Estimation of oxidative stress, Oxidized LDL and some trace element in type 2 diabetes mellitus

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Abstract

Oxidative stress is a physiological state in which high numbers of reactive oxygen species (ROS) and free radicals are produced. It has been demonstrated that oxidative stress plays a role in the development of diabetes. We aimed in this study to assists oxidative stress markers in diabetic patients, such as the oxidation-reduction process and OxLDL, as well as the relationship between OxLDL and the traditional risk factors for type 2 diabetes (LDL, FBS). In addition, various trace metals (copper, zinc, and chromium) were evaluated as antioxidants. The study was conducted at the Specialized Center for Endocrinology and Diabetes in Babylon province, Iraq from the period January- March 2021. In this research a 90 participants were selected, 45 were diagnosed diabetes type 2 and 45 were normal individuals were selected from relative and medical staff with age range (30-70 years). A significant increase of the mean OxLDL of patients group were (598.31 ± 103.61) versus (406.35 ± 90.63) of control group with (P-value= 0.00). The mean LDL of patients were (113.48 ± 27.47) versus (93.59 ± 18.20) of control group, statistically this differences was highly significant (P-value= 0.00). There was a positive association of Ox- LDL, LDL and FBS. The finding revealed that there were significant variances in (zinc, copper and chromium) levels between patients compared with control. A decrease in mean level of zinc and chromium was observed in diabetic patients, while the level of copper in diabetic patients increased compared with the healthy control groups ($p < 0.01$).

Keywords: *T2DM; Oxidized LDL; oxidative stress; trace elements.*

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تقدير الإجهاد التأكسدي و LDL المؤكسد وبعض العناصر النزرة في داء السكري من النوع 2

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الخلاصة

الإجهاد التأكسدي هو حالة فسيولوجية يتم فيها إنتاج أعداد كبيرة من أنواع الأكسجين التفاعلية (ROS) والجذور الحرة. لقد ثبت أن الإجهاد التأكسدي يلعب دورًا في تطور مرض السكري. هدفنا في هذه الدراسة هو مساعدة علامات الإجهاد التأكسدي في مرضى السكري ، مثل عملية تقليل الأكسدة و OxLDL ، وكذلك العلاقة بين OxLDL وعوامل الخطر التقليدية لمرض السكري من النوع 2 (LDL، FBS). بالإضافة إلى ذلك ، تم تقييم المعادن النزرة المختلفة (النحاس والزنك والكروم) كمضادات للأكسدة. أجريت الدراسة في المركز التخصصي للغدد الصماء والسكري في محافظة بابل ، العراق في الفترة من كانون الثاني (يناير) إلى آذار (مارس) 2021. في هذا البحث ، تم اختيار 90 مشاركًا ، وتم تشخيص 45 منهم بمرض السكري من النوع 2 ، وتم اختيار 45 فردًا عاديًا من الأقارب و كادر طبي في الفئة العمرية (30-70 سنة). كانت الزيادة المعنوية في متوسط OxLDL لمجموعة المرضى (598.31 ± 103.61) مقابل (406.35 ± 90.63) لمجموعة التحكم مع (قيمة P = 0.00). كان متوسط LDL للمرضى (113.48 ± 27.47) مقابل (93.59 ± 18.20) للمجموعة الضابطة ، وكانت هذه الفروق ذات دلالة إحصائية عالية (قيمة P = 0.00). كان هناك ارتباط إيجابي بين Ox- LDL و LDL و FBS. أظهرت النتائج وجود فروق معنوية في مستويات (الزنك والنحاس والكروم) بين المرضى مقارنة مع مجموعة السيطرة. لوحظ انخفاض في متوسط مستوى الزنك والكروم في مرضى السكري ، بينما ارتفع مستوى النحاس في مرضى السكري مقارنة بمجموعات الضبط الصحية (p <0.01).

الكلمات المفتاحية : *داء السكري النوع الثاني ؛ LDL المؤكسد ؛ الاجهاد التأكسدي ؛ العناصر النزرة.*

Introduction

Diabetes mellitus (DM) is a long-term metabolic condition defined by hyperglycemia as a result of insulin insufficiency (type 1 DM) or insulin resistance (type 2 DM). It's a widespread disease that's becoming more widespread over the world [1]. Diabetes is still a problem that both emerging and advanced countries should be concerned about. In 2017, it was predicted that 451 million people globally (ages 18–90) had diabetes. By 2045, these numbers were predicted to rise to 693 million [2]. Hyperglycemia is linked to a number of significant consequences, including microvascular and macrovascular complication [3]. Excess free radicals and other oxidant species in the body, which are formed from oxygen, nitrogen, and other chemical components, induce lipid and protein oxidation. Because LDL particles are very vulnerable to oxidative damage, oxidized LDL (ox-LDL) is the most common modified form of native LDL [4]. LDL oxidation is a multi-step process in which lipids undergo oxidative modifications via enzymatic and non-enzymatic processes, resulting in complicated products. Ox-LDL production is thought to occur mostly within the extracellular area of the vascular wall

