

## HUMAN PARAOXONASE AND HDL-CHOLESTEROL IN PAKISTANI PATIENTS WITH ACUTE MYOCARDIAL INFARCTION AND NORMAL HEALTHY ADULTS

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### ABSTRACT

**Objective:** Human serum paraoxonase is a high density lipoprotein (HDL)-bound enzyme exhibiting antiatherogenic properties. The aim of this study was to investigate any relationship between serum paraoxonase activity and serum levels of HDL-cholesterol in Pakistani patients with acute myocardial infarction (AMI) compared to normal healthy subjects and to examine possible association between serum paraoxonase activity and AMI in Pakistani population.

**Methodology:** In a case-control study, serum paraoxonase activity and serum levels of HDL-cholesterol and LDL-cholesterol were monitored in 164 Pakistani patients with AMI and 106 normal healthy adults matched for gender, BMI and age within 10 years.

**Results:** Mean serum concentration of HDL-cholesterol and mean serum paraoxonase activity in AMI patients were not significantly different from the corresponding values in normal healthy subjects. Mean serum paraoxonase activity value was significantly lower in normal healthy subjects with low HDL-cholesterol (serum levels < 40mg/dl) compared to the value in those with normal levels of HDL-cholesterol (P=0.04). In AMI patients, paraoxonase activity was lower in subjects with low HDL-cholesterol compared to those with normal levels of HDL-cholesterol, however, the decrease was not statistically significant. Correlation analyses of the data revealed a moderate association of paraoxonase activity with HDL-cholesterol (Pearson's  $r=0.225$ ,  $P<0.01$  for AMI patients and  $r=0.281$ ,  $P<0.01$  for normal healthy controls). Seventy three percent of normal healthy subjects and 65% of AMI patients in this study had low HDL-cholesterol.

**Conclusion:** Low serum paraoxonase activity and high prevalence of low HDL-cholesterol in Pakistani population could be contributing to the high rates of coronary heart disease in this population.

**KEYWORDS:** Acute myocardial infarction, Coronary heart disease, Low HDL-cholesterol, Normal healthy adults, Pakistani, Serum paraoxonase.

Pak J Med Sci October - December 2007 (Part-I) Vol. 23 No. 5 659-664

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- \* Received for Publication: June 27, 2007
- \* Revised: August 10, 2007
- \* Revision Accepted: August 12, 2007

### INTRODUCTION

Human paraoxonase (EC. 3.1.8.1) is an arylesterase synthesized in the liver; it associates with high density lipoprotein (HDL) and has been shown to be responsible for the anti-oxidative property of HDL.<sup>1,2</sup> Since this enzyme plays an important role in preventing low density lipoprotein (LDL) oxidation, it is considered to protect against the development of coronary heart disease (CHD).<sup>3</sup> Mackness et al. have recently shown an association between activity of serum paraoxonase and CHD.<sup>4</sup> Moreover, populations at greater risk of

developing CHD have diminished serum paraoxonase levels.<sup>5</sup> Similarly, low HDL-cholesterol (serum levels <40mg/dl), a component of the metabolic syndrome, is an established risk factor for development of atherosclerosis.<sup>6,7</sup> The physical association of paraoxonases with HDL-cholesterol suggests that some of the protective effects of HDL against CHD could be due to paraoxonase activity.<sup>7</sup> Keeping this hypothesis in mind, it would be logical to assume that individuals with "low HDL-cholesterol" (<40mg/dl) would have lower activity of serum paraoxonase and therefore, could be at a greater risk of developing CHD. The present study was undertaken to investigate any relationship between serum levels of HDL-cholesterol and paraoxonase activity in Pakistani patients with acute myocardial infarction (AMI) compared to normal healthy subjects and to examine possible association between serum paraoxonase activity and AMI in Pakistani population.

#### PATIENTS AND METHODS

One hundred and sixty four consecutive Pakistani patients with AMI (age range: 30-74 years) admitted to the Armed Forces Institute of Cardiology, Rawalpindi from August 2003 to September 2003, were included in this study. They were enrolled within the index admission after confirmation of AMI diagnosis based on WHO criteria of clinical history suggestive of myocardial ischaemia, ECG indications of myocardial damage, and elevation of biochemical markers (creatinine kinase and creatine kinase-MB). All patients were assessed as having risk factors for coronary artery disease (CAD), such as diabetes mellitus, hypertension, obesity and hypercholesterolemia. Criteria for diabetes were set as an abnormal fasting blood glucose level > 125mg/dl at admission, or having taken hypoglycemic medications. All those with systolic blood pressure greater than 140mmHg and/or diastolic blood pressure of 90mmHg or those on regular antihypertensive medications were classified as hypertensive. Those having a serum cholesterol level greater than 200mg/dl were considered to have hypercholesterolemia. A body mass index (BMI)

of greater than 30 was classified as obese. Individuals who were pregnant, using antiepileptics, taking oral contraceptives, having malabsorption syndrome, suffering from tuberculosis, liver disease, uremia, or cancer were excