Evaluation of Serum Cholinesterase Activity, Lipid Peroxidation and Lipids Profile in Type 2 Diabetes Mellitus

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Abstract

The study was designed to evaluate serum cholinesterase activity, lipid peroxidation, lipids profile and nitric oxide production, in sera of diabetes type 2 and the control group to show whether there are changes in their levels.

The results of this study showed significant elevations ($P< 0.001$) in each of serum cholinesterase and lipid peroxidation end product represented by serum MDA concentrations in type 2 diabetic patients as compared to control group.

Although, this study showed a non-significant elevation in serum nitrite, the nitrate was elevated significantly ($P< 0.001$) in diabetic type 2 patients as compared to control group. The lipids profile of diabetic type 2 patients showed significant changes represented by significant elevations ($P< 0.001$) in each of TG, t. cholesterol, LDL and VLDL, and a significant decrease ($P< 0.001$) in HDL as compared to control group.

Using regression analysis showed a significant positive correlation between serum cholinesterase ($P< 0.001$) and each of FBS ($r = 0.698$), MDA ($r = 0.69$), NO^x ($r = 0.51$), t. cholesterol ($r = 0.487$), TG ($r = 0.691$), LDL ($r = 0.575$), and VLDL ($r = 0.455$), and a significant negative correlation($P< 0.001$) with HDL ($r = - 0.861$).

Conclusion can be made that diabetes mellitus is a low grade systematic inflammation associated with high oxidative stress, lipid peroxidation and serum cholinesterase activity, which may in turn, affect lipoproteins metabolism.

الخلاصة

لقد صممت هذه الدراسة لتقدير فعالية انزيم الكولين استريز في مصل الدم أضافة الى مستويات أكسدة الدهون وملف الدهون وأوكسيد النتريك في مصول دم مرضى السكري النوع الثاني ومجموعة السيطرة من اجل معرفة فيما اذا كانت هناك تغيرات في مستوياتها.

وقد أظهرت نتائج الدراسة وجود ارتفاعات معنوية ($P < 0.001$) في مستويات كل من انزيم الكولين استريز واكسدة الدهون lipid peroxidation المتمثلة في MDA, في مرضى السكري مقارنة بمجموعة السيطره. هذا وبالرغم من ارتفاع مستويات النتريت بصورة غير معنوية, فان النترات قد ارتفعت بشكل معنوي ($P < 0.001$). أما بالنسبة الى ملف الدهون فقد اظهرت النتائج وجود تغيرات معنوية متمثلة بارتفاعات معنوية ($P < 0.001$) في مستويات كل من TG و الكوليستيرول الكلي و LDL و VLDL , وانخفاضا معنويا ($P < 0.001$) في مستويات HDL في مرضى السكري مقارنة بمجموعة السيطره.

وان نتائج التحليل الانحداري قد اظهرت وجود علاقة موجبة معنوية ($P < 0.001$) بين فعالية الانزيم ومستوى السكر الصيامي FBS ($r = 0.698$) و MDA ($r = 0.69$) وأوكيد النتريك NO^x ($r = 0.51$) والكوليستيرول الكلي ($r = 0.487$) و TG($r = 0.691$) و LDL ($r = 0.575$) و VLDL ($r = 0.455$),وعلاقة سلبية ومعنوية ($P < 0.001$).

يمكننا الاستنتا من خلال نتائج الدراسة عبارة عن التهاب نظامي قليل الدرجة مصحوب بجهد تأكسدي عالي واكسدة مرتفعة للدهون وارتفاعا في فعالية انزيم الكولين استريز في مصل الدم, والذي ربما يؤثر في ايض البرتينات الدهنية.

Introduction

1- Cholinesterases:

Cholinesterases are enzymes capable of hydrolyzing choline esters. In (1964), the enzyme commission recommended [Acetylcholine Acetylhydrolase; E.C.3. 1.1.7], Acetylcholinesterase (AChE) for the 'true' and 'specific' cholinesterase; the enzyme capable of hydrolyzing acetylcholine faster than other choline esters. The other cholinesterases were collectively termed as 'pseudo' cholinesterase [Acylcholine Acylhydrolase; E.C.3.1.1, 8]. The pseudo cholinesterases included Butyrylcholinesterase (BChE) (defined by its capacity of hydrolyzing butyrylcholine (BCh) faster than any other choline esters) and Propionylcholinesterase (defined by its capacity of hydrolyzing propionylcholine faster than any other choline esters). Hence on the basis of substrate specificity and inhibitor sensitivity, it is possible to differentiate the 'true' and 'pseudo' forms of cholinesterase[1].

