

## Original Article

### Study on paraoxonase 1 in type 2 diabetes mellitus

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#### Abstract

Type 2 Diabetes Mellitus (T 2 D M) occurring as a result of reduced insulin action, is seen in a larger section of population. It is also a condition of Oxidative Stress utilizing the antioxidant resources of the body. One such antioxidant is the Paraoxonase1 (PON1) enzyme associated with High Density Lipoprotein. So, the activity of PON1 may be reduced in T 2 D M. Hence, this study was taken up to analyze the status of PON I activity in patients with Type 2 Diabetes Mellitus, attending the Diabetic OP of Sree Balaji Medical College and Hospital (SBMCH). The study included 93 Type 2 Diabetic patients and 89 age and sex matched healthy controls. Paraoxonase 1 activity was assayed by Fluorimetry using the Invitrogen Molecular probes kit. There was a significant reduction in PON1 activity ( $p$  value  $>0.001$ ) along with a decrease in HDL cholesterol among the Type 2 D M patients compared to healthy controls. The progression of Diabetes Mellitus through the years reflected in a much more reduction in PON1 activity as shown by the Pearsons' correlation analysis. The results were analyzed using SPSS statistical package. It is concluded that Type 2 D M being a condition of Oxidative stress has resulted in the reduction of the antioxidant activity of enzyme PON1.

#### Introduction

Diabetes mellitus (DM) is defined as a metabolic disorder due to complete or relative insulin deficiency characterized by chronic hyperglycemia, disturbance of fat and protein metabolism. The insulin deficiency may either be due to defect in secretion or action. Type 1 DM is insulin dependent and is mainly immune mediated. Type 2 DM is non-insulin dependent diabetes mellitus. In Type 2 DM the

circulating insulin level is normal or slightly elevated or decreased, depending on the stage of the disease (1). The global epidemic is mainly Type 2 Diabetes. Hyperglycemia in Type 2 Diabetes Mellitus generates free radicals. These free radicals induce oxidative stress and in turn impair the antioxidant defense system. Antioxidant defense mechanisms include both enzymatic and non-enzymatic strategies. Common antioxidants include the vitamins A, C, E, glutathione, and the enzymes superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase (2) and the latest in the list is Paraoxonase 1 enzyme closely associated with High Density Lipoprotein. Paraoxonase is a mammalian antioxidant enzyme associated with high-density lipoprotein ("good cholesterol") in serum. It helps in preventing and reverting LDL oxidation (3). Other antioxidants

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include  $\alpha$  lipoic acid, mixed carotenoids, coenzymes  $Q_{10}$ , several bioflavonoids, antioxidant minerals (copper, zinc, manganese and selenium) and the coenzymes (folic acid, vitamins  $B_1$ ,  $B_2$ ,  $B_6$ ,  $B_{12}$ ) (4). They work in synergy with each other and against different types of free radicals. Each antioxidant has different mechanism of reducing oxidative stress, enzyme PON1 is shown to prevent and revert HDL and LDL oxidation (3). Aim of the study was to see the Serum PON1 activity in South Chennai Diabetic and healthy population and to see the relation of the enzyme activity with the duration of Type 2 DM.

## Materials and Methods

A total of ninety three (93) - type 2 diabetic patients who were not on any antioxidant therapy in the age group 30-50 yrs attending the Diabetic OP of SBMC&H were included in the study and were compared with eighty nine (89) age and sex matched healthy controls. The sample size was calculated by statistical method to be  $71 \pm 10$  in each group.

**Exclusion criteria:** a. Patients with any other chronic illness like hypertensive patients, patients with cardiovascular diseases, chronic allergy etc. b. Post-menopausal women; smokers and alcoholics.

Ethical clearance was obtained from the Ethical Clearance Committee of SBMC&H. Informed consent was signed by patients and healthy controls.

Venous blood - 4 ml was drawn from the patients in the fasting condition for the following investigations: Paraoxonase1, Fasting Plasma Sugar (FPS), lipid profile, urea, creatinine, and uric acid. Part of this blood was transferred to heparinized tube for estimation of Glycated Hemoglobin ( $HbA_{1c}$ ).

