

# Paraoxonase Activity In Type 2 Diabetes Mellitus Patients With And Without Complications

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## ABSTRACT

**Introduction:** Type 2 diabetes mellitus (DM) is a disease of metabolic dysregulation. The current study was undertaken to understand the relationship between the fasting lipid profile and the paraoxonase 1 (PON1) activity in type 2 diabetic patients, with and without complications. **Materials and methods:** The study group consisted of a total of 155 subjects, which included non-diabetic healthy control subjects (n = 50) and diabetic patients with complications (group I, n = 66) and without complications (group II, n=39). PON1 activity was measured in all the subjects, based on spectrophotometric methods and the fasting lipid profile and the fasting plasma glucose (FPG) levels were determined by using a clinical chemistry analyzer, Hitachi 912. **Results:** FPG ( $p<0.001$ ) and triacylglycerides

(TAG) ( $p<0.001$ ) were significantly increased and high-density lipoprotein-cholesterol (HDL-C) ( $p<0.001$ ) and PON1 ( $p<0.001$ ) were significantly decreased in group I patients as compared to the normal controls. In group II patients, FPG ( $p<0.001$ ), TAG ( $p<0.001$ ) and total cholesterol (TC) ( $p<0.05$ ) were significantly increased and HDL-C ( $p<0.05$ ) was significantly decreased as compared to the normal controls. By using Pearson's correlation, HDL-C was found to be positively correlated with PON1 in group I patients ( $r=0.317$ ,  $p<0.01$ ). **Conclusion:** Type 2 DM patients with complications have significantly decreased HDL-C levels and PON1 activity, possibly indicating their decreased biochemical roles in these patients.

**Key Words:** PON1, Fasting lipid profile, Diabetes, Anti-atherogenesis.

## INTRODUCTION

Type 2 diabetes mellitus (DM) is a disease of metabolic dysregulation, involving the impaired uptake and the utilization of glucose, altered lipid metabolism, the accumulation of various lipid species in the circulation and in the tissues, and the disruption of metabolic signaling pathways that regulate insulin secretion from the pancreatic beta-cells.[1] Previous studies have shown that increased levels of oxidative damage to lipids in diabetes, and their presence correlated with the development of complications.[2], [3] Several studies have demonstrated that the increased susceptibility of low density lipoprotein (LDL) to oxidation and higher levels of oxidized LDL in DM correlated with an increased risk of cardiovascular complications. [3-5]

Monitoring the trends in cardiovascular complications via Paraoxonase 1 (PON1) is of critical importance in managing patients with type 2 DM.[6] PON1 is an ester hydrolase which is present in serum and in the liver. Serum PON1 is located in a subfraction of high density lipoprotein (HDL) that contains apoA-I and clusterin (apoJ). It has been suggested that, this subfraction of HDL is principally responsible for the breakdown of lipid peroxides, and that it consequently protects against lipoprotein oxidation. [7], [8] Low serum PON1 activity, independent of genotype, has been reported with diseases which are known to be associated with cardiovascular diseases (CVD) such as DM, hypercholesterolemia, and renal failure. In the case of diabetes, serum PON1 activity is decreased even before the onset of clinical CVD and in animal models of diabetes.[9]

This study was conducted to establish the relationship between PON 1 activity and fasting lipid profile in type 2 diabetic patients with and without complications.

## MATERIALS AND METHODS

### Subjects

The study group consisted of a total of 155 subjects, which included non-diabetic healthy control subjects (n=50) and type 2 DM patients (n = 105). The duration of diabetes in all the patients was  $10\pm4$  years. We grouped the type 2 DM patients into two groups. Group I (n=66) consisted of type 2 DM patients with complications of nephropathy, neuropathy, retinopathy, ischaemic heart disease, diabetic gangrene, hemiplegia, and paraplegia, and group II (n=39) consisted of type 2 DM patients without any clinically demonstrable complications. On taking the treatment history, 17 patients were found to be on a diabetic diet and lifestyle modifications, 23 were on insulin therapy, 42 were on oral hypoglycaemic drugs, and 23 were on both insulin and 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.

### SAMPLE AND REAGENTS

Under aseptic conditions 5 mL each of fasting blood samples were drawn into plain and EDTA-NaF containing vacutainers from the antecubital vein, from type 2 DM patients and healthy controls, for the assessment of the lipid profile and fasting plasma glucose (FPG), respectively. The vacutainers containing the blood samples were kept at room temperature for 30 min and were centrifuged at 2000g for 15 min for the clear separation of serum or plasma. All assays were performed immediately after the serum or plasma was separated. The special chemical, Paraoxone, was obtained from Sigma Chemi. Co. (St Louis, MO). All other reagents were of the analytical grade.

## BIOCHEMICAL DETERMINATIONS

PON1 was estimated spectrophotometrically by the method which is described elsewhere, with minimal modifications.[10] Briefly, the assay mixture consisted of 500 µl of 2.2 mmol/l paraoxone substrate in 0.1 mol/l tris-HCl buffer, pH 8.0, containing 2 mmol/l CaCl<sub>2</sub> and 50 µl of fresh serum specimen. The absorbance was monitored at 405 nm, at 25 °C. The PON 1 activity was expressed in international units (IU). One IU was defined as 1 µmol of p-nitrophenol which was formed/min/L at 25 °C.