Serum cholinesterase is pseudo form and mainly butyrylcholinesterase (BChE) [2]. The function of BChE in higher vertebrates is unclear. Human lacking BChE activity does not show any pathology, except patients undergoing surgery when exposed to succinylcholine as a curarizing agent, fail to recover breathing at the end of anesthesia, if this compound is not hydrolyzed by BChE. BChE present as a soluble form in the plasma of mammals is suggested to serve as a safeguard against the diffusion of ACh into the bloodstream and / or against orally ingested toxic compounds, since this enzyme has a broad range of substrates [3]. BChE is used in inactivation of some drugs such as cocaine, aspirin, and amitryptyline, or in activation of others such as bambuterol, and heroin. In addition, BChE has a function in the development and progression of Alzheimer disease [4].

2- Diabetes Mellitus:

World Health Organization (WHO) and the American Diabetes Association (ADA) have therefore, adopted that individuals with a fasting plasma glucose (FPG) of 3.5-5.5mmol/L are considered as healthy but anything greater than 7mmol/L will be considered as diabetic. According to the classification of WHO and ADA,

there are two major types of diabetes: type 1(IDDM) and type 2 (NIDDM) [5].

Type 2 diabetes, or adult-onset diabetes, is a term used for individuals who have insulin resistance and usually have relative (rather than absolute) insulin deficiency. Most patients with this form of diabetes are obese, and obesity itself causes some degree of insulin resistance [6]. This form of diabetes frequently goes undiagnosed for many years because the hyperglycemia develops gradually and at earlier stages is often not severe enough for the patient to notice any of the classic symptoms of diabetes [7].

During diabetes, persistent hyperglycemia causes increased production of free radicals especially reactive oxygen species (ROS), in all tissues from glucose auto-oxidation, lipid peroxidation and protein glycosylation [8,9]. Several conditions were known to disturb the balance between ROS production and cellular defense mechanism. This imbalance can result in cell dysfunction and tissue injury, particularly relevant and dangerous for beta islet, which is among those tissues that have the lowest levels of intrinsic antioxidant defenses[8-10].

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 [8].

3- Nitric Oxide and Its Metabolites:

Nitric oxide NO is an endogenous gaseous free radical with a short half-life *in vivo* of a few seconds or less. Therefore, the levels of the more stable NO metabolites, nitrite (NO_2^-) and nitrate (NO_3^-), have been used in the indirect measurement of NO in biological fluids [11].

The biological activities of nitric oxide (NO) were first widely appreciated when it was identified as the endothelial-derived relaxing factor responsible for the potent vasodilating properties of stimulated endothelia. Since then, NO has been recognized as a pleiotropic biological mediator, regulating divers activities ranging from neuronal function to immune system regulation [12].

Nitric oxide (NO) is generated by the conversion of L-arginine and possibly other basic amino acids and polypeptides, perhaps by oxidative metabolic pathways that could involve polyunsaturated fatty acid-derived oxygen radicals, by the action of a diverse family of nitric oxide synthase (NOS). [13-16]. The NOS family include neuronal (nNOS), endothelial (eNOS) and inducible (iNOS) [17].

Nitric oxide plays an important role as an effecter molecule in β-cell destruction. It can also combine with oxygen to produce potent cellular killer such as the highly toxic hydroxyl radical ($OH^{\cdot}$) and peroxynitrite ($ONOO^{\cdot}$) which is the powerful oxidant [18].

Altered levels of NO have been shown to be associated with sepsis, infection, hypertension, exercise, type 2 diabetes, hypoxia, and cancer [12, 19, 20].

Although NO can be measured by many direct and indirect means (e.g., gas and liquid chromatography, electron paramagnetic resonance, mass spectrometry, electrochemistry, ..etc), the short half-life and low concentrations of NO in vitro reduce the practicality of this methods for evaluation of biological samples. The difficulties inherent to

quantitation of NO can be eliminated by measuring its stable metabolities, in particular, nitrite and nitrate [11, 12, 14].

4- Lipid Peroxidation:

Lipid peroxidation is a well-established mechanism of cellular injury in human, and is used as an indicator of oxidative stress in cell and tissues. Lipid peroxides, derived from polyunsaturated fatty acids, are unstable and decompose to form a complex series of compounds. These include reactive carbonyl compounds, which is the most abundant malondialdehyde (MDA) [21]. MDA attacks the lysine amino acid in protein which results in proteolysis. Therefore, measurement of MDA is a widely used as an indicator of lipid peroxidation, as well as hydroperoxides and conjugated dienes[22- 25].

Increased levels of lipid peroxidation products have been associated with a variety of chronic diseases in both humans and model systems [21], such as diabetes [22], myocardialinfarction [24], respiratory disorders, inflammation [26], β-thalassemia [27], ulcerative colitis [28], blunt trauma [29], liver diseases, atherosclerosis, and apoplexy [30].

