

Paraoxonase Activity as a Biochemical Indicator of Dyslipidaemia in Diabetes Mellitus Type 2 and Ischemic Heart Disease

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ABSTRACT

Mammalian paraoxonases (PONs) are a group of enzymes of calcium-dependent, esterases consisting of Paraoxonase-1, 2 and 3 Paraoxonase. But, PON1 has the dominant PON activity in blood. It is synthesized by the liver and found in the circulation associated with high-density lipoproteins (HDL). It protects low density lipoprotein (LDL) and HDL from lipid peroxidation. Alterations in circulating PON1 level have been reported in a variety of diseases involving oxidative stress including non-insulin dependent diabetes mellitus (NIDDM) and ischemic heart disease (IHD). The aim of this work has been planned to demonstrate PON1 activity in different diseases associated with dyslipidemia as NIDDM and IHD. This study was carried out on 20 Patients with NIDDM (NIDDM group) and 20 patients admitted to the Cardiac Care Unit with IHD (IHD group) in addition to 20 healthy subjects free from any disease as control group. All groups were selected from Suez Canal University Hospital. They were subjected to full history taking and assay of fasting blood sugar, lipid profile and liver function tests using colorimetric methods. In addition, PON1 activity was measured using paraoxon as a substrate and apolipoprotein A1 (ApoA1) was measured using nephelometric method. Our results showed that patients with NIDDM and IHD had higher BMI, they were dyslipidemic where cholesterol, triglycerides and low density lipoprotein were higher and HDL was lower in the two patients groups compared to the control. In addition PON1 activity and ApoA1 were decreased in the two patients groups compared to the control. A positive correlation was found between PON1 and ApoA1 in the two patients' groups. Conclusions: PON1 activity and ApoA1 were reduced in Diabetes mellitus and Ischemic heart disease patients compared to the controls. Dyslipidemia is associated with patients of mentioned diseases with higher body mass index.

Key words: Apolipoprotein A1; Dyslipidemia; Non-insulin dependent diabetes mellitus; Ischemic heart disease; Oxidative stress, Paraoxonase1.

INTRODUCTION

Diabetes mellitus type 2 is a disease of metabolic dysregulation involving the impaired uptake and utilization of glucose, altered lipid metabolism, accumulation of various lipid species in circulation and in tissues, and disruption of the metabolic signaling pathways that regulate insulin secretion (Randle, 1998). It causes oxidative stress due to enhanced production of mitochondrial reactive oxygen species (ros) (Brownlee, 2001), nonenzymatic glycation of proteins (brownlee, 2000) and glucose autoxidation (Wolff *et al.*, 1991). Increased levels of free fatty acids (FFAs) can contribute to oxidative stress by promoting mitochondrial uncoupling and β oxidation (Evans *et al.*, 2002). Oxidative stress induced by hyperglycemia and FFAs leads to the activation of stress-sensitive signaling pathways which worsen both insulin secretion and action and promote the development of overt type 2 diabetes mellitus (Evans *et al.*, 2002). In addition, oxidative stress may be a cause for evaluation of complications in diabetes mellitus (Tabak *et al.*, 2011).

Ischemic heart disease, Coronary artery disease, is a condition in which fatty deposits (Atheroma) accumulate in the cells lining the wall of the coronary arteries. These fatty deposits build up gradually and irregularly in the large branches of the two main coronary arteries which encircle the heart and are the main source of its blood supply leading to narrowing or

hardening of the blood vessels supplying blood to the heart muscle (Maton *et al.*, 1993). Narrowing of these arteries eventually block the flow of blood which reduces the amount of oxygen supplied to the heart muscle. The reduction in blood flow may result in a number of symptoms, which can vary in intensity among individuals (Maton *et al.*, 1993). Part of these leads to myocardial infarction which accounts for complete occlusion of the blood vessels (Who, 1980).

Elevated concentrations of ldl in the arterial wall are considered to be the initiator of atherosclerotic plaque formation (Lusis, 2006). Atherosclerosis is an abnormal response of some areas of vascular wall to the cumulative and often collective effects of episodic injury repair processes, causing permanent arterial damage (Lusis, 2000). Artery wall cells can produce oxidative species via multiple pathways which seed LDL trapped in the subendothelial space and

Initiate lipid oxidation (Witztum, 1994), the biological properties of oxidized LDL in vitro appear to be dependent on the degree to which the lipid and protein components are oxidized. Enzymes associated with hdl particles, including paraoxonase in its major isoform pon1, platelet activating factor acetylhydrolase (PAF-AH) and lecithin cholesterol acyl-transferase (LCAT) can cleave oxidized lipids and thereby inhibit ldl oxidation (Navab *et al.*, 2004). Apoai, the major hdl apolipoprotein, can remove oxidized lipids from LDL.

