

Peroxidase Activity and Other Biochemical Parameters in Female with Type 2 Diabetes Mellitus with and without Coronary Arteriosclerosis

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Abstract

Oxidative stress reduces insulin secretion and increases insulin resistance in some experimental models and may thus play a causal role in the pathogenesis of diabetes. The purpose of this study was to examine the relationship between serum Peroxidase activity in serum of patients with Type II Diabetes Mellitus and with and Without Coronary Arteriosclerosis and healthy control. In addition to the antioxidant parameters, levels of fasting blood glucose, lipid profile, urea, total protein were determined in diabetic patients and controls. The results indicated that Peroxidase activity levels reduced in diabetic patients.

Introduction

The term diabetes mellitus refers to the family of metabolic conditions associated with the loss of normal glucose regulation resulting in hyperglycemia. In 2000, there were 171 million diabetics worldwide comprising 2.8% of the population; this number is projected to reach 366 million (4.4% of the population) by 2030 [1]. Type 1 diabetes, also known as insulin-dependent diabetes mellitus (IDDM), typically results from autoimmune destruction of pancreatic islet cells responsible for insulin secretion within the first few decades of life. In contrast, type 2 diabetes, or non-insulin-dependent diabetes mellitus (NIDDM), involves progressive insulin resistance as target tissues become insensitive to insulin resulting in chronic hyperglycemia and hyperinsulinemia.[2]

Hyperglycaemia, which is a characteristic for all types diabetes, is a comparatively or entirely lack of insulin action, insulin resistance, and germination of diabetes-specific pathology in the peripheral nerve, retina, and renal glomerulus. Accelerated atherosclerotic disease is also associated with diabetes by 2-4 folds[2], affects arteries that equipped heart, lower extremities, and brain. Heart disease is the major diabetes complication.[3]

Hyperglycemia causes tissue damage through 5 major mechanisms: (1) increased flux of glucose and other sugars through the polyol pathway; (2) increased intracellular formation of AGEs (advanced glycation end products); (3) increased expression of the receptor for AGEs and its activating ligands; (4) activation of protein kinase (PK)C isoforms; and (5) overactivity of the hexosamine pathway.[2]

This leads of hyperglycaemia has been confirm by a huge number of studies for both DMT1, and DMT2[3,4], Although every cell in human body with diabetes is exposed to abnormally high glucose levels, but hyperglycaemia selectively damage specific cell type, this could be due to the failure in down regulation of these cells for glucose uptake when extracellular glucose concentration is increased. Vascular endothelial cells, in contrast to other cells which are not directly susceptible to hyperglycaemia damage revealed no significant change in glucose transport rate when is elevated, causing in intracellular hyperglycemia. [5]

Accelerated CVD risk in female diabetic patients is thundering than men, because productive years in women life time proses them a protection from CVD, and this protection is missing in diabetics [6].

Both, Type 1 and type 2 diabetic patients show a similar atherosclerotic plaque profile with an increase in necrotic core size and a decrease in the fibrotic cap size [7, 8]. However, type 2 diabetics show enhanced atherosclerotic plaque burden with more distal plaques compared to type 1 diabetics [8].

Hyperglycemia stimulates specific processes for oxidative stress and pro-inflammatory state, especially, an increase in the production of reactive oxygen species (ROS) in mitochondria's matrix. This overproduction of ROS is one of the key steps in the pathogenic process leading to cardiovascular complications in diabetic patients, leading to multiple atherogenic stimuli promote endothelial cell dysfunction and activation [9, 10].

Several ROS play vital roles in endothelial pathophysiology including the hydroxyl radical (OH^\bullet), free radicals superoxide ($\text{O}_2^{\bullet-}$) and as well as the peroxynitrite (ONOO-/ONOOH), non-free radical species hydrogen peroxide (H_2O_2), and hypochlorous acid (HClO). Endothelial cell dysfunction is stimulated by ROS scavenging NO directly or oxidative modification of tetrahydrobiopterin resulting in eNO uncoupling [11]. Thus, ROS reduce endothelial cell NO production (scavenging, eNOS uncoupling) while activating both direct (NF- κ B activation) and indirect mediators (LDL oxidation) of endothelial cell activation [11].

The presence of ROS, under normal conditions induces the expression of antioxidant enzymes as a defence mechanism. This is not a rule under diabetes condition. For instance, in fibroblasts from T1D patients with overt nephropathy, the exposure to hyperglycemia led to an increase in lipid peroxidation without a compensatory increase in the level of the antioxidant enzymes like: Peroxidase, Cu-Zn superoxide dismutase, catalase, and glutathione peroxidase [12]. Even patients with a short diabetes duration and without chronic complications present less antioxidant plasma capacity and uric acid levels suggesting that the oxidative stress occurs early in the disease [12].

