Levels of Glucose-6-phosphate Dehydrogenase in Type 1 Diabetes Mellitus patients with Nephropathy and Cardiovascular disease complication

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Received 20, December, 2012
Accepted 18, December, 2013

Abstract:

The aim of this study is to evaluate oxidative stress in diabetes mellitus (DM) Type1 by the measurement of Glucose-6-phosphate Dehydrogenase (G-6-PD), an enzyme expressed in human $RBC_{s,}$ is important in the generation of reduced glutathione which is the key product in oxidative stress controls. The Study was carried on 80 samples of blood and serum of National Diabetes Center (NDC). The study groups under fasting conditions and they divided as:
20 samples of diabetes mellitus patients without complications and 20 samples of diabetes mellitus with cardiovascular (CV) complications and 20 samples of diabetes mellitus with Nephropathy (Neph) complications compared with 20 control group with average age (13-67) years.. The results showed an elevation in the lipid profile and urea levels in patients groups compared with control group and a decrease in glucose-6-phosphate Dehydrogenase, HDL levels in all patients groups compared with control group.

Key words: diabetes mellitus disease, oxidative stress, G6PD

Introduction:

Diabetes mellitus, a common metabolic disorder resulting from defects in insulin secretion or action or both, is characterized by hyperglycemia often accompanied by glycosuria, polydipsia, and polyuria [1]. Type I (insulin-dependent) diabetes mellitus is caused by an autoimmune process that leads to inappropriate inflammation directed at the pancreatic islets [2]. During diabetes, persistent hyperglycemia causes increased production of free radicals for all tissues from glucose auto-oxidation and protein glycosylation increases the cytosolic NADH:NAD^+ ratio, which can be activated by hyperglycemia via increased production of Reactive oxygen spieces ROS and DNA strand breaks .

Free radicals are generated as by-products of normal cellular metabolism; however, several conditions are known to disturb the balance between free radical production and cellular defense mechanisms the increase in the level of free radical in diabetes could be due to their increased production and/ or decreased destruction by enzymic antioxidants. The level of these antioxidant enzymes critically influences the susceptibility of various tissues to oxidative stress and is associated with the development of complications in diabetes. Also this is particularly relevant and dangerous for the beta islet cell, which is among those tissues that have the less levels of intrinsic antioxidant defenses[3]., Diabetes produces disturbances of lipid profile, especially an increased

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susceptibility to lipid peroxidation [4] which is responsible for increase incidence of atherosclerosis [5]a major complication of diabetes mellitus[6]

Much attention has been focused on the role of oxidative stress, and it has been reported that oxidative stress may constitute the key and common event in the pathogenesis of secondary diabetic complications [7].Diabetic nephropathy is a kidney disease that occurs as a result of diabetes. Cardiovascular and renal com-plications share common risk factors The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction and failure of various organs, especially the eyes, kidneys, nerves, heart and blood vessels. Diabetes affects the kidney in stages. At the onset of diabetes, the kidney grows large and the glomerular filtration rate (GFR) becomes disturbed. Most recent basic and clinical researches have pointed toward sclerosis and kidney failure [8].

Subjects, Instruments, Materials and Methods

A questionnaire was designed with different questions including duration of diabetes mellitus, family history, smoking, usage of drugs, drug duration, height, weight, heart disease. All diabetic patients with and without cardiovascular and nephropathy complications were treated with insulin injection. Diabetic patients were examined by an endocrinologist in National Diabetes Center (NDC**),** Patients with thyroid function disease, and hormonal abnormalities were excluded from the study. Patients and controls were classified according to the following:

Subjects: Include eighty (80) samples were devided as:

A- Patients: DM without CV and nephropathy Complications Group: this group consists of 20 patients, DM with Cardiovascular Complications Group: this group consists of 20 patients and DM with Nephropathy **(**NP) Complications: this groups consists of 20 patients.

B- Controls: Twenty healthy women individual were included in this study as control group matched in age and gender with other groups. None of the controls were diabetic, alcoholic, smoker, or having a history of coronary heart disease, thyroid or other metabolic disease before taking part in this study.

Table (1): The numbers and Age Mean of patients in DM patients with and without complications , and controls group

Groups	Female No.	Age Mean (Years)
DM without complications	20	33.65±6.3
DM with CV complications	20	43.45±16.14
DM with NP complications	20	50.65±13.56
Controls	20	51±12.06

Collection of Blood Samples:

5 ml of blood were obtained by vein puncture, using a 5 ml disposable syringe. The blood samples were divided into two aliquots: The first aliquot 3ml was dispensed for tube containing ethylene diaminetetracetic acid (EDTA). The second aliquot 2ml was dispensed in a plain tube and left for around an hour to clot at room temperature. Then, it was centrifuged at 3000 rpm for 10 minutes to collect serum stored in the freezer (-20ºC) until use.

