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# ASSOCIATION OF PARAOXONASE (PON1) 55 AND 192 POLYMORPHISM WITH TYPE 2 DIABETES MELLITUS AND ITS COMPLICATIONS

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**ABSTRACT**

**Introduction:** India has more than 77 million Diabetic individuals. Environmental and genetic factors are involved in the pathogenesis of T2DM. **Aim:** to study association of paraoxonase (PON1) 55 and 192 polymorphism with type 2 diabetes mellitus and its complications. **Methods:** A cross sectional study conducted on 60 previously diagnosed type 2 diabetes mellitus patients and 30 controls in the Diabetic Care and Research Center, Department of Medicine, S.P. Medical College attached to P.B.M. Associated Group of Hospitals, Bikaner. The PCR products were analysed by agarose gel electrophoresis. Specific PCR products were purified and subjected to bi-directional sequencing with primers, to identify any alteration of sequence. **Results:** The mean age of diabetic subjects is 53.83±11.56 years and that of non-diabetic subject is 55.6±11.27 years. in diabetic subjects PON1-L55M and PON1-Q192R polymorphism present in 46.67% and 48.33% respectively. In non-diabetic subjects PON1-L55M and PON1-Q192R polymorphism present in23.33% and 26.67% respectively. In diabetic subjects having positive PON1-L55M polymorphism and negative PON1-L55M polymorphism, we found a statistically significant difference in fasting sugar, HbA1c and lipid profile (P-value<0.05). We do not find a statistically significant correlation in PON1-L55M and PON1-Q192R polymorphism and presence of nephropathy (P-value 0.672; 1.0). We found a statistically significant correlation in positive PON1-L55M polymorphism and PON1-Q192R polymorphism and presence of retinopathy (P-value 0.0413; 0.038 respectively). In cases having neuropathy a statistically significant correlation found in positive PON1-L55M polymorphism and presence of neuropathy (P-value 0.030). **Conclusion:** Presence of PON1 polymorphism may reflect a reduced capacity to detoxify pro-inflammatory oxidized lipids, leading to an increased susceptibility to develop type 2 diabetes mellitus and its complications.

**Keywords:** Paraoxonase polymorphism, type 2 diabetes mellitus, complications.

## INTRODUCTION:

India has more than 77 million Diabetic individuals. Globally, 9.3% of adult population is suffering from Diabetes mellitus.[1] The prevalence of diabetes among adults has reached approximately 20% in urban populations and approximately 10% in rural populations.[2]

As environmental and genetic factors are involved in the pathogenesis of T2DM. The majority of genes involved play a role in β-cell function. Genetic polymorphisms that have impacts on important proteins that participate in glucose metabolism and insulin secretion may also affect susceptibility to T2DM. The paraoxonase (PON) gene family is composed of three members (PON1, PON2, PON3) that are located adjacent to each other on the long arm of chromosome 7q21–22 in humans.[3,4] PON1 and PON3 are predominantly located in the circulation associated with high density lipoprotein (HDL) particles, while PON2 is an intracellular enzyme and is not present in the circulation.[4,5]

PON1, a high-density lipoprotein-associated esterase/lactonase, also endowed with the capacity to hydrolyze organophosphates, but all the three proteins prevent oxidative stress and fight inflammation. They therefore seem

central to a wide variety of human illnesses, including atherosclerosis, diabetes mellitus, mental disorders and inflammatory bowel disease.[6]
PON1 activity was found to be decreased in cardiovascular diseases[7] and in diabetes mellitus[7]. Diabetes mellitus is characterized by increased oxidative stress and damage, possibly due to the result of glycosylation of LDL by glucose.[8]Several factors may take part in these changes. Firstly, oxidative stress is accelerated and thus lipid peroxidation may contribute to vascular wall impairment[9]. Secondly, glycation of proteins including enzymes may decrease their activities in diabetes[10]

