LIPID PROFILE RELATED TO OXIDATIVE STRESS AND ANTIOXIDANTS IN DIABETIC PATIENTS .

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Abstract
The concentration of antioxidant such as glutathione, high density lipoprotein (HDL) were measured to assay the oxidative stress associated with Diabetes millitus(DM) compared with healthy controls, Glutathione was found to be reduced when compared with those of healthy controls, this depletion in antioxidant concentration may be due to their protective role against oxidative stress. The other finding is that Diabetes has lower HDL levels, but higher in low density lipoprotein(LDL), very low lipoprotein(vLDL), total Cholesterol(tCH) and Triglycerides(TG).

الخلاصه:
تم في هذه البحث قياس تراكيز عدد من مضادات الاكسده لمعرفه تاثير الجهد التاكسدي وعلاقته بمرض السكري وتبين من النتائج انخفاض مستويات الكلوتاثيون قياسا بنماذج السيطره وقد يعزى الانخفاض الى صفته في مقاومه الجهد التاكسدي المتكون نتيجه المرض وتم التوصل الى ان هذه التاثيرات قد ادت الى انخفاض في مستويات البروتين الدهني عالي الكثافه وارتفاع في مستويات البروتين الدهني واطى الكثافه اضافه الى ثلاثي الكليسيرايد والكوليستيرول الكلي.

Introduction

Diabetes mellitus can be defined as a group of metabolic disorders of carbohydrate metabolism due to glucose under utilized, producing hyperglycemia [(1).]

Generally developed at later time from patient, the case of type II is insulin resistance[(2)]. The reasons behind this resistance to insulin involve environmental causes and genetic causes(DM). The diagnosis of diabetes mellitus depends only on proof of hyperglycemia (Table 1). The diagnosis of type I is easy because hyperglycemia appears severe and accompanied with serious metabolic disorders.

In type II the diagnosis is difficult because glucose levels in serum may be mild; its importance to identify this type because the development of the complications accompanying it.

Table (1) Criteria for the diagnosis of diabetes mellitus [3,4]

Diabetes mellitus in non pregnant adults Any one of the following is diagnostic:-	
1-	Classical symptoms of diabetes and unequivocal elevation of plasma glucose
2-	Elevated fasting glucose on more than one occasion venous plasma ≥140 mg/dl
3-	Sustained elevated glucose concentration during the OGTT on move than one occasion venous plasma ≥ 200 mg/dl
Impaired Glucose tolerance in non pregnant adults three criteria must be met:-	
1-	Fasting plasma glucose ≥ 140 mg/dl
2-	½-hr,1-hr or(1.5-hr OGTT plasma glucose ≥ 200 mg/dl
3-	2-hr OGTT plasma glucose concentrations between 140and 200 mg/dl
Diabetes mellitus in children Either 1 or 2 is considered diagnostic of diabetes:-	
1-	Classical symptoms and a random plasma glucose > 200 mg/dl
2- A B -	In asymptomatic individuals:- A fasting glucose value of ≥140 mg/dl and Sustained elevated glucose concentration (≥ 200 mg/dl) during the OGTT on more than one occasion.
Impaired glucose tolerance in children Two criteria should be met:-	
1-	Fasting plasma glucose concentration of < 140 mg/dl
2-	Plasma glucose value 2 hr after receiving oral glucose of > 140 mg/dl

Antioxidant System

Antioxidants are substances (molecule or atom) found in food or in the body at low concentration. An antioxidant has more than one function to prevent the oxidative sequence. They may act by preventing the generation of radical or inhibiting any free radicals that are generated[5, 6]. Tissue's defense against free radical include primary antioxidant (catalase, superoxide dismutase, glutathione peroxidase)secondaryantioxidant (glutathione, albumin, uric acid... etc) and essential radical scavengers such as vitamin C (in aqueous phase) and vitamin E (in the lipo-soluble phase)[6].

