

## Glutathione, Vitamin C, Malonodialdehyde Oxidized Low-Density Lipoprotein and Lipid Profile Levels in Type 2 Diabetic Iraqi Males

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### Abstract

Diabetes is a devastating disease throughout the world. It is associated with several mechanisms, one of which is oxidative stress. Oxidative stress plays an important role in the pathogenesis and the complications of diabetes. Hyperglycemia results in overproduction of oxygen free radicals, which contributes to the progression of diabetes. This study was designed to evaluate the status of serum Glutathione, Vitamin C, Malonodialdehyde and Oxidized Low-Density Lipoprotein levels in male patients with type 2 diabetes mellitus. A total study subject of 82 were examined, including 46 patients with type 2 DM and 36 healthy individuals as a control group matched for age, sex, and body mass index (BMI). Blood samples were drawn after an overnight fasting for the determination of serum glucose, HbA1c, urea, uric acid, insulin, GSH, MAD, OX-LDL, Vit C, and lipid profile. It was found that total diabetic patients had higher FSG, HbA1c, urea, FSI, OX-LDL, MDA, TC, TG, LDL, and VLDL levels and lower UA, GSH and Vit C levels when comparing with control groups. The prevalence oxidative stress among type 2 DM patients is very high with deslipidemia is being most common continuous monitoring of glucose and lipid profile in diabetic patients is essential to reduce the mortality rate.

Keywords: Diabetes mellitus, Glutathione, Malonodialdehyde, Vitamin C.

### Introduction

Type 2 diabetes mellitus (DM) is explained by genetic, and environmental factors, and insulin resistance.<sup>[1]</sup> HbA1c level provides an index of the average blood glucose concentration over the previous 2-3 months.<sup>[2]</sup> Presently, HbA1c is the most acceptable and widely used measure of chronic glycemia.<sup>[3]</sup>

Type 2 diabetes is associated with reduced life expectancy, significant morbidity due to the specific diabetes related microvascular complications (retinopathy, nephropathy and neuropathy), and the increased risk of macrovascular complications (ischemic heart disease, stroke and peripheral vascular disease).<sup>[4]</sup>

Diabetes produces disturbances of lipid profiles, especially an increased susceptibility to lipid peroxidation, which is responsible for increased incidence of atherosclerosis, a major complication of diabetes mellitus.<sup>[5]</sup>

The development of these complications impacts on quality of life. Normally, levels of reactive oxygen species (ROS) are regulated by variety of enzymatic and non-enzymatic antioxidant systems. But increased production and/or inadequate removal of ROS may cause oxidative stress, imbalance between

prooxidant and antioxidants which can lead to oxidative damage to biological macromolecules.<sup>[6]</sup>

Under normal conditions free radicals are formed in minute quantities and are rapidly scavenged by natural cellular defense mechanism. Lipidperoxidation products Malonodialdehyde (MDA), which increases in clinical and experimental diabetes, are important results of oxygen derived free radicals stress. These products may be important in the pathogenesis of vascular complications in diabetes mellitus.<sup>[7]</sup>

Glutathione (GSH) is the major cellular antioxidant that protects against environmental toxicants as well as reactive oxygen species (ROS) mediated cell injury. GSH detoxifies ROS, reduces peroxides and detoxifies multiple compounds through glutathione-S-transferase (GST) conjugation.<sup>[8]</sup> Vitamin C is an important antioxidant in human, capable of scavenging oxygen-derived free radicals. Several studies showed decreased basal vitamin C level in diabetic patients and also it is suggested that oxidative stress is increased in diabetes.<sup>[9]</sup>

Several studies have shown that diabetes mellitus (types I and II) is accompanied by

increased formation of free radicals and decreased antioxidant capacity, leading to oxidative damage of cell components.<sup>[10-12]</sup> Patients with T2DM exhibit low HDL-C levels.<sup>[13]</sup>

LDL oxidation initiates a series of events that ultimately will lead to increased acquisition of LDL by macrophages, the formation of foamy cells and the development of atherosclerosis.<sup>[14]</sup>