FPG and fasting lipid profile were estimated by an enzymatic kinetic assay method by using an automated analyzer, Hitachi model 912. FPG was determined by the modified glucose oxidase/peroxidase method. [11] Total cholesterol estimation was done by the cholesterol oxidase method; HDL cholesterol was estimated by the same method after precipitating the low-density lipoproteins (LDLs), very-low density lipoproteins (VLDLs), and the chylomicrons. [12] Triglycerides were estimated by using an enzymatic mixture containing lipoprotein lipase, glycerol kinase, glycerol-3-phosphate oxidase and peroxidase. [13] Low density lipoprotein levels were calculated by using Friedewald's formula. [14]

## STATISTICAL ANALYSIS

The results were expressed as mean±standard deviation (SD). A  $p < 0.05$  was considered to be statistically significant. Statistical analysis was performed by using the Statistical Package for Social Sciences (SPSS-16, Chicago, USA). 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

As shown in [Table/Fig 1], in group I, FPG ( $p < 0.001$ ), TAG ( $p < 0.001$ ) and total cholesterol (TC)/HDL-C ratio ( $p < 0.001$ ) were found to be significantly increased, and levels of HDL-C ( $p < 0.001$ ) and PON1 ( $p < 0.001$ ) were found to be significantly decreased as compared to the normal controls. In group II patients, FPG ( $p < 0.001$ ), TAG ( $p < 0.001$ ), TC ( $p < 0.05$ ) and TC/HDL-C ratio ( $p < 0.001$ ) levels were found to be significantly increased and HDL-C ( $p < 0.05$ ) was found to be significantly decreased as compared to the normal controls. HDL-C was found to be positively correlated with PON1 in the group I patients ( $r = 0.317$ ,  $p < 0.01$ ).

	Type 2 DM patients with complications (Group I, n=66)	Type 2 DM patients without complications (Group II, n= 39)	Normal controls (n=50)
Age	58±12	54±9	51±11
Sex (M/F)	39/27	21/18	31/19
FPG (mg/dL)	193.86±75.83***	178.56±54.99***	75.46±8.9
TC (mg/dL)	161.48±45.51	170.54±35.94*	154.5±16.2
TAG (mg/dL)	166.73±96.39***	158.87±77.44***	70.02±13.23
HDL-C (mg/dL)	35.24±14.58***	43.07±19.04*	50.14±4.21
LDL-C (mg/dL)	96.86±40	99.13±27.08	91.8±14.5
TC/HDL-C ratio	5.27±2.31***	4.63±1.87***	3.1±0.3
PON 1 (IU)	118.1±37.76***	157.36±43.90	141.97±12.27

[Table/Fig 1]: Demographic and biochemical characteristics in normal controls and type 2 DM patients with (Group I) and without complications (Group II) (expressed in mean±SD).

\* $P < 0.05$  compared to normal controls.

\*\* $P < 0.01$  compared to normal controls.

\*\*\* $P < 0.001$  compared to normal controls.

## DISCUSSION

Significantly high levels of FPG ( $p < 0.001$ ) in the group I patients indicated a poor glycaemic control in these patients, which led to the increased glycation of proteins and other biomolecules. Prolonged dysglycaemia in these patients might have caused increased damage to the biomolecules and the biomembranes, thus leading to various diabetes associated complications. [15] A significant increase in TAG and the TC/HDL-C ratio and a significant decrease in HDL-C and PON1 activity in the group I patients indicated diabetes dyslipidaemia. Especially, significantly decreased HDL-C in these patients significantly affected the activity of PON1.

After the further classification of the group I patients based on the prospective treatment history from the case records, 23 patients were started on both insulin and oral hypoglycaemic agents, on their first presentation to the hospital, their FPG being 203.35±94.59, HDL-C being 30.56±12.63, and PON1 being 76.21±24.27. This data indicates the poor glycaemic control and significantly decreased HDL-C and PON1 levels, especially in patients with various diabetes related complications when compared to the group II patients and the normal healthy controls.

This fact was further evidenced by the correlation data of our study, where HDL-C was found to be positively correlated with PON1 in group I patients ( $r = 0.317$ ,  $p < 0.01$ ). This decrease in the activity of PON1 might prevent the normal physiological function of HDL-C and its anti-atherogenesis function, thereby leading to accelerated atherogenesis and its related complications in these patients. [9] Although there was increase in the TAG and the TC levels and the TC/HDL-C ratio, LDL-C levels were not significantly increased. There was a marginal decrease in HDL-C in the group II patients, but this did not alter the activity of PON1 in these patients. This may possibly indicate the significant physiologically active functioning of PON1 in these patients, thus protecting them from diabetes related complications. But, a further, prospective, well designed, clinical and pathophysiological study has to be undertaken to establish this fact.

It can be concluded that type 2 DM patients with complications have significantly decreased HDL-C levels and PON1 activity, possibly indicating their decreased biochemical roles in these patients.

## ACKNOWLEDGEMENTS

We thank our Dean Dr. Sripathi Rao, and Dr. S Sudhakar Nayak, professor and head, department of biochemistry for financial support.

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**DECLARATION ON COMPETING INTERESTS:** No competing Interests.

Date of Submission: **05/10/2010**  
 Peer Review Completion: **04/12/2010**  
 Date of Acceptance: **10/01/2011**  
 Date of Publication: **06/02/2011**