Hyperglycemia and Free Radicals

Although the diabetes control and complications trial describe hyperglycemia as a risk factor for the development of diabetes complication[7]. There is no consensus about the pathogenic link between hyperglycemia and complication [8]. There are number of hypotheses on the origin of complications, including advanced glycation end product (AGE) hypothesis [9,10] oxidative stress [11,12,13], reductive stress [14].In this work, an attempt has been made to understand the relation between oxidative stress and diabetes complications.

Free Radical in the Pathogenesis of IDDM

IDDM appears as a result to the autoimmune beta cell destruction in pancreatic islets . At normal conditions, beta cells secrete insulin in response to the increase of serum glucose. The decrease and finally total loss of insulin secretion is linked to destruction of β cell. Increasing evidence suggests that free radicals play-as" important factors in the β cell destruction and loss insulin [15] .

Free Radicals in Pathogenesis of NIDDM

The etiology of NIDDM has far more diversity than IDDM [16] ,however there are many factors, which may induce beta cells malfunction and / or insulin resistance such as genetic abnormalities, environmental factors or obesity. These factors can cause mild hyperglycemic and cause alternations in free radicals generation and antioxidant enzyme[17] .

Glutathione

Glutathione is a major intracellular peptide sulfhydryl compound. Glutathione has many biological functions such as maintenance of membrane protein sulfhydryl groups in its reduced form and it functions in catalysis, metabolism, transport and in the protection of cells against foreign compounds, free radicals and ROS , Glutathione is an active compound in reactions that destroy H_2O_2 and other peroxides [18]. Glutathione acts also as a cofactor for many enzymes such as glutathione peroxidase, which catalyzes detoxification of intracellular. Thus, it maintains of glutathione levels in its natural state for cellular defense against oxidative injury and for cellular integrity [18]. The physiological role of glutathione as an antioxidant described and substantiated in studies of numerous disorders reflecting the increased oxidation result of abnormal glutathione [19] .

Lipid Profiles and Oxidation Hypothesis

Fatty acids (R-COOH) are straight- chain compounds, which have varying length, may be saturated or unsaturated. In the blood, fatty acids are carried mainly in conjugated form (bound to albumin) .There are four types of the lipids present in plasma, cholesterol, cholesterol ester, triglyceride and phospholipids. These lipids are relatively insoluble in water but are carried in the body fluids as soluble protein complexes known lipoproteins [20, 21] .

Lipid Peroxidation

Poly unsaturated fatty acid (PUFA) are very important components of cell membrane.

They form a double layer structure of membrane. They are arranged to be the non-polar tail faces towards the inner side of the double layer structure [22]. Oxidant can react with polyunsaturated fatty acid in cell membrane to form toxic metabolites. Lipids, which contain two or more unsaturated carbon-carbon double bond (C=C) can be attached by reactive oxygen species (ROS)[23] .

Materials & Methods

All chemicals were use as supplied without further purification.

Collection of Blood and Serum Preparation

Five ml of blood has been with drown and the blood allowed to clot for 15 minutes, the clot shrinks and serum can be obtained by centrifuging for approximately 10 minutes at a relative centrifugal force (RCF) of 1000 x g to 2000 x g [24] .

Patients and Controls

Thirty patients (21 males, 9 female) with diabetes mellitus type I and twenty-four (15 males, 9 females) with diabetes mellitus type II has been subjected to the present study, as well as fifty apparently healthy individuals as a control (3 1 males, 19 females) after having been asked about their health. (All the samples is collected from The laboratories of Mergan hospital in Hilla city)

Determination of serum reduced glutathione (GSH)

More than one type of analytical methods are used to determine serum glutathione (GSH) depending on the action of sulfhydryl groups. Thus methods include photometric, enzymatic, flourometric and HPLC are used [25,26,27].

