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# ASSOCIATION OF PARAOXONASE-1 LEVELS AND G ALLELE (192 A/G POLYMORPHISM) IN OCCURRENCE AND RECURRENCE OF MYOCARDIAL INFARCTION

**Biochemistry**

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## ABSTRACT

**Background and objective:** Oxidative stress plays an important role in atherosclerosis and myocardial infarction (MI). Due to antioxidant properties of Paraoxonase-1, we studied the implication of Paraoxonase-1 gene polymorphism (192A/G) on occurrence and recurrence of MI.
**Material and Methods:** 200 patients of acute MI and 100 age and sex-matched controls were included. Paraoxonase-1 genotypes were evaluated by Polymerase Chain Reaction and Restriction Fragment Length Polymorphism. Serum Paraoxonase-1 levels were estimated by ELISA.
**Result and Discussion:** Our findings were that significantly low serum Paraoxonase-1 concentration (p-value=<0.0001) was observed in patients with acute MI as compared to controls. Allelic frequency of A was more in the population that we studied but we observed that the frequency of G allele was more in patients of MI as compared to controls. Our results further suggest that individual with GG genotype were more prone to develop MI even if there is no family history of MI thus indicating that GG genotype has significant role in development of MI.
**Conclusion:** The G allele was more frequent in the MI patients than in the healthy subjects, and also there are significant reduced levels of Paraoxonase-1 in patients with G allele, thus suggesting that it may play a role in the pathogenesis of cardiac ischemia in our Indian population.

## KEYWORDS

Oxidative stress; Atherosclerosis; myocardial infarction (MI); Paraoxonase-1; polymorphism

## INTRODUCTION

Coronary Heart Disease is one of the most important contributors to the rising burden of Cerebro Vascular Disease[1]. The most common type of Coronary Heart Disease is the myocardial infarction. Recently, the incidence of myocardial infarction has been decreased in the industrialized nations due to better health systems and achievement of efficient public health strategies , however the rates are still rising in the developing countries[2].

The oxidative alteration of low density lipoprotein (LDL) in the arterial wall is thought to be the key mechanism behind the initiation and further development of atherosclerosis and thus contributes to coronary heart disease[3].

Paraoxonase-1 an aryl esterase, is a high density lipoprotein (HDL) associated enzyme which is responsible for its antioxidant properties[3]. The Paraoxonase-1 gene in humans is located on the long arm of chromosome 7, and it is a calcium-dependent antioxidant glycoprotein with a molecular mass of 43kDa and is found in serum as a component of the HDL[3]. This enzyme is synthesized in the liver and plays a significant role in preventing LDL oxidation[3].

Variable results have been seen on association of Paraoxonase-1 levels and risk of myocardial infarction. It has been reported that HDL associated Paraoxonase-1 blocks inflammatory processes by inhibiting oxidation of LDL. A significant decrease in the Paraoxonase-1 levels , accounts for the failure of HDL to protect LDL from oxidation. The genetic source of the inter-individual variability of Paraoxonase-1 activity has been attributed to the presence of an A-G polymorphism in the coding region of the gene coding for this enzyme[4]. The A/G polymorphism corresponds to arginine / glutamine polymorphism at amino acid position 192[4]. Individuals homozygous for arginine at position 192 show a significantly higher serum Paraoxonase-1 activity than those homozygous for Glutamine[5] . Another polymorphism at amino acid 55, a leucine to methionine substitution also affects Paraoxonase-1 activity[5]. Various studies investigating the association between Paraoxonase-1 genetic polymorphisms and the presence of myocardial infarction has also been reported[6,7]. Some studies reports no relationship while others suggests association between Paraoxonase-1 genetic polymorphism and myocardial infarction[8]. The relative contribution of Paraoxonase-1 192(A/G) to development of myocardial infarction in Indian population and their association with serum Paraoxonase-1 levels have not been adequately addressed. Since association between Paraoxonase-1 polymorphism and myocardial infarction varies among population with different ethnic background the objective of the present study was to look at the genotype frequencies of Paraoxonase-1 192(A/G) in Indians for risk evaluation. And also to estimate the serum Paraoxonase-1 levels to predict occurrence and recurrence of MI so that it can be applied in patient care.