5- **Plasma lipid Profiles:**

The common four types of lipid molecules present in plasma include triglycerides, cholesterol, cholesterol esters and phospholipids.

Diabetes is associated with increased mortality and morbidity due to vascular complications [31]. Many studies have shown that diabetes is consistently associated with changes in plasma lipids and lipoproteins, and these alterations are of interest because of their possible role in the etiology of the increased cardiovascular disease associated with diabetes [23, 32, 33].

Aim of The Study:

This study is directed to accomplish the following aims:-

1- The measurement of serum levels of cholinesterase of healthy control and patients with type 2 DM, to find whether there are significant differences between their levels.

2- Several biochemical variables related to oxidative stress in DM where taken in consideration in this work such as, nitric oxide (NO) production, lipid peroxidation represented by malondialdehyde MDA, and lipids profile, to point the changes in their levels and to find whether correlation exist between each of these variables.

Materials and Methods:

The study was conducted in Basrah Governorate, Basrah General Hospital, and Science College of Basrah University. 60 patients suffering from type 2 diabetes mellitus (30 males, and 30 females) aged between (34)to(65) years with a mean age of (44.11 ± 12.03) were included in this study. The diabetic patients were diagnosed on the basis of WHO criteria [34]. All patients have a history of duration of disease more than one year and no one of them have a hypertension.

The control group comprised of (60) healthy individuals (30 males and 30 females), aged between (30) to (66) years with a mean age of (45.1± 11.87). The control subjects were selected from the staff of Basrah General Hospital and from the staff and the students of various colleges of Al-Basrah University. They had

no history of diabetes mellitus or any other disease.

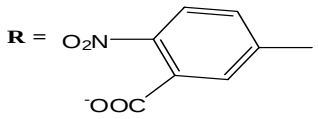
The studied samples of patients were collected from Basrah General Hospital. About 10 ml of the blood was drawn from a forearm vein of fasting patients recently diagnosed with diabetes (type 2), and healthy subjects were used as a control group. The blood samples were allowed to clot for 10 minutes at room temperature, and then centrifuged at (402 Xg for 10 minutes). The separated serum was drawn and divided into four parts, and stored in a deep freezing (-12C) until they are used.

In the present study we used the Pointe Kit, Canton MI 48188. USA to determine serum glucose levels. Whereas, serum cholinesterase was determined colorimetrically by Ellman's Method [35], which is the most popular method as it is sensitive for kinetic and enzymological studies. The principle of this method is the measurement of the rate of production of the thiocholine as the acetylthiocholine is hydrolyzed. This is accomplished by the continuous reaction of the thiol with 5,5-dithiobis-2-nitrobenzoate (DTNB)(I) to produce the yellow anion of 5-thio-2-nitrobenzoic acid(II). The rate of color production is measured at (405nm)in a photometer. ELISA Reader and printer was used for this purpose [36].

$$H_2O + (CH_3)_3N^+CH_2CH_2SCOCH_3 \xrightarrow{(enzyme)} (CH_3)_3N^+CH_2CH_2S^- + CH_3COO^- + 2H^+$$

$$(CH_3)_3N^+CH_2CH_2S^- + RSSR \longrightarrow (CH_3)_3N^+CH_2CH_2SSR + RS^-$$

I II



Serum nitric oxide end products; nitrite and nitrate, were measured colorimetrically using ELISA reader, according to Miranda KM, et al, 2001, assay. The assay combines reduction of nitrate by vanadium(III) and measurement of nitrite by Griess reaction in a single step.

Zinc sulfate was used in deproteinization of serum samples [11].

Malone dialdehyde (MDA) in serum was determined by colorimetric thiobarbituric acid (TBA) method. Under the acid and heating condition of the reaction, the lipid peroxides break down to form MDA, which complexes with TBA to form a coloured red compound that can be measured spectrophotometrically at 535 nm [37, 38].

Serum triglycerides concentrations were determined by the Fossati-enzymatic-colorimetric method [39], using (TG-PAP-150)-bioMerieux SA-France kit.

Serum total cholesterol was estimated by enzymatic colorimetric method, using BIOLABO SA cholesterol CHOD PAP-kit, France. Serum high density lipoprotein HDL was estimated by enzymatic colorimetric method [40], using BIOLABO SA HDL-cholesterol PTA-kit, France.

Serum very low density lipoprotein concentration was calculated according to the conventional Friedewaled equation [41].

Serum VLDL concentration = 0.2 x serum TG concentration

Serum low density lipoprotein concentration was calculated according to the following equation [41]:

LDL conc. = total cholesterol conc. – (HDL conc. + VLDL conc.)

