ORIGINAL ARTICLE

Study of Serum Paraoxonase and High Density Lipoprotein Fractions in Diabetes

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Abstract

Objective: Significant alteration in lipid profile and antioxidant system occurs in response to diabetes mellitus (DM). Paraoxonase (PON) is a family of three enzymes PON1, PON2 and PON3 associated with high density lipoprotein (HDL). The HDL in human plasma consists of two main sub-fractions HDL2C and HDL3C. We studied the HDL subclasses and HDL associated enzyme paraoxonase with respect to diabetes.

Materials and Methods: The study was conducted in a tertiary care referral hospital in India. A total of 80 subjects were included in the study. Lipid profile, PON1 arylesterase (ARE), PON1 lactonase (LACT) and HDL fractions were estimated. Regression analysis was applied.

Results: PON1 ARE, LACT and HDL fractions are found to be decreased among cases than in controls. PON1 ARE & LACT showed negative correlation with blood glucose levels and HDL 3C while positive correlation with HDL 2C.

Conclusion: PON1 ARE and PON1 LACT activities reduction are due to increased oxidative stress. PON1 as well as HDL fraction levels are oxidative stress subjects. Among the HDL fractions, HDL2C is the more variable fraction and reflects changes in HDL. The study suggested that the protective role of total HDL against oxidative damage and complications is mainly mediated through HDL2C fraction.

Keywords: PON1, Arylesterase, Lactonase, HDL2, HDL3

Introduction

Diabetes mellitus is one of death major causes worldwide. It is characterized by relative or absolute deficiency of insulin secretion and/or insulin resistance that causes chronic hyperglycemia and impaired carbohydrates, lipids and proteins metabolism. The majority of cases of diabetes mellitus result from 2 major etiologic mechanisms. (1) First, type 1 diabetes mellitus is an autoimmune disease in which absolute deficiency of insulin because of immune-mediated destruction of pancreatic β cells causes hyperglycemia. Second, type 2 diabetes mellitus (T2DM) is characterized by insulin

resistance. The main risk factors for T2DM include obesity, excess calories consumption and reduced physical activity. A strong genetic component is associated with vulnerability to T2DM.

Epidemiological data showed increase of T2DM prevalence throughout the world and more so in India. (2,3) The complications of T2DM compromise health and reduce the life span in affected individuals. It also impose substantial financial burden to individual and healthcare delivery systems. Cardiovascular disease is a major cause of morbidity and mortality in T2DM patients; therefore, identification of fundamental biochemical and molecular pathways that lead to diabetic complications is essential. (4,5)

Moreover, T2DM is also characterized by micro-angiopathy complication such as retinopathy, nephropathy and neuropathy. (6) Several studies demonstrated that neutralization of reactive molecules significantly were able to inhibit the development and progress of endothelial dysfunction; cardiomyopathy, retinopathy, nephropathy, and neuropathy in patients with T2DM. (7) There are various molecules which are either the reasons or result of diabetic complications. Their detailed study can help in prevention and timely interception of diabetic complications in future.

Paraoxonase (PON) enzymes were originally discovered as enzyme hydrolyzing exogenous organophosphate compound such as insecticide paraoxon. Paraoxonase is a family of three enzymes PON1, PON2 and PON3. (8,9) PON is an enzyme located in a sub-fraction of HDL containing ApoA1 and cluster in Apo J and it is anchored to HDL by its hydrophobic N-terminal end and also bound to ApoA1. It contributes most of the anti-atherogenic activity of the HDL molecule. This enzyme has paraoxonase arylesterase (ARE) and lactonase (LACT) activity and by virtue of its hydrolytic action, prevents accumulation of lipid peroxides in LDL, and gives protection against lipoprotein oxidation. (10,11)

