PARAOXONASE 1 ACTIVITY AS A PREDICTOR OF CARDIOVASCULAR DISEASE IN TYPE 2 DIABETES

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Abstract. The role of paraoxonase 1 in cardiovascular disease complications in type 2 diabetes mellitus is not fully understood. We studied paraoxonase activity towards paraoxon in 188 non-diabetic and 140 diabetic subjects using general linear models and univariate analysis. Adjusting for age revealed a reduction in activity towards paraoxon was associated with a significant increase in risk (p=0.023) for cardiovascular disease complications in diabetic patients. Multivariate analysis of two plasma measures of paraoxonase activity using paraoxon and diazoxon also showed reduced paraoxonase activity towards paraoxon was associated with a significant increase in risk (p=0.045) for cardiovascular disease complications in diabetic patients. These analyses showed that a reduced paraoxonase activity towards paraoxon was associated with ethnicity. Based on multivariate analysis, subjects of Malay ethnic origin have significantly higher than expected activity (p=0.008, compared to Indians), towards paraoxon than subjects of Chinese origin who in turn had higher than expected paraoxonase activity (p=0.028, compared to Indians) Indian subjects.

Key words: paraoxonase 1, cardiovascular disease, type 2 diabetes

INTRODUCTION

Paraoxonase 1 (PON1) is associated with the development of atherosclerosis (Mackness *et al*, 2010). The hydrolytic active site of PON1 has been shown to mediate two major anti-atherogenic functions: protection of low-density lipoprotein (LDL) from oxidation and stimulation of high-density lipoprotein (HDL)-mediated macrophage cholesterol efflux (Rosenblat *et al*, 2006). There is also increasing evidence that highlights the association between PON1 and diabetes mellitus (DM) (Hofer *et al*, 2006). PON1 is physically associated with HDL. Individuals with type 2 DM may present with low HDL levels (Sarkar *et al*, 2006) or may present with levels comparable with healthy subjects (van Wijk *et al*, 2006; Saeed *et al*, 2007). However, the HDL apoproteins may be highly glycated in diabetics compared to those in normoglycemic individuals (Norata *et al*, 2006). Glycation of HDL appears to inhibit PON1 activity, implying that insulin resistance is associated with impaired PON1 activity (Hedrick *et al*, 2000). When PON1 becomes impaired, its ability to impede LDL oxidation is reduced, leading to accelerated atherosclerosis. Hyperglycemia also induces oxidative stress via several mechanisms resulting in

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increased reactive oxygen species (ROS) generation (Jay *et al*, 2006). Although endogenous ROS help to maintain homeostasis, their excess over a prolonged period of time may cause oxidative stress leading to proatherogenic events.

Several studies have shown lower levels of PON1 activity in diabetics compared to healthy subjects (Mastorikou *et al*, 2006). A post-prandial reduction in HDL-cholesterol (HDL-C) and PON1 activity was observed in type 2 diabetics who were given fresh cream (van Wijk *et al*, 2006). In healthy individuals the consumption of similar fat results in increased PON1 activity (Sutherland *et al*, 1999). These findings suggest decreased protection of lipoproteins against oxidation in diabetics. However, the mechanism leading to decreased post-prandial PON1 activity in diabetics is not fully known. A negative correlation has also been found between PON1 activity and oxidized LDL concentration in healthy subjects, but not in type 2 diabetics (Mastorikou *et al*, 2006). Poor glycemic control in diabetics has been correlated with high glycation and low PON1 activity, leading to a decreased ability to metabolize oxidized LDL.

The present cross-sectional study was carried out based on the hypothesis HDL-associated PON1 decreases accumulation of oxidized LDL *in vitro*, contributing to the attenuation of atherosclerosis in type 2 DM. This study evaluates PON1 activity as a parameter for prognosis of cardiovascular diseases (CVD) in type 2 diabetes.

MATERIALS AND METHODS

Subjects

A group of subjects comprising outpatients who attended the Diabetic Clinic of the University of Malaya Medical Centre, Kuala Lumpur, Malaysia and healthy volunteers residing in Selangor and Kuala Lumpur, were selected. The demographic characteristics of these subjects were recorded.

This study was approved by the Medical Ethics Committee and informed consent was obtained from each subject included in the study.

Blood sampling

Venous blood was obtained by a trained phlebotomist under sterile conditions from subjects. Blood was collected in appropriate vacutainers for glucose, lipid profile, and enzyme activity. The plasma for enzyme activity was collected after centrifugation of the blood for 5 minutes at 500*g* within three hours of sampling.