Results an Discussion

1- The Effect of DM on Serum Cholinesterase Activity:

Table-(1): Serum cholinesterase activity in relation to sex in type-2 DM patients and Control groups.

Serum cholinesterase activity(µmol/l/min)						95% C.I		Range	
Sex	**group**	**n**	**Mean**	**±SD**	**SE**	**Lower**	**Upper**	**Min.**	**Max.**
Males	**Control**	30	6.532	1.436	0.262	5.995	7.068	4.63	8.82
	Type2DM	30	10.5**	1.459	0.266	9.955	11.046	7.76	13.38
Females	**Control**	30	6.317	1.204	0.219	5.867	6.766	4.41	8.82
	Type2DM	30	10.04**	1.379	0.251	9.526	10.566	7.43	12.79

** $P < 0.001$

Table (1) shows a significant elevation in serum cholinesterase in both, males and females patients with diabetes mellitus type-2 comparing with that of control, and the males showed higher levels than females in both cases.

The measurement of serum cholinesterase activity is of diagnostic value because of; a significant alteration of the normal value was occurred in various diseases including diabetes mellitus [42, 43]. Several studies assessed serum cholinesterase activity in diabetic patients. For example, Turecky L, et al, 2005, reported that the activity of serum cholinesterase was significantly higher in

group of patients with diabetes mellitus compared to a control group (65.05 vs.73.33 µkat/l) [44]. Dave KR and Katyare SS, 2002, showed that BChE activity was increased only in male diabetic rats (2.3-fold), whereas for females the result was reversed [45].

The elevated levels of serum cholinesterase, that were investigated in this study, as shown in tables (1), is in a compatible with the above researches. This study showed also a significant positive correlations between serum cholinesterase and fasting blood sugar in both males ($r = 0.737$, $p < 0.001$), and females ($r = 0.652$, $p < 0.001$).

Inacio LG, et al, 2006, attributed this elevation in the serum cholinesterase activity in diabetic patients to a possible interference of this disease in the catalytic mechanism of the serum cholinesterase enzyme [46]. Rao AA, et al, 2007, in a reliable explanation suggested that, type-2 diabetes mellitus is a low-grade systemic inflammatory condition, and acetylcholine (ACh) has anti-inflammatory action [43]. Hence, the determined elevated serum cholinesterase concentrations in diabetic patients were enhanced by the high acetylcholine levels that were detected in type-2 diabetic patients.

2- **Serum Nitric Oxide Metabolites NO^x:**

a- **Serum Nitrite:**

Table (2) showed non-significant elevation in serum nitrite concentration of diabetic women only as compared to the control subjects.

Table-(2): Serum NO_2^- in relation to sex in type 2 diabetic patients and control

Serum NO_2^- (µM)						95% C.I		Range	
sex	**group**	**n**	**Mean**	**SD**	**SE**	**Lower**	**Upper**	**Min.**	**Max.**
Males	Control	30	5.76	1.83	0.33	5.08	6.45	3.00	9.00
	Type2DM	30	5.70	2.40	0.45	4.76	6.63	2.00	11.00
Females	Control	30	5.70	2.39	0.43	4.80	6.59	2.00	11.00
	Type2DM	30	6.36	2.28	0.41	5.51	7.21	2.00	10.00

b- Serum Nitrate:

Table-(3): Serum NO_3^- in relation to sex in type 2 diabetic patients and control groups.

Serum NO_3^- (µM)						95% C.I		Range	
sex	**group**	**n**	**Mean**	**SD**	**SE**	**Lower**	**Upper**	**Min.**	**Max.**
Males	Control	30	24.93	4.29	0.78	23.32	26.53	18.00	32.00
	Type2DM	30	30.86*	3.83	0.69	29.43	32.29	26.00	38.00
Females	Control	30	23.61	2.53	0.46	22.67	24.56	18.00	28.50
	Type2DM	30	27.51*	3.15	0.57	26.33	28.68	24.00	36.00

- $P < 0.001$

Table (3) show that serum nitrate concentration was significantly increased in both sexes of diabetic patients as compared to the control subjects.

c- Serum Total Nitric Oxide Metabolites NO^x:

As shown in table (4) the concentration of serum NO^x was significantly increased in the group of patients with type 2 diabetes mellitus ($p < 0.001$) , as compared to the control groups.

Table-(4): Serum NO^x in relation to sex in type 2 diabetic patients and control groups.

