood sample was centrifuged at 3000 rpm for 15 mins. The one milliliter of serum was used for measurement of fasting blood glucose. Two milliliters of plasma was gained, then used for TAS and GR assay. The remained was used for SOD and GPx assay. All blood Samples assays were performed at 37°C using a Hettich – Germany centrifugal analyzer (Tietz, 1999).

Laboratory Investigations

Physio-biochemical Analysis

Fasting blood glucose measured according to the principle methods for enzymatic oxidation presence (GOD) using (RANSEL RS 505, Randox Laboratories, UK kit) (Kumar, et al. 2009). An activity of erythrocyte glutathione peroxidase (GPx) was measured using a kit supplied by (RANSEL RS 505, Randox Laboratories-UK). GPx activity was measured using sample volume of 5 µl in a total reaction volume of 285 µl and the wavelength used was 340 nm. Superoxide dismutase (SOD) was measured by enzymatic reagent kits provided from (RANSOD SD 125, Randox Laboratories-UK). Following a standard procedure, the serum samples were diluted to 1:5 in the sample buffer, 10 µL of the sample have been added to 200 µL of the radicals' detector (1:400 dilution). After slow agitation, 20 µL of xanthine oxidase was then added to the wells. The microplate was incubated for 20 minutes at room temperature. The absorbency was read at 440 wavelength of nm. The levels are reported in IU/mL. Total Antioxidant Status (TAS) was measured using a kit supplied by Randox Laboratories Ltd. (Cat. No.

Table 1. Anthropometric data in two groups, the healthy (control) and diabetic group. *t- test is significant at the 0.05 level. BMI: Body mass index is calculated as weight in kilograms divided by the square of height in meters.

Variables	Control Group n=44	Diabetic Group n=45	P-value
Age (Years)	35.18±1.72	48.66±2.30	0.001*
BMI (Kg/m2)	23.45±0.37	33.65±1.10	0.001*
Waist Circumference (cm)	82.75±1.32	119.46±0.07	0.09*

Table 2. Clinical data in different groups. (p<0.05) significantly different from control. t-test.*P ≤ 0 .05. Std. Error: Standard error

Variables	Group	N	Mean	Std. Error Mean	Sig.
Fasting blood glucose (mmol/l)	Diabetic	45	7.29	0.44	0.001*
	Control	44	4.20	0.12	
Glutathione Reductase(GR)(U/l)	Diabetic	45	66.91	2.75	0.73
	Control	44	72.34	2.80	
Glutathione Peroxidase(GPx)(U/l)	Diabetic	45	308.22	17.09	0.002*
	Control	44	461.36	25.22	
Total Antioxidant Status(TAS) (mmol/l)	Diabetic	45	1.20	0.06	0.002*
	Control	44	1.49	0.07	
Superoxide Dismutase(SOD)(U/ml)	Diabetic	45	1.99	0.12	0.05*
	Control	44	2.02	0.10	
Glycated hemoglobin (HbA1c)(%)	Diabetic	45	8.13	0.15	0.04*
	Control	44	5.00	0.13	

NX2332). Then 4 µl plasma sample volume was added in a total assay volume of 405 µl. Color production is measured at 600 nm with a read time of 5 min. Glutathione reductase (GR) was measured using a kit provided by Randox Laboratories Ltd. (Cat. No. GR2368) at 340 nm. The assay requires a sample volume of 10 µl in a total reaction volume of 310 µl.

Statistical Analysis

Statistical analysis was performed by using SPSS version 15, independent sample T-test and one–way ANOVA. Data were represented as mean ± Standard error. Bivariate correlations were performed using the person correlation coefficient.P value (P<0.05) was considered statistically significant.

RESULTS

A total of 89 individuals, 45 patients diagnosed with type II diabetes and 44 healthy subjects (control group) were studied. Anthropometric evaluation has been performed, which is indicator that used to investigate the prediction of chronic and acute diseases (Villareal, et al. 2005. Mohammed, Mokif, & Hadwan, 2019). Anthropometric values considered according to both group's age, body mass index (BMI) and waist circumferences (**Table 1**). Anthropometric measurements showed that patients group was significantly older (P = 0.001*) with remarkable high BMI (P = 0.001*) comparing to control group. Overall age mean was 35.18±1.72 years for healthy group, while overall age mean was 48.66±2.30 years for diabetic group. Anthropometric measurements showed statistics differed (P= 0.09) in terms of waist circumference respectively among both healthy and diabetic groups. BMI mainly used to assign unhealthy diet and obese in both groups while waist circumference was used to determine individuals with possible health risks.