Determination of glucose and lipid profile

The measurements of glucose, total cholesterol, triglycerides and HDL-C were carried out using the Dimension® clinical chemistry system (Dade Behring, Deerfield, IL). LDL-C was calculated using the Friedewald formula. Analyses were carried out at the Clinical Diagnostic Laboratory, University of Malaya Medical Centre.

Determination of PON1 enzymatic activity

Paraoxonase (POase) and diazoxonase (DZOase) activities were analyzed spectrophotometrically. Paraoxon (1.2 M) (Sigma Chemical, St Louis, MO) and diazoxon (1.0 M) (Chem Service, West Chester, PA) were used as substrates, respectively, in a Tris buffer (0.1 M, pH 8.5) containing 2 M NaCl and 2 mM $CaCl_2$ (Richter *et al*, 2004). A blank determination of basal assay mixture without plasma was performed to observe the spontaneous hydrolysis of paraoxon and diazoxon solutions (Senti *et al*, 2001). One milliter of substrate solution was

placed in a cuvette. Upon addition of the neat plasma sample (10 μl for paraoxonase and 5 μl for diazoxonase) to the substrate solution, the reaction was monitored for 2 minutes at 25ºC in a UV-Vis spectrophotometer (Varian Cary 50, CA). Measurement of activity was performed using the "Kinetics" application. Paraoxonase and diazoxonase activities were expressed as 1 μmol of substrate hydrolyzed per minute per liter of plasma. The extinction coefficients for *p*-nitrophenol (hydrolysis product for paraoxon) and 2-Isopropyl-4-methyl-6-hydroxypyrimidine (for diazoxon) were 18 and 3 $mM^{-1}cm^{-1}$ at pH 8.5, respectively. A two-dimensional plot of initial rates of diazoxon hydrolysis on the y axis versus rates of paraoxon hydrolysis on the x axis was used for the simultaneous determination of paraoxonase and diazoxonase activity genotyping in addition to phenotyping.

Statistical analysis

Univariate analysis was performed for predictors of continuous variables, *ie* PON1 activity. Two models were constructed for the prediction of PON1 activity. The general linear model procedure was used to evaluate the factors and covariates that were significant predictors of PON1 activity in the study groups. The variables included in Model 1 were *PON1* polymorphisms $PON1_{192QR}$ and $PON1_{55LM}$, ethnicity, alcohol status, smoking status, age, systolic blood pressure (SBP), diastolic blood pressure (DBP), fasting blood glucose, glycated hemoglobin (HbA_{1c}), body mass index (BMI), HDL-C, LDL-C, total cholesterol (TC), and triglyceride (TG) levels based on studies by Rahmani *et al* (2002), Hofer *et al* (2006) and Parra *et al* (2006). Variations in these two *PON1* polymorphisms with the same samples have been reported previously (Poh *et al*, 2007). The variables in Model 2 were those that were used in Model 1 but excluded the two *PON1* polymorphisms. The final variables that showed significant values taken together with the means, pairwise comparisons and Bonferroni *post hoc* (categorical variables) and parameter estimates (continuous variables) were determined to be predictors of PON1 activity. A large regression coefficient, as denoted by B, means a particular risk factor strongly influenced the probability of the outcome in question. A near-zero regression coefficient means the risk factor had little influence on the probability of that outcome.

A multivariate analysis of variance (MANOVA) was performed on predictors of two continuous dependent variables which are PON1 activities towards paraoxon and diazoxon. The tests examined for differences in the dependent variables POase and DZOase activities simultaneously.

Multinomial regression was performed for prediction analyses in categorical outcomes (test groups). Two multinomial logistic regression models were fitted to evaluate risk factors in association with complications in type 2 DM based on a study by Sarkar *et al* (2006). The variables included in Model 1 were ethnicity, diabetic status, smoking status, alcohol status, age, BMI, and DZOase and POase activities. The variables included in Model 2 were the *PON1* polymorphisms ($PON1_{192QR}$ and $PON1_{55LM}$) ethnicity, diabetic status, smoking status, alcohol consumption status, age, BMI, and DZOase and POase activities, to observe whether *PON1* polymorphisms improved the predictive value of PON1 activity in the study groups. If the resultant model-fitting-information was significant, then the stepwise method using the forward-entry procedure was performed. The final variables that showed significant values based

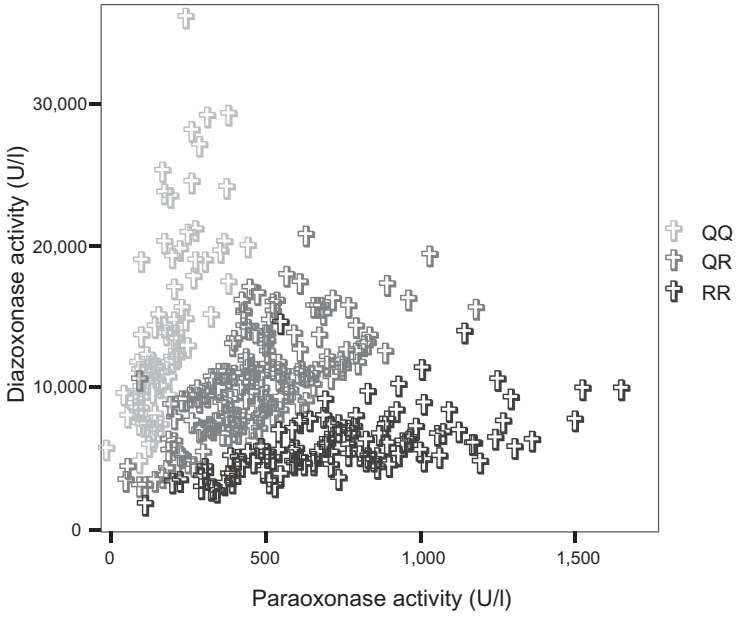

Fig 1–Plot of $PON1_{192}$ bivariate phenotype versus genotype in the determination of the functional genomics of plasma PON1. The plot gave rise to three sub-populations representing three genotypes as indicated by the three shades.

on the likelihood-ratio-tests and Wald statistic, taken together with odds ratio Exp(B) and 95% confidence interval for categorical variables and parameter estimates for continuous variables, were determined to be predictors of CVD complication in type 2 DM.

Analysis was performed using SPSS 13.0 for Windows statistical software. A *p*-value of <0.05 was taken to be statistically significant.

RESULTS

Demographic characteristics of the sample population

The sample population consisted of 199 females and 129 males. The mean age was 52. ± 10.9 years old. The distribution of the sample population according to study groups was as follows: Group 1 (control) – 46.6%, Group 2 (diabetics) – 13.4%, Group 3 (CVD) – 10.7%, and Group 4 (diabetic and CVD) – 29.3%. When Groups 1 and 3 were combined, the total was 57.3% for non-diabetics, and 42.7% for diabetics (Groups 2 and 4).

Status of PON1

The rates of spontaneous hydrolysis for paraoxon and phenylacetate were less than 0.0001% for a typical sample of plasma.

There was no significant difference in PON1 activity between paraoxon in males (541 ± 271 U/l) and in females (552 ± 327 U/l) when the independent samples *t* test was performed (p=0.749). PON1 activity towards diazoxon was higher (p=0.008) in females (10,061 ± 5,102 U/l) than in males (8,527 ± 5,060). When the rate of hydrolysis of diazoxon versus paraoxon was plotted, the population fell into three groups (Fig 1): individuals functionally homozygous for $PON1_{192Q}$ (*QQ*), heterozygotes ($PON1_{Q/R192Q/R}$; *QR*) and individuals homozygous for $PON1_{192R}$ (*RR*).

When PON1 activities towards paraoxon and diazoxon were demarcated by tertiles to classify as being low, intermediate or high, the thresholds for the first and second tertiles were at 368 and 622 U/l for overall PON1 activity towards paraoxon, and 6,690 and 10,640 U/l for PON1 activity towards diazoxon, respectively. When PON1 activities towards paraoxon and diazoxon were demarcated by tertiles within the genotypes of the $PON1_{192}$ polymorphism, the thresholds were 184 and

Table 1
Effect of ethnicity on PON1 activity towards paraoxon and diazoxon.

Ethnicity	POase activity[a], U/l	DZOase activity[b], U/l
Malay	592 ± 308	10,238 ± 5,806
Chinese	583 ± 289	8,404 ± 4,479
Indian	457 ± 295	9,371 ± 4,458
Total	540 ± 304	9,459 ± 5,054

Values are mean ± SD; Equal variances were assumed; One-way ANOVA, [a]$p<0.001$, [b]$p=0.029$; Tukey's *post hoc* test was used

Table 2
PON1 activity towards paraoxon and diazoxon by study group.