### Patients and Methods

The present study was carried out in the National Diabetes Center for Treatment and Research at Al-Mustansiriyah University between July 2012- March 2013. A total of 46 patients of type 2 diabetes mellitus (males of age group 38-68 years.), who were already diagnosed to have type 2 diabetes mellitus based on the criteria of the expert committee on the diagnosis and classification of diabetes mellitus. 36 age and sex matched (males) healthy individuals served as controls who attended for routine health check up at the center. None of the healthy control was taking any medicine or dietary supplement; they were selected after detailed physical examination and laboratory tests. Samples collection: After 12 hrs fasting 6 ml. venous blood sample was collected in plain tubes, the samples were allowed to clot for half an hour following which a sample was centrifuged for 15 minutes at 4000 rpm. Serum was stored immediately at -20°C until analysis. Serum glucose was determined by a glucose oxidase method (Randox Company, U.K.).<sup>[15]</sup> Creatinine, uric acid and urea were measured by colorimetric method (LINER Chemicals/Spain). Vitamin C was determined by high-performance liquid chromatography (HPLC-UV).<sup>[16]</sup> Total cholesterol, triglycerides and HDL cholesterol were measured by enzymatic analytical chemistry (CHOD-PAP method, Roche Diagnostics, Milan, Italy).<sup>[17]</sup> Serum Malondialdehyde (MDA) was measured by enzyme linked immunosorbent assay ELISA (KA1206, Taipei City, Taiwan). Glutathione (GSH) level was performed using a quantitative sandwich ELISA immunoassay (CSB-E09495h, Wuhan, Hubei, China). Serum insulin level was performed using a quantitative sandwich ELISA immunoassay

(DRG diagnostics Company, Germany). ox-LDL level in human serum was performed using a quantitative sandwich ELISA immunoassay (Human Company, Germany). Glycated hemoglobin was measured by using the Variant Hemoglobin A1C program developed by BIO-Rad.

### Statistical Analysis

All data have been presented as mean  $\pm$  SD. One-way analysis of variance (ANOVA) was performed on each variable and the Bonferroni statistics employed to compare the mean values from the different groups. Unpaired t-test was used to assess the effect between groups. Differences were considered significant at  $P < 0.05$ . All statistical analyses were performed using SPSS statistical software (version 19).

### Results and Discussion

Clinical and biochemical characteristics of the study population, i.e. control and type 2 diabetic men are given in Table (1), Figs.(1,2). Waist shows significant differences between the two groups in waist circumference ( $p < 0.05$ ) but no significant differences between the two groups in hip circumference.

The mean value of fasting serum glucose  $187.90 \pm 48.16$  mg/dl, HbA1c  $8.13 \pm 1.74\%$ , serum urea  $37.06 \pm 5.90$  mg/dl, serum creatinine  $1.25 \pm 0.20$  mg/dl in diabetic males and  $93.13 \pm 6.93$  mg/dl,  $4.85 \pm 0.31\%$ ,  $31.32 \pm 4.65$  mg/dl,  $1.02 \pm 0.16$  mg/dl in control group, respectively.

There was a highly significant increase in fasting serum insulin  $5.69 \pm 2.50$   $\mu$ IU/ml in control and  $17.73 \pm 6.27$   $\mu$ IU/ml in the patients of type 2 diabetes, ( $p < 0.0001$ ).

**Table (1)**  
**Baseline characteristics of the study Parameters.**

Parameters		Mean±S.D	Cohen's d	t	P-value
Waist cm	cases	113.00±10.19	0.581	-2.540	0.013
	controls	107.16±9.92			
Hip cm	cases	112.96±9.83	0.145	-0.650	0.518
	controls	111.42±11.33			
F.S.G mg/dl	cases	187.90±48.16	2.755	-10.863	0.000
	controls	93.13±6.93			
HbA1c%	cases	8.13±1.74	2.616	-10.340	0.000
	controls	4.85±0.31			
F.S.I μIU/ml	cases	17.73±6.27	2.524	-10.197	0.000
	controls	5.69±2.50			
S. Urea mg/dl	cases	37.06±5.90	1.080	-4.609	0.000
	controls	31.32±4.65			
S. Creatinine mg/dl	cases	1.25±0.20	1.248	-5.354	0.000
	controls	1.02±0.16			
Total. C mg/dl	cases	197.57±51.16	1.207	-4.832	0.000
	controls	151.71±16.42			
T.G mg/dl	cases	167.14±74.72	1.216	-4.820	0.000
	controls	101.39±16.16			
HDL mg/dl	cases	43.76±6.70	1.420	6.013	0.000
	controls	52.10±4.90			
LDL mg/dl	cases	119.61±47.53	1.151	-4.616	0.000
	controls	78.74±16.25			
VLDL mg/dl	cases	33.37±14.73	1.222	-4.844	0.000
	controls	20.35±3.15			
MDA nmol/ml	cases	6.38±1.25	2.753	-11.476	0.000
	controls	3.52±0.78			
OX-LDL U/ml	cases	24.70±11.12	0.171	-6.252	0.000
	controls	10.40±2.69			
GSH μg/ml	cases	1.49±0.37	1.575	7.478	0.000
	controls	2.38±0.71			
Vitamin C mg/dl	cases	1.03±0.24	1.223	5.731	0.000
	controls	1.44±0.41			
S.U.A mg/dl	cases	4.51±0.70	1.295	5.589	0.000
	controls	5.37±0.61			