Serum NO^x (µM)						95% C.I		Range	
sex	**group**	**n**	**Mean**	**SD**	**SE**	**Lower**	**Upper**	**Min.**	**Max.**
Males	Control	30	30.70	4.73	0.86	28.93	32.46	21.00	40.00
	Type2DM	30	36.56*	5.13	0.93	34.65	38.48	29.00	47.00
Females	Control	30	29.31	4.04	0.73	27.80	30.82	20.00	39.50
	Type2DM	30	33.87*	3.32	0.60	32.63	35.11	27.00	44.00

$P < 0.001$

Nitric oxide has been recognized as a biological mediator, regulating diver's activities ranging from neuronal function to immune system regulation. It is a gaseous

free radical with a short half-life *in vivo* of a few seconds or less. Therefore, the levels of the more stable nitric oxide metabolites (NO^x), nitrite (NO_2-) and nitrate (NO_3^-), have been used in the indirect measurement of nitric oxide in biological fluid [11, 12].

Excessive formation of oxygen-derived free radicals can damage the synthesis and release of endothelium derived relaxing factor (nitric oxide) and might explain the etiology of diabetes-induced decreases in endothelium-dependent relaxation [47]. For this purpose, several studies were made to evaluate the NO^x levels in variety of diseases related to oxidative stress like diabetes. The results of these studies can be classified into two main groups. The first group comprises the studies that found a decrease in NO^x levels in diabetes mellitus [13, 48].

The second major group of researchers showed significant or non-significant elevations in serum nitric oxide metabolites in patients with diabetes type 2 as compared to controls [19, 20, 49- 54].

Results of the present study, as shown in tables (2-4) are in agreement with the work of the above group of researchers, in that a non-significant increase in serum nitrite levels of women and a significant increases in serum nitrate and NO^x levels of both, males and females, with diabetic type 2 as compared to control.

It was reported that increased serum levels of NO^x indirectly reflect the presence of either endothelial dysfunction or vascular injury [20,49]. Several hypotheses have been raised to explain the increased serum levels of NO^x in diabetic patients. Since diabetes mellitus is associated with increased cytosolic [Ca^{2+}] and the enzymes eNOS and nNOS are constitutively expressed and activated by Ca^{2+}-Calmodulin. Hence, increased production of NO is expcted [55- 57]. It was reported that the HbA_{1c} concentration was significantly and positively related to NO_2^- and NO_3^- serum content [54]. Whereas, others have suggested that the raised levels of NO^x could reflect the negative feedback with cGMP [50], since NO^x interacts with soluble guanylate cyclase, leading to elevation of cGMP concentrations. In this instance, vasodilation would be blunted in diabetic subjects, not because of an inability to produce NO^x, but rather due to an inhibition of the action of NO^x presumably secondary to the generation of cGMP which is reported to be low [53]. Since diabetes mellitus type-2 is a low-grade systemic inflammation [43], so one cannot be discard the other sources of NO^x induced by the inflammatory cells (macrophages, neutrophils, and vascular muscle cells, among others) on the nitric oxide synthase [19].

Moshage H, et al, 1995, reported that nitrite in a whole blood is very rapidly (> 95% in one hour) oxidized to nitrate [11], and therefore plasma nitrite determination alone is meaningless. So, results of the present study; no-change in serum nitrite of diabetic males, non-significant elevation in serum nitrite of diabetic females, and high significant elevations in serum nitrate of both diabetic males and females as compared to controls, in agreement with the above study because most of nitrite was converted to nitrate during the collection of blood samples and the predetermination time.

The results of the present study showed a significant positive correlation between serum NO^x and FBS for both, males ($r = 0.466$, $p < 0.001$), and females ($r = 0.434$, $p = 0.001$) and significant ($p < 0.001$) positive correlation with serum cholinesterase ($r = 0.51$).

3- Malondialdehyde (MAD) and Diabetes Type 2:

Table(5): Serum MDA in relation to sex in type2 diabetic patients and control groups

Serum MDA(µM)						95% C.I		Range	
sex	**group**	**n**	**Mean**	**SD**	**SE**	**Lower**	**Upper**	**Min.**	**Max.**
Males	Control	30	1.21	0.19	0.035	1.13	1.28	0.86	1.50
	Type2DM	30	1.79*	0.22	0.041	1.70	1.87	1.08	2.10
Females	Control	30	1.12	0.18	0.033	1.05	1.19	0.85	1.41
	Type2DM	30	1.64*	0.16	0.03	1.57	1.70	1.37	2.10

* $P < 0.001$

Table (5) shows that serum MDA concentration was significantly increased in both sexes of diabetic patients as compared to the control subjects.

Malondialdehyde (MDA) measurement is widely used as an indicator of oxidative stress and lipid peroxidation in degenerative diseases like diabetes [21]. The results of this study as shown in table (5) are in agreement with the results of previous several studies [8, 22, 59].