Study group	POase activity[a], U/l	DZOase activity[b], U/l
Group 1	581 ± 315	10,006 ± 4,692
Group 2	583 ± 342	8,679 ± 3,666
Group 3	647 ± 307	11,000 ± 6,100
Group 4	442 ± 244	8,378 ± 5,766
Total	548 ± 306	9,458 ± 5,133

Values are mean ± SD; Equal variances were assumed; One-way ANOVA, [a]$p<0.001$, [b]$p=0.017$; Tukey's *post hoc* test was used

261 U/l for the *QQ* genotype, 431 and 573 for the *QR* genotype, and 640 and 913 U/l for the *RR* genotype, respectively.

Relationship of ethnicity to PON1 activity

PON1 activity towards both substrates differed significantly by ethnicity (Table 1). Malays and Chinese had significantly higher PON1 activity towards paraoxon than Indians (ANOVA, $p<0.01$), and Malays had higher PON1 activity towards diazoxon than Chinese subjects ($p=0.022$).

Association of PON1 activity with diabetic status

The difference in PON1 activity towards the two substrates by diabetic status was significant (Table 2). The *post hoc* test showed Groups 1-3 had similar PON1 activity toward paraoxon, which was higher than Group 4. There was no pairwise difference in PON1 activity towards diazoxon between Group 1 and the other groups. There was also no difference between Group 2 and the other groups. However, Group 3 had higher PON1 activity towards diazoxon than Group 4 ($p=0.046$).

When the univariate analysis was performed, ethnicity, $PON1_{55LM}$ and $_{-192QR}$ polymorphisms, age, and TG, TC, HDL-C and LDL-C levels were significant predictors of PON1 activity towards paraoxon. Diabetic status and HDL-C level were not significant predictors ($p=0.1$) in Model 1 (test results not shown). Model 2, which did not include the two *PON1* polymorphisms, showed ethnicity, diabetic status and HDL-C level were significant predictors of PON1 activity (Tables 3a, b). This

suggests the *PON1* polymorphisms were confounding factors in Model 1.

Model 2 predicts Malays and Chinese would have higher PON1 activity towards paraoxon than Indians. The three groups comprised of control subjects, and those with DM and CVD (Groups 1-3) were predicted to have higher PON1 activity compared to those with DM having complications (Group 4). The model also indicated that higher HDL-C level predicted higher PON1 activity. In summary, the prediction of PON1 activity in Model 2 is governed by the following equation:

PON1 activity = 239 + 100 (Malay)
+ 95 (Chinese) + 84 (Control)
+ 123 (DM) + 133 (CVD)
+ 147 (HDL)

Tables 4a and b show the model predicts activity against diazoxon. Age, TG, TC and LDL levels were significant predictors of activity against diazoxon (p=0.05), while ethnicity and CVD status were not significant (p=0.1). Based on this model, Chinese are predicted to have lower DZOase activity than Indians. DZO activity was predicted to decrease with age, TG and LDL levels, and increase with higher TC levels. CVD status does not appear to predict DZOase activity.

When multivariate analysis was performed, ethnicity and diabetic status were significant predictors of POase activity, while ethnicity, smoking status, age, TG, TC and LDL-C levels were significant predictors of DZOase activity (Tables 5a, b).

Table 3a
Tests of between-subjects effects for prediction of PON1 activity (Model 2).

Source	F	*p*-value	Observed power[a]
Ethnicity	4.111	0.017	0.726
Diabetic status	2.601	0.052	0.636
HDL-C	9.450	0.002	0.865

Dependent variable, POase activity
[a]Computed using α=0.05

Table 3b
Parameter estimates for prediction of PON1 activity (Model 2).

Variable	Subgroup	B	SE	*t*	*p*-value	Observed power[a]
	Intercept	238.637	54.507	4.378	0.000	0.992
Ethnicity	Malay	100.035	37.602	2.660	0.008	0.756
	Chinese	95.420	43.208	2.208	0.028	0.596
	Indian	0[b]				
Study group	Control	84.231	42.338	1.990	0.047	0.509
	DM	123.323	53.838	2.291	0.023	0.627
	CVD	132.744	60.643	2.189	0.029	0.588
	DM and CVD	0[b]				
HDL-C	-	147.378	47.943	3.074	0.002	0.865

General linear model was used. Dependent variable, POase activity
[a]Computed using α=0.05; [b]This parameter is set to zero because it is the reference category.

Table 4a
Tests of between-subject effects for prediction of DZOase activity.

Source	F	*p*-value	Observed power[a]
Ethnic	2.621	0.074	0.520
Age	8.677	0.003	0.836
TG	14.964	0.000	0.971
Total cholesterol	15.812	0.000	0.977
LDL	11.908	0.001	0.931
CVDstat	2.523	0.082	0.504

Dependent variable, DZOase activity. [a]Computed using α=0.05.