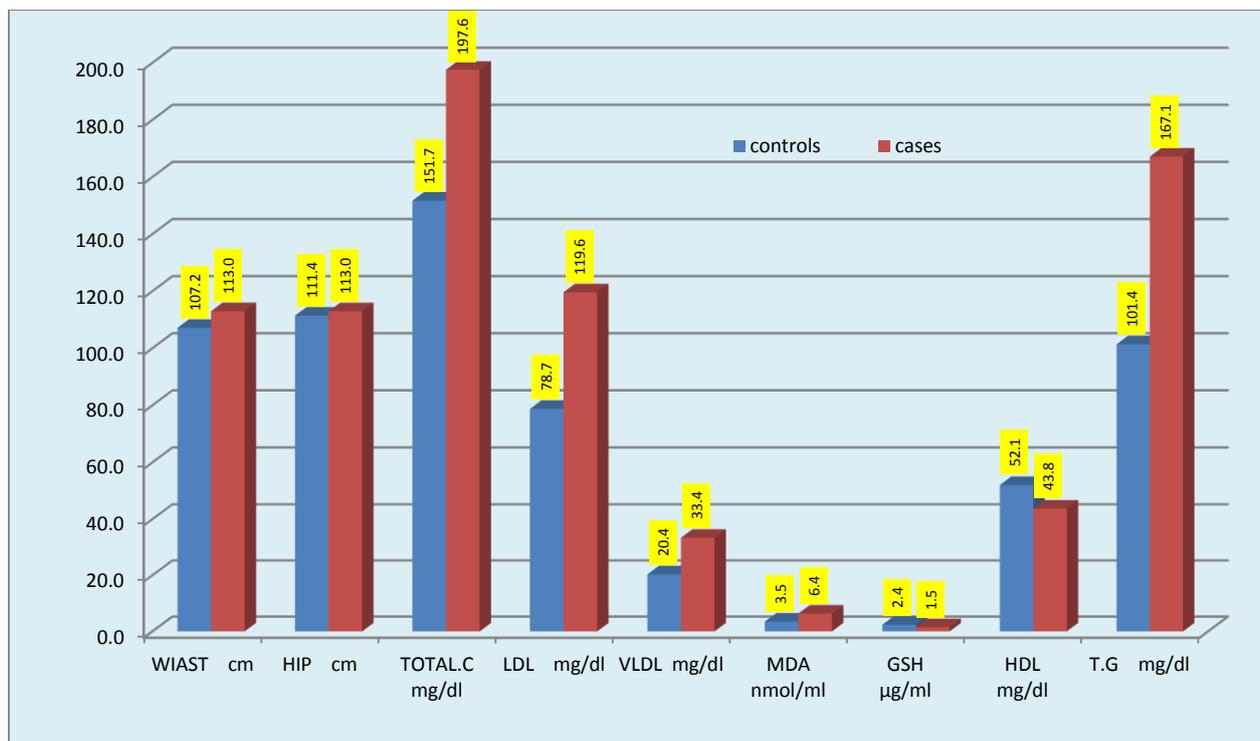
There was a highly significant increase in FSG, HbA1c, S. urea and S. creatinine in patient group compared to the control group ( $p<0,0001$ ).