Materials and methods:

Determination of Serum Glucose-6-phosphate Dehydrogenase (G6PD) level:

Serum G6PD is measured by G6PD kit [9]

Determination of Serum Fasting Glucose (S.FG) level:

Fasting Glucose was determined using an enzymatic colorimetric method with a commercially available kit [10].

Determination of Serum Total Cholesterol (S.T.C.) level:

Serum cholesterol was measured by an enzymatic method using cholesterol kit [11]

Determination of Serum Triacylglycerol (S.TG) level:

Serum Triacylglycerol was measured using an enzymatic method by TG Kit [12]:

Determination of Serum High Density Lipoprotein (S.HDL) level:

Serum HDL was measured by HDL kit [13].

Determination of Serum Low Density Lipoprotein (S.LDL) level:

LDL was very difficult to isolate and measure .Hence, LDL level is most usually derived by the Friedwalds formula as follows [14]:

LDL = Total cholesterol – [HDL+ TG/5]

Determination of Serum S.VLDL-level:

Very low-density lipoprotein-cholesterol was estimated by using formula of Friedwalds [15]:

VLDL = TG/5

Determination of Serum Urea level:

Enzymatic determination of urea level (urease –modified Berthelot reaction [16],

Statistical Analysis

Results are expressed as Mean±SD. and significant differences between means were assessed by student t-test using the available statistical software packages (Microsoft Excel XP), statistical significance was set at $P<0.05$.Correlation analysis was used to test the linear relationship between parameters.

Result

Data demonstrated in Table (2) shows the characteristics Lipid profile in DM groups compared to control groups, Total serum cholesterol levels were significantly (p=0.04) higher in DM patients with nephropathy complications with mean (182.9±45.6) when compared with controls (154 ± 30.3mg/dl) . Total serum cholesterol in DM without complications and DM with CV complications was (157.05±28.4) and (238.5±44.9mg/dl) showed no significant difference (P>0.05) when compared with that of control (154 ± 30.3) as shown in Table (2)

Table (2): Lipid profile in DM groups compared to control groups

Mean±SD	Controls	DM without complications	DM with Nephropathy	DM with CV	P .value
S.cholesrol (mg/dl)	154 ± 30.3	157.05±28.4	182.9±45.6	238.5±44.9	0.04
Triglycerid (mg/dl)	96.88±21.3	106±41.5	142.6±58.2	198±102.8	0.0003
HDL (mg/dl)	51.62±2.95	50.5±5.6	45.68±6.07	40.7±5.68	0.0008
LDL (mg/dl)	77.29±24.72	82.6±28.3	100.7±50.7	153.7 ±44.3	0.08
VLDL (mg/dl)	20±5.09	24.8±15.2	28.15±11.8	42.6±21.53	0.0001
Atherogenic Index (LDL/HDL)ratio	1.53±0.54	1.58±0.55	2.15±1.37	3.82±1.17	0.08
LDL Size Index (TG/HDL) ratio	1.91±0.81	2.1±1.06	3.29±1.71	5.21±3.03	0.0001

Triglyceride level was found to be significantly higher (p=0.0003) in DM patients with CV complications with means of (198±102.8mg/dl) compared to control with a mean of (96.88±21.3 mg/dl) . Serum Triglyceride in DM without complications and DM patients with nephropathy complications were

(106±41.5 mg/dl) and (142.6±58.2mg/dl) respectively complications with no significant difference (P>0.05) could be detected when compared to control as shown in Table (2)

The mean level of HDL was significant (P=0.0008) in the DM patients with nephropathy complications of mean (45.68±6.07) when compared to that found in the control group (51.62±2.95mg/dl) while no deference significant in respectively DM patients without complication and DM patients with CV complications (50.5±5.6 mg/dl) and (40.7±5.68mg/dl) respectively when compared to control as shown in Table (2)

LDL levels were not significantly (P>0.05) higher in DM patients without complications when compared with controls (82.6±28.3mg/dl) vs. (77.29±24.72mg/dl) respectively while in DM patients with nephropathy complications and DM patients with CV complications (100.7±50.7mg/dl) and (153.7 ±44.3mg/dl) respectively also no significant difference (P>0.05) were observed when compared to control as shown in Table (2)