Increased serum MDA was shown in a variety of diseases, like in patients with ulcerative colitis [28], respiratory diseases[26], and β-thalassemia [27]. These increases in serum MDA, which reflects the increased of lipid peroxidation, may be a useful marker of oxidative stress [22], which may be resulted from high free radical production [26,60], or due to decreased activity of antioxidant defense systems or both reasons [21].

Results of this study showed a significant ($p < 0.001$) positive correlation between serum MDA and fasting blood sugar for both males ($r = 0.975$) and females ($r = 0.657$). A significant positive ($p < 0.001$) correlation with serum cholinesterase ($r = 0.69$).

4- Lipids Profile and Diabetes Type 2:

As shown in tables (6-10) the concentrations of serum TG, t. cholesterol, and LDL were significantly increased, whereas HDL was significantly decreased, in the groups of patients with type 2 DM as compared to the control groups.

Table (6): Serum TG in relation to sex in type 2 diabetic patients and control groups

Serum TG (mmol/l)						95% C.I		Range	
Sex	**group**	**N**	**Mean**	**SD**	**SE**	**Lower**	**Upper**	**Min.**	**Max.**
Males	Control	30	1.72	0.08	0.014	1.69	1.75	1.55	1.80
	Type2DM	30	2.28*	0.20	0.037	2.20	2.35	2.00	2.65
Females	Control	30	1.79	0.16	0.029	1.73	1.85	1.50	2.10
	Type2DM	30	2.38*	0.31	0.056	2.26	2.49	1.85	2.97

- $P < 0.001$

Table (7): Serum t. cholesterol in relation to sex in type 2 diabetic patients and control groups

Serum cholesterol (mmol/l)						95% C.I		Range	
Sex	**group**	**n**	**Mean**	**SD**	**SE**	**Lower**	**Upper**	**Min.**	**Max.**
Males	Control	30	3.33	0.40	0.07	3.18	3.48	2.53	4.20
	Type2DM	30	4.13*	0.65	0.12	3.88	4.37	3.20	5.10
Females	Control	30	3.77	0.57	0.10	3.56	3.99	2.42	4.50
	Type2DM	30	4.53*	0.26	0.04	4.43	4.63	4.20	4.99

- $P < 0.001$

Table-(8): Serum LDL in relation to sex in type 2 diabetic patients and control groups.

Serum LDL (mmol/l)						95% C.I		Range	
sex	**group**	**n**	**Mean**	**SD**	**SE**	**Lower**	**Upper**	**Min.**	**Max.**
Males	Control	30	1.74	0.32	0.05	1.61	1.86	1.10	2.40
	Type2DM	30	2.81*	0.77	0.14	2.52	3.10	1.70	4.80
Females	Control	30	2.14	0.52	0.09	1.95	2.33	1.00	2.70
	Type2DM	30	3.20*	0.28	0.05	3.09	3.30	2.61	3.60

* $P < 0.001$

Table-(9): Serum VLDL in relation to sex in type 2 diabetic patients and control groups

Serum VLDL(mmol/l)						95% C.I		Range	
sex	**group**	**n**	**Mean**	**SD**	**SE**	**Lower**	**Upper**	**Min.**	**Max.**
Males	Control	30	0.24	0.07	0.01	0.21	0.26	0.1	0.35
	Type2DM	30	0.38*	0.11	0.02	0.33	0.42	0.22	0.70
Females	Control	30	0.29	0.06	0.01	0.27	0.32	0.20	0.40
	Type2DM	30	0.36*	0.06	0.01	0.34	0.38	0.27	0.51

- $P < 0.001$

Table-(10): Serum HDL in relation to sex in type 2 diabetic patients and control groups.

Serum HDL (mmol/l)						95% C.I		Range	
sex	**group**	**n**	**Mean**	**SD**	**SE**	**Lower**	**Upper**	**Min.**	**Max.**
Males	Control	30	1.34	0.13	0.02	1.29	1.39	1.20	1.81
	Type2DM	30	1.01*	0.11	0.02	0.96	1.05	0.80	1.20
Females	Control	30	1.32	0.07	0.01	1.29	1.35	1.20	1.40
	Type2DM	30	0.96*	0.08	0.01	0.93	0.99	0.80	1.12

* $P < 0.001$

Diabetes is associated with increased mortality and morbidity due to vascular complications [31]. Most of the effects of diabetes mellitus type 2, on the macrovasculature are the result of an acceleration of atherosclerosis and increase thrombosis [61]. Atherosclerosis occurs earlier in diabetics than non-diabetics, its severity is often greater, and its distribution is more diffuse [62]. Many studies have shown that diabetes is consistently associated with changes in plasma lipids and lipoproteins, and these alterations are of interest because of their possible role in the etiology of the increased cardiovascular disease associated with diabetes [32].