### 1.Methodology:

**Place of study:**
The study is being conducted in the Department of Biochemistry, Lady Hardinge Medical College & associated SSKH in collaboration with the Department of Cardiology, G B Pant Hospital New Delhi.

**Study population:**
We enrolled 200 patients of Myocardial Infarction presenting within 24 hours of the event to Cardiology department / Emergency of GB Pant hospital. The subjects are enrolled after confirmation of the diagnosis by clinical evaluation, ECG and blood tests as per accepted criteria.

**Group A: Patients of MI who do NOT have a family history of MI**
So far we have enrolled 100 patients after informed consent, which are further sub –grouped
Group A1: Patients who had a MI for the first time (n= 63 enrolled)

Group A2: Patients who had a recurrent MI (n= 37 enrolled)

**Group B**: **Patients of MI who have a family history of MI**
So far we have enrolled 100 patients after informed consent, which are further sub –grouped

Group B1: Patients who had a MI for the first time (n= 54 enrolled)

Group B2: Patients who had a recurrent MI (n=nrolle 46 ed)

**Inclusion Criteria applied :** Myocardial Infarction within 24 hours

**Exclusion criteria applied for the selection of cases:**
Patients of Diabetes Mellitus, Cancers, Patients of Chronic liver Disease, Chronic Kidney Disease, Diseases of inflammatory

pathology like arthritis.

**2. Collection and processing of blood samples**

Five milliliter (5 ml) of peripheral blood was collected using all aseptic procedures, from all the participants within 24 hours of MI after confirmation of the diagnosis by clinical evaluation, ECG. Informed consent was taken before sampling. The sample were taken in two vacutainers, 2 ml of whole blood was used for the total DNA isolation and remaining volume of blood sample was used for the serum separation at 2000 rpm for 10 minutes. Serum was stored at minus 20 degrees (in suitable aliquots) before it was batch analyzed for various ELISA test as detailed below.

- Paraoxonase-1

Boster's human Paraoxonase-1 ELISA Kit is based on standard sandwich enzyme-linked immune-sorbent assay technology was used. Standards and test samples are added to the wells, a biotinylated detection polyclonal antibody for Paraoxonase-1 is added subsequently and then followed by washing with PBS or TBS buffer. Avidin-Biotin-Peroxidase Complex is added and unbound conjugates are washed away with PBS or TBS buffer. HRP substrate TMB is used to visualize HRP enzymatic reaction. TMB is catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the human PON1 amount of sample captured in plate.

Upon analysis, the standard graph was obtained which was further used to determine the amount in each sample.

**B) DNA isolation and Genetic Study-**

DNA was extracted from peripheral lymphocytes using the commercially available nucleic isolation kit QIAamp DNA Mini and Blood Kit (Qiagen, Chatsworth, CA, USA) following the manufactures protocol and stored at –20°C.

Polymorphisms/ mutations were assessed by PCR-RFLP using appropriate primers and restriction enzymes (*BspPI)* . The band pattern observed after RFLP for individual genotypes were: for homozygous AA only a single band i.e. 100 bp appeared on the gel, for homozygous GG two bands i.e. 65 bp, 40 bp appeared on gel and for heterozygous AG all 3 bands i.e.100 bp, 65 bp and 40 bp appeared on the gel.

**Results:**

**Table 1 : Comparison of serum Paraoxonase-1 (pg/ml) levels in various groups:**

| | | | |
|---|---|---|---|
| Family history vs without family history | Family history | 3.72±2.62 | 0.397 |
| | Without family history | 3.15±1.89 | |
| Family 1st MI vs family recurrent MI | Family 1st MI | 3.00±0.98 | 0.137 |
| | Family recurrent MI | 4.25±3.28 | |
| Without family 1st MI vs without family recurrent MI | Without family 1st MI | 3.06±2.35 | 0.723 |
| | Without recurrent MI | 3.28±0.87 | |
| Family 1st MI vs Without family 1st MI | Family 1st MI | 3.00±0.98 | 0.922 |
| | Without family 1st MI | 3.06±2.35 | |
| Patients of MI vs Control | Patients | 3.25±1.73 | <0.0001 |
| | Control | 6.18±2.07 | |