Principle

5,5- Dithiobis (2-nitrobenzoic acid)(DTNB) is a dichromogen that is readily reduced by sulfhydryl group of GSH to produce an intensely yellow compound. Reduced chromogen has maximum absorbance at 412 nm and is directly proportional to GSH concentration

Determination of Serum Total Cholesterol [28]

Total cholesterol in the serum was measured by enzymatic method, with the biomerux kit, France.

Determination of Serum Triglyceride [29]

Total triglycerides in the serum were measured by enzymatic with the biomerieux kit, France.

Determination of Serum High Density Lipoprotein-Cholesterol (HDL-C)[30]

HDL-Cholesterol in the serum were measured by enzymatic method using biomeriex kit, France.

Determination of Serum Low Density Lipoprotein Cholesterol LDL

In lipoprotein fractionation the widely accepted method for determining the LDL-cholesterol is the beta quantification procedure. Very low-density lipoprotein (vLDL) is separated by ultra centrifugation and high-density lipoprotein (HDL), by precipitation. Because of ultra centrifugation is unavailable in most routine laboratories and the procedure is expensive, time consuming and technically demanding, the nearly universal approach in clinical laboratories has been to estimate LDL-cholesterol from the formula

of (Friedewaid et al. 1972)[31,32]. After the measurement of total cholesterol, triglycerides and HDL-cholesterol, LDL-cholesterol is calculated from the equation:-
LDL= TC - VLDL (estimated as triglyceride ÷2.2) - HDL

Determination of Serum Lipid Peroxidation

The concentration of lipid peroxides in the plasma was determined by the colorimetric thiobarbituric acid (TEA) method. Under the acid and heating conditions of the reaction the lipid peroxides break down to form Malondialdehyde (MDA) which is complex with (TEA). The resulting MDA-TBA chromogen can be measured spectrophotometrically at 532 nm [33,34].

Statistical analysis

The results are expressed as number, range, confidence interval C.I 95% and whenever possible as mean + SD (SE) of number of observations. The data are analyzed by using student's "t" and correlation test taking $P \leq 0.05$ as the lowest limit of significance.

Results and Discussion

In the present study, the control values of different variants were determined in sera of apparently healthy individuals, compared with different in-patients group. Then, the determination of different variants was done in sera of patients with the two types of DM. The age of healthy controls and patients subjected to the present study are shown in Table (2). Groups, sex, No., mean (years), SD (upper value, lower value).

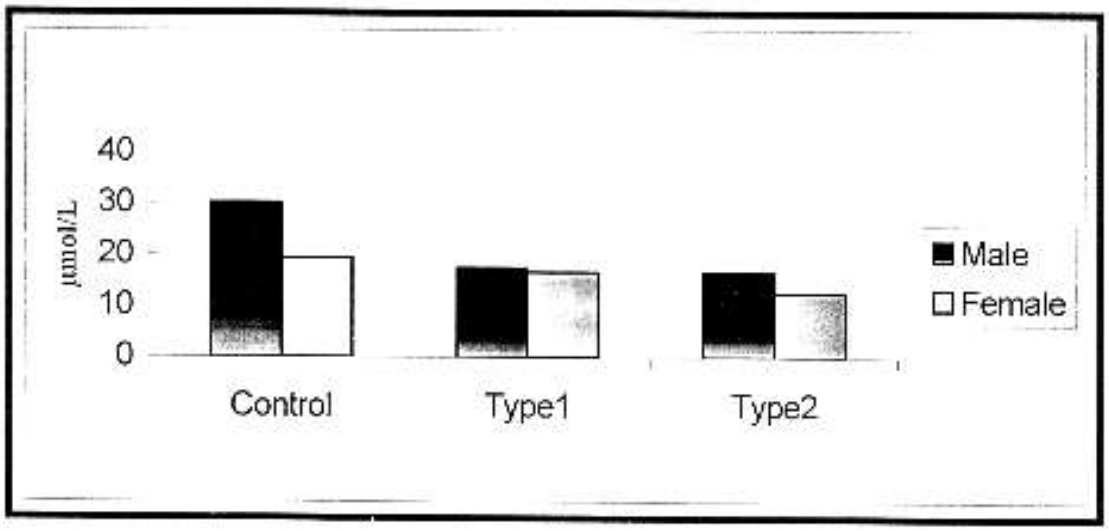
Table (2) The age of healthy controls and patients with diabetes mellitus.

