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ORIGINAL ARTICLE

# Serum Paraoxonase Activity In Normolipidaemic Patients With Acute Myocardial Infarction

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## ABSTRACT

**Background:** Although studies have demonstrated that HDL-C associated paraoxonase is involved in protection of LDL-cholesterol and other lipoproteins from the deleterious effects of oxygen free radicals in ischaemia and reperfusion, there are controversial data on the correlation between paraoxonase activity and the ischaemia process.

**Aim:** The present study was planned to evaluate the paraoxonase activity in patients with acute myocardial infarction (AMI), with normal lipid profile.

**Setting and Design:** Serum paraoxonase activities were determined in 165 normolipidaemic patients diagnosed with AMI, and 165 age and sex matched healthy volunteers served as controls.

**Material and Methods:** Serum Paraoxonase activities were measured by using an enzymatic kit manufactured by the Zeptomatrix Corporation, New York, USA, for AMI patients and controls. Also, the lipid profile was analyzed enzymatically in these subjects.

**Statistics:** The values were expressed as means ± standard deviation (SD), and data from patients and controls was compared by using student's 't'-test.

**Results and Conclusion:** Serum paraoxonase activity was significantly decreased in AMI patients as compared to controls ($p<0.001$). Also, total cholesterol levels, TC/ HDL-C ratio, triglyceride levels, LDL-cholesterol levels, LDL-C/ HDL-C ratio and TG/HDL-C ratio were significantly higher ($p<0.001$), and HDL-cholesterol levels were significantly lower in AMI patients ($p<0.001$). No correlation was observed between PON1 activity and HDL-C levels in patients and controls. These findings suggest that decreased paraoxonase activity could be due to increased oxidative stress in AMI.


**Key Words:** Acute Myocardial Infarction, Normal Lipid Profile, Paraoxonase

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## Introduction

With the explosive rise in the incidence of Coronary Artery disease (CAD), it is estimated that this will be the leading cause of morbidity and mortality in the developing world by the year 2015[1]. People hailing from the Indian subcontinent have a higher probability of death due to CAD. It is a multifactorial disease, and some predisposing factors are heredity, hyperlipidaemia, obesity, hypertension, environmental factors and life style variables like stress, smoking, alcohol consumption, etc[2].Diet, especially fat, plays an important role the development of CAD, and the risk further increases in the presence of dyslipidaemia. The lipoprotein profile has been investigated extensively in recent years, which is found to be deranged in a large proportion of CAD patients; especially in Asians showing a mixed picture of dyslipidaemia. Low density lipoprotein cholesterol (LDL) is considered as the most important risk factor of CAD. However, a significant proportion of patients have a normal lipid profile [3]. The oxidation of LDL is believed to have a central role in atherogenesis. Subendothelial accumulation of foam cells plays

a key role in the initiation of atherosclerosis. These foam cells, which may be generated by the uptake of oxidized LDL by macrophages via scavenger receptors, accumulate in fatty streaks that evolve to more complex fibro fatty or atheromatous plaques[4]. Oxidized LDL may also be involved in atherogenesis by inducing smooth muscle cell proliferation[5]. and smooth muscle foam cell generation. Under oxidative stress, not only LDL, but other serum lipids are exposed to oxidation. High density lipoprotein (HDL) is one of the most important independent protective factors for arteriosclerosis, which underlies coronary heart disease (CHD). The HDL associated Paraoxonase (PON1) enzyme is known to have protective effects on lipid peroxidation[6].Numerous cohort studies and clinical trials have confirmed the association between a low HDL-cholesterol concentration and an increased risk of CHD. Though many factors may play a role in its pathogenesis, low PON1 activity could be an independent risk factor[7]. PON1 activity is inversely related to the risk of developing an atherosclerotic lesion, which contains cholesterol-loaded macrophage foam cells. Although experimental studies have demonstrated the reduction in PON1 activity due to oxygen free radicals in ischaemia and reperfusion[8], there are controversial data on correlation between PON1 HDL-C and the ischaemia process. As it is well established that dyslipidaemia is an important contributory factor for AMI, and PON1 activity is decreased in dyslipidaemia, and so the present study was undertaken with the objective of studying the PON1 activity in normolipidaemic AMI patients, and also to observe whether PON1 activity could be an independent risk factor in this group of subjects.

## Materials and Methods

One hundred sixty five patients (males 123; females 42) with AMI and 165 age-sex matched healthy volunteers were taken for this study. The study was conducted for a period of four and half years, from April 2002 to August 2006. Informed consent was taken. Smoking habits, systolic and diastolic blood pressure, and family history of coronary heart diseases were recorded after clinical confirmation of AMI.

### Diagnostic Criteria of Patients

All the patients had their first episode of MI with diagnostic criteria: typical chest pain, specific abnormalities for MI on electrocardiogram, elevated serum creatine phosphokinase (CPK-MB) and aspartate aminotransferase enzyme levels.

### Exclusion Criteria

Patients with diabetes mellitus, renal insufficiency, hypertension, current smokers, hepatic disease, or taking lipid lowering drugs or antioxidant vitamin supplements were excluded.