Aim of the study: was to examine the association of change of Peroxidase activity and the onset of cardiovascular events in a selected sample of patients admitted to the hospital with type 2 DM with evidence of coronary arteriosclerosis (C.A.), without any complication which were compared to healthy control group.

Material and Methods

Subjects

The study group of this study consist of a total 47 subjects, which Included non-diabetic healthy control subjects (n =11) and type2 DM subjects (n = 36) which were previously diagnosed and collected from the Centre of Diabetic Research in Baghdad , Iraq from Nov. 2012 till Feb. 2013 . We grouped the DM subjects into two groups. Group I (n=25) consisted of DM patients with complications of ischaemic heart disease with diabetic duration was (7.78 ± 2.789) years, and group II (n=11) consisted of DM patients without any clinically demonstrable complications (8.7 ± 2.92) years. Regarding the history of the treatment history, and lifestyle modifications, 22 were on insulin therapy, 14 were on oral hypoglycaemic drugs. The healthy controls were not on any kind of prescribed medication or dietary restrictions. Informed consent was taken from all subjects and this study was approved by the institutional ethics committee.

Blood Samples Collection

A venous blood sample was acquired from every fasting subject into non- heparinized tubes, serum were separated by centrifugation at 4000 g for 10 min, aliquoted, coded, frozen and stored at -20°C until analysis. All chemical reagents were of the analytical grade (NaH_2PO_4 , Na_2HPO_4 , 4-aminoantipyrine, DMSO, H_2O_2 , Phenol) were obtained from BDH company.

Peroxidase Assay

Peroxidase was estimated spectrophotometrically by the Tindler's method (1966) with modifications. The reaction rate is determined by measuring an increase in absorbance at 510 nm resulting from the decomposition of hydrogen peroxide. Briefly, the assay mixture consisted 700 μl s. Of AAP (4- aminoantipyrine) (250mg AAP in 10mls. H_2O_2), 10 μl s. Phenol (250mg in 5mls. DMSO) , 40 μl s of phosphate buffer pH=7(0.4 mls in 19.6mls. phosphate buffer pH=7) , and 50 μl s of serum. The mixture was incubated in spectrophotometer at 25°C for 3-4 minutes to achieve temperature equilibration and establish blank rate, if any. Add 0.1 ml of diluted enzyme and record the increase in A510 for 4-5 minutes. Calculate $\Delta\text{A}_{510}/\text{minute}$ from linear portion of the curve.

Calculation

$$\text{Units/mg} = \frac{\Delta A_{510}/\text{min}}{6.58 \times \text{mg enzyme/ml reaction mixture}} \\ \text{mgP HPOFF per milliliter} = A_{278} \times 1.22$$

Biochemical Determinations

In addition to peroxidase assays, other biochemical measurements including fasting plasma glucose, plasma lipid profile, Urea, and total protein were measured enzymatically by using Biosystem Kits, Spain.

Statistical Analysis

The results were expressed as mean±standard deviation (SD). A $p < 0.005$ was considered to be statistically significant. Statistical analysis was performed by using Excel 2010, One-way analysis of variance (ANOVA) was used to compare the mean values. Pearson's correlation was applied to correlate between the parameters.

Results

Table (1) shows the medium and SD of age, duration of disease, lipid profile, TC/HDL-C, LDL-C/HDL-C, total protein, urea, and peroxidase activity for 47 subject are presented in table 1.

As apparent from table 1 there were a highly significant increment in the level FBG of both patients groups as compared to control group ($p < 0.0001$) which could be due to a poor glycaemic control in these patients, or to insulin resistance in some patients which led to the increased glycation of proteins and other biomolecules. Prolonged hyperglycaemia in these patients might have caused accelerates damage to the biomolecules and the bio membranes, thus leading to various diabetes associated complications. (13)

Significant increments were seen in both TG, and VLDL and TG/LDL ratio in group 2 as compared to control group associated with insignificant decrement in HDL level in group 2 as compared to the two other groups, in the other hand an insignificant increment were shown in other lipid profile parameters in both patients groups, the results could be due to dyslipidemia status in especially diabetic patients with cardiovascular complication, Each of these dyslipidemic features is associated with an increased risk of cardiovascular disease. Increased hepatic secretion of large triglyceride-rich VLDL and impaired clearance of VLDL appears to be of central importance in the pathophysiology of this dyslipidaemia. Small dense LDL particles arise from the intravascular processing of specific larger VLDL precursors. Typically, reduced plasma HDL levels in type 2 diabetes are manifest as reductions in the HDL_{2b} subspecies and relative or absolute increases in smaller denser HDL_{3b} and HDL_{3c}. (14)

Peroxidase activities decline were shown in both T2DM groups, especially for group 2 as compared to healthy group. This decrease could be due in decreases in the level of antioxidative enzymes (SOD and GPx) which is proportional to FBG level, and would lead to increase in hydrogen peroxide level which will inhibit peroxidase enzyme due to acidic environment (acidosis) (15). It has been suggested that diabetic's blood are more exposed to lipid peroxidation due to the impaired antioxidant defence system. Oxidative stress is an imbalance between free radical production and lipid peroxidation on one hand, and the antioxidant defence system on another. The pro-oxidant/antioxidant balance in diabetes may be due to either acceleration of cellular reactions leading to increased free radical production, such as non-enzymatic protein glycation, glucose, oxidation and increased sorbitol pathway, or reduced antioxidant defence potential (16).