The physiological role of PON1

PON1 is an esterase and lactonase synthesized by the liver and found in the circulation associated with HDL. It is a glycoprotein of 354 amino acids with a molecular mass of 43 kDa (Primo-parmo *et al.*, 1996). It is a calcium dependant, containing APOA1 and APO J (Blatter *et al.*, 1993). It has been reported to confer antioxidant properties by decreasing the accumulation of lipid peroxidation products (Sozmen *et al.*, 2001) and hydrolyzing the organophosphate substrate paraoxon (the toxic metabolite of the insecticide parathion); it could hydrolyze pro inflammatory oxidized lipids which are present in LDL and ruins their potentially atherogenic characteristics (Watson *et al.*, 1995). In addition it can hydrolyze homocysteine thiolactones (Jakubowski, 2000). Because homocysteine thiolactones may lead to endothelial dysfunction and vascular damage, the detoxification of the homocysteine thiolactone by pon1 may play a role in cardioprotection (Jakubowski, 2005). Increased invivo glycation of pon1 may be responsible for the derangement of membrane hydroperoxide metabolism found in HDL from people with type 2 diabetes and those with CHD. Ayub *et al.*, (1999) suggested that pon activity shown to be inversely related to the risk of coronary heart diseases, hypercholesterolemia and diabetes.

Apolipoprotein A-I

Apoa1 is a 29 kDa protein comprising 243 amino acids. It is produced in the liver and intestine and secreted as the predominant constituent of nascent HDL particle (Navab *et al.*, 2006). Defects in the gene encoding it are associated with hdl deficiencies (Florvall *et al.*, 2006). It has a specific role in lipid metabolism; promotes cholesterol efflux from tissues to the liver for excretion, defines the size and shape of HDL, solubilizes its lipid components and helps to clear cholesterol from arteries (Dastani *et al.*, 2006). It is a cofactor for lcat which is the best predictor for ihd mortality. The aim of this work is to demonstrate PON1 activity in different diseases associated with dyslipidemia such as NIDDM and IHD.

MATERIALS AND METHODS

This study was carried out on 20 Patients from Diabetes Clinic, suffering from None Insulin Dependent Diabetes Mellitus for at least 5 years (NIDDM group), 20 patients admitted to the Cardiac Care Unit, suffering from Ischemic Heart Disease (IHD group), in addition to 20 healthy subjects from the outpatient clinic free from any disease as control group. All groups were taken from Suez Canal University Hospital. All patients were diagnosed by the faculty members of the mentioned departments, their age ranged from 40 to 60 years old.

All patients and healthy subjects were subjected to full history taking including age, sex, height, weight and biochemical investigations. Five ml of fasting blood samples were collected from each of them. Sera were separated and stored at -20°C till the time of their

measurements for the following biochemical parameters: fasting blood sugar according to the method described by Burrin, and Price (1985); lipid profile including cholesterol (Allain *et al.*, 1974), triglycerides (Fossati and Prencipe, 1982), HDL (Burstein *et al.*, 1970) and LDL (Rifai *et al.*, 2001). Liver function tests were also carried on the same samples including ALT and AST (Bergmeyer *et al.*, 1986), albumin (Doumas and Peters 1971), total and direct bilirubin (Balistreri and Shaw, 1987) in addition PON1 activity (La Du and Eckerson, 1984) and ApoA1 (Rifai, 1986).

Statistical Analysis

The results were expressed as mean ± standard error (SE). A p<0.05 was considered to be significant. Statistical analysis was performed by using the SPSS computer program. One-way analysis of variance (ANOVA) was used to compare the mean values, followed by multiple comparison post hoc tests. Pearson’s correlation was applied to correlate between the parameters.

RESULTS

The demographic data of each studied group including weight, height and BMI are shown in table (1).

