Paraoxonase-1 activity in type 2 diabetes mellitus with and without nephropathy

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Background
Paraoxonase-1 (PON-1) is an enzyme synthesized in the liver that has antioxidant functions as it binds to the HDL particles and prevents the oxidation of LDL, which possibly plays a role in the prevention of atherosclerosis and coronary artery disease.

Objectives
To determine PON-1 activity in type 2 diabetic patients with and without diabetic nephropathy and its correlation with the lipid profile, disease duration, and glycemic status.

Patients and methods
This study was carried out on 30 patients with type 2 diabetes mellitus who attended the diabetes and endocrine clinic at Kasr Al Ainy Hospital, Cairo University, including 20 patients with evidence of diabetic nephropathy and 10 patients without diabetic nephropathy as well as 15 healthy age-matched control participants. Fasting blood sugar, 2 h postprandial blood sugar, total cholesterol, HDL, LDL, triglycerides, and serum creatinine were measured. PON-1 activity was detected using a colorimetric method.

Results
PON-1 activity was reduced significantly in diabetic patients with and without nephropathy, with mean 226.1 ± 135.4 and 221.7 ± 119.6 nmol/ml/min, respectively, versus 758.5 ± 353.9 nmol/ml/min in the control group ($P < 0.001$). PON-1 activity was not significantly different between diabetic patients with and without nephropathy. PON-1 activity was correlated negatively with HDL ($r = -0.496$, $P = 0.026$) in diabetic patients with nephropathy. PON-1 activity was not correlated significantly with disease duration and glycemic status.

Conclusion
PON-1 activity was significantly reduced in type 2 diabetes, but did not differ between diabetics with or without nephropathy.

Keywords:
diabetic nephropathy, oxidative stress, paraoxonase-1, type 2 diabetes

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Introduction

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia because of defects in insulin secretion, insulin action, or both [1]. Chronic hyperglycemia can induce reactive oxygen species and increase the oxidative stress, which may be a result of glycation and glucoxidation. Increased oxidative stress induces lipid oxidation and formation of lipid peroxides, which have been implicated in the pathogenesis of diabetic complications [2].

Diabetic nephropathy is one of the most leading causes of end-stage renal disease in Egypt after hypertensive nephropathy [3]. The pathogenesis of diabetic nephropathy is still unclear and complex. Previous studies have documented the important role of oxidative stress in the development of diabetic nephropathy [4,5].

Human serum paraoxonase-1 (PON-1) is an enzyme synthesized in the liver and released in blood binding to HDL [6]. It is an antioxidant enzyme that hydrolyzes the toxic peroxides in the oxidized lipids in both LDL and HDL; thus, it has been implicated in the prevention of atherosclerosis and cardiovascular diseases [7]. Serum PON-1 activity and concentrations vary widely in the population, PON-1 can vary by more than 40-fold, and the PON-1 protein levels by more than 13-fold in a single PON-1 genotype [8]. Decreased PON-1 activity, independent of genotype, has been documented in diabetes, hypercholesterolemia, and renal failure [9]. It was noted that patients with low PON-1 activity are more vulnerable to diseases involving increased oxidative damage and lipid peroxidation compared with patients with high PON-1 activity [10].

Diabetic complications are a major cause of morbidity and early mortality; therefore, early detection and treatment of lipoprotein-related disorders may facilitate early recognition and treatment of diabetic patients [11].

This study was carried out to determine the PON-1 activity in type 2 diabetic patients with and without nephropathy and its correlation with the lipid profile, disease duration, and glycemic status.

Patients and methods

Participants

This study was carried out on 30 patients with type 2 diabetes mellitus who had attended the diabetes and endocrine clinic at Kasr Al Ainy Hospital, Cairo University, and 15 healthy age-matched control participants. The study was carried out from January 2014 to June 2014. Diabetes and diabetic complications were defined according to WHO 1999 [12]. All patients had been diagnosed with diabetes mellitus for at least 5 years. Diabetic patients were subdivided into 20 with diabetic nephropathy and 10 with no evidence of nephropathy. All patients were subjected to an assessment of history, clinical examination, retinal examination, ECG, urine analysis, serum creatinine, fasting blood sugar, 2 h postprandial blood glucose, total cholesterol (TC), triglycerides (TAG), LDL-cholesterol (LDL-C), HDL-cholesterol (HDL-C), and determination of estimated glomerular filtration rate. Determination of serum PON-1 activity was performed using the calorimetric method.

Exclusion criteria

Patients receiving renal replacement therapy, kidney transplant recipients as well as those with liver disease, congestive heart failure, and cerebrovascular disorders were excluded.