The most common type of lipid abnormalities encountered in a patients with diabetes mellitus are elevated levels of triglycerides (TG), VLDL, LDL and total cholesterol, and decreased high-density lipoprotein cholesterol (HDL) concentrations [63].

The results of the present study in tables (6- 10) are in agreement with several studies made previously [48,51, 52, 64, 65].

TG, t. cholesterol, LDL and VLDL, in this study showed a significant positive correlation with fasting blood sugar in both, males ($r = 0.826, 0.575, 0.629$ & 0.515) ($p < 0.001$) and females ($r = 0.569, 0.458, 0.559$ & 0.365) ($p < 0.001$ respectively, except for VLDL $p = 0.004$). Whereas HDL showed a significant negative correlation in both, males ($r = -0.731$) and females ($r = -686$) ($p < 0.001$).

References

1- Legay C., ***Microscopy Research and Technique,*** 2000, **49**: 56-72.

2- Soreq H. & Seidman S. ***Nat. Rev. Neurosci***., 2001, **2(9)**: 670.

3- Massouli J. ***Neurosignals***, 2002, **11**: 130-143.

4- Cokugras AN., ***Turk. J. Biochem***., 2003, **28 (2)**: 54-61.

5- Davidson MB. "Diabetes Mellitus: diagnosis and treatment" W. B. Saunders company, Los Angeles, California, 2007.

6- Bacha F, Saad, Gungor N, & Arslanian SA., ***Diabetes Care***, 2006, **29**: 1599-1604.

7- Asfandiyarova-Kolcheva N, Ryazantsev I, & Ryanzantsev V., ***Diab. Vasc. Res***., 2006, **3**: 57-60.

8- Moussa SA., ***Romanian J. Biophys***., 2008, **18(3)**: 225-236.

9- Robertson RP., ***J. Biol. Chem.***, 2004, **279(41)**: 42351-42354.

10- West IC., ***Diabetic Med.***, 2000, **17**: 171-180.

11- Moshage H, Kok B, Huizenga JR, & Jansen PLM., ***Clin. Chem***., 1995, **41(6)**: 892-896.

12- Miranda KM, Espey MG, & Wink DA., ***NITRIC OXIDE: Biology and Chemistry***, 2001, **5(1)**: 62-71.

13- Huynh NT, & Tayek JA., ***J Am. Coll. Nutr***., 2002, **21(5)**: 422-427.

14- Ghasemi A, Hedayati M, & Biabani H., ***J. Med. Sci. Res***., 2007, **2**: 29-32.

15- Pacher P, Beckman JS, & Liaudet L., ***Physiol. Rev***., 2007, **87**: 315-424.

16- Ignarrol LJ., ***FASEB J.***, 1989, **3**: 31-36**.**

17- Asano K, Chee CB, Gaston B, et al., ***Proc. Natl. Acad. Sci. USA,*** 1994, **91(21)**: 10089-10093.

18- Spinas AG., ***News. Physiol. Sci***., 1999, **14**: 49-54.

19- Pereira FO, Frode TS, & Medeiros YS., ***Mediators Inflamm.***, 2006, **2006(1)**: 39062-69.

20- Ozden S, Tatlipinar S, Bicer N, et al., ***Canadian Journal of Ophthalmology,*** 2003, **38(5)**: 393-396.

21- Saraymen R, Yazar S, Kilic E, & Ozbilge H., ***Saudi. Med. J.***,2004, **25(12)**: 2046-2052.

22- Kalaivanam KN, Dharmalingam M, & Marcus SR., ***Int. J. Diab. Dev. Ctries***., 2006, **26(Issue 1):** 30-32.

23- Hanachi P, Moghadam RH, & Latiffah AL., ***European J Sci. Res***., 2009, **28(1)**: 6-13.

24- Kumar A, Sivakanesan R, & Gunasekera S., ***Pak. J. Med. Sci***., 2008 **(part 1), 24(5)**: 689-693.

25- Wang AW, Sha S, Lesniak W, et al., ***Antimicrobial Agents and Chemotherapy***, 2003, **47(6)**: 1836-1841.

26- Rai RR, & Phadke MS., ***Ind. J. Cli. Biochem***., 2006, **21(2)**: 161-164.

27- Livrea MA, Tesoriere L, Pintaudi AM, et al., ***Blood***, 1996, **88(9)**: 3608-3614.

28- Baskol G, Baskol M, Yurci A, et al., ***Cell Biochem. Funct***., 2006, **24(3)**: 283-6.

29- Girotti MJ, Khan N, & McLellan BA., ***J. Trauma***., 1991, **31(1)**: 32-35.