Plasma HDL particles are highly heterogeneous in their physicochemical properties, intravascular metabolism and biologic activity. (12) It is a well-known fact that HDL has anti atherogenic property. HDL originates as discoidal complexes of apo-lipoprotein and phospholipids which are secreted from liver. The surface of HDL also contains varying amounts of other apo-lipoproteins: apo A-IV, the apo-lipoproteins, apo-D, apo-E and apo-J as well as some of the plasma factors which are involved in remodeling. (13,14)

The HDL in human plasma consists of two main sub-fractions HDL2C and HDL3C. HDL2C is considerably larger and less dense than HDL3C. (15) HDL3C, generated from discoidal HDL by the action of LCAT, accepts cholesterol from the tissues via the SR-B1 and the cholesterol is then esterified by LCAT, increasing the size of the particles to form the less dense HDL2C.

Therefore we studied the HDL subclasses and HDL associated enzyme paraoxonase with respect to diabetes. The study is aimed to know the effect of various factors on diabetes their interrelation.

Materials and Methods

The study was conducted in a tertiary care referral hospital in India. It was approved by the Institutional Ethics Committee. A total of 80 subjects were included in the study. Sample size is calculated using 2-sided confidence interval 95% and power 80%. Using the mean and standard deviation form our pilot study, sample size was 35. Therefore, a total of 40 subjects were enrolled each as cases and controls. Cases were selected randomly from patients visiting the hospital to avoid the selection bias. Randomization is done using random number generator program. Cases were selected according the American diabetes association criteria. (16) Subjects with newly diagnosed T2DM were included as cases. Patients with diabetes microvascular complication and patients on treatment were excluded from the study. Age and sex-matched

controls were selected from the population attending the regular medical checkup in the hospital. Written informed consent was taken from all the participants. With all aseptic precautions, early morning fasting blood samples were collected by venipuncture from all patients. From control subjects, blood samples were collected at the time of their routine clinical visits. The samples were analyzed immediately after processing. Serum PON1 arylesterase and lactonase activities were measured as described in literatures. (17,18) Serum total cholesterol (TC), HDL-C, LDL-C and triglyceride (TG) were estimated using enzymatic diagnostic kits on a Smartlab Auto Analyzer (ERBA, Mannheim, Germany). HDL subclasses were determined by dextran sulphate/MgCl2 precipitation procedure. (19) Normal istribution of the ARE and LACT was assessed by the Shapiro-Wilk test. The two-sample t-test was applied for hypothesis testing. The results were expressed as mean ± standard deviation (mean ± SD) for all continuous variables. The statistical significance level was set at 0.05. There were no differences between the two groups with regard to age and sex.

We also used the linear regression for the analysis. The result of linear regression is given in terms of R2 which is a statistical measure of how close the data are to the fitted regression line. It is also known as the coefficient of determination. It indicates the proportion of the variance in the dependent variable that is predictable from the independent variable. In general, the higher the R2, the better the model fits your data. The results obtained were analyzed by using MyStat statistical software.

Results

Serum PON1 ARE activity reduced significantly in patients with T2DM than in controls. Similarly, serum PON1 LACT reduced significantly in patients with T2DM. The results showed that ARE and LACT are inversely correlated with glucose control and their combination even more so. The study findings were shown in the Table 1. Figures 1 and 2 showed the distribution of the PON1 ARE and LACT respectively in study subjects. Figure 3 showed distribution of HDL and its subclasses.

HDL cholesterol is statistically insignificant among cases and controls. But when HDL2C and HDL3C were taken separately, they were statistically significant among cases and controls.

Figures 4 and 5 showed the correlation of HDL with PON1 ARE and PON1 LACT respectively. Both figures showed there was positive correlation of HDL2C with PON1 activities while the HDL3C has negative correlation with PON 1 activities.