Biochemical lipid profile characteristics (TC, TG, HDL-C, LDL-C and VLDL) are shown in Table (1), Fig.(1). There was a highly significant increase in serum TC, TG, LDL-C, VLDL-C and a highly significant decrease in HDL-C in the patient group compared to the control group ( $p<0,0001$ ).

There was a highly significant increase in serum MDA and ox-LDL in patient group compared to the control group ( $p<0,0001$ ).

The mean value of MDA  $6.38\pm 1.25$  nmol/ml, ox-LDL  $24.70\pm 11.12$  U/ml in diabetic males and  $3.52\pm 0.78$  nmol/ml,  $10.40\pm 2.69$  U/ml. in control group, respectively.

Serum vitamin C, GSH and uric acid levels are significantly lower in the diabetic men there was significant increase in blood urea and serum creatinine in diabetic control subjects. Similar results were observed in Diabetic Nepalese. <sup>[20]</sup> Due to high blood sugar.



**Fig.(1): The waist, Hip and serum Total. C, LDL, VLDL, HDL, TG, MDA, GSH levels in T2DM compared with control samples.**

Compared to the control group ( $p < 0.0001$ ) with  $1.03 \pm 0.24$  vs.  $1.44 \pm 0.41$  (mg/dl),  $1.49 \pm 0.37$  vs.  $2.38 \pm 0.71$  ( $\mu\text{g/ml}$ ),  $4.51 \pm 0.70$  vs.  $5.37 \pm 0.61$  (mg/dl), respectively.

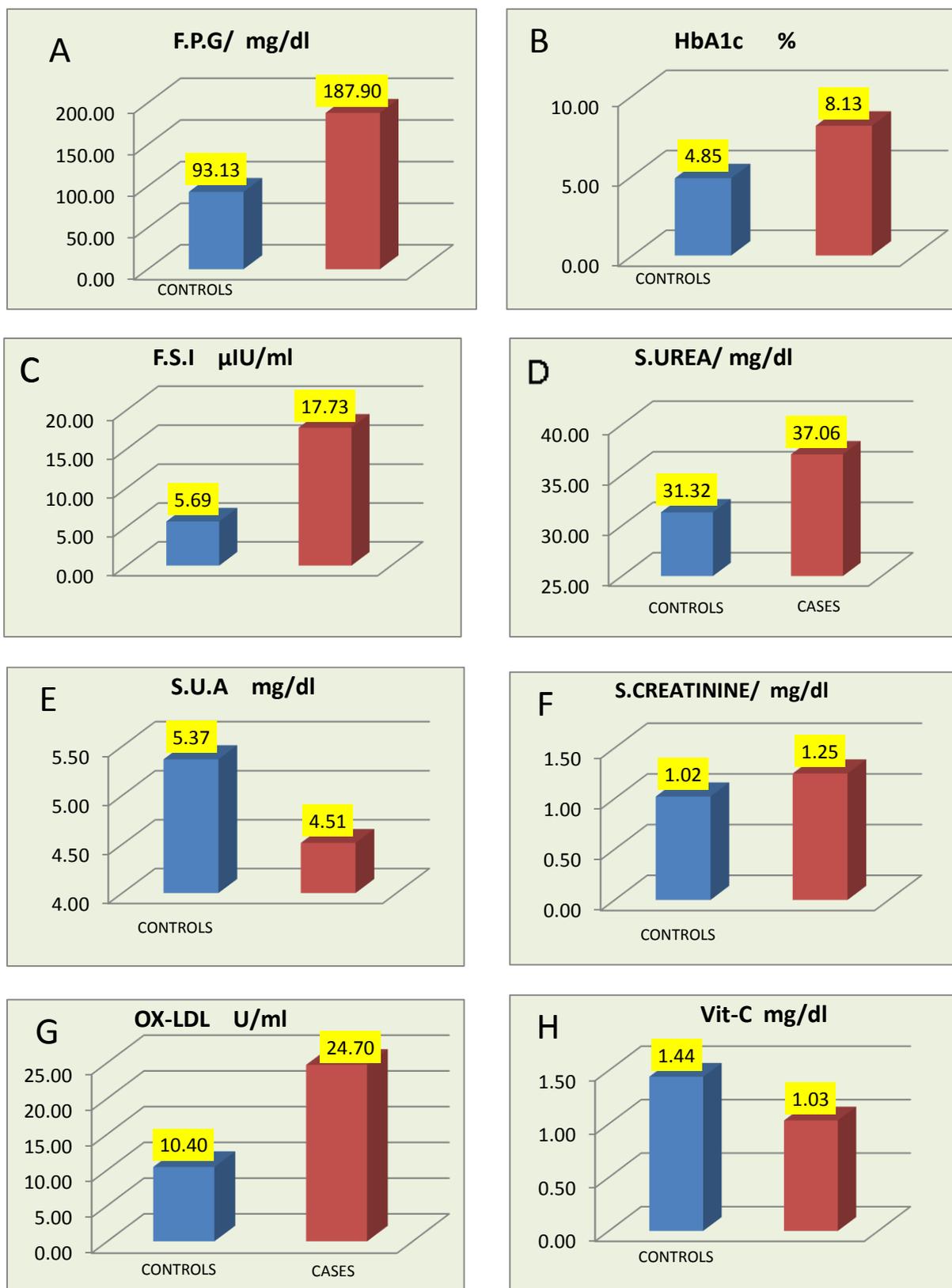
This study reveals high prevalence of hypercholesterolemia, hypertriglyceridemia, high LDL-C, VLDL-C. and low level of HDL as compared to non diabetic patients. Similar study among young adult population was done by Sawant et al [18] showed similar results. Insulin affects the liver apolipoprotein production. It regulates the enzymatic activity of lipoprotein lipase (LpL) and Cholesterol ester transport protein. All these factors are likely cause of dyslipidemia in Diabetes mellitus. Moreover, insulin deficiency reduces the activity of hepatic lipase and several steps in the production of biologically active LpL may be altered in DM. The main disorder in lipid metabolism was hypertriglyceridemia in our study. This finding is in concord with our previous study.[19] there is damage to the nephrons.