There are two common and functional single nucleotide variants in the PON1 gene: glutamine (Q) to arginine (R) substitution at codon 192 (Q192R; rs662) and leucine (L) to methionine (M) substitution at codon 55 (L55M; rs854560)[11]. According to studies, genetic variability of the PON1 position 192 and 55 and PON1 phenotype affect risk for cardiovascular disease (CVD) and cerebrovascular disease[12]. A number of studies have addressed the role of PON1 in diabetes mellitus (DM). According to studies, further association studies of the new variants will need to evaluate their role in the complex disease, T2DM and in different populations, particularly populations with a high prevalence of diabetes[13]. PON1 and PON2 have been shown to play important roles in protecting against CVD, which is the principal cause of mortality in patients with T2DM, and so it seem worthy of further investigation. Studies that have simultaneously evaluated the functional variants in two gene of PON family in T2DM are limited. It seems that there is a need for determining the genetic variants of these enzymes in different ethnic populations, because some variants were associated with diabetes, its cardiovascular complications, and could potentially influence beta cell function.[14]

**Aim:** to study association of paraoxonase (PON1) 55 and 192 polymorphism with type 2 diabetes mellitus and its complications.

**Methods:**
A cross sectional study conducted on 60 previously diagnosed type 2 diabetes mellitus patients and 30 controls in the Diabetic Care and Research Center, Department of Medicine, S.P. Medical College attached to P.B.M. Associated Group of Hospitals, Bikaner.
Subjects who gave informed consent for genetic study and previously diagnosed case of type 2 diabetes mellitus who fits in ADA 2022 guideline for diabetes were included as case in study.
Subjects not suffering from any chronic illness or autoimmune illness like CKD, CLD, CLL, Sjogren syndrome, Rheumatoid arthritis and give consent were taken as control in the study. Either case or control suffering from any acute illness in present or in past one month were excluded from study.

**Criteria for study**

1. **ADA 2022 guideline for diabetes –**
   - FPG $\geq$126 mg/dl (7.0 mmol/L). Fasting is defined as no caloric intake for 8 hours
     or
   - '2-h PG $\geq$200 mg/dl (11.1 mmol/L) during OGTT. The test was performed as described by WHO, using a glucose load containing the equivalent of 75g anhydrous glucose dissolved in water
     or
   - $HbA_{1C}\geq$6.5% (48 mmol/mol). The test was performed in a laboratory using a method that is NGSP certified and standardized to the DCCT assay
     or
   - In a patient with classic symptoms of hyperglycemia, a random plasma glucose $\geq$200 mg/dl (11.1 mmol/L).
2. **Retinopathy :** By detailed fundus examination and classified according to diabetes retinopathy study (DRS) and early treatment diabetic retinopathy study (ETDRS).
3. **Nephropathy :** Urine for albuminurea tested by urine dipstick test. Overt nephropathy was confirmed by estimation of level of blood urea, serum creatinine and macroalbuminurea.
4. **Neuropathy :** By history of numbness, paraesthesia, tingling sensation and confirmed by touch sensation with 10gm monofilament, vibration sense by biothesiometer and ankle reflex.

After taking ethical clearance patient's demographic and behaviour variables, anthropometric, clinical characteristics was recorded and filled in the proforma with the consent of patients. Blood sample was taken in three vial as 2ml sample in EDTA vials was used for genetic variant study in gene. Another 2ml sample in EDTA vial was used for HbA1c and complete blood count analysis and 3ml sample in plain vial was used for biochemical study and C-

Reactive protein quantitative assessment. blood glucose was done by glucose oxidase method and lipid profile and biochemical profile estimated enzymatically using a Hitachi automatic analyser 7600. Estimation of creatinine was done by Jaffe method and glycated haemoglobin (Hba1c) by ion exchange chromatography method.
2ml venous blood sample was taken in EDTA vial and DNA preparation was done on that. Isolated DNA was quantified by spectrophotometry method at 260-280 mm and quantified DNA was then diluted with $T_{10}$ buffer to 25nm

- **Primer used are:(Gtex portal)**
- L55M

5'-TTGAGGAAAAGCTCTAGTCCA-3'
5'-GAAAGACTTAAACTGCCAGTCC-3'

- Q192R

5'-TTGTTGCTGTGGGACCTGAG-3'
5'-AATCCTTCTGCCACCACTCG-3'

PCR amplification were carried out using thermo scientific green master mix in 25ul reaction volumes containing about 100ng of genomic DNA, 10pmol of each primer, 2mM of each Dntp, 0.5U of Taq polymerase with a standard buffer containing 1.5mM MgCl2. The PCR products were analysed by agarose gel electrophoresis. Specific PCR products were purified and subjected to bi-directional sequencing with primers, to identify any alteration of sequence.