Discussion

There are the increase of oxidative stress at cellular level in T2DM and metabolic syndrome. PON1 activities reduce in T2DM and its complications. There is a negative correlation between oxidative stress and PON1 activities. It is hypothesized that the oxidative damage to the lipoproteins especially HDL leads to PON1 activity reduction. Present study coincides well with this hypothesis as PON1 activities are reduced significantly in diabetes mellitus. PON1 activities reduce in diabetes and its complications as disease

Table 1. Study findings in cases and controls (mean ± SD)

Parameter	Cases (n = 40)	Controls (n = 40)	*P*-value
Age (Yrs)	57.9 ± 7.09	58.17 ± 7.95	0.871
Fasting glucose (mg/dl)	136.70 ± 8.94	92.40 ± 12.42	0.001*
PON1 arylesterase (kU/L)	127.5 ± 25.09	156.85 ± 16.42	0.009*
PON1 lactonase (U/L)	6.91 ± 1.27	10.6 ± 1.90	0.005*
HDL cholesterol (mg/dl)	34.27 ± 5.24	37.35 ± 7.36	0.035*
HDL2C cholesterol (mg/dl)	21.10 ± 3.19	26.05 ± 5.61	0.0006*
HDL3C cholesterol (mg/dl)	13.18 ± 3.48	11.10 ± 2.57	0.003*

* Statistically significant

progresses. There are reports of oxidative stress increase in LDL and HDL of diabetes patients which is associated with HDL and PON activity decrease. The lower PON activity and the compositional changes in HDL and LDL could contribute the greater risk of cardiovascular disease. (20,21)

Some researchers found that PON1 activities are correlated well with HDL concentration.