As a result, kidneys unable to maintain the fluid and electrolytes homeostasis. There is rise in plasma concentration of serum creatinine and urea. In severe hyperglycemia, there is formation of advanced glycation end-products (AGEs) has long been recognized to cause cellular injury. AGEs accelerates

increased vascular permeability, basement membrane thickening and mesangial fibrosis. This process leads to renal failure. [21]

In the present study, we noticed a marked increase in HbA1c level in diabetic patients, which could be due to excessive glycosylation of hemoglobin.

Type 2 diabetes mellitus is associated with endothelial dysfunction and oxidative stress. Chronic exposure to elevated glucose and hyper lipidemia can cause damage in different types of cells by variety of mechanisms. Lipids and proteins are the primary target of oxidative stress.[22] Lipid peroxidation of the cellular.



**Fig.(2): The Fasting Serum Glucose, HbA1c, FSI, Urea, UA, Creatinine, ox-LDL and Vit-C levels in T2DM males compared with control sample.**

Structures, a consequence of increased oxygen free radicals is thought to play an important role in atherosclerosis and micro

vascular complications of diabetes mellitus.<sup>[23]</sup> Our study showed that there are increase in serum MDA, ox-LDL and decrease in serum

GSH, Vitamin C, UA concentrations in diabetic subjects which is in line with many previous studies.<sup>[24]</sup> Increased non enzymatic glycosylation and auto oxidation of glucose is the possible mechanism in the excessive production of free radicals in diabetes mellitus which induce lipid peroxidation and increased MDA concentration. MDA is a highly toxic by-product formed in part by oxidation derived from free lipid radicals, and studies have shown considerably raised concentrations in DM. MDA reacts both irreversibly and reversibly with proteins and phospholipids with profound effects.<sup>[25]</sup> One of the more prominent markers of oxidative stress in DM is oxidized LDL (ox-LDL). Increased production of free oxygen radicals, higher ox-LDL levels, along with stimulation of ox-LDL uptake by macrophages and the ensuing promotion of early atherosclerotic changes might be contributed by insulin resistance (IR). hyperglycemia is associated by increased inflammatory burden and increased lipid peroxidation, all leading to enhanced macrophage foam cell formation, low density lipoprotein (LDL) oxidation by macrophages was increased due to the activation of several pro-oxidant systems, as well as the depletion of antioxidants .<sup>[26]</sup> Moreover, hyperglycemia may lead to intracellular changes in redox state resulting indepletion of cellular NADPH+H pool, leading to increased tendency for oxidative stress and high level of oxidized lipoprotein, especially LDL.<sup>[27]</sup> In addition, the macrophages express scavenger receptors for modified lipoprotein (ox-LDL) permitting them to ingest lipids and become foam cells, the activated macrophage can generate reactive oxygen species that augment oxidant stress.<sup>[28]</sup>