Mean VLDLthere was a significant (p=0.0001) difference in DM patients with CV complications (42.6±21.53.2mg/dl) than in the control group (20±5.09mg/dl),no a significant difference(P>0.05) between in DM patients without complications and DM patients with nephropathy complications (20±5.09mg/dl) and(28.15±11.8mg/dl) when compared with control as shown in Table (2)

serum Atherogenic mean values were (1.58±0.55) for DM without complications and control (1.53±0.54) with no difference significant. Serum Atherogenic value showed also no difference significant increase in DM patients with nephropathy complications(2.15±1.37) group and the mean of DM patients with CV complications(3.82±1.17) compared to control, Table (2)

Serum TG/HDL are shown in Table (2). In mean DM patients without complications (2.1±1.06) mg/dl, the means of Serum TG/HDL-c were no significant than in mean control (1.91±0.81) subjects .also there was no significant difference between the means of DM patients with nephropathy complications (3.29±1.71) with the mean of control (1.91±0.81) ,but the mean of DM patients with CV complications(5.21±3.03)showed significant difference (p=0.0001) when compared with to mean controls (1.91±0.81).

Also The means (±SD) of DM duration are shown in Table (2). Showed no significant difference between the means of duration in DM patients with/without complications compared with control. Also the means of fasting glucose were greater than in the control subjects. There was not a significant difference (P>0.05) between the means of DM with/without complications compared with control groups as shown in Table (2)

Table (3): Fasting Glucose (FG) level (mg/ml), Age, duration of DM in DM groups compared to control groups.

FG(mg/ml)	control	DM without complications	DM with nephropathy	DM with CV	p.value
Number of sample	20	20	20	20	
Duration of DM	-	6.5±5.13	7.45±5.32	8.4±5.69	0.6
Mean±SD glucose	88.63±8.47	229.05±91.63	231.1±97.84	241.1±105.51	0.9

Body Mass Index (BMI) was found to be increased significantly [p=0.03] in
DM patients without complications with a mean of (29.28± 7.22 Kg/m^2) compared to controls with a mean of (24.19±2.57 Kg/m^2), and a significant difference was found (p=0.01) between DM patients with nephropathy complications (28.25±4.14 Kg/m^2) and control (24.19±2.57Kg/m^2) ,also significant difference (p=0.0005) was found between DM patients with CV complications (31.53±6.22Kg/m^2) and mean control (24.19±2.57Kg/m^2) as shown in Table (4)

Table (4): Body Mass Index (BMI) in DM groups and control groups.

Mean±SD	Controls	DM without complications	DM with nephropathy	DM with CV
BMI (Kg/m2)	24.19±2.57	29.28± 7.22	28.25±4.14	31.53±6.22
P.Value compared with control	-	0.03	0.01	0.0005

The means (±SD) of Percent Body Fat (PBF) are shown in table (6). In all patients groups, the means of PBF were greater than in the control subjects, . There was significant difference (p=0.0008) between the means of PBF in DM patients without complications (40.14±10.58) with the mean of control (30.13±5.67). The mean of DM patients with nephropathy complications (40.16±6.08) was not significantly difference(p=0.9) as compared to mean controls (30.13±5.67), also the mean of DM patients with CV complications (44.50±7.86) not significantly difference(p=0.9) when compared with mean controls (30.13±5.67) as shown in Table(5)

Table (5): Percent Body Fat (PBF) in DM groups and control groups.

Mean±SD	Controls	DM without complications	DM with Nephropathy	DM with CV
PBF	30.13±5.67	40.14±10.58	40.16±6.08	44.50±7.86
P .Value compared with control	-	0.0008	0.9	0.9

Glucose- 6- P –Dehydrogenase (G6pd) level was found to be not significantly different [p=0.1] in DM patients without complications with a mean of (0.61±0.59 u/ml) compared to controls with a mean of (0.91±0.76u/ml), and a significant difference was found (p=0.002) between DM patients with nephropathy complications (0.28±0.24) and control (0.91±0.76) ,also significant difference (p=0.002) was found between DM patients with CV complications (0.27± 0.29) and mean control (0.91±0.76 u/ml) as shown in Table (6)

Table (6): Glucose- 6- P – Dehydrogenase (G-6-PD) in DM groups compared to control groups.