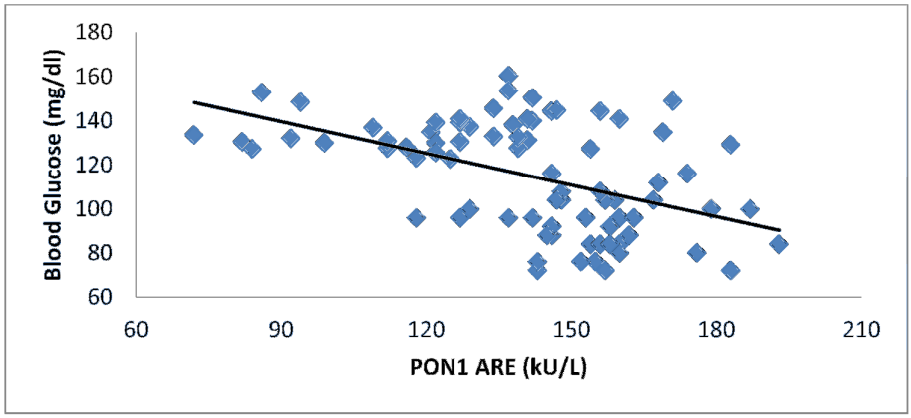

Figure 1. Correlation of PON1 ARE with blood glucose level

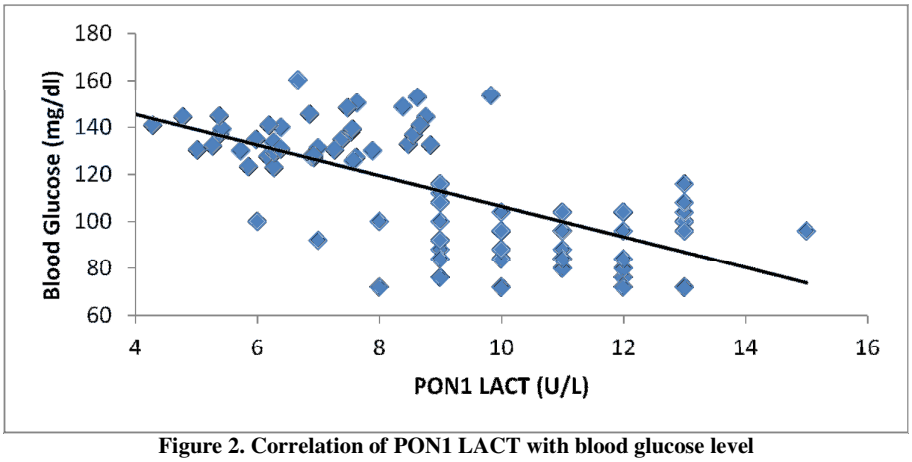

Figure 2. Correlation of PON1 LACT with blood glucose level

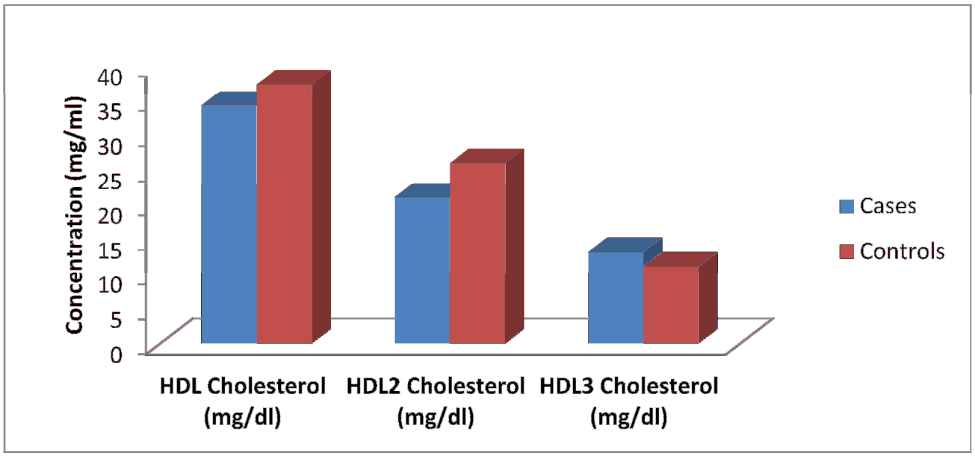

Figure 3. Distribution of HDL and Its Subclasses

Dysfunctional or modified HDLC is responsible for the reduced antioxidant capacity. As PON1 prevents oxidation of LDL and HDL, qualitative changes in lipoproteins coupled with reduced HDLC. A study reported that anti-atherogenic activity of HDL3C significantly altered in diabetes as a result of profound alterations in HDL metabolism and composition which is similar to results of present study. (22) A research have shown that antioxidant activity of HDL3C was reduced in metabolic syndrome. (23) HDL2C shows positive correlation with PON1 activities. Also HDL2C levels are found to be more in controls as compared to case of diabetes. Kupio Study showed that HDL2C is inversely correlated with risk of atherosclerosis which is consistent with present study. Similarly Quebec City Suburbs Study showed HDL2C was inversely associated with risk of cardiovascular disease. (24)

One study revealed that decreased HDL2C levels are associated with increased risk of vascular events through changes in lipoprotein composition. (25) Similar results were also obtained from Physicians' Health Study, which demonstrated protective effects of HDL2C. (26) A study showed that HDL2C is the more variable subclass and reflects changes in HDL. This suggests that the protective role of total HDL against oxidative damage and complications is mainly mediated through HDL2C fraction. (27) Higher levels of HDL2C are more consistently preventive for diabetic complications than HDL3C. (28,29) This is similar to our findings.

Findings of present study could be explained in the following way. In metabolic syndrome