Plasma HbA1c concentrations and FBS were statistically significantly in patients group (P = 0.04* and 0.001*) as shown in **Table 2**. Anti-oxidation enzymes parameters results are also shown in **Table 2**. Data of anti-oxidation enzymes such as (GPx) and (SOD) were significantly lower in patients group (P < 0.002* and P < 0.05*). Concentrations of plasma Total Antioxidant Status (TAS) was also lower in patients than control group (P = 0.002*), while Glutathione Reductase(GR) level did not changed in patients groups (P = 0.73).

There are significant differences (P≤ 0.05) in mean of age, BMI, waist circumference, FBS, (GPx), (TAS), (HbA1c) in both female and male of patients and healthy groups as shown in **Table 3**. There were no other statistically significant differences (P ≥ 0.05) between gender of control and patient groups in relation to the serum levels of Glutathione Reductase(GR) and Superoxide Dismutase(SOD) enzymes. In addition, data showed there was no significant correlation (P ≥ 0.05) between (HbA1c) and antioxidant enzymes concentration in type II diabetes patients as shown in **Table 4**.

DISCUSSION

In the present study, anthropometric measurements showed that patients with type II diabetes were significantly older, as known for type II diabetes, it is an adult onset and the symptoms appears gradually. Patients also had a significantly higher BMI than the control subjects, as reported before that type II diabetes is insulin resistant where the genetic factors produce susceptibility to the diseases but the life style also believed to play an important role, such as obesity, inactive life style and unhealthy diets, they are all associated with type II diabetes. This finding is consistent with many other studies in which patients with diabetes were shown a remarkable increase as a consequence of aforementioned factors (Decode-Decoda Study Group, & European Diabetes Epidemiology Group. (2003).

Table 3. Comparison between Diabetic and control subjects for both genders. LSD.*P ≤ 0 .05. Std. Error: Standard error

Parameters	Control Group n=44		Diabetic Group n=45		p-value of genders
	Male (n=19) Mean ±Std. Error	Female (n=25) Mean ±Std. Error	Male (n= 21) Mean ±Std. Error	Female (n=24) Mean ±Std. Error	
Age (Years)	34.84±2.84	35.44±2.18	44.23±3.79	52.54±2.57	0.001*
BMI (Kg/m2)	22.91±0.31	23.86±0.61	34.46±1.54	32.95±1.59	0.001*
Waist Circumference (cm)	82.36±2.41	83.04±1.49	119.90±1.13	118.37±1.75	0.001*
Fasting blood glucose (mmol/l)	4.38±0.16	4.06±0.18	6.41±0.40	8.06±0.72	0.001*
Glutathione Reductase (GR)(U/l)	73.92±4.35	70.26±3.12	67.76±4.38	66.16±3.53	0.40
Glutathione Peroxidase (GPx)(U/l)	463.15±31.72	452.80±32.34	358.57±21.69	309.16±21.62	0.001*
Total Antioxidant Status (TAS) (mmol/l)	1.52±0.11	1.47±0.10	1.24±0.10	1.16±0.08	0.01*
Superoxide Dismutase (SOD) (U/ml)	1.90±0.16	2.10±0.13	2.17±0.15	1.82±0.18	0.76
Glycated hemoglobin (HbA1c) (%)	5.20±0.20	4.73±0.14	7.85±0.19	8.37±0.21	0.001*

Table 4. Correlation between glycated hemoglobin and antioxidant enzymes in type 2 diabetes patients. *. Correlation is significant at the 0.05 level (2-tailed).TAS: Total Antioxidant Status, GPx" glutathione peroxidase; GR: Glutathione Reductase , SOD :superoxide dismutase, Correlation coefficient (r) ,correlation is significant (P)≤ 0.05 level