Postprandial Plasma sugar (PPPS) sample was collected as 1 ml of venous blood for blood sugar estimation. The serum was centrifuged at  $4^\circ\text{C}$  and stored in  $-80^\circ\text{C}$  for the assay of Paraoxonase.

FPS & PPPS were estimated using Glucose Oxidase Peroxidase (GOD POD) Method (Enzymatic method). The glucose (GOD-POD) KIT is based on Trinder's method in which glucose oxidase

and peroxidase are used along with phenol and 4-aminoantipyrine.

**Glycated hemoglobin:** Estimated by Ion exchange resin method.

**Total Cholesterol:** using CHOD POD liquid (Cholesterol oxidase and peroxidase). A colorimetric method.

**Triglycerides:** Colorimetric method using Glycerol Phosphate Dehydrogenase and Peroxidase (GPO-POD).

**HDL:** Diagnostic reagent for precipitation of non HDL lipoproteins in tests for determination of high density lipoprotein cholesterol (HDL-C)

**LDL:** Fried Wald formula: Total cholesterol - (VLDL+HDL -C)

**VLDL:**  $TGL/5$

**Paraoxonase 1:** was assayed by a highly sensitive Fluorimetric assay for the organophosphatase activity of Paraoxonase 1, based on the hydrolysis of a fluorogenic organophosphate analog. The assay was done using Enzchek Paraoxonase Assay kit. (Invitrogen Molecular probes, Eugene). The reagents were constituted and assay performed as per manufacturer's instructions.

**Excitation/emission maxima – 360/450 nm.**

**Statistical Analysis:** The results were expressed as mean  $\pm$  Standard Deviation. A  $p < 0.001$  was considered to be statistically significant. Statistical analysis was performed using Statistical Package for Social Sciences. One way analysis of variance (ANOVA) was used to compare the mean values. Pearson's correlation was applied to correlate between the parameters.

## Results

The Glycemic status was assessed through the analysis of Fasting plasma sugar (FPS), Post

prandial Plasma sugar (PPPS) and the Glycated hemoglobin (HbA1c). Values of all the parameters were expressed as Mean±S.D. The FPS values in diabetic subjects were (169.63±69.43 mg/dl) compared to the healthy controls (88.22±13.25 mg/dl). The difference is strongly significant. In the present study the PPPS values among diabetic subjects was (244.46±88.19 mg/dl) and in controls was (112±24.35 mg/dl). According to WHO the Normal Glycated Hemoglobin level ranges from 5-6.4%. Hb<sub>A1C</sub> of 6.5% or more is considered to be Diabetic level. Glycated Hemoglobin level in the Diabetic patients of our study was 7.22±1.03 and in controls the value was 5.61±0.64.

**The lipid profile**

Our diabetic subjects had significantly increased triglyceride levels as 185±24.56 mg/dl in comparison to controls who had 108±24.56 mg/dl. With respect to HDL though there is significant decrease in HDL among diabetic cases (36.67±5.75 mg/dl) in comparison with healthy controls of the study (40.37±3.51 mg/dl), the levels are in the normal range only. Mean LDL levels were 134±49.04 mg/dl in diabetics when compared to the healthy controls where the mean value was 109.48±27.84 mg/dl. The VLDL levels also showed significant increase among diabetics (30.36±10.12) in comparison to healthy

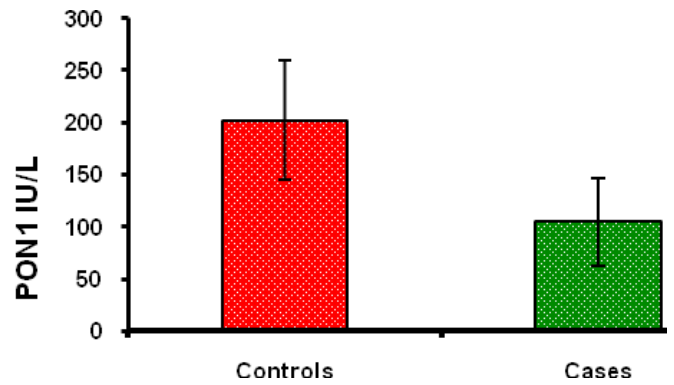
controls (21.89±4.94). The raised TGL & VLDL result in the increased LDL which is a metabolite of VLDL.