Table (1): Demographic Data of All Studied Group

Variable		NIDDM	IHD	Control
Gender	F	9	11	8
	M	11	9	12
Weight(kg)	F	81.556±2.625	79.091±2.544	71.625±1.546
	M	79.273±0.954	80.333±2.147	77.25±0.856
	T	80.3 ±1.274	79.65 ±1.658	75±1.21
Height(m)	F	1.639 ±0.016	1.646 ± 0.014	1.655±0.008
	M	1.68 ±0.01	1.682 ± 0.01	1.678±0.012
	T	1.662 ±0.008	1.662±0.009	1.669±0.01
BMI(kg/m2)	F	30.359±0.922#	29.175±0.761	26.273±0.566
	M	28.114±0.46	28.386±0.562	27.426±0.316
	T	29.124±0.537*	28.818±0.483*	26.97±0.33

All values are in mean ± S.E,* p<0.05 is statistically significant against control

#p<0.05 is statistically significant comparing F against M in the same group

F, female; M, male; T, total; m; meter, kg; kilogram, BMI; Body mass index

NIDDM; Non insulin dependent diabetes mellitus, IHD; Ischemic heart disease

The results shown in Table (1) demonstrate the mean± SE of BMI of each group in the study. BMI of NIDDM group was found significantly increased when compared to those of the control group. These findings came in agreement with the results obtained by Nobecourt *et al.* (2005), Mastorikou *et al.* (2008) and Tabak *et al.* (2011). Arafa and Amin (2010) reported that, BMI plays an important role in increasing the rate of the prevalence of Diabetes among both males and females while

Kouchak *et al.* (2011) proposed that BMI (kg/m²) of diabetes group had approximately same BMI compared to the controls.

BMI of IHD group was found significantly increased compared to the control group. These results came in agreement with the result obtained by Frikke - Schmidt *et al.* (2008) and Jayakumari and Thejaseebai (2009) while the mean \pm S.E of male and female BMI of each group showed that female BMI of NIDDM group is increased significantly when compared to those of male BMI of the same group while there was no significant difference between female and male of IHD group.

Biochemical and Clinical Data of the Studied Groups

Table (2) demonstrates the biochemical characteristics of each group in the present study. NIDDM group showed high levels of FBS with high levels of lipid profile and normal liver function tests compared to the control group. IHD patients demonstrated high levels of lipid profile with normal levels of FBS and liver function tests compared to the control group

Table (3) demonstrates lipid profile, PON1 activity and ApoA1 value of the studied groups. Cholesterol, TG, LDL were found significantly higher compared to the control group in both NIDDM and IHD patients while HDL was found significantly lower compared to the control group in the two patients groups. In addition PON1 activity and ApoA1 value were found

significantly decreased compared to the control group. It is clear from lipid profile results that both of diabetes and IHD patients were dyslipidemic. Results of NIDDM patients in this study came in agreement with results obtained from Fawzy *et al.* (2002), Nobecourt *et al.* (2005) and Rasic-Milutinovic *et al.* (2011) while Mastorikou *et al.* (2008) reported that HDL was non-significantly decreased and cholesterol was non-significantly increased when compared to their respective controls. Maritim *et al.* (2003) reported that dyslipidaemia is a prominent feature of type2 diabetes. Eckardstein and Sibling (2011) proposed that LDL inhibits glucose- stimulated insulin secretion and proliferation of β -cells by LDL-receptor dependent and independent mechanisms, respectively.

In addition, ApoA-I and HDL stimulate insulin secretion and also inhibit apoptosis of β -cells. So, LDL, HDL and cholesterol regulate the function and survival of β -cells. They also reported that HDL exerts anti-obesity and insulin-sensitizing effects. Thus dyslipidaemias may not only be consequences but also contributors to the pathogenesis and hence targets for prevention of T2DM.

Our results also came in agreement with those obtained by Sheikh-Ali *et al.* (2011) who reported that Diabetes leads to depletion of the cellular antioxidant defense system and is associated with an increase in the production of free radicals.

Table (2): Biochemical and Clinical Data of Each Studied Groups.

	DM		Lipid Profile		
	FBS,mmol/L	Chol,mmol/L	TG,mmol/L	HDL,mmol/L	LDL,mmol/L
Control	5.18 \pm 0.08	4.19 \pm 0.07	1.038 \pm 0.03	1.49 \pm 0.046	2.219 \pm 0.094
NIDDM	10.953 \pm 0.6	5.749 \pm 0.136	2.37 \pm 0.075	0.943 \pm 0.02	3.788 \pm 0.129
IHD	5.022 \pm 0.105	6.12 \pm 0.193	2.283 \pm 0.117	0.695 \pm 0.042	4.37 \pm 0.164
	Liver Function Tests				
	ALT, U/L	AST, U/L	Alb,g/L	D.B, μ mol/L	T.B, μ mol/L
Control	16.75 \pm 0.833	13.9 \pm 0.784	42.41 \pm 1.077	0.76 \pm 0.104	10.82 \pm 0.612
NIDDM	19.35 \pm 1.592	15.05 \pm 1.553	38.57 \pm 0.742	1.314 \pm 0.192	12. \pm 0.78
IHD	21.65 \pm 1.726	48.55 \pm 7.635	35.205 \pm 0.556	1.573 \pm 0.235	11.62 \pm 1.016