	No.	Sex	Mean	SD	Upper	Lower
Control	31	Male	35.2	9.22	60	22
Control	19	Female	32.6	8.7	57	22
Type 1	21	Male	30.8	10.7	55	28
Type 1	9	Female	31.07	9.3	56	27
Type 2	15	Male	38.7	10.2	62	36
Type 2	9	Female	37.11	10	55	38

The Effect of Diabetes on Glutathione

Glutathione (GSH), cysteine and ergothionine are three compounds, which make up the non-protein thiol compounds (NPSH)[35]. Many investigators using methods, which measure NPSH, have interpreted the result as GSH because GSH makes at least 90% of the NPSH [36].GSH plays a central role in the defense against a variety of diseases. Its function includes the detoxification of carcinogens, free radicals and peroxides, regulation of immune function; and maintenance Compared with healthy controls, GSH concentrations were found to be significantly decreasing in sera of patients with different types of Diabetes mellitus. In the present study it was found that GSH is decreased

statistically significantly as shown in fig (1). Previous studies have shown low levels of GSH in patients with renal failure, liver failure [38], and CHD [39] vitamin B12 deficiency [40] and multiple-organ failure[41].



Figure(1): The levels of GSH in healthy controls and patients with DM

The decrease of GSH in diabetes may be attributed to

(1) Decreasing the synthesis of GSH: by using C^{14}-glycine, the synthesis of GSH by liver slices from the diabetic rat is found to be below the normal rate[42] .

(2) Increasing the rate of producing free radicals in diabetes mellitus; in this case GSH acts as scavenger and that is due to the decrease in GSH. (Alloxan destroys B-cell via producing free radicals; a single dose of GSH inhibited the function of Alloxan)[35].

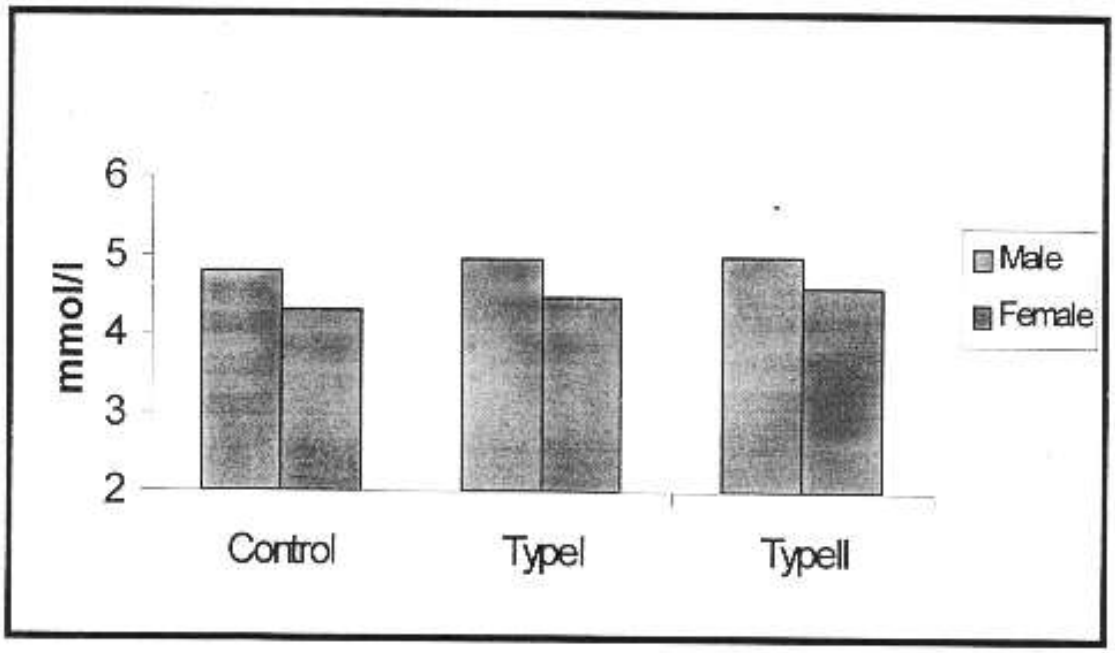
The depletion of GSH levels in the present study is compared with healthy controls supports the hypothesis that considers GSH as protective factor against the development of different types of Diabetes mellitus.