Under *in vivo* conditions, GSH acts as an antioxidant and its decrease was reported in diabetes. We have observed a significant decrease in GSH content in diabetic erythrocytes. The decrease in GSH content represents increased utilization due to oxidative stress. Glutathione (GSH) is an important cell antioxidant that acts as an electron donor to reduce ROS and acts as a cofactor for NO synthesis from L-arginine.

Depressed GSH levels contribute to decreased NO synthesis. Hyperglycemic states

also deplete the NADPH necessary for GSH regeneration, leading to increased oxidative stress. Vitamin C is a hydrophilic antioxidant in plasma, because it disappears faster than other antioxidants when plasma is exposed to reactive oxygen species. The observed significant decrease in the level of serum vitamin C could be caused by increased utilization of vitamin C as an antioxidant defense against reactive oxygen species or by a decrease in GSH, which is required for the recycling of vitamin C. Free radicals, the main causes of oxidative stress, may react with variety of biomolecules including lipids, proteins, nucleic acids and macromolecules of connective tissues. Most important physiological antioxidants are vitamin C and uric acid, while bilirubin and thiol-containing molecules make a comparatively small contribution. Vitamin C can reduce superoxide, hydroxyl, peroxyxynitrite radical and other reactive oxidants and may reduce a protective physiological role by preventing lipid peroxidation.<sup>[29]</sup>

## Conclusions

Results of the present study showed that there were increased lipid peroxidation in type 2 diabetic individuals. Free radical formation along with antioxidant deficiency in type 2 diabetes mellitus Iraqi males which play an important role in the development of diabetic complications like diabetic nephropathy, retinopathy, neuropathy, peripheral arterial disease and atherosclerosis.

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### الخلاصة

البول السكري واحد من اكثر الامراض انتشارا على مستوى العالم وهو يرتبط مع عدة اليات واحدة منها الاجهاد التأكسدي الذي يلعب دور مهم في نشوء وتعقيدات المرض. من اهم اسباب ارتفاع مستوى السكر في الدم هو زيادة انتاج الجذور الأوكسجينية الحرة والتي تساهم في زيادة التعقيدات الناتجة من المرض. تهدف الدراسة الحالية لتقييم المستويات المصلية لبعض مضادات الاكسدة (GSH, Vitamin C UA) وكذلك بعض الاصناف الأوكسجينية الفعالة (MDA, ox-LDL) لدى الرجال العراقيين المصابين بداء السكري من النوع الثاني ومقارنتها مع الاشخاص الاصحاء. لذلك تم اخذ ٤٦ رجل مصابون بداء السكري من النوع الثاني مع ٣٦ رجل نسبة السكر في الدم لديهم طبيعية (اصحاء). تم سحب عينات الدم صيما لكلتا المجموعتين لقياس المتغيرات الحيوية التالية ( FSG, FSI, HbA1c, urea, creatinine, UA, GSH,

(Vitamin C, OX-LDL, MDA). اختبار (t) استعمل لتقييم الاختلاف في قيمة المتوسط للفحوصات المختارة بين المجاميع. تم ملاحظة ان المستوى المصلي للمتغيرات التالية (FSG, FSI, HbA1c, urea, creatinine, OX-LDL, ) كان اعلى بشكل مهم احصائيا في مرضى السكري مقارنة بالمجموعة الضابطة. اما المستوى المصلي للمتغيرات (GSH, Vitamin C,UA,HDL) كان منخفضا بشكل مهم احصائيا في مرضى السكري مقارنة بالمجموعة الضابطة. الامر الذي اوضح الرجال المصابون بداء السكري من النوع الثاني يعانون من حالة اجهاد تأكسدي تبدو واضحة للعيان بشكل كبير كما ويعانون من ارتفاع مستوى الدهون الضارة لذلك ينبغي عليهم مراقبة مستويات السكر والدهون في الدم بشكل مستمر للحفاظ على حالتهم الصحية بشكل جيد.