FBS, fasting blood sugar; chol, cholesterol ; TG, triglycerides; HDL, high density lipoprotein; LDL, low density lipoprotein; ALT, alanine amino transferase; AST, aspartate aminotransferase; PON1, paraoxonase 1; ApoA1, apolipoprotein A1; NIDDM, non insulin dependent diabetes mellitus; IHD, ischemic heart disease

Table (3): Lipid profile, PON1 Activity and ApoA1 in the Studied Groups.

	Control	NIDDM	IHD
Chol, mmol/L	4.19 \pm 0.07	5.749 \pm 0.136 *	6.12 \pm 0.193*
TG, mmol/L	1.038 \pm 0.03	2.37 \pm 0.075*	2.283 \pm 0.117*
HDL, mmol/L	1.49 \pm 0.046	0.943 \pm 0.02*	0.695 \pm 0.042*
LDL, mmol/L	2.219 \pm 0.094	3.788 \pm 0.129*	4.37 \pm 0.164*
PON1, U/L	48.979 \pm 2.2	20.4 \pm 1.493*	12.05 \pm 0.59*
ApoA1, g/L	1.83 \pm 1.71	1.06 \pm 0.05 *	0.835 \pm 0.032

Chol, cholesterol; TG, triglycerides; HDL, high density lipoprotein; LDL, low density lipoprotein PON1, paraoxonase1; ApoA1, apolipoprotein A1; NIDDM, non insulin dependent diabetes mellitus IHD, ischemic heart disease; *Significant (p< 0.05).

Oxidative stress can be the result of multiple pathways. Some of these are related to substrate-driven overproduction of mitochondrial reactive oxygen species, advanced glycation end product formation, glucose autoxidation, and depletion of micronutrients and cellular elements with antioxidative properties which came in agreement with our study where PON1 activity and ApoA1 value were found significantly decreased in diabetic patients compared to the control group. These findings came also in agreement with the results obtained by Gupta *et al.* (2011), Rasic-Milutinovic *et al.* (2011) and Patrick *et al.* (2012). The results of IHD group obtained in the present study came in agreement with the results obtained from Gocmen *et al.* (2004), Mastorikou *et al.* (2008) and Jayakumari and Thejaseebai (2009) who reported that patients with CAD had significantly lower HDL, ApoA-I and PON1 activity and higher LDL compared to their respective controls. Mackness *et al.* (2000) and Mackness *et al.* (2001) reported that the reduction in the PON1 activity might be due to decreased synthesis or inactivation under oxidative stress.

Oxidants are products of normal metabolism and inflammatory response, inactivation and removal of these ROS depend on reaction involving antioxidant defense systems, which constitute a diverse group of compounds with different properties. In this study, patients with CAD were found to have inadequate antioxidant status due to depletion of PON1 activity. The lower activity of PON1 depresses the ability of circulating HDL particle in protection of LDL from oxidation, participates in reverse cholesterol transport and inhibits monocyte-endothelial cell interaction which confirms the results obtained in this study. (Duell *et al.*, 1991 and Cavallero *et al.*, 1995)

Results in Table (4) demonstrates a negative correlation between PON1 activity and TG levels which give a good support to these obtained by Mastorikou *et al.* (2008) and Patrick *et al.* (2012) and demonstrated positive correlation between PON1 and each of HDL and ApoA1 which give a good support to these obtained by Navab *et al.* (2004) and Mackness and Mackness (2004). In diabetic cells, the amount of glucose oxidized in the Krebs tricarboxylic acid cycle is increased; leading to an increase in generation of ROS. The generated ROS increasing oxidative stress which plays a key role in the pathogenesis and progression of diabetes and diabetic complications (Kaneto *et al.*, 2005; and Pamplona and Barja, 2007). To protect molecules from ROS and free radicals, cells have developed an antioxidant defense system including SOD, CAT, and GSH-P (Haron, 1991). Navab *et al.* (2004) reported that several protein components of HDL particles possess anti-oxidative properties, these include PON1 and ApoAI. Mackness and Mackness (2004) reported that PON1 is almost entirely associated with HDL and is

thought to contribute to their anti oxidative, anti-atherogenic properties which came in consistence with the results shown in Table (4).