**STATISTICAL ANALYSIS**
Appropriate statistical analysis was applied as and when required using SPSS software version 17.0. Unpaired 't' test, chi square test, were applied. A p value <0.05 is considered as significant.

**RESULTS:**
The mean age of diabetic subjects is 53.83±11.56 years and that of non-diabetic subject is 55.6±11.27 years. There 51.67% male in diabetic and 43.33% male in non diabetics. Majority of subjects from urban area (81.67%) followed by 18.33% from rural areas {as prevalence of diabetes is increasing with urbanization}.

**Table 1. Sociodemographic**

| Age | Diabetic (N=60) | | Non-Diabetic (N=30) | |
|---|---|---|---|---|
| | N | % | N | % |
| 31-40 years | 7 | 11.67 | 1 | 3.33 |
| 41-50 years | 22 | 36.67 | 11 | 36.67 |
| 51-60 years | 17 | 28.33 | 10 | 33.33 |
| ≥61 years | 14 | 23.33 | 8 | 26.67 |
| **Gender** | | | | |
| Male | 31 | 51.67 | 13 | 43.33 |
| Female | 29 | 48.33 | 17 | 56.67 |
| **Area** | | | | |
| Urban | 49 | 81.67 | 17 | 56.67 |
| Rural | 11 | 18.33 | 13 | 43.33 |

Here, in diabetic subjects PON1-L55M and PON1-Q192R polymorphism present in 46.67% and 48.33% respectively. In non-diabetic subjects PON1-L55M and PON1-Q192R polymorphism present in23.33% and 26.67% respectively. This difference is found to be statistically significant with p-value 0.0332 and 0.05 respectively.

**Table 2 Polymorphism**

| Polymorphism Positive | Diabetic (N=60) | | Non-Diabetic (N=30) | | P-value |
|---|---|---|---|---|---|
| | N | % | N | % | |
| **PON1-L55M** | 28 | 46.67 | 7 | 23.33 | 0.0332 |
| **PON1-Q192R** | 29 | 48.33 | 8 | 26.67 | 0.05 |

Here, in diabetic subjects having positive PON1-L55M polymorphism and negative PON1-L55M polymorphism, we found a statistically significant difference in fasting sugar, HbA1c and lipid profile (P-value<0.05).

**Table 3 Association of PON 55 polymorphism in diabetic patients**

| PON1-L55M-DB | | N | Mean | Std. Deviation | Sig. (2-tailed) |
|---|---|---|---|---|---|
| FBS | Positive | 28 | 182.63 | 40.05 | 0.0038 |
| | Negative | 32 | 154.79 | 31.35 | |
| HbA1c | Positive | 28 | 9.73 | 1.32 | 0.03 |
| | Negative | 32 | 9.01 | 1.26 | |
| TC | Positive | 28 | 199.86 | 42.69 | 0.013 |
| | Negative | 32 | 170.31 | 45.94 | |
| TG | Positive | 28 | 185.36 | 40.04 | 0.000 |
| | Negative | 32 | 143.56 | 44.38 | |
| HDL | Positive | 28 | 51.06 | 7.38 | 0.024 |
| | Negative | 32 | 58.16 | 15.50 | |
| LDL | Positive | 28 | 97.71 | 20.84 | 0.0098 |
| | Negative | 32 | 82.21 | 23.71 | |
| VLDL | Positive | 28 | 37.07 | 7.69 | 0.003 |
| | Negative | 32 | 28.49 | 9.41 | |

Here, in diabetic subjects having positive PON1-Q192R polymorphism and negative PON1-Q192R polymorphism, we found a statistically significant difference in fasting sugar, HbA1c and lipid profile (P-value<0.05) except HDL (P-value 0.124).