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when the body is under oxidative stress [5]. Ox-LDL is directly implicated in the beginning and progression of atherosclerotic lesions in coronary arteries, which can lead to coronary artery disease (CAD). LDL oxidation in the arterial wall is the first step of atherosclerosis process. Because endothelial cells produce reactive oxygen and nitrogen species (ROS and NOS), oxidative changes will be important in the clinical characteristics of coronary artery disease, such as endothelial dysfunction and plaque Copper (Cu), zinc (Zn), and chromium (Cr) are trace elements that activate or inhibit enzymatic activities in a variety of biological processes [7]. Zinc is an important and dynamic component in the digestion of glucose. It aids in the maintenance of many body tissues' efficacy as well as the speeding up of the operation of particular enzymes. It is important for maintaining insulin secretion and synthesis, besides the conformational reliability of insulin in the hexametric formula and its ability to modify insulin receptor intracellular events that regulate glucose tolerance and the ability to bolster the normal beta cell response to glucose contents [8]. It is required for proper insulin handling and storage, and its absence in weaker beta cells and increased insulin resistance implies increased oxidative stress [9]. Copper is required because it can act as an electron receiver or conductor. Copper aids in oxidation-reduction reactions in basic metabolic processes such as mitochondrial respiration, is a component of the antioxidant enzyme superoxide dismutase (Cu, Zn-SOD), and aids in iron stability as an adjuvant to ceroloplasmin [10]. Chromium, an important trace element and a powerful antioxidant, aids glucose and lipid metabolism by relieving glucose intolerance and lowering elevated lipids. Chromium deficiency has an impact on maintaining appropriate glucose tolerance and lipid profiles [11]. disruption [6].

Subjects and Methods

Ninety participants were enrolled in this study that attended the Specialized Center for Endocrinology and Diabetes in Babylon province, Iraq from the period January- March 2021. Their ages range from (30-70) years. They divided into two groups (45 patients with type 2 diabetes involved 22 males &23 females and 45 as the control set involved 23 males &22females.Venous blood samples (5 ml) were taken from all the study groups by means of disposable syringes and divided into two parts. First part of blood was transferred into poly ethylene plane tubes, to obtain serum to perform biochemical parameters. The second part transferred into an anticoagglutination (EDTA) tube to perform the oxidation-reduction reaction by cyclic voltammetry.

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Cyclic Voltammetry Devices are consist from NuVant Systems Inc.'s EZstat series (potentiostat/glvanostat) that introduced electrochemical technology in the United States. To perform cyclic voltammetry (CV), electrochemical workstations of a Bioanalytical system were linked to a personal computer. Glassy carbon electrode (GCE), Ag/AgCl (3M NaCl), and Platinum wire (1 mm diameter) were used as working, reference, and counter electrodes, correspondingly. Before being used in the CV cell, the surface of GCE was cleaned by polishing with alumina solution to abolish contaminants.

Procedure

Each participant's blood sample (approximately 1 ml) was diluted with 9 ml deionized water, then placed in a 10 ml quartz cell with three - electrode (glassy carbon electrode as working electrode, Ag/AgCl as reference electrode, and plantinum wire as counter electrode). The three electrodes were linked to a potentiostat to determine the results with cyclic voltammetry using the computer. Serum glucose was measured using an enzymatic colorimetric approach and (SPINREACT, Spain) kit. Auto analyser spectroscopy was used to estimate serum copper, zinc, and chromium levels. OxLDL was measured using an approach. For statistical analysis, (SPSS 20) the Statistical Package for Social Sciences version 20 software was utilized.

Results and Discussion

Table 1 shows an evaluation of the mean of fasting blood sugar for patients and control groups. The mean FBS for (Type-2 DM) patients was (191.9765.40) compared to (88.486.99) for the control group, which was statistically significant (P-value 0.01).

Table (1): Mean of FBS (mg/dl) according to studied group (Type-2 DM) and control group.

Variables	Study Groups	N	Mean	Std. Deviation	T-Test	P-value
FBS (mg/dl)	Controls	45	88.48	6.99	10.55	0.00
	Patients	45	191.97	65.40		

These findings corroborate those of Jyothirmayi, who reported that FBS levels in diabetes patients were higher than in controls. In persons with T2DM, insulin insufficiency or insulin resistance are common reasons of elevated blood sugar [12]. The resistance of beta cells to produce insulin is the cause of the rise in fasting blood glucose levels in diabetic patients. Insulin

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resistance is a common characteristic of type 2 diabetes, and it plays a key role in the disease's improvement [13].