The Effect of Diabetes on Total Cholesterol

Unsaturated fatty acid in phospholipids and also cholesterol in cell membranes act as a susceptible to free radical and singlet oxygen (O_2–) mediated per oxidative modification, during which lipid hydro peroxide (LOOH) derivatives may accumulate [43,44].

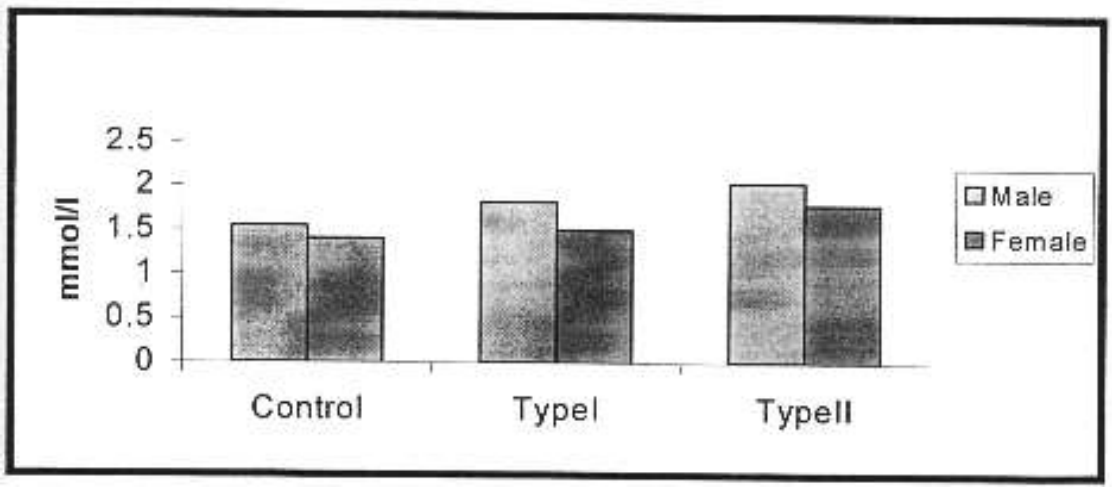
There are many causes which make the LOOH noxious to a cell; these reasons include (1) relatively high polarity, which can perturb membrane structure (2) long life time compared with free radical precursors or products [45], (3) involvement in oxidative stress signaling that could culminate in a peptic cell death [46],Compared with healthy controls. Total cholesterol concentrations were found to significantly increase in sera of patients with type I and type II Diabetes mellitus as shown in Fig (2).

Previous studies have shown that the role of diabetes on cholesterol synthesis is unclear [47,48,49].The effects on cholesterol metabolism of normalizing blood glucose levels with insulin have varied from decrease[50] to unchanged[47]. The increment of total cholesterol in the present study, when compared with healthy control, supports the hypothesis that considers glucose increasing is due to increase in cholesterol synthesis.



Fig(2): The levels of TC in sera of healthy controls and patients with diabetes mellitus.

The Effect of Diabetes on Triglyceride Compared with healthy controls, triglycerides levels were found to significantly increased in sera of patients with different types of DM, as in Fig (3) .



Fig(3): The levels of TG in sera of healthy controls and patients with diabetes mellitus.