**PON1 Values**

The values of activity are expressed as mean±SD. The PON1 activity is significantly decreased in diabetic patients as 104.92±42.2 IU/L. In case of healthy controls the values are 201.98±57.31 IU/L with strongly significant p value of < 0.001.

TABLE III : A Comparison of Study outcome variable in two of groups.

Parameter	Controls	Cases	Significance
PON1 U/mL	201.98±57.31	104.92±42.21	t=13.020; P<0.001**



Graph 1 : A Comparison of PON1 activity two of groups.

TABLE IV : Pearson correlation of PON1 with study variables cases.

Pair	Cases	
	r value	p value
PON1 vs. Age in years	-0.608	<0.001**
PON1 vs. FPS (mg/dl)	-0.118	0.260
PON1 vs. PPPS	-0.100	0.342
PON1 vs. HbA1c	-0.136	0.198
PON1 vs. HDL	0.096	0.361

TABLE I : A Comparison of sugar parameters in two groups.

Parameters	Controls	Cases	Significance
FPS (mg/dl)	88.22±13.25	169.63±69.43	t=10.753; P<0.001**
PPPS (mg/dl)	112.33±24.35	244.46±88.19	t=13.499; P<0.001**
HbA1c	5.61±0.64	7.22±1.03	t=12.452; P<0.001**

Student t test.

TABLE II : A Comparison of Lipid parameters in two groups.

Parameters	Controls	Cases	Significance
Total Chol (mg/dl)	171.36±27.44	185.19±33.12	t=3.0405; P=0.003**
Triglycerides (mg/dl)	108.57±24.56	153.35±51.76	t=7.333; P<0.001**
HDL (mg/dl)	40.37±3.51	36.67±5.75	t=5.168; P<0.001**
LDL (mg/dl)	109.48±27.84	134.18±49.04	t=4.117; P<0.001**
VLDL(mg/dl)	21.89±4.94	30.36±10.12	t=7.053; P<0.001**

Student t test.

**Discussion**

The PON1 activity is genetically predetermined and has marked racial and inter individual variation and this may be the reason for different activity range among various studies (5, 6, 7). Studies across the globe show that serum PON1 activity has been found to be significantly decreased in type 1 and type 2 diabetes compared to the healthy control subjects (8, 9, 10). The protective effect of PON1 against

lipid per oxidation may be decreased in diabetic patients because of the lower enzyme activity. A study in Japan has shown decreases PON1 activities in patients with Type 2 Diabetes Mellitus though the concentration of the enzyme remains the same (11). We have seen a significant decrease in PON1 activity with the increase in duration of Diabetes Mellitus. Increased levels of Fasting Plasma Glucose in the Diabetic group indicate a poor glycemic control in these patients. This in turn leads to the increased glycation of proteins and other biomolecules. Prolonged hyperglycemia in these patients might have caused increased free radical synthesis depleting the total antioxidant stores (12). Furthermore, an increased prooxidative status in turn leads to reduced PON-1 activity due to the inhibition of PON-1 by its substrates, i.e., lipid peroxides (3).

In a study conducted at South Karnataka, Suvarna *et al* report that the levels of HDL-C and PON1 activity are decreased significantly in diabetic patients with complications in comparison to diabetics without complications (14). Further, in a study among North West Indian Punjabi diabetic population also there is decreased PON1 activity in comparison to healthy population (15).

## Conclusion

In conclusion the PON1 activity decreases in diabetes. This decrease may be due to the increasing oxidative stress in diabetes. Since the PON1 has antioxidant activity it could have been used up in the process.

## Limitations and utility

The above assays could be employed to detect complications early and revert the conditions. This would increase the longevity and quality of life of patients with diabetes. The Limitations of this study was that the assay is expensive.

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