PON1 is tightly bound with the hydrophobic N-terminal domain to ApoA1 of HDL and circulates as part of its components in the blood of humans and other vertebrates (Mackness and Durrington, 1995 and Watson *et al.*, 1995) which may be confirmed by presence of a positive correlation between ApoA1 and PON1. ApoA1 demonstrated negative correlation with each of FBS, Chol and TG and demonstrated a positive correlation with HDL.

Table (4): Pearson's Correlation Coefficients of Serum PON1 Activity and ApoA1 with Clinical Variables in NIDDM Group

	PON1Activity	ApoA1
Chol	- 0.341	- 0.453*
TG	-0.724*	- 0.735*
HDL	0.721*	0.695*
LDL	- 0.335	-0.431
PON1	1	0.938*
ApoA1	0.938*	1
FBS	- 0.416	- 0.453*

Alchol, cholesterol; TG, triglycerides; HDL, high density lipoprotein; PON, paraoxonase1, ApoA1, apolipoprotein A 1; FBS, fasting blood sugar; * p<0.05

Table (5) shows a direct relationship between PON1 and HDL but this was not significant ($r=0.339$), which can be explained partly by the fact that certain HDL characteristics such as particle size, surface phospholipids, and apo proteins are most important to hold and stabilize PON1 in HDL particle. There was a negative correlation between PON1 and LDL which came in agreement with the results obtained by Rozek *et al.* (2005) who reported that PON1 activity in patients with severe carotid artery disease was strongly correlated with LDL, increasing LDL levels; may be due to inactivation of PON1 activity with increasing oxidative stress.

Table (5): Pearson's correlation coefficients of serum PON1 activity and ApoA1 with clinical variables in IHD group.

	PON1	ApoA1
Chol	- 0.281	- 0.200
TG	- 0.384	- 0.237
HDL	0.339	0.461*
LDL	- 0.455*	- 0.279
PON1	1	0.742
ApoA1	0.742*	1

Chol, cholesterol; TG, triglycerides; HDL, high density lipoprotein, PON, paraoxonase1; ApoA1, apolipoprotein; FBS, fasting blood sugar; *p<0.05

Azarsiz *et al.* (2003) and Granér *et al.* (2006) reported that PON1 activity was significantly lower in CAD group compared with their controls and correlated with ApoA1 ($p < 0.001$). La Du and Novais (1989) showed that it was extremely difficult to remove ApoA1 from PON1 during purification from human serum, which has led to the suggestion that ApoA1 and PON1 are closely associated. Our results were in consistence with these findings and were in line with population studies that have shown a statistical association of PON1 activity with HDL and with ApoA1 (Abbott *et al.*, 1995) except for PON1 and HDL in our study as reported before which was decreased by decreasing PON1 but not significant. Elevated plasma levels of ApoA1 and HDL are important protective factors for atherosclerosis and coronary heart disease (Livshits *et al.*, 1998). Systemic and atherosclerotic plaque inflammation markers were significantly reduced by the Recombinant ApoA1 administration (rApoA1); rApoA1 administration enhances reverse cholesterol transport and induces a clearance from the overall inflammatory status associated with atherosclerotic disease (Cimmino *et al.*, 2009).

Several explanations for the decreased activity of serum PON1 were suggested; the first is; glycation of ApoA1 or PON1 itself may alter the function of PON1 on HDL and reduce its activity, the second is; derived certain cytokines from vascular wall may have secondary effect on the function of circulating PON1 (Ikeda *et al.*, 1998), the third is; there may be some inhibitors against the enzyme activity of PON1 in circulating blood of diabetic patients such as glycosylated proteins (Inoue *et al.*, 2000) and the fourth one is; modification of lipoprotein composition or conformation due to the impairment of lipoprotein metabolism. PON1 bound to modify HDL may be disturbed to interact with substrates (Bucala *et al.*, 1994).

CONCLUSION AND RECOMMENDATION

PON1 activity and ApoA1 values were significantly decreased in NIDDM and IHD patients. Dyslipidemia was associated with these patients. A positive correlation was demonstrated between PON1 and ApoA1 in the two groups. Consequently, PON1 might be useful in following up the patients with these diseases and can be used as a good parameter in evaluating the severity of these diseases. However, repeating the study with large populations may be recommended, in addition, study the genetic polymorphism distribution should be considered to achieve a compact overview that can explain the variability in PON1 activity and its relationship with the other factors that associate the disease and its complications.

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