Comparative study of oxidative stress in diabetes mellitus

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Abstract:

The aim of the study was comparative between oxidative stress in diabetes mellitus using the measurement of some biophysical and biochemical parameters on two groups of diabetic patients, were conducted in the Al-Yarmuk Teaching Hospital,30 patients insulin dependent diabetes mellitus (IDDM) or type 1 ,their ages ranged between (15-45) and30 patients non-insulin dependent diabetes mellitus (NIDDM) or type 2,their ages ranged between (42-65).This study has been compared with 30 healthy subjects.The present study was demonstrated to evaluate the alteration in oxidative stress as measured by plasma and red blood cells Malondialdehyde (MDA) andchanges in antioxidant mechanism as measured by plasma and red blood cells Glutathione (GSH) in patients with diabetes mellitus type 1 and type 2,in compares to healthy control group. The results showed significant increment in serum Malondialdehyde (MDA) levels ,and significant decrease in serum glutathione (GSH) levels of Diabetic patients (IDDM), (NIDDM)),compared with control.Total cholesterol (TC), triglyceride (TG) and low density lipoprotein (LDL), high density lipoprotein (HDL), Diabetic patients (IDDM),and (NIDDM)patients showed significant increases in LDL levels. LDLs and very-low-density lipoproteins (VLDLs) the so-called "bad" cholesterols. Unlike HDLs, LDLs and VLDLs are high-cholesterol particles and significant decrease in HDL compared with control Oxidative stress results when free radicals increase more than antioxidants which is naturally synthesis in the body, then causes changing in the cells accure by oxidative stress.

Keywords: Antioxidants; Diabetic complications; Lipid peroxidation; Malondialdehyde; Oxidative stress; Type 1 diabetes mellitus; Type 2 diabetes.

Introduction:

Diabetes mellitus is a worldwide health problem predisposing to markedly increased cardiovascular mortality and morbidity [1]. Lipid abnormalities significantly contribute to the increased risk of cardiovascular disease and other morbidity in diabetics [2].**Type 1 diabetes ,** which used to be called insulin dependent or juvenile diabetes, Type 1 diabetics make very little or no insulin . This type of diabetes, the body does not make insulin. It usually starts in child or young adult, but it can occur at any age. It is treated by taking daily insulin shots or using an insulin pump and by following a special meal plan. About 5 to 10 percent of cases of diabetes are type 1.This type of diabetes; the body makes some insulin but cannot use it properly [3]

Type 2 diabetes patients, which used to be known as non-insulin dependent diabetes, do make their own insulin but it is either not in a sufficient amount to meet their needs or their body has become resistant to its effects [4]. Oxidative stress induced by reactive oxygen species (ROS), which is generated by hyperglycaemia[5].Diabetes mellitus is

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characterized by hyperglycaemia together with biochemical alterations of glucose and lipid peroxidation. It has been demonstrated by high levels of serum TC, triglycerides, LDL, VLDL, low concentration of HDL [6].

Several studies have evaluated free radical induced lipid peroxidation and the antioxidants in diabetic patients [7, 8]. Some complications of diabetes mellitus are associated with increased activity of free radical-induced lipid peroxidation and accumulation of lipid peroxidation products [9] Abnormally high levels of peroxidation and the simultaneous decline of antioxidant defense mechanisms can lead to damage of cellular organelles and lead to oxidative stress [10]. Under normal physiological conditions, there is a critical balance in the generation of oxygen free radicals and antioxidant defense systems used by organisms to deactivate and protect themselves against free radical toxicity [11]. Impairment in the oxidant/antioxidant equilibrium creates a condition known as oxidative stress. Oxidative stress is known to be a component of molecular and cellular tissue damage mechanisms in a wide spectrum of human diseases [12]

Diabetes is associated with a number of metabolic alterations and principal among these is hyperglycemia. Increased extra cellular matrix production and vascular dysfunction have all been implicated in the pathogenesis of vascular disease in type 1 and type 2 diabetes [13]. A variety of natural antioxidants exist to scavenge oxygen free radicals and prevent oxidative damage to biological membranes. One group of these antioxidants is enzymatic (intracellular), which include super oxide dismutase, glutathione peroxidase and catalase. In addition to enzymatic antioxidants, the major natural antioxidants, most of them derived from natural sources by dietary intake are vitamin A, vitamin C and vitamin E and carotenoids. Also, numerous small molecules are synthesized or produced within the body that has antioxidant capacity (e.g. glutathione and uric acid) [14, 15].