### Criteria for Normolipaedemics

Normal lipid profile was defined if LDL was <130mg/dl, HDL was ≥ 35 mg/dl, Total cholesterol (TC) was <200 mg/dl and Triglycerides (TG) were <150 mg/dl[9].

Venous blood was collected after overnight fasting, EDTA was added, and samples were processed for lipid profiles.

For PON1 activity studies, blood samples were collected from patients who were admitted in the Intensive Care Unit, 4-6 hours after onset of AMI. Only normolipidaemic AMI patients were included in the study. Lipid profile (Total cholesterol, triglycerides, and HDL-cholesterol) were analyzed enzymatically using a kit obtained from (Randox Laboratories Limited, Crumlin, UK). Plasma LDL-cholesterol was determined from the values of total cholesterol and HDL-cholesterol using the following formula:

$$\text{LDL-c} = \text{TC} - \frac{\text{TG}}{5} - \text{HDL-c (mg/dl)}$$

For PON1 studies, an assay kit manufactured from Zeptometrix Corporation 872 Main Street, Buffalo New York 14202 (ZMC catalogue 0801199), was used[10],[11]. This assay is based on the principle that PON1 catalyzes the cleavage of phenyl acetate, resulting in phenol. The rate of formation of phenol is measured by monitoring the increase in absorbance at 270 nm, at 25°C. One unit of arylesterase activity is equal to 1 µM of phenol formed per minute. The activity is expressed in kU/L, based on the extinction coefficient of phenol of 1310 $M^{-1}cm^{-1}$ at 270 nm at pH 8.0 and at 25 °C. Blank samples containing water were used to correct for non-enzymatic hydrolysis.

## STATISTICAL ANALYSIS

Data on lipid profile and PON1 activity was entered in Microsoft excel for Windows 2000. The mean ± SD was obtained using excel software. The two-sample-t-test value was obtained between the patients and the control. The distribution of 't' probability was calculated depending on 'n', and the significance of the test was obtained. P values <0.001 were considered as highly significant.

## Result

PON1 activity was significantly decreased in patients with AMI as compared to controls [Table/Fig-1], [Table/Fig-2] and [Table/Fig-3] (p<0.001). Total cholesterol levels, TC/ HDL-C ratio, triglyceride levels , LDL-cholesterol levels, and LDL/ HDL-C ratio, were higher in AMI subjects as compared to controls [Table-1], (p<0.001). Also, significant differences were seen in HDL-C levels between AMI patients and controls (p<0.001). Total cholesterol levels, TC/ HDL-C ratio, and triglyceride levels were higher in both genders of AMI subjects as compared to controls [Table/Fig2]and[Table/Fig3] (p<0.001).Significant differences were seen in the HDL-C levels between AMI patients and controls, only in females[Table/Fig 3] (p<0.001). No correlation was observed between PON1 and HDL-C levels in patients and controls. LDL-cholesterol levels and LDL/ HDL-C ratio were higher in male AMI subjects as compared to controls [Table/Fig 2] (p<0.001)

(Table/Fig 1): Paraoxonase activity and lipid profile in patients and healthy controls (mean ±SD)

| Variables | Controls (n=165) | Patients (n=165) | P value (95%CI) |
|---|---|---|---|
| Age | 60.55 ± 3.98 | 61.84 ± 3.80 | 0.0037( 61.26-62.42) |
| Total Cholesterol † | 168.58 ± 12.16 | 186.44 ± 13.95 | <0.001(184.31-188.56) |
| HDL-Cholesterol † | 50.51 ± 6.78 | 41.27 ± 4.62 | <0.001(40.56-41.97) |
| TC: HDL-C* | 3.39 ± 0.36 | 4.57 ± 0.58 | <0.001(4.48-4.65) |
| Triglycerides † | 107.84 ± 11.51 | 128.96 ± 12.19 | <0.001(127.10-130.82) |
| LDL-Cholesterol † | 83.59 ± 11.95 | 119.37 ± 14.05 | <0.001(17.22-21.51) |
| LDL:HDL-C* | 1.90 ± 0.31 | 2.93 ± 0.51 | <0.001(2.85-3.00) |
| TG: HDL-C* | 2.17 ± 0.35 | 3.16 ± 0.49 | <0.01(3.086-3.234) |
| Paraoxonase activity( kU/L) | 98.42 ± 6.15 | 69.66 ± 9.99 | <0.001(68.13-71.18) |

* ratio † (mg %)