**Table 4. Association of PON 192 polymorphism in diabetic patients**

| PON1-Q192R-DB | | N | Mean | Std. Deviation | Sig. (2-tailed) |
|---|---|---|---|---|---|
| RBS | Positive | 30 | 222.20 | 49.14 | 0.001 |
| | Negative | 30 | 151.20 | 39.75 | |
| FBS | Positive | 30 | 199.60 | 72.90 | 0.001 |
| | Negative | 30 | 139.00 | 31.84 | |
| HbA1c | Positive | 30 | 9.81 | 1.38 | 0.015 |
| | Negative | 30 | 8.99 | 1.15 | |
| TC | Positive | 30 | 192.13 | 44.62 | 0.029 |
| | Negative | 30 | 176.07 | 47.73 | |
| TG | Positive | 30 | 169.00 | 23.52 | 0.035 |
| | Negative | 30 | 157.13 | 20.30 | |
| HDL | Positive | 30 | 53.59 | 10.23 | 0.124 |
| | Negative | 30 | 55.15 | 14.21 | |
| LDL | Positive | 30 | 98.45 | 25.13 | 0.026 |
| | Negative | 30 | 83.04 | 26.53 | |
| VLDL | Positive | 30 | 39.83 | 5.53 | 0.001 |
| | Negative | 30 | 31.42 | 5.65 | |

Here, we do not find a statistically significant correlation in PON1-L55M and PON1-Q192R polymorphism and presence of microalbuminuria (P-value 0.672; 1.0). In our study we have 60 diabetic cases and out of them 36 cases has microalbuminuria and out of those 36 cases 16cases have positive PON1-L55M polymorphism and 18 have positive PON1-Q192R polymorphism.

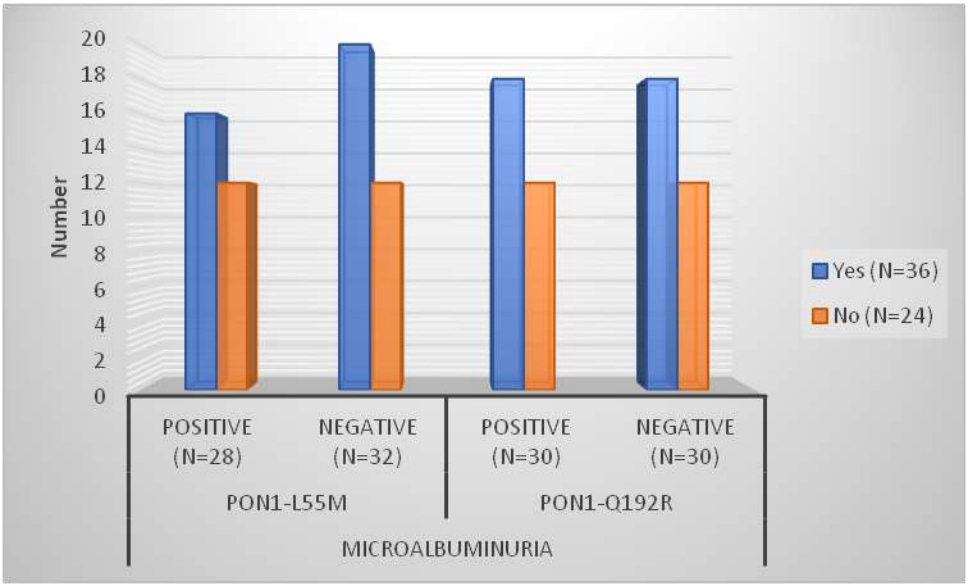


**Fig. 1 Association with Nephropathy**

Here we found a statistically significant correlation in positive PON1-L55M polymorphism and PON1-Q192R polymorphism and presence of retinopathy (P-value 0.0413; 0.038 respectively). In our study we have 60 diabetic cases and out of them 28 cases have retinopathy and out of those 28 cases 17 cases have positive PON1-L55M polymorphism and 18 has positive PON1-Q192R polymorphism. In cases having neuropathy a statistically significant correlation found in positive PON1-L55M polymorphism and presence ofneuropathy (P-value 0.030).