Ethical aspects

Research protocols were approved by the medical ethics committee of Kasr Al Ainy Medical School, Cairo University. All participants provided a written informed consent after the research protocols were carefully explained to them. Informed consent was obtained from all the study participants and their approval was obtained by a signature.

Methods

All laboratory investigations were performed on a Roche Diagnostics Hitachi 917 automated analyzer. LDL-C was calculated using the method of Friedewald *et al.* [13]. Urinary albumin excretion was calculated as 24 h urine collection. Levels of albuminuria lower than 30 mg/24 h were reported to be negative, levels of 30–300 mg/24 h were reported as microalbuminuria, and levels above 300 mg/24 h were reported as macroalbuminuria. Estimated glomerular filtration rate was determined on the basis of the MDRD 4-variable equation [14].

Paraoxoase activity in enzymatic assay was assessed on the basis of the method described by Sampson *et al.* [15]. As recommended by Abbott *et al.* [16] before the analysis of PON-1 activity, serum from controls and diabetics was preincubated with 5 μmol/l serine for 10 min at room temperature to inhibit serum butyrylcholinesterease activity, which is elevated in diabetes and would otherwise interfere with the determination of PON-1 activity in the serum from individuals with diabetes.

Paraoxon (*O,O*-diethyl *P*-nitrophenyl phosphate; 1.0 mmol/l; Sigma chemical Co., St Louis, Missouri, USA) was used as a substrate, and enzyme activity was measured at 37°C in 50 mmol/l Tris/HCL buffer (pH 6.8) containing 1.0 mmol/l $CaCl_2$. The rate of *P*-nitrophenol generation was determined on a spectrophotometer RA50 (Bayer, Pittsburgh, Pennsylvania, USA) by recording the increase in A_{405} at 37°C.

Statistical analysis

Data were analyzed using the statistical package of social science software program (SPSS Inc., Chicago, Illinois, USA), version 21.

Data were summarized as range, mean, SD, and median for quantitative variables or frequency and percentage for qualitative variables.

Comparison between groups was performed using one-way analysis of variance (if parametric) or Mann–Whitney and Kruskal–Wallis tests (if nonparametric) for quantitative variables and χ^2 with Fisher's exact test for qualitative variables.

Spearman correlation coefficients were calculated to signify the association between different parametric quantitative variables and Pearson's test for nonparametric variables.

Receiver operating characteristics curve analyses were carried out to explore the discriminant ability of PON-1 activity to differentiate patients with diabetes mellitus.

P values less than 0.05 were considered statistically significant.

Results

The demographic and laboratory data of the studied groups are shown in Table 1. Using the analysis of variance test, paroxonase-1 activity was significantly lower in both

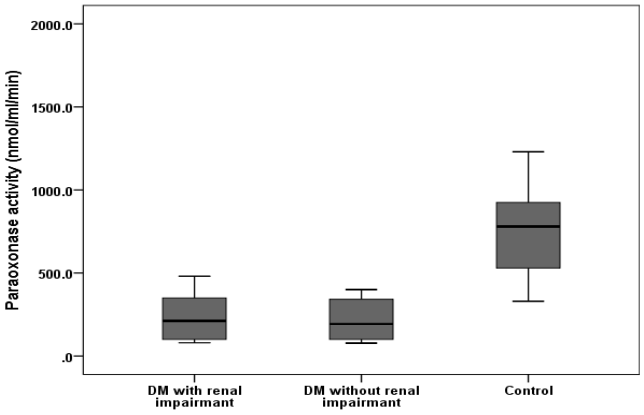
diabetic patients groups compared with nondiabetic controls ($P < 0.001$) (Table 1 and Fig. 1). There was no significant difference between paroxonase activity levels among those with and without nephropathy. TC and TAG were elevated significantly in the diabetic nephropathy group compared with the other groups ($P < 0.001$) but HDL-C was not significantly different between the three groups ($P = 0.4$) (Table 1). Paroxonase activity was not correlated to any of the parameters measured apart from the HDL levels in patients with diabetic nephropathy (Table 2). Table 3 and Figure 2 show the cut-off point level of PON-1 activity to discriminate between diabetic patients and nondiabetic controls.

Discussion

In our study, the mean PON-1 activity was significantly decreased in the diabetic patients with nephropathy and the diabetic patients without nephropathy compared with the control participants. We found that the cut-off point up to 500 nmol/ml/min was the level of PON-1 activity to discriminate the diabetic patients from the control group using receiver operating characteristic curve analysis. These results were in agreement with previous studies that reported low PON-1 activity in type 2 diabetes [16–18].