Patients and methods:

A total of 90 (30 IDDM)patients age range between (15-45), (30NIDD) patientsage range between (42-65) and30 normal healthyage range between (15-66) were conducted in the Al-Yarmuk Teaching Hospital. Based on preliminary survey, 30 patients are treated with insulin, had no other medications and they had no supplemental intake of vitamins or other nutrients. The remaining 30(15 male and 15 female) age range between (15-66) were chosen from the community and used as control subjects.

The duration period of the disease was graded into:

1- 30 IDDM (15 male and 15 female) from (12-23) years.
2- 30NIDD (15 male and 15 female) from (2-15) years.

Blood samples were collected from both types (30 IDDM + 30NIDD) patients and the control healthy groups before treatment according to [16].The serum was separated after centrifugation for 15 minutes at 2000 rpm and the resulted serum were preserved frozen at (-18C^{o}) unless immediate analysis was indicated. Erythrocytes were separated from plasma by centrifugation and the erythrocytes were washed twice with isotonic saline containing 2 mls sodium azide to inhibit catalase activity [17]

Measurement of erythrocytes MDA based on the reaction of thiobarbituric acid (TBA) forming TBA .MDA adduct ,was carried out using the modified method of Stocks and Dormandy[17]

Plasma MDA assay procedure was the same as that described for erythrocytes MDA.

Erythrocytes GSH content was determined by the method of Godin *et al.,* [18] Serum

glutathione is determined by a modified procedure utilizing Ellman's reagent[18.] Total concentration of cholesterol was measured by enzymatic method with commercially available kit (BioMerieux/France) according to the conventional Friedwald equation [19] Serum low density lipoprotein is determined according to the following equation [20.]. Low density lipoprotein (LDL) = Total cholesterol– (Very low density lipoprotein + High density lipoprotein). Total serum triglycerides concentration was measured by enzymatic method with commercially available kit (BioMetricux, France) [19.] HDL was measured by enzymatic method, with commercially available kit (BioMerieux – France) [20.]

Statistical analysis:

The data of this study, were compiled into the computerized data file and the frequency, distribution and statistical description (mean, rang, and SD) were derived using SPSS statically software.

We used statistical analysis of variance (ANOVA) test and least significantly difference (LSD) test by probability of less than 0.05 ($P<0.05$) according to [21]

Results and Discussion:

The biochemical parameters of the patients and subjects with IDDM and non-diabetic are given in (Table 1). The subjects with diabetes had duration of the disease ranged from 15-45years in IDDM patients and from42-65 years in NIDDM patients.

Table (1) Clinical parameters in diabetic patients compared to control

Parameters	Control	IDDM	NIDDM
N	30	30	30
Female/male	15/15	15/15	15/15
Age (years)	15-66	5415-	5642-
Duration of diabetes (years)		12-23	512-

Table (2) Oxidative and antioxidant parameters in plasma of diabetic patients compared to control

Parameters	Control(mean±SD)	IDDM(mean±SD)	NIDDM(mean±SD)
MDA µmol/L	1.3±0.97	3.1±0.25	2.7±0.12
GSH mmol/L	3.6±0.87	2.6±0.21	2.4±0.35

Table (3) Oxidative and antioxidant parameters in Erythrocytes of diabetic patients compared to control

Parameters	Control(mean±SD)	IDDM(mean±SD)	NIDDM(mean±SD)
MDA µmol/L	2.6±1.53	4.95±0.55	3.97±0.12
GSH mmol/L	5.4±0.73	2.43±1.13	3.44±1.23