Glycated hemoglobin (HbA1c) (%)	Antioxidant enzymes							
	TAS (mmol/l)		(GR)(U/l)		(GPx)(U/l)		(SOD)(U/ml)	
	r	p	r	p	r	p	r	p
	-0.05	0.44	0.06	0. 67	-0.18	0.22	0.07	0.63

The increased plasma levels of antioxidant enzymes in patients with type II diabetes normally refer to patients subjected to higher oxidative stress especially for newly diagnosed patients when compared to control subjects (Naudi, et al. 2012). The current study measured the total antioxidant activity of plasma because of its established ability to withstand oxidative stress. In addition, we found that plasma HbA1c concentrations and Fasting Blood Glucose were significantly higher in patients than in controls. Because all participants had poor glucose and HbA1c control, this was considered to be indicative of poorer glycemic control. Poor glycemic control is when patient is unable to improve their diet by reducing the amount of carbohydrate intakes in daily meals, unable to lose weight and follow regular exercise and eventually access to the medications that help to control blood sugar. HbA1c values reflect overall glycemic exposure over the past 2–3 months and are determined by fasting (FPG) level (Saudek, Kalyani, & Derr, 2005).

Here, the total anti-oxidation enzymes of plasma were investigated because they can act as indicators for oxidative stress. However, here the total plasma antioxidant state was significantly lower in patients with diabetes compared with control subjects. This could be as a result of low GSH (reduced glutathione). Although this study did not investigate the level of GSH, but it is well known that GSH is a substrate and a co-factor of GPx enzyme. Another reason for lower level of GPx can be a sever oxidation stress which cause inactivation of this enzyme (Rahbani-Nobar, et al. 1999).

It is common that having high blood glucose is causing damage to our body. Having high blood sugar causes oxidation of glucose in the reaction causing glycation of proteins. This reaction can cause tissue damage and thus creates many of free radicals that attacking normal cells. This also decreases the activity of super oxide dismutase, which is the only body's own antioxidant enzyme (Maritim, Sanders, & Watkins 2003). This oxidation and glycation reaction is affecting the energy production entities in the cells that known as mitochondria. Thus, the damaged mitochondria will produce less energy than the normal mitochondria as they cannot use the glucose in a normal way to produce the energy (Fei, et al. 2014). Detecting high level of antioxidant enzymes in the blood of newly diagnosed type II patients is an indicator of the body's response to the damage caused by high blood glucose level such as releasing huge amount of free radicals in the blood stream that cause inflammation which is one of the diabetes complications.

The mechanism in which free radicals released and cause inflammation is complicated. It believes that it start after sustain production of insulin over the time. Then, large amount of another hormone-like substance by beta cells can be produce. White blood cells are responding to this hormone and consume it. High level of free radicals production will occur inside the white blood cells as a result of high blood glucose combined to the hormone.

The free radicals activate the sentinel proteins that originally involved in the protection from microbial infections. The newly active sentinel protein aggregates with other protein to form a ring. Other adaptor protein then bind to the ring and will act as a platform to interact inactive proteins from the surrounding cell fluids and brining two hubs together to form an active hub which is then released. The newly active proteins convert another small signaling protein into their active form. This considers as a key active proteins involved in

inflammation in the body. By the time increase the level of blood glucose and decrease the amount of insulin causes damage to some tissues in our body (Martinon, 2010). As common known that there is a relationship between oxidative stress (reduced glutathione) and glutathione peroxidase, glutathione Reductase level. However, in the present study uncompensated response to the oxidation stress may report, the level of Glutathione Reductase(GR) did not show any significant differences between healthy objectives and patients groups. Glutathione Reductase plays important role in protecting RBC and the hemoglobin from oxidative damage that causes severe hemolytic anemia (Waggiallah, & Alzohairy, 2011). The reason for why the level of this enzyme remained same between the patients and healthy subjects is unclear. It can be a sign for reduced amount of ROS that did not lead to any adaptation of anti-oxidant defense (Gawlik, et al. 2016). This will required more investigation. In conclusion, this study has reported uncompensated response to the oxidation stress as shown by lower antioxidant level in the blood serum of the patient comparing to the healthy control subjects.

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