An insignificant increment was shown in urea's level in patients with coronary arteriosclerosis, while an insignificant decrement was shown in T2DM patients without complication. On the other hand an insignificant increment of Total protein was seen in both patients groups, which are considered as significant markers of renal dysfunction, or may be due to increased protein catabolism. (16, 17).

These facts were further evidenced by the correlation data of our study. Table (2) shows the correlation between peroxidase level in three groups and other biochemical parameters. A positive correlation was shown between peroxidase activity and FBG level in group 2 while showing a negative with the other parameters in the same group. However different correlations were shown between peroxidase and other parameters in group 1 and healthy control group. A positive correlation were observed with TG/HDL, and LDL/HDL ratios as compared to a negative correlation in control group. Urea, and total protein levels showed a negative correlations with Prx activity as compared to positive correlation in control group.

Elevated extra and intra-cellular glucose concentration results in an oxidative stress. When diabetic complications are developed, an increase in oxidative damage and subsequently emaciation of antioxidant defence systems is observed (18). Changes in oxidant and antioxidant systems are related with duration of disease and become more important as complications develop. Finding of several studies demonstrated that overproduction of peroxides along with emaciation of antioxidant defence systems causes oxidative damage and these events in type 2 diabetic patients are observed in an earlier stage, before diabetic complications develop. (19)

Table 1: Biochemical Parameters of Control Healthy Group and Group1 Type2 DM without Complication and Group2 Type 2 DM with Coronary Arteriosclerosis

Groups Biochemical parameters	Group1: patients with type2 DM without complication N=11	Healthy control N= 11	Group2: patients with type 2 DM with coronary arteriosclerosis N=25
Age(yrs)	53.5 ±4.29	52± 6.25	56.21± 7.66
Duration of disease	4.7±1.16		11.75±2.79
FBG gm/dl	235.5±116.13****	88.6±14.83	202.33±78.56*****
CHO gm/dl	171.8±25.41	178.51±50.68	205.33±55.12
TG gm/dl	100.9±80.95	80.95±28.93	163.460±84.26****
VLDL gm/dl	20.7±5.50	16.0±5.77	33.5±18.39***
LDL gm/dl	102.3±26.93	113.1±44.35	125.92±53
HDL gm/dl	52.1±5.43	48.7±14.35	44.625±8.27
TG/HDL	1.98±0.69	1.83±0.83	4.03±2.63**
LDL/HDL	1.91±0.57	2.25±1.45	3.21±1.12
Urea gm/dl	26.4±4.25	27.5±9.68	32.375±8.3
Total protein gm/dl	68.3±6.0	66.42±6.89	70.375±6.41
Peroxidase u/L	4.68±2.162	10.88±3.29	5.43±1.104

*** p< 0.001 ; **** p< 0.0001; ***** p<0.00001

Table 2: R Value for the Correlation between Peroxidase Activity and Other Biochemical Parameters of Control Healthy Group and Group1 Type2 DM without Complication and Group2 Type 2 DM with Coronary Arteriosclerosis

Groups Biochemical parameters	Group1: patients with type2 DM without complication N=11	Healthy control N= 11	Group2: patients with type 2 DM with coronary arteriosclerosis N=25
	Peroxidase U/L		
	r value =		
Age(yrs)	0.1578	-0.0988	0.0824
Duration of disease	-0.4489		-0.1844
FBG gm/dl	-0.01486	0.021	0.0647
CHO gm/dl	-0.4964	0.39754	-0.2323
TG gm/dl	0.6406	0.2285	- 0.1207
VLDL gm/dl	0.511	0.225	- 0.1443
LDL gm/dl	-0.5480	0.2913	- 0.2290
HDL gm/dl	-0.5115	0.4360	0.2430
TG/HDL	0.7183	-0.222	-0.1655
LDL/HDL	0.45166	-0.17862	-0.3721
Urea gm/dl	-0.251	0.4092	-0.1609
Total protein gm/dl	-0.266	0.4051	-0.0269

Conclusion

In conclusion, decreased enzymatic antioxidant activity and increased lipid profile were observed with patients with type 2 diabetes mellitus, which demonstrate the acceleration of cardiovascular disease complication.

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