Table 4b
Parameter estimates in prediction of DZOase activity.

Parameter	B	SE	*t*	*p*-value	B
Intercept	9,022.387	2,271.115	3.973	0.000	0.977
Malay	35.027	640.959	0.055	0.956	0.050
Chinese	-1,466.563	722.549	-2.030	0.043	0.525
Indian	0(b)				
Age	-83.895	28.480	-2.946	0.003	0.836
TG	-1,312.597	339.323	-3.868	0.000	0.971
Total cholesterol	3,383.169	850.810	3.976	0.000	0.977
LDL	-3,212.277	930.862	-3.451	0.001	0.931
Hypertensive	1,313.215	918.068	1.430	0.154	0.297
Non-HT	-78.083	928.424	-0.084	0.933	0.051
CVD and HT	0(b)				

General linear model was used. Dependent variable, DZOase activity
[a]Computed using α=0.05; [b]This parameter is set to zero because it is the reference category. Non-HT included diabetics and non-diabetic controls who did not have HT).

Based on this multivariate analysis, Chinese and Malays were predicted to have higher POase activity than Indians. POase activity was also predicted to be higher in type 2 diabetics without CVD than diabetics with CVD complications. Chinese were predicted to have lower DZOase activity than Indians. Control subjects had lower DZOase activity than diabetics with complications. Non-smokers were predicted to have higher activity than smokers. DZOase activity was predicted to decrease with age, TG and LDL levels, and increase with higher TC levels.

Hence, both univariate and multivariate analyses appeared to concur regarding predictors of activity towards paraoxon and diazoxon.

Predictors of complications in type 2 DM

Based on Model 1, the model-fitting-information indicates the presence of significant predictors, which were shown by the likelihood-ratio-test to be age, BMI and

Table 5a
Tests of between-subject effects on multivariate analysis.

Source	Dependent variable	F	*p*-value	Observed power[a]
Ethnicity	POase activity	3.079	0.047	0.591
	DZOase activity	3.494	0.032	0.650
Diabetic status	POase activity	1.505	0.213	0.396
	DZOase activity	1.683	0.171	0.439
Smoking status	POase activity	0.413	0.521	0.098
	DZOase activity	3.296	0.070	0.440
Age	POase activity	0.013	0.911	0.051
	DZOase activity	10.201	0.002	0.890
TG	POase activity	0.542	0.462	0.114
	DZOase activity	2.965	0.086	0.404
TC	POase activity	0.500	0.480	0.109
	DZOase activity	5.244	0.023	0.627
LDL	POase activity	0.209	0.648	0.074
	DZOase activity	4.582	0.033	0.569

[a] Computed using α=0.05

PON1 activity towards paraoxon, in that order (p<0.001). Parameter estimates in this model indicate the older the person is, the higher the likelihood he will have DM, followed by DM and CVD (Tables 6a,b). Similarly, the higher the BMI, the higher the likelihood he will present with DM, then CVD, followed by DM and CVD. On the other hand, the lower the PON1 activity towards paraoxon, the higher the likelihood the person will present with CVD, DM and DM with CVD, in order of descending likelihood.

In Model 2, the addition of *PON1* polymorphisms as variables factors did not improve the predictive value of PON1 activity toward paraoxon, since the parameters of the B coefficients, Wald statistics and *p*-values after regression analysis remained identical to those in Model 1.

Summary of predictors

Based on univariate (Model 2) and multivariate analyses, ethnicity, diabetic status and HDL-C level had an impact on PON1 activity toward paraoxon. Based on Model 1 from logistic regression analysis, age, BMI and PON1 activity toward paraoxon had an impact on the study groups. Among the variables, diabetic status and PON1 activity toward paraoxon were mutually present as a significant predictor of the other. This is consistent with the initial ANOVA that showed there was a significant difference in PON1 activity toward paraoxon among the four study groups.

DISCUSSION

Spontaneous hydrolysis of paraoxon and diazoxon did not affect PON1 activity in this study. Therefore, assays of PON1 activity were not corrected for spontaneous hydrolysis. Eckerson *et al* (1983) showed spontaneous, or nonenzymatic, hydrolysis is not significant except in samples having extremely low activity.

Table 5b
Parameter estimates on multivariate analysis.