Table (4) Lipid profile in diabetic patients compared to control

Parameters	Control	Mean±SD	IDDM	Mean±SD	NIDDM	Mean±SD
Cholesterol mg/dl	30	143.5±0.72	30	245.2±0.02	30	204.7±0.26
Triglycerides mg/dl	30	95.6±0.52	30	143.8±0.56	30	115.3±0.45
HDL Cholesterol mg/dl	30	45.5±0.30	30	24.8±0.85	30	30.7±0.34
LDL Cholesterol mg/dl	30	170±0.52	30	242±0.77	30	212±0.22
VLDL mg/dl	30	19±0.12	30	28±0.13	30	23±0.52

The results of this study showed clearly a significant difference in the oxidative stress as measured by plasma MDA between the patients and the control, and the mean plasma MDA was different in various types of diabetes patients (IDDM and NIDD).The mean erythrocyte and plasma MDA was higher in patients with diabetes mellitus than control(Table 2). The mean plasma GSH varied between 3 groups, but the healthy control had significant higher mean GSH than of diabetes patients (IDDM and NIDD. However erythrocytes GSH was higher in control group compare with the diabetes patients (IDDM and NIDD)(Table 2).This may suggest the oxidative stress correlated with disease activity and treatment [22]A statistically significant increment ($P < 0.05$) in values MDA as lipid peroxides were observed in diabetic patients and a significant reduction ($P < 0.05$) in GSH. There was a decrease in the antioxidant levels with corresponding increased protein and lipid oxidation (tabl 2).Lipid oxidation induces the overproduction of oxygen free radicals and consequently increases the protein oxidation and lipid oxidation [22]. A significance difference in the mean plasma concentration of total antioxidant status was observed in IDDM patients and in NIDDM patients[23]. Mechanisms involved in the increased oxidative stress in diabetes include not only oxygen free radical generation due to no enzymatic glycosylation (glaciation), autoxidation of glaciation products, but also changes in the tissue content and activity of antioxidant defense systems. Increased levels of the products of oxidative damage to lipids have been detected in serum of diabetic patients, and their presence correlates with the development of complications [24].

The lipid profile of IDDM and NIDDM is statistically significant changes with non diabetics. However, the (lipid peroxidation) levels increased diabetics compared with healthy control (Table4). Significant differences were found between patients with type 1 diabetes and type 2 diabetes and control subjects in the concentration of total, HDL, LDL cholesterol and triglycerides ((Table4). Results were in accordance with those of previous finding showed clearly the increased glucose levels induces diabetes, the overproduction of oxygen free radicals and consequently increases the protein oxidation and lipid oxidation (Table 2). Plasma MDA levels was significantly higher, which would indicate the free radical mediated oxidative damage of lipids and proteins are produced at in diabetics [25]. There are several studies evaluated the free radical induced lipid peroxidation and the antioxidants in diabetic patients. Many of these studies assessed individual antioxidants that act cooperatively in vivo to provide greater protection to the organism against free radical damage than could be provided by any single antioxidant acting alone. Controversial reports have been reported concerning the antioxidant status in diabetic patients [26].

The findings of this study suggest that diabetes in an altered metabolic state of oxidation-reduction and are convenient to give therapeutic interventions with antioxidants [27]. The results of this study have been confirmed with others [24].Which demonstrated that insulin treatment nearly corrects the oxidative stress in type 1 diabetics but only improves it in type 2 diabetics, the authors suggested the existence of metabolic differences between the two types of diabetes [28].