**Table 2:-Association of Paraoxonase-1(pg/ml) with genotype.**

| Paraoxonase 1(pg/ml) | A/A (n=54) | A/G (n=24) | G/G (n=20) | Total | P value | Test performed |
|---|---|---|---|---|---|---|
| Mean ± Stdev | 3.85 ± 2.82 | 2.92 ± 1.12 | 2.86 ± 1.19 | 3.43 ± 2.29 | 0.058 | Kruskal Wallis test;Chi square=5.684 |
| Median(IQR) | 3.34 (2.618-4.158) | 2.62 (2.254-2.907) | 2.8 (1.96-3.384) | 3.03 (2.257-3.948) | | |
| Range | 0.84-18.33 | 1.69-5.14 | 1.4-6.08 | 0.84-18.33 | | |

**Table 3:-Association of Paraoxonase 1(pg/ml) with alleles.**

| Paraoxonase 1(pg/ml) | A (n=108) | G (n=52) | Total | P value | Test performed |
|---|---|---|---|---|---|
| Mean ± Stdev | 3.7 ± 2.62 | 2.88 ± 1.14 | 3.43 ± 2.28 | 0.006 | Mann Whitney test;2062 |
| Median(IQR) | 3.19 (2.507-4.158) | 2.77 (2.058-3.384) | 3.03 (2.257-3.948) | | |
| Range | 0.84-18.33 | 1.4-6.08 | 0.84-18.33 | | |

**Discussion**

In recent years several studies has been done suggesting a role of Paraoxonase-1 in atherogenesis and coronary heart disease. Our current findings were that significantly low serum Paraoxonase-1 concentration (p-value=<0.0001) was observed in patients with acute MI as compared to controls (table 1).

Allelic frequency of A was more in the population that we studied but we observed that the frequency of G allele was more in patients of MI as compared to controls. Our results further suggest that individual with GG genotype were more prone to develop MI even if there is no family history of MI thus indicating that GG genotype has significant role in development of MI.

In our study population G allele was more frequent in the MI patients than in the healthy subjects. The frequency of the alleles was similar to that in some Asian[6] and Saudi Arabian[7] populations, while the frequency of the Paraoxonase-1 192 A allele was significantly higher in MI patients in some African black populations[8]. Some case-controlled studies have shown that the Paraoxonase-1 G allele is very common in MI patients, indicating that the Paraoxonase-1 192 polymorphism may be a risk factor for atherosclerosis[6], because it is seen that individuals with G allele have significantly reduced serum levels of Paraoxonase-1(table 3).

Also it was observed that there are increased chances of recurrence of MI in patients having family history of MI with GG genotype, thus suggesting that genetic susceptibility can be an explanation for recurrence of MI.

Our results also showed that the individuals carrying GG genotype have significantly low levels of Paraoxonase-1 as compared to AA and AG genotype (table 2). Previous studies, including ours, suggest that the Paraoxonase-1 GG polymorphism provides the lowest protection against LDL and HDL oxidation compared to other Paraoxonase-1 polymorphisms[3]. Moreover, Paraoxonase-1 confers antioxidant activity on HDL , therefore, when serum Paraoxonase-1 levels decreases, HDL loses its antioxidant properties which is significantly associated with the GG genotype. Consequently, it results in deposition of oxidized LDL, which further escalates the inflammatory and thrombotic process leading to MI.

In summary, the G allele was more frequent in the MI patients than in the healthy subjects, and also there are significant reduced levels of Paraoxonase-1 in patients with G allele, thus suggesting that it may play a role in the pathogenesis of cardiac ischemia in our Indian population. And also there is genetic susceptibility for recurrence of MI in individuals with GG genotype. But this study needs to be done in larger sample size to establish and strengthen this association and to be used in patient care.

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**Conflict of interest:** None declared

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