Table (2) from cyclic voltammetry, the potential cycling of the oxidation-reduction reaction current for the CNT/GCE working electrode was performed in blood samples from diabetes and control groups. Table (2) displays the reliability of the anode current peak (Ipa) current and the mean ± SD of control groups (41.43 ± 2.44) compared to patients groups (42.69 ± 2.11) (p value 0.05), and the cathode current peak (Ipc) current and the mean ± SD of control groups (33.17 ± 5.96) compared to patients groups (35.11± 2.25) (p value 0.005).

Table (2): Means of Ipa –oxidation (u A) and Ipc- reduction according to studied group cases (Type-2 DM) and control.

Variables	Study Groups	N	Mean	Std. Deviation	T-Test	P-value
Ipa –oxidation (u A)	Controls	45	41.43	2.44	2.61	0.01
	Patients	45	42.69	2.11		
İpc –reduction (u A)	Controls	45	33.17	5.96	2.03	0.04
	Patients	45	35.11	2.25		

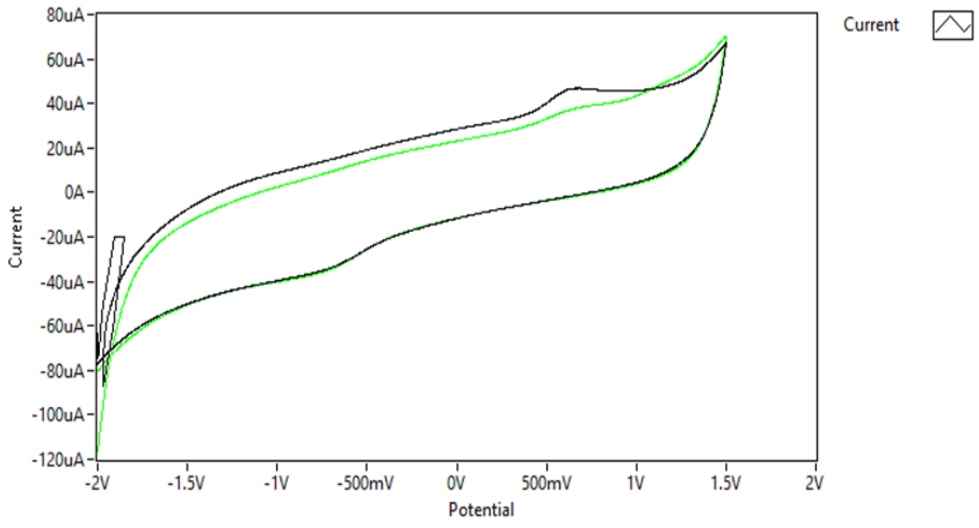

Fig. (1): Cyclic voltammetry of oxidation and reduction reaction current peak of blood sample at ten times cyclic on CNT/GCE electrode, scan rate 0.1V/sec versus Ag/AgCl as a reference electrode.

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Oxidation is a biological process that involves the loss of electrons and is required for natural living since it helps to generate cellular energy. Oxidative Stress, on the other hand, arises when cellular damage is caused by excessive oxidation. Because of its complexity, this process cannot be measured or defined by a single variable [14]. Hyperglycemia has been hypothesized to cause chronic oxidative stress via the glucose oxidation route, resulting in an excess of mitochondrial superoxide generation; thus, mitochondria are the predominant endogenous source of ROS production [15].

Table (3) demonstrated that increased serum Ox- LDL in T2DM patients versus control were (598.31103.61), (406.3590.63) respectively. This difference was statistically significant (P- value =0.00).

Table (3): Distribution of Ox LDL (pg/ml) according to studied group patients and control.

Variables	Study Groups	N	Mean	Std. Deviation	T-Test	P-value
Ox LDL (pg/ml)	Controls	45	406.35	90.63	9.35	0.000
	Patients	45	598.31	103.61		

These findings are comparable to those of Heidri et al., who discovered that OxLDL levels were higher in diabetes [16]. Compared to non-diabetics, patients with diabetes had significantly greater levels of OxLDL, according to Trpkovic et al. Oxidized low-density lipoprotein (OxLDL) promotes an inflammatory state and fat deposition in the artery, which contributes to atherogenesis. Inflammatory disorders, such as diabetes, are characterized by oxidative modification of lipids and proteins [17]. Type 2 diabetes mellitus is marked by abnormalities in lipoprotein metabolism, which are linked to plaque development and ox-LDL buildup during atherosclerosis [18]. Table (4) shows that the mean LDL of (Type-2 DM) patients was (113.48 ± 27.47) compared to (93.59 ± 18.20) in the control.