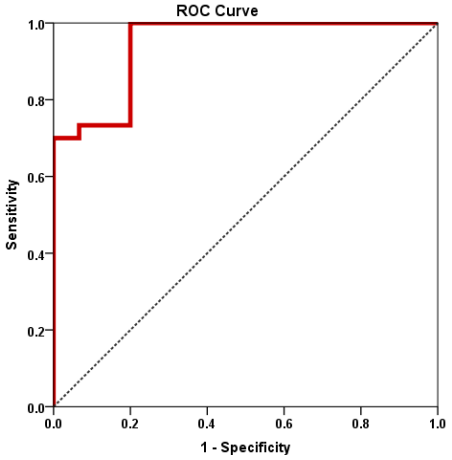
In contrast to these results, Valabhji *et al.* [19] did not report any difference in PON-1 activity in type 2 diabetes and controls and this may have been because of the technique used to detect PON-1 activity in that study, which used substrates such as phenyl acetate, which may not be the most appropriate substrate to assess PON-1 activity. Also, the study carried out by Kopporasch *et al.* [20] did not find a significant

Figure 1



Box plot showing the distribution of paraoxonase-1 activity among the studied groups.

Figure 2



ROC curve analysis to explore the ability of paraoxonase-1 activity to discriminate diabetic cases from controls. ROC, receiver operating characteristic.

Table 1 Demographic and laboratory data of the studied groups

ANOVA test	DM+ nephropathy ($n = 20$)	DM only ($n = 10$)	Control ($n = 15$)	P value
Sex [n (%)]				
Male	10 (50.0)	6 (60.0)	8 (53.3)	0.9
Female	10 (50.0)	4 (40.0)	7 (46.7)	
Age	50.4 ± 3.3 (A)	51.9 ± 2.7 (A)	49.3 ± 3.8 (A)	0.2
Duration of diabetes (years)	9.6 ± 4.0 (A)	10.0 ± 4.7 (A)		0.8
FBS (mg/dl)	158.4 ± 45 (A)	156.6 ± 54 (A)	100.8 ± 5.4 (B)	<0.001*
2 h PPBG (mg/dl)	315 ± 61.2 (A)	302.4 ± 64.8 (A)	135 ± 5.4 (B)	<0.001*
Creatinine (mg/dl)	1.6 ± 0.4 (A)	1.0 ± 0.2 (B)	0.9 ± 0.1 (B)	<0.001*
Total cholesterol (mg/dl)	202.8 ± 50.0 (A)	179.0 ± 36.1 (AB)	143.3 ± 19.7 (B)	<0.001*
TAG (mg/dl)	136.9 ± 59.2 (A)	87.8 ± 20.4 (B)	72.7 ± 13.6 (B)	<0.001*
HDL-C (mg/dl)	46.2 ± 2.1 (A)	45.2 ± 2.4 (A)	45.0 ± 3.7 (A)	0.4
LDL-C (mg/dl)	150.8 ± 46.6 (A)	133.8 ± 35.1 (A)	98.5 ± 19.7 (B)	0.001*
Paraoxonase activity (nmol/ml/min)	226.1 ± 135.4 (A)	221.7 ± 119.6 (A)	758.5 ± 353.9 (B)	<0.001*
eGFR (ml/min/1.73 m^2)	43.9 ± 11.4 (A)	80.6 ± 13.0 (B)		<0.001*

Groups with different letter labels are significantly different at a P value of 0.05. Values are expressed as mean ± SD; ANOVA, analysis of variance; DM, diabetes mellitus; EGFR, estimated glomerular filtration rate; FBS, fasting blood sugar; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; PPBG, postprandial blood glucose; TAG, triglycerides; *$P < 0.05$ is significant.

difference in PON-1 activity in impaired glucose-tolerant patients and early diabetes compared with the controls, and they suggested that the decrease in PON-1 activity may occur later in the course of diabetes mellitus [20].

There may be several explanations for the decreased PON-1 activity in type 2 diabetes. This may have been because of the conformational changes of the enzyme as a result of glucoxidation or lipoxidation [21]. Ferretti *et al.* [22] found that in-vitro incubation of HDL with glucose decreased the activity of HDL-associated paraoxanase; thus, glycated HDL leads to physiochemical modification in the properties of HDL. Also, Hedrick *et al.* [23] found that in-vitro glycation of purified paraoxanase protein might cause a 40% reduction in its enzymatic activity and Deakin *et al.* [24] found that the decrease in the size of HDL particles and the accumulation of unesterified cholesterol in the HDL particles that occur in diabetes may affect the ability of HDL to release PON-1 from cells and may also affect its stabilization. These decreases in PON-1 activity prevent the antioxidant function of HDL and accelerate atherogenesis and related complications [9].