**Table 5 Association with Diabetic Ratinopathy and Neuropathy**

| Diabetic Retinopathy | PON1-L55M | | PON1-Q192R | |
|---|---|---|---|---|
| | Positive (n=28) | Negative (N=32) | Positive (N=30) | Negative (N=30) |
| Yes (N=28) | 17 | 11 | 18 | 10 |
| No (N=32) | 11 | 21 | 11 | 21 |
| P-value | 0.0413 | | 0.038 | |
| **Neuropathy** | | | | |
| Yes (N=36) | 20 | 14 | 20 | 14 |
| No (N=24) | 8 | 18 | 10 | 16 |
| P-value | 0.03 | | 0.118 | |

**DISCUSSION**

In our study mean age of all subjects is 54.92 years with mean age of diabetic subjects is 53.83 years and that of non-diabetic subject is 55.6 years. There are 51.67% male in diabetic, majority of subjects from urban area (81.67%) and belongs to middle class (75.56%).

Here, in diabetic subjects PON1-L55M and PON1-Q192R polymorphism present in 46.67% and 48.33% respectively (p-value=0.0332). In non-diabetic subjects PON1-L55M and PON1-Q192R polymorphism present in 23.33% and 26.67% respectively (P=0.05). The mean age of subjects having positive polymorphism of PON1-L55M is 56.15years and that of PON1-Q192R is 56.76 years, which is significantly higher (P-value 0.014; 0.022) from subjects having no polymorphism of both gene loci (50.71 years; 50.79 years respectively). Similarly, we also found that PON1-L55M polymorphism is more in higher BMI group both in diabetic and non-diabetics.

Here, in diabetic and non-diabetic subjects and having positive PON1 gene polymorphism and negative PON1 gene polymorphism the difference in lipid profile (P-value<0.05).

Concordance with our results **Durrington et al**[15] stated that serum PON1 activity is associated with HDL-cholesterol. **Mackness et al**[4] reported that PON1 paraoxonase activity in healthy subjects from Belfast was associated with total and HDL-cholesterol.

**Namitha et al**[16] positive correlation is found between HDL and salt stimulated PON activity in diabetics ($P < 0.05$), indicating decreased HDL level further decreases PON activity.

In our study we found a statistically significant correlation in PON1-L55M and PON1-Q192R gene polymorphism and presence of retinopathy (P-value 0.0413; 0.038 respectively). We do not found a statistically significant correlation in positive polymorphism of PON1-L55M and PON1-Q192R gene and presence of microalbuminuria (P-value 0.672; 1.0).

In consistent with our results **Wang et al**[16] meta-analysis showed that PON1 polymorphism was significantly associated with retinopathy. However, such an association was not detected in nephropathy, which may be due to the limited studies, various phenotypes and heterogeneity in the genetic susceptibility between nephropathy and retinopathy.

**Tsuzura et al**[17] suggest a critical role for gene polymorphism in the development of diabetic retinopathy, although, the exact molecular mechanisms remain elusive. The lower PON1 activity determined in type 2 diabetic patients has been associated with micro- and macrovascular complications of DM. **Kopprasch et al**[18] has been reported that PON1 activity is decreased in diabetic nephropathy and neuropathy. A study by **Gowda et al**[19] showed a significant decrease in PON activity in Type 2 DM patients with nephropathy but not in Type 2 DM patients without any complications.

In cases having neuropathy a statistically significant correlation found in positive polymorphism of PON1-L55M gene and presence of neuropathy (P-value 0.030). **Abbott et al**[20] reported that paraoxonase specific activity in both type 1 and type 2 diabetic populations with clinical peripheral neuropathy was significantly lower than in either of the diabetic populations without neuropathy or in the non- diabetic control subjects. The reason for this is at present unclear but was not due to a difference in glycemic control between the populations.

**CONCLUSION:**

In conclusion, presence of PON1 polymorphism may reflect a reduced capacity to detoxify pro-inflammatory oxidized lipids, leading to an increased susceptibility to develop type 2 diabetes mellitus and its complications. Present study showed a significant correlation between PON1 polymorphism and Type 2 DM and a positive correlation with HDL. Causes for the development of diabetic complications are multifactorial and increased oxidative stress precipitated by reduced PON activity adds to the pathogenesis of diabetic complications.

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