The Effect of DM on Lipoproteins:-

The role of lipid abnormalities and the need for early detection of risk factors in insulin-resistance patients with or without diabetes is associated with cardiovascular disease[51].

The typical dyslipoproteinemia of type 2 diabetes is characterized by increased VLDL, small ((dense)) LDL particles[52], The most important factor in DM is the size of LDL particle. The percentage of patients having small LDL is increased by at least two folds in type II DM[53].

Compared with healthy controls, HDL levels were found to significantly decrease in sera

of patients with different types of DM, where as LDL, and vLDL levels were found to significantly increase in sera of patients with different types of DM, as shown in Fig (4) and Table(3).

Fig(4): Lipo-protein levels in sera of healthy controls and patients with DM

Table(3) Lipo-protein levels in sera of healthy controls and patients with diabetes mellitus.

	Sex	Mean mmol/l	SD	Upper	Lower	SE	95% I.C		P	Sign
							Upper	Lower		
Control	M	1.17	0.099	1.3	0.9	0.017	1.208	1.13	--	--
	F	1.239	0.159	1.35	1.11	0.09	1.59	1.39	--	--
T_1	M	0.905	0.197	1.2	0.7	0.035	0.98	0.82	0.001	Sign
	F	1.04	0.12	1.2	0.994	0.021	1.089	0.994	0.001	Sign
T_2	M	1.13	0.2	1.33	0.75	0.06	1.28	0.95	0.001	Sign
	F	1.147	0.39	1.4	0.89	0.019	1.187	1.04	0.001	Sign
C	M	2.1	0.36	2.6	1.4	0.06	2.29	2.00	--	--
	F	2.2	0.6	2.7	2.0	0.14	2.7	2.00	--	--
T_1	M	3.3	0.8	3.7	2.00	0.153	3.9	2.9	0.001	Sign
	F	2.9	0.7	3.0	2.0	0.24	3.7	2.6	0.001	Sign
T_2	M	2.9	0.8	3.1	1.97	0.15	3.9	3.3	0.001	Sign
	F	2.6	1.0	2.9	2.05	0.3	3.00	2.11	0.001	Sign
C	M	0.709	0.05	0.8	0.53	0.01	0.76	0.636	--	--
	F	0.545	0.04	0.65	0.38	0.07	0.6	0.497	--	--
T_1	M	0.85	0.13	0.98	0.68	0.02	0.94	0.704	0.001	Sign
	F	0.688	0.076	0.9	0.5	0.025	0.815	0.559	0.001	Sign
T_2	M	0.877	0.15	1.1	0.55	0.03	0.98	0.681	0.001	Sign
	F	0.81	0.14	1.51	0.51	0.048	098	0.67	0.001	Sign

Decreased HDL cholesterol levels are noticed in 74.6% of patients with type 1 diabetes and 53% of patients with type II diabetes, especially in association with hypertriglyceridemia. Patients who used (Daonil) have (HDL) levels more than patients that used other drugs in NIDDM.

Pervious studies have shown that vLDL triglyceride is not an important source of plasma FFAs. Wolf et al administered biosynthetic labeled vLDL to dogs and found that >95% of vLDL-triglyceride fatty acids were taken up directly into tissues without traversing the plasma FFA pool. On the other hand, glycerol produced from VLDL by the action of LPL was released into the circulation [54], determine lipoprotein subclass distribution by using nuclear magnetic resonance (NMR) spectroscopy.

They found that NIDDM increased the large VLDL particle concentration, decreased in LDL size and decreased HDL size as a result of depletion of HDL particles. Most studies have concluded that oxidized low-density lipoprotein is responsible for atherosclerosis[55]. The macrophage engulfed oxidized LDL form foam cell. The accumulation of foam cells in sub endothelial space is due to endothelial cell injury and thereby the atherosclerotic was initiated[56,57]. LDL can be oxidized either in presence of transition metals, or in metal independent system.

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