Dependent variable	Parameter	B	SE	*t*	*p*-value	Observed power[a]
POase activity	Intercept	127.701	270.729	0.472	0.637	0.076
	Malay	85.506	40.844	2.093	0.037	0.551
	Chinese	95.305	45.163	2.110	0.036	0.557
	Indian	0[b]				
	Control	60.605	63.636	0.952	0.342	0.158
	DM	115.271	57.244	2.014	0.045	0.519
	CVD	91.256	73.881	1.235	0.218	0.234
	DM & CVD	0[b]				
	Non-smoker	-54.170	84.295	-0.643	0.521	0.098
	Smoker	0[b]				
	Age	.211	1.880	0.112	0.911	0.051
	TG	-23.188	31.509	-0.736	0.462	0.114
	Totalchol	55.500	78.522	0.707	0.480	0.109
	LDL	-36.298	79.476	-0.457	0.648	0.074
DZOase activity	Intercept	12,223.792	4,492.346	2.721	0.007	0.774
	Malay	223.859	677.744	0.330	0.741	0.063
	Chinese	-1,662.132	749.420	2.218	0.027	0.599
	Indian	0[b]				
	Control	-1,792.341	1,055.950	1.697	0.091	0.395
	DM	-1,053.248	949.884	1.109	0.268	0.197
	CVD	30.402	1,225.948	0.025	0.980	0.050
	DM & CVD	0[b]				
	Non-smoker	2,539.283	1,398.746	1.815	0.070	0.440
	Smoker	0[b]				
	Age	-99.635	31.196	3.194	0.002	0.890
	TG	-900.372	522.846	1.722	0.086	0.404
	Totalchol	2,983.791	1,302.954	2.290	0.023	0.627
	LDL	-2,822.800	1,318.787	2.140	0.033	0.569

General linear model used. Dependent variables, POase and DZOase activities.
[a]Computed using α=0.05. [b]This parameter is set to zero because it is the reference category.

In the present study, diabetics with complications were found to have significantly lower PON1 activity than diabetics without complications. PON1 activity was also found to be lower in diabetics with CVD than those without CAD (Tsuzura *et al*, 2004; Kosaka *et al*, 2005; Inoue *et al*, 2006; Lakshman *et al*, 2006; Moldoveanu *et al*, 2006). Investigations of diabetic complications in relation to PON1 activity have also been carried out, suggesting the role of PON1 in the attenuation of premature atherosclerosis in diabetes (Mackness *et al*, 2002). The percentage of patients with lower HDL-C was higher in diabetic patients with CVD than in diabetics without CVD or healthy subjects (Moldoveanu *et al*, 2006).

Table 6a
Likelihood ratio tests on logistic regression analysis.

Effect	Model fitting criteria	Likelihood ratio tests		
	-2 Log likelihood of reduced model	Chi-square	df	*p*-value
Intercept	762.183	79.119	3	0.000
Age	772.538	89.475	3	0.000
BMI	708.853	25.790	3	0.000
PON	697.540	14.476	3	0.002

The chi-square statistic is the difference in -2 log-likelihoods between the final model and reduced model. The reduced model is formed by omitting an effect from the final model.

Table 6b
Parameter estimates for logistic regression analysis.

Diabetic status[a]		B	SE	Wald	df	*p*-value	Exp(B)	95% Confidence interval for Exp (B)	
								Lower bound	Upper bound
DM	Intercept	-8.164	1.799	20.588	1	0.000			
	Age	0.078	0.019	16.225	1	0.000	1.081	1.041	1.123
	BMI	0.111	0.043	6.561	1	0.010	1.118	1.026	1.217
	PON1	0.000	0.001	0.005	1	0.942	1.000	0.999	1.001
CVD	Intercept	-8.929	1.974	20.461	1	0.000			
	Age	0.067	0.021	9.878	1	0.002	1.070	1.026	1.116
	BMI	0.138	0.046	8.993	1	0.003	1.147	1.049	1.255
	PON1	0.001	0.001	1.231	1	0.267	1.001	0.999	1.002
DM & CVD	Intercept	11.872	1.730	47.073	1	0.000			
	Age	0.142	0.018	61.197	1	0.000	1.152	1.112	1.194
	BMI	0.175	0.040	19.363	1	0.000	1.192	1.102	1.288

Multinomial logistic regression model was used. [a]The reference category is Control.