(Table/Fig 2): Paraoxonase activity and Lipid Profile in Male patients and healthy controls (mean ±SD)

| Variables | Control Male(n=123) | Male Patients (n=123) | P value(95%CI) |
|---|---|---|---|
| Age | 60.68 ± 4.14 | 61.53 ± 3.28 | 0.0366(60.95-62.10) |
| Total Cholesterol † | 168.09 ± 12.10 | 183.84 ± 13.65 | <0.001(182.41-186.25) |
| HDL-Cholesterol † | 49.90 ± 7.30 | 41.78 ± 4.88 | 0.0801(40.91-42.64) |
| TC: HDL-C* | 3.42 ± 0.30 | 4.45 ± 0.58 | <0.001(4.34-4.55) |
| Triglycerides† | 105.02 ± 10.31 | 126.22 ± 11.74 | <0.001(124.14-128.29) |
| LDL-Cholesterol † | 79.88 ± 7.98 | 116.82 ± 13.76 | <0.001(114.38-119.25) |
| LDL:HDL-C* | 1.92 ± 0.25 | 2.84 ± 0.52 | <0.001(2.74-2.93) |
| TG:HDL-C* | 2.15 ± 0.37 | 3.06 ± 0.47 | <0.01 (2.97-3.14) |
| Paraoxonase activity( kU/L) | 98.00 ± 6.29 | 69.43 ± 10.20 | <0.001(67.61-71.23) |

*ratio † (mg %)

(Table/Fig 3): Paraoxonase activity and Lipid Profile in Female patients and healthy controls (mean ±SD)

| Variables | Control Female (n=42) | Patients Female (n=42) | P value(95%CI) |
|---|---|---|---|
| Age | 60.52 ± 2.93 | 62.73 ± 4.97 | 0.0356(61.22-64.23 ) |
| Total Cholesterol † | 170.00 ± 12.35 | 194.03 ± 13.03 | <0.001(190.08-197.97 ) |
| HDL-Cholesterol † | 52.31 ± 4.58 | 39.77 ± 3.37 | <0.001(38.75-40.78 ) |
| TC: HDL-C* | 3.28 ± 0.47 | 4.96 ± 0.44 | <0.001(4.82-5.09 ) |
| Triglycerides † | 116.11 ± 10.96 | 136.99 ± 9.81 | <0.001(134.02-139.95 ) |
| LDL-Cholesterol † | 94.47 ± 14.81 | 126.86 ± 12.22 | <0.05 (123.16-130.55 ) |
| LDL:HDL-C* | 1.83 ± 0.44 | 3.21 ± 0.40 | <0.001 (3.08-3.33 ) |
| TG:HDL-C* | 2.23 ± 0.28 | 3.47 ± 0.41 | <0.001(3.34-3.59 ) |
| Paraoxonase activity( kU/L) | 100.07 ± 5.45 | 70.33 ± 9.44 | <0.001(67.47-73.18 ) |

*ratio † (mg %)

## Discussion

Involvement of oxygen free radicals (OFRs) in the pathophysiology of inflammation, ischaemia and reperfusion causing damage in tissues and organs, have been reported earlier[12].Indirect evidence of OFR generation in AMI patients is substantiated by measuring PON1 activity in them [13], and lowering of PON1 activities in AMI patients have been reported earlier[14], [15].Decrease in PON1 activity could be due to the overwhelming production of OFRs at the time of infarction. Diabetes is one of the major risk factors of AMI, as lowering of PON1 activities are observed in diabetic patients. The lack of protective effect of the PON1 enzyme on OFR's, aggravates the risk of Coronary Artery Disease in diabetes[16]. Reduction in infarct size in animal models of temporary coronary artery occlusion and reperfusion has been tried by various means of anti-free radical interventions[17].Increased OFRs generated in the early stage of MI causes decreased PON1 activity during that phase[18].In the present study, lower PON1 activities were observed in patients as compared to controls. Decreased

PON1 activities might be due to enhanced protection from free radical damage in AMI during the ischaemic process. Due to increased production of toxic free radicals, the efficiency to hydrolyze lipid peroxides by PON1 is lowered, which further decreases its specific activity, and the same were observed in the present study. Despite data linking OFR generation in reperfusion injury, scientific studies failed to prove the beneficial effect of oxygen radical scavengers or other agents on infarct size reduction[19].The present study has its limitations, as the study was conducted only on normolipidaemic AMI patients. If the PON1 activity was compared between normolipidaemic and dyslipidaemic AMI patients, then it could have been better to draw a conclusion as an independent risk factor in normolipidaemic patients, and whether there is a significant change in PON1 activities between those two groups. The decreased enzymatic antioxidants could be a compounding factor, predisposing lowered PON1 activities in AMI patients. One might even ponder that the decreased activity of PON1 might be due lower HDL levels, as PON1 is associated with HDL, but the prediction can be over ruled as the present study didn't observe any correlation between HDL and PON1 activities. The study concludes that due to overwhelming production of free radicals, there is a decreased activity of PON1, and due to its incapability to scavenge free radicals, AMI patients become more susceptible to infarction. As PON1 is associated with HDL, and as it breaks down lipid peroxides and free radicals associated with the lipoprotein, so its activity must be intact, acting as the first line of defense against the oxidized LDL molecules which is the known cause of atherosclerosis. If PON1 activities were normal in these normolipidaemic AMI patients, the probability of infarction could be decreased or delayed. Future research could be carried out on oxidative stress parameters, PON1, and inflammatory markers in both normolipidaemic and dyslipidaemic AMI patients. Measurement of PON1 activities at the baseline after two days and few months after infarction is necessary to arrive at the final conclusions about the role of PON1 in AMI.

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