Mean G-6-PD IU/ml	Control	DM without complications	DM with nephropathy	DM with CV
Valid N	20	20	20	20
Mean±SD	0.91±0.76	0.61±0.59	0.28±0.24	0.27± 0.29
P value		0.1	0.002	0.002

Urea level was found to be significantly increased [p=0.02] in DM patients without complications with a mean of (26.25±3.84) compared to controls with a mean of (23.25±4.03 mg/dl), and a significant difference was found (p=0.01) between DM patients with nephropathy complications (41.9±6.07 mg/dl) and control (23.25±4.03 mg/dl) ,also significant difference (p=0.005) was found between DM patients with CV complications (26.6±2.92 mg/dl) and mean level of control (23.25±4.03 mg/dl) as shown in Table (7)

Table (7): Mean of urea level (mg/dl) in DM groups compared to control groups

urea(mg/ml)	Control	DM without complications	DM with nephropathy disease	DM with CV
Number of sample	20	20	20	20
Mean±SD	23.25±4.03	26.25±3.84	41.9±6.07	26.6±2.92
P value compared with control		0.02	0.01	0.005

Discussion:

This study revealed of increased levels of TC, TG, LDL, and decreased levels of HDL compared with control female diabetic patients had significantly higher levels of cholesterol , The Hyperlipidemia in females may be attributed to the effects of sex hormones on body fat distribution, leading to this differences [17]. Also High HDL levels protect against CV development, as patients with high HDL tend to have lower prevalence of CV risk factors. On the other hand, patients with low levels of HDL are more likely to develop CV disease [18].

Serum Atherogenic LDL/HDL ratio value were found to be elevated in diabetes mellitus patients as compared with control, Several large clinical studies have found the LDL/HDL ratio to be an excellent predictor of CV risk [19].

Also serum of TG/HDL ratio show increase in mean of DM patients compared with control this agreement with study [20]**.**The presence of hypertriglyceridemia, low HDL concentrations, and high TG/HDL ratio always associated with insulin resistance because insulin affects TG and HDL metabolism [21]. Add of that G6pd level showed a decrease in DM patients when compared to that of controls which may be an indication to increased oxidative stress in diabetes contributes to the development of diabetic complications. Oxidation of lipids in plasma lipoprotein and cellular membranes is associated with the development of CV disease in diabetes[22] .

In all patients groups, the means of PBF were greater than in the control subjects Clinical evidence suggests that the association of diabetes with central obesity is stronger than the association with general fat . Central obesity has been associated with decreased glucose Tolerance, reduced metabolic clearance of insulin, and decreased insulin-stimulated glucose disposal . With the rapidly increasing diabetic population in our country [23].Increased BMI in DM patients compared to controls was found. Although BMI is a measure of overall adiposity, it is often considered an indicator of body fatness; it is a surrogate measure of body fat because it measures excess weight rather than excess fat [24]. In the present investigation, diabetes associated nephropathy (DM+NP) . Clinical abnormalities are often detected 5–10 years after onset or diagnosis of DM. The patients to be Nephropathy DM [25]

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مستويات إنزيم الكلوكوز 6 فوسفات ديهايدروجينيز في مرضى السكري من النوع الاول مع مضاعفات الكلى والامراض الوعائيه

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الخلاصة:

الهدف من هذه الدراسه هو تقدير قياس مستوى إنزيم الكلوكوز 6 فوسفات لمرضى السكري من النوع الاول بواسطه قياس مستوى انزيم G6PD الموجود في كريات الدم الحمراء والمسؤول عن اختزال الكلوتاثايون المفتاح الرئيسي للسيطره على الجهد التاكسدي اجريت الدراسه على80 عينه من الدم والمصل من النساء وقد تم تقسيمهم كالاتي:

20عينه للمرضى المصابات بالسكري ،20 عينه للمرضى المصابات بالسكري مع مضاعفات الامراض الوعائيه و20 عينه للمرضى المصابات بالسكري مع المضاعفات الكلويه مقارنه ب 20 عينه من الاصحاء سريريا وقد تمت الدراسه في المركزالوطني لابحاث السكري ومركز بحوث التقنيات الاحيائيه /جامعه النهرين وكان معدل اعمارهم مابين (13-67) سنه

اظهرت النتائج وجود ارتفاع في مستوى الدهون واليوريا في مرضى السكري من النوع الاول مقارنه بمجموعة الاصحاء(مجموعه السيطره) كما اظهرت الدراسه وجود انخفاض في نسبه انزيم G6PD و HDLفي مريضات السكري من النوع الاول مقارنه بمجموعة السيطره .