The reduced activity of PON1 and reduced HDL levels in diabetics with CVD may be due to the increased glycation of HDL. Glycation of HDL increases its turnover and reduces its efficiency during cholesterol transport (Hedrick *et al*, 2000). The effect of glucose *in vitro* on PON1 lactonase activity and HDL was studied by Rosenblat *et al* (2007). Sera from healthy subjects were incubated with glucose in increasing concentrations. This resulted in a significant glucose dose-dependent reduction in PON1 activity in both the serum and HDL fraction. The ability of HDL to induce macrophage cholesterol efflux was reduced. Western blot analysis demonstrated glucose caused the dissociation of PON1 from HDL to a free PON1 form in a glucose dose-dependent manner. PON1 lactonase activity was 60% lower in

Table 7
Comparison of POase and DZOase activity in human subjects.

Subject population	Sample	POase activity (U/l)[a]	DZOase activity (U/l)[b]	Reference
Healthy	Sera	140 ± 43 - 1,008±244[c]	-	Eckerson *et al*, 1983
Healthy	Sera	144 (129-160)[d]-Geneva 142 (122-164)[d]-Manchester	-	Blatter-Garin *et al*, 1994
Healthy	Sera	142 (72-283)[d]	-	Abbott *et al*, 1995
Healthy	Sera	215 (26-621)[d]	-	Mackness *et al*, 1998
DM		164 (8-467)[d]		
Healthy	Sera	181±134	-	James *et al*, 1998
Healthy	Sera	44[d]; 82[c]	-	Sozmen *et al*, 1999
Type 2 DM		44[d]; 91[c]		
Type 2 DM with HPT		35[d]; 74[c]		
Healthy	Sera	162	4,323	Inoue *et al*, 2000
Healthy control	Plasma	685[c]	10,009	
Vascular disease		546[c]	8,493	Jarvik *et al*, 2000
Diabetic retinopathy	Sera	113 (3-415)[d]	-	Mackness *et al*, 2000a
Healthy	Sera	226[c]	-	Senti *et al*, 2001
CAD diabetics and non-diabetics	Sera	81[d]	-	Rahmani *et al*, 2002
Healthy	Sera	286[c]	6.5	Yamada *et al*, 2001
Healthy	Sera	688 ± 412[c]; 412 ± 153[d]	-	Ferré *et al*, 2002
Healthy	Sera	-	14.17	Cherry *et al*, 2002
DM	Sera	238 ± 80	-	Tsuzura *et al*, 2004
DM with CVD		259 ± 93		
Familial hypercholesterolemia	Sera	732 (2 M NaCl)	6,648	van Himbergen *et al*, 2005
Healthy	Sera	340 ± 37[d]	-	Rosenblat *et al*, 2005
DM		183 ± 35[d]		
Hypercholesterolemia		208 ± 12[d]		
Peripheral arterial disease	Sera	294[d]	-	Pasqualini *et al*, 2005
DM	Sera	331 ± 29[c]	-	van Wijk *et al*, 2006
Healthy control	Sera	300	-	Lakshman *et al*, 2006
DM without CVD		200		
DM with CVD		200		
Healthy control	Sera	269[d]	-	Mastorikou *et al*, 2006
DM		114[d]		
CHD		151[d]		
Healthy adults	Plasma	1,024[c]	-	Holland *et al*, 2006
Healthy newborns		315[c]	-	
Healthy control	Sera	413[c]		Juretic *et al*, 2006
DM		464[c]		
Healthy control	Sera	60 ± 27[d]	-	Hashim and Zarina, 2007
DM		21 ± 3[d]		

[a]Units in nmol/min/l; [b]Units in μmol/min/l; [c]With 1 M NaCl; [d]Without NaCl

diabetics than in healthy subjects. Free PON1 and HDL-bound PON1 inactivation rates were higher in diabetics than in healthy subjects. The authors concluded the inactivation and dissociation of PON1 from HDL by high glucose concentrations may step up the atherosclerotic process in diabetic patients.

In this study, healthy subjects, diabetics and CVD patients had similar activity towards paraoxon, and this was higher than PON1 activity in diabetics with complications. Hashim and Zarina (2007) reported similar results. Mastorikou *et al* (2006) showed PON1 activity in healthy subjects was higher than in diabetics, while diabetics had lower activity than CHD patients. The present study also showed comparable activity towards paraoxon between diabetics without complications and healthy subjects. Rahmani *et al* (2002) showed there was no significant difference in activity towards paraoxon in their study groups, which consisted of coronary artery disease (CAD) patients with diabetes, CAD patients without diabetes, and healthy subjects matched for age and sex. In some instances, PON1 activity was higher in diabetics than healthy subjects. Juretic *et al* (2006) found PON1 activity was slightly higher in diabetics, which they associated with higher cholesterol levels. They also found the $PON1_{192R}$ allele was present more commonly in diabetics. Since hydrolysis of paraoxon by this allele is more efficient than the $PON1_{192Q}$ allele, this may have contributed to the higher PON1 activity measured. A study on variation in *PON1* polymorphism showed a higher frequency of the $PON1_{192R}$ allele in the general population (Poh and Maniandy, 2007). In a study by Lakshman *et al* (2006), diabetic patients who had undergone coronary artery bypass surgery were more aggressively treated than were DM-CVD and DM+CVD groups, receiving a higher mean dose of statins which might have contributed to the additional antioxidant potential as translated by the associated activity of PON1. The authors demonstrated a negative correlation for both serum paraoxonase (POase) and homocysteine thiolactonase (HTLase) activity with the degree of CVD. There was a 30% decrease in POase activity in diabetic patients without overt CVD compared to healthy subjects. Similarly, HTLase activity in diabetics without overt CVD was 11% lower than healthy subjects. Increased angiographic atherosclerotic activity, as indicated by the Gensini Score, was associated with decreased PON1 activity. The authors attributed the lower PON1 activity to diabetic pathophysiology rather than *PON1* polymorphism, since there was no difference in the distribution of genotypes between the groups. They also found the protective role of HDL against LDL oxidation was negatively correlated with the degree of CVD. The shorter lag time of LDL oxidation kinetics in diabetics with CVD compared to diabetics without CVD, could be due to lower PON1 activity; this suggests combined accelerated atherogenesis with diabetic pathology. Table 7 compares the relationship between disease status and PON1 activity towards paraoxon and diazoxon. Variation in PON1 activity may have been due to several factors, such as variation in the salt introduced into the activity assay buffer, whether sera or plasma was used and the pH of the buffer.

The present study also showed that CVD patients had higher PON1 activity towards diazoxon than diabetics with complications across the $PON1_{192}$ genotypes. When stratified, activity towards diazoxon was higher in healthy subjects and diabetics without complications than

diabetics with complications for $PON1_{192RR}$ subjects. Studies of activity towards diazoxon in relation to diabetic complications are currently lacking. Yamada *et al* (2001) reported that there was a positive association between insulin resistance and activity towards diazoxon in the non-diabetic population. Their results suggest hyperinsulinemia is a factor contributing to variability in PON1 activity. However, there was no association noted between common carotid artery intima-media thickness (a measure of early atherosclerosis) and activity towards diazoxon (van Himbergen *et al*, 2005).

However, some studies have shown no discrimination in PON1 activity when diabetics were compared with normal subjects. Hofer *et al* (2006) found no significant associations between PON1 activities and diabetes complications. In a study of diabetics in a Croatian population, PON1 activity towards paraoxon and phenylacetate was measured (Juretic *et al*, 2006). The authors found that total cholesterol and LDL-cholesterol in female diabetics and triglyceride concentration in male diabetics were higher than in normal subjects. PON1 activity, however, was similar between diabetics and normal subjects.

In the present study, the results of stepwise multinomial regression analysis confirmed that age, BMI and PON1 activity towards paraoxon were associated with complications in type 2 diabetes. The results of univariate analysis showed that ethnicity, diabetic status and HDL-C level had an impact on PON1 activity. Thus diabetic status and PON1 activity were mutually present as significant predictors, independent of *PON1* polymorphisms. These findings are in agreement with those of Tsuzura *et al* (2004), where diabetics with complications had lower PON1 activity than diabetics without complications. These observations support the hypothesis that PON1 prevents LDL lipid peroxidation, thus protecting against diabetic complications (Lakshman *et al*, 2006). The attenuation of PON1 activity could result in higher susceptibility to LDL oxidation, especially in diabetics suffering oxidative stress (Tsuzura *et al*, 2004). However, the direct effect of PON1 on diabetes development has not been fully studied (Rozenberg *et al*, 2008). HDL-C was a significant contributor to the variation in PON1 activity towards paraoxon. These findings are consistent with those of Phuntuwate *et al* (2005). Furthermore, the association between PON1 activity and clinical events was observed in a follow-up study spanning 9 years (Inoue *et al*, 2006). It was found that PON1 activity retained a significant inverse association with CVD, whereby an increased incidence of CVD was detected in diabetic patients with low PON1 levels. A low PON1 concentration may be a useful predictor of CVD in diabetic patients.

This is the first evaluation of paraoxonase and diazoxonase activities in three major Malaysian ethnic groups. We found that diabetics with CVD complications had lower PON1 activity against paraoxon than diabetics without such complications. Monitoring PON1 activity as a parameter of vascular risk could improve the prognosis of complex vascular disease in type 2 diabetes.

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