In addition, contributed that to Malondialdehyde (MDA) concentrations, were measured serving as a more conventional index of lipid oxidation. Many researches referred

that antioxidant status in patients with type 1 diabetes were determined by measuring the total antioxidant capacity (TAOC) as well as the concentration of some individual antioxidants in plasma and serum samples [29]. Diabetic patients have been generally described by others as having high levels of oxidative stress [27]. Oxidative stress generally causes damage to the membrane polyunsaturated fatty acids leading to the generation of MDA, a thiobarbituric acid reacting substance (TBARS), that agreed with this study. Increased lipid peroxidation products in diabetic subjects with vascular complications, have been reported [28]. Some authors have shown that high concentration of glucose may be associated with the presence of oxidative stress as reflected by the increase of intracellular lipid oxidation [29]. Serums MDA levels are higher in patients with newly diagnosed type 2 diabetes mellitus, and its concentration is elevated in poorly controlled type 2 diabetic patients [31]. The relationship between free radicals is supported by our results demonstrating an association between Malonodialdehyde MDA and Glutathione GSH, in patients with IDDM and NIDDM patients (table 2). The decreased in glutathione in diabetic patients also supports the hypothesis of radical mediated injury in this disease [30]Oxidative stress is known to be a component of molecular and cellular tissue damage mechanisms in a wide spectrum of human diseases [12]. A lipid profile is a direct measure of three blood components: cholesterol, triglycerides, and high-density lipoproteins (HDLs). Cholesterol is a vital substance that the body uses to produce such things as digestion-aiding material, hormones, and cell membranes. It is both produced by the body and absorbed from some of the foods when eat. Cholesterol and triglycerides are transported in the blood by combinations of lipids and proteins called lipoproteins. HDLs, the so-called "good" or "healthy" cholesterol, are lipoproteins made mostly of protein and little cholesterol. HDLs can help to clear cholesterol deposits in blood vessels left by another blood component called low-density lipoproteins, or LDLs[31]..The study found significant increment ($P < 0.05$) in cholesterol and LDLs, so significant decrease ($P < 0.05$) in HDLs in two types (IDDM) patients ,and (NIDDM) patients these results agreed with Bonnefont, *et.al*[31].

Conclusion

It can be conclude from this study that the free radicals are high in (IDDM) patients and almost lase in(NIDDM) patients similarly the antioxidants are decreased in(IDDM) patients than in(NIDDM) patients.

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دراسة مقارنة فرط الاكسدةعند المصابين بمرض السكري

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الخلاصة:

الهدف منهذهالدراسة المقارنة بين فرط الاكسدة لكلا النوعين من مرضى السكر (30)من النوع الاول المعتمد على الانسولين في العلاج تتراوح اعمارهم بين(15-45) و(30)من النوع الثاني الغير معتمد على الانسولين في العلاج من مرض السكر تتراوح اعمارهم بين(45-65) و(30)من الشخاص المعافين بعد اجراء الفحوص اللازمة لهم في مستشفى اليرموك التعليمي . صممت الدراسة الحالية لتقييم فرط الاكسدة في قياس معدل الملانودايلدهايد في مصل الدم وكريات الدم الحمر والتغيرات الحاصلة في مضادات الاكسدة بقياس معدلات الكلوتاثيونفي مصل الدم وكريات الدم الحمر في المرضى ومقارنتها مع مجموعة السيطرة . اظهرت الدراسة ارتفاعا معنويا بمعدل الملانودايلدهايد الناتج عن فرط الاكسدة لكلا النوعين من مرضى السكر,كما اظهرت الدراسة انخفاضا معنويا في نسبة الكلوتاثيون وهو احد مضادات الاكسدة التي تتكون في خلايا الجسم لكلا النوعين من مرضى السكر مقارنة مع مجموعة المقارنة.

اظهرت الدراسة فضلا عن ذلك ارتفاعا معنويا لبعض مكونات الدهون الضارة للجسم ومنها الدهون قليلة الكثافة لكلا النوعين من مرضى السكر,وانخفاضا معنويا في نسبةالدهون الجيدة مثل الدهون عالية الكثافة لكلا النوعين من مرضى السكر مقارنة مع مجموعة السيطرة . فرط الاكسدة يحصل نتيجة لزيادة الجذور الحرة التي تسبب فرط الاكسدة على مضادات الاكسدة التي تتكون بشكل طبيعي في الجسمونتيجة لذلك تحصل تغيرات في مكونات الخلايا نتيجة لاكسدة الدهون .