30- Hristozov DV, Gadjeva T, Volykova S, et al., ***Archives of Physiol. And Biochem***., 2001, **1091(4)**: 331-336.

31- Williams SB, Cusco JA, Roddy MA, et al., ***J. Am. Coll. Cardiol***., 1996, **27**: 567-574.

32- Howard BV., ***Diabetes Care***, 1993, **16**: 284-291.

33- Pryor WA, & Squandrito GL., ***Am. J. Physiol.***, 1995, **268**: L695-L722.

34- Mayne P. "Clinical Chemistry in Diagnosis and Treatment" 6th$^{Ed.}$, 2002.

35- Elman GL, Courtney KD, Andres V, & Featherstone RMA., ***Biochem. Pharmacol***., 1961, **7**: 88-95.

36- Gouri A., "Acetylcholinesterase activity and isozyme pattern in normal and lithium-treated developing chick brain" (thesis), M.Sc., India, Mumbai University, 2004.

37- Lunec J., ***J. Ann. Clin. Biochem.***, 1990, **27**: 173-182.

38- Guidet B & Shah SV., ***Am. J. Physiol***., 1989, **257**: F440-5.

39- Fossati P, & Prencipe L., ***Clin. Chem.,*** 1982, **28(10)**: 2077-2080.

40- Tietz NW., "Text book of clinical chemistry" 3rdEd., 1999: 819-861.

41- Harris N, Neufeld Et, Ticho B, et al., ***Clin. Chem.***, 1996, **42(8)**: 1182-1188.

42-Bergmeyer HU., "Methods of Enzymatic Analysis" 1965;765-770.

43- Rao AA, Sridhar GR, Das UN, et al., ***Medical Hypothesis***, 2007, **69(6)**: 1272-6.

44- Turecky L, Kupcova V, Mojto V, et al., ***Bratisl. Lek. Listy.***, 2005, **106 (8-9)**: 266-269.

45- Dave KR & Katyare SS., ***Journal of Endocrinology***, 2002, **175**: 241-250.

46- Inacio LG, Stefanello F, Sausen LD, et al., ***Diabetes Res***., 2006, **72(1)**: 28-32.

47- Piper GM, Jordan M, Dondlinger LA, et al., ***Diabetes***, 1995, **44**: 884-889.

48- Singhania N, Puri D, Medhu SV, & Sharma SB., ***Q. J. Med***., 2008, **101**, 449-455.

49- Matata BM, & Galinanes M.., ***Diabetes***, 2001, **50**,2603-2610.

50- Aydin A, Orhan H, Sayal A, et al., ***Clin. Biochem***., 2001, **34(1)**,65-70.

51- Rani HS, Madhavi G, Rao VR, et al., ***Ind. J. Clin. Biochem***., 2005, **20(2)**, 75-80.

52- Khan MA., "Nitric oxide and lipid peroxidation status in diabetes mellitus and hypertension" (thesis), Ph. D., India, University of Karachi, 2000.

53- Piatti PM, Monti LD, Zavaroni I, et al., ***J. Clin. Endocrinol. & Metab***., 2000, **85**, 2416-2420.

54- Chiarelli F, Cipollone F, Romano F, et al., ***Diabetes***, 2000, **49**, 1258-1263.

55- Szkudelski T., ***Physiol. Res***., 2001, **50**: 536-548.

56- Alderton WK, Cooper CE, & Knowles RG., ***Biochem. J***., 2001, **357,** 593-615.

57- Voet D & Voet J. "Biochemistry" 3rd Ed.', 2004.

58- Jain SK, Levine SN, Vie RM, et al., ***Diabetes Care***, 1999, **22(7):** 1171-1175

59- Al-Fartosy AJ., "Determination of new biochemical marker of uric acid and its derivatives from oxidative stress in type 2 diabetic patients" (thesis), Ph. D., Iraq, University of Basrah, 2008.

60- Nader MM, Eissa LA, Gamil NM, & Ammar EL-S. M., ***Saudi Pharmaceutical Journal,*** 2007, **15(1),** 23-32.

61- Feener EP, & King Gl., ***Lancet,*** 1997, **350**, 9-13.

62- Johnstone MT, Creager S J, Scales KM, et al., ***Circulation***, 1993, **88**, 2510-2516.

63- Solano MP, & Goldberg RB., ***Clin. Diab***., 2006, **24(1),**27-32.

64- Rai V, Lyer U, Mani I, & Mani UV., ***Int. J. Diab. Dev. Countries,*** 1997**, 17**, 33-37.

65- Kim NH, Oh JH, Choi KM, et al., ***Yonsei Med. J***., 2000, **41(3):** 387-392.