Table 2 Correlation of PON-1 activity with different parameters within each group

	Paraoxonase activity (nmol/ml/min)		
	DM+ nephropathy (n = 20)	DM only (n = 10)	Control (n = 15)
Age			
r	0.140	−0.050	−0.344
P	0.555	0.890	0.209
Duration of diabetes (years)			
r	−0.274	0.268	NA
P	0.243	0.454	
FBS (mg/dl)			
r	0.229	−0.626	0.043
P	0.332	0.053	0.878
2 h PPBG (mg/dl)			
r	0.325	−0.618	−0.095
P	0.162	0.057	0.738
Creatinine (mg/dl)			
r	−0.295	0.595	−0.086
P	0.206	0.070	0.760
Total cholesterol (mg/dl)			
r	−0.317	−0.614	0.485
P	0.173	0.059	0.067
TAGs (mg/dl)			
r	−0.443	−0.388	0.372
P	0.051	0.268	0.172
HDL-C (mg/dl)			
r	**−0.496**	−0.525	0.417
P	**0.026***	0.119	0.122
LDL-C (mg/dl)			
r	−0.148	−0.608	0.343
P	0.534	0.062	0.211
eGFR ml/min/1.73 m²			
r	0.330	−0.463	
P	0.156	0.177	

DM, diabetes mellitus; EGFR, estimated glomerular filtration rate; FBS, fasting blood sugar; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; NA, not applicable; PON-1, paraoxonase-1; PPBG, postprandial blood glucose; *r*, correlation coefficient; TAG, triglycerides; **P* < 0.05 value is significant.

In our study, there was no significant difference in PON-1 activity between diabetics with or without nephropathy. This was in agreement with Ikeda *et al.* [25], who did not report differences in PON-1 activity in type 2 diabetes with and without nephropathy, although there was a significant difference between patients with and without retinopathy. This was in contrast to the previous two studies [26,27], which found a significant decrease in diabetic patients with nephropathy compared with diabetics without nephropathy. In the study by Khodeir and colleagues, the patients with diabetic nephropathy were older and had a longer duration of diabetes compared with diabetic patients without nephropathy. In the other study, the duration of diabetes was not reported and this may have been a drawback that could have affected the results of that study. In our study, the mean duration of diabetes in patients with and without nephropathy was longer, 9.7 ± 4.2 years, and was not significantly different between both groups, which may explain the similarity in PON-1 activity among patients in the two groups.

Seres *et al.* [10] reported a progressive decrease in PON-1 activity in elderly patients and this decrease was related to the occurrence of oxidative stress that develops with aging. In our study, there was no significant difference in PON-1 activity in terms of age; this may be related to our selected participants, who were in the middle age group 50 ± 3 years, and this was in agreement with Khodeir *et al.* [26].

In our study, we found a negative correlation between duration of diabetes in patients with nephropathy and PON-1 activity, but this was not significant. Also, we found a nonsignificant negative correlation between fasting blood sugar and 2 h postprandial blood glucose and PON-1 activity in

Table 3 ROC curve analysis

Tested variable	AUC	95% CI	*P* value	Cut-off point	Sensitivity (%)	Specificity (%)
Paraoxonase-1 activity	0.944	0.878–1.000	<0.001	≤500	100.0	80.0

AUC, area under the curve; CI, confidence interval; ROC, receiver operating characteristic.

diabetic patients without nephropathy, and this was in agreement with Elattar *et al.* [28]. In contrast to these results, Kordonouri *et al.* [29] reported a negative correlation between blood glucose levels and PON-1 activity.

In our study, there were no significant correlations between TC, TAG, LDL-C, and PON-1 activity in any of the groups and this was in agreement with Elattar *et al.* [28], who also did not find any significant correlations in the lipid profile and PON-1 activity in both the control and the diabetic group.

However, we found a negative correlation between HDL and PON-1 activity in the diabetic patients with nephropathy. The relationship between PON-1 and lipids has shown conflicting results in different studies. Some studies have shown a positive correlation [30–32], whereas others have found no correlation [16]. This may be explained by the association of PON-1 with HDL particles [33] and the consequent impact of HDL levels [24] and drug intake, for example statins on the levels of PON-1 [34]. Moreover, the PON-1 genotype is a major determinant of serum lipids and lipoprotein concentrations [35]. Nonetheless, Mackness *et al.* [36] have suggested that PON-1 is associated exclusively with a discrete subpopulation of HDL particles, namely, apo-A1 and clusterin, which explains the poor correlation between PON-1 and HDL found in the population studies.

Conclusion

Our study supports previous observations that type 2 diabetes mellitus patients have significantly decreased PON-1 activity, which did not differ between diabetic patients with and without nephropathy.

Acknowledgements

Conflicts of interest

There are no conflicts of interest.

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