Table (4): Distribution of LDL (mg/dl) according to studied group patients and control

Variables	Study Groups	N	Mean	Std. Deviation	T-Test	P-value
LDL (mg/dl)	Controls	45	93.59	18.20	4.04	0.00
	Patients	45	113.48	27.47		

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Abdul Majid et al's findings are comparable to our results in that diabetic patients have high LDL levels. Diabetics with dyslipidaemia are more likely to develop CHD and other atherosclerotic problems [19]. Diabetic dyslipidemia may be caused by insulin resistance and hyperglycemia, which cause changes in lipoprotein metabolism, resulting in increased lipolysis and increased TG and LDL synthesis by the liver, as well as increased HDL breakdown [20]. Table (5) revealed that Ox-LDL levels were considerably associated with LDL levels, as evidenced by a higher score of (r=0.307, P-value=0.03), and a significant positive link with FBS.

Table (5): Simple correlation coefficient between Ox- LDL, LDL and FBS among studied groups.

Variable		Ox-LDL	LDL	FBS
Ox-LDL	r	1	.307**	.453**
	P-value		.003	.000(HS)
	N	90	90	90
****. Correlation is significant at the 0.01 level (2-tailed).**				

Glycemic management was found to be more important than the occurrence of diabetic complications in determining the level of oxidation of LDL-c [21]. According to [22], ox-LDL has a substantial correlation with all traditional lipid indicators. Fasting glucose levels were found to have positive and extremely significant relationships with oxLDL in a finding by Njajou [23]. Hyperglycemia, dyslipidemia, and lipoprotein oxidation are the main risk factors for CVDs in people with T2DM, which affects roughly 10-72 percent of the diabetic population [24]. Table (6) shows that (serum zinc) levels in T2DM patients were considerably lower than in the control group. This is a statistically significant difference (p value 0.01). In comparison to control groups (96.22 ± 16.40), the mean ± SD of patients was 72.53 ± 88.89.

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Table (6): Serum trace elements (Zinc, Chromium and Copper) levels among the studied groups.

Variables	Study Groups	N	Mean	Std. Deviation	T-Test	P-value
Zinc (µg/dl)	Controls	45	96.22	16.40	8.51	0.000
	Patients	45	72.53	8.89		
Chromium (µg/dl)	Controls	45	0.173	0.022	12.09	0.000
	Patients	45	0.107	0.02		
Copper (µg/dl)	Controls	45	113.77	21.07	7.97	0.000
	Patients	45	148.97	20.81		

These findings are consistent with those of Puri, et al and Devi, et al, which-found a decrease in serum zinc in diabetic compared to normal participants [25, 26]. Zinc is a cofactor for hundreds of enzymes that control carbohydrate, lipid, and protein metabolism. Zn is widely known for its function in the production, packing, and secretion of insulin [27]. Because of the impaired renal function associated with DM, hyperglycemia is frequently linked with hyperzincuria and significant urine zinc loss. Hyperglycemia is thought to impact zinc transport in the tubules [28]. There are significant differences in plasma chromium (0.107 ± 0.02) of the patients versus (0.173 ± 0.022) the control group, $p < 0.0001$. This difference was statistically significant. Chromium can rise the number of insulin binding receptors and the rate at which they are phosphorylated, enabling glucose transport into the liver, muscle, and adipose tissue simpler [29]. Chromium reduces lipid peroxidation by increasing the synthesis of antioxidant enzymes through different biological processes. Furthermore, chromium's antioxidant properties may aid in the reduction of oxidative stress and inflammation in T2DM patients [30].

According to the findings, the mean Copper of (Type-2 DM) patients was (148.97 ± 20.81) greater than the control group (113.77 ± 21.07). Persistent hyperglycemia, which increases glycation and induces the release of copper ions from copper binding sites in proteins, could possibly be to blame for the elevated copper level in diabetes mellitus. The oxidative stress is exacerbated by the copper ions released into the bloodstream [31]. Copper is a highly cytotoxic element in its free form because to its chemical feature of participating in redox

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processes. To generate reactive oxygen species and free radicals, free copper readily participates in redox processes such as the Heiber-Weiss reaction and the Fenton reaction [32].

Conclusion

The serum Ox-LDL level was found to be strongly linked to type 2 diabetes in this investigation. The present study concluded that, besides the traditional lipid criteria, Ox-LDL as a residual lipid marker should be included when assessing cardiovascular risk and managing lipid in type 2 diabetic patients. Changes in the amounts of copper, zinc, and chromium in type 2 diabetes patients may be major contributors in raising blood sugar levels and, as a result, increasing the risk of diabetes complications.

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