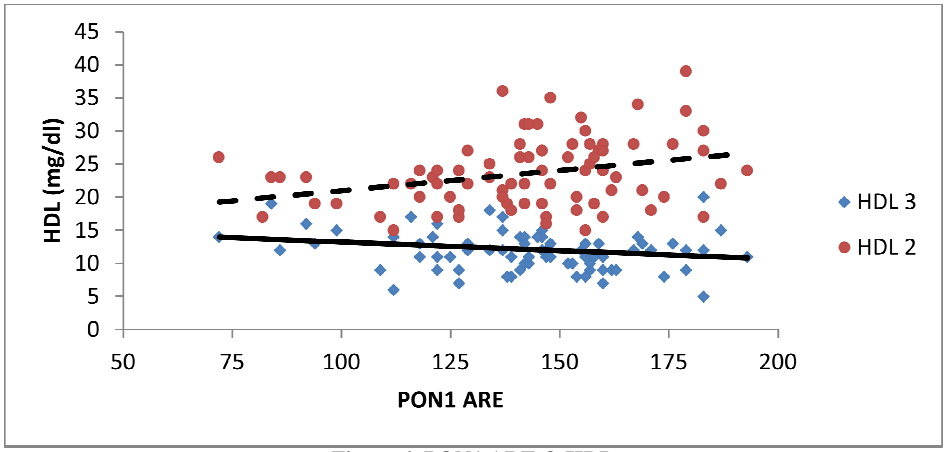

Figure 4. PON1 ARE & HDL

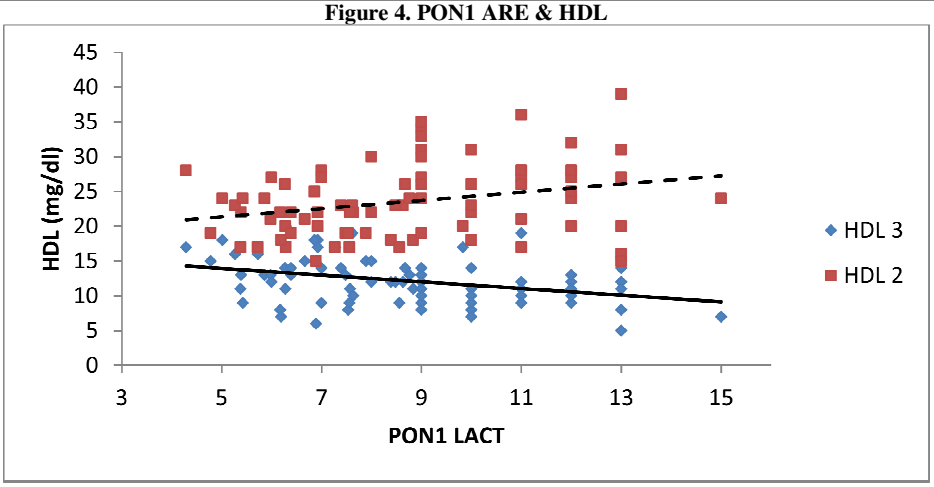

Figure 5. PON1 LACT & HDL

as the quality of HDL is impaired, antioxidant, anti-inflammatory, cytoprotective, anti-thrombotic activities which are owed to HDL2C are also deranged. The altered PON1 arylesterase and lactonase activities in diabetes could have two possible explanations. One is that serum PON1 activities may be lowered as a result of an altered synthesis or secretion of HDL-cholesterol. The other one is the overproduction of the free radicals. Free radicals are disproportionately formed in metabolic abnormalities, such as chronic hyperglycemia and dyslipidemia, by oxidation, and the subsequent oxidative degradation of proteins. Insulin resistance seems to stimulate endothelial superoxide anion production via nicotinamide adenine dinucleotide phosphate hydrolase adherence, can worsen the degree of oxidative stress. The activation of oxidative-stress pathways is a key component in the development and progression of diabetic complications. (30)

Conclusions

PON1 as well as HDL fraction levels are oxidative stress subject. Among the HDL fractions, HDL2C is the more variable fraction and reflects changes in HDL. This suggests that the protective role of total HDL against oxidative damage and complications is mainly mediated through HDL2C fraction. Higher levels of HDL2C are more consistently preventive for diabetic complications than HDL3C. check of HDL fraction levels are more effective than mere HDL cholesterol. This will provide more comprehensive picture of the oxidative stress in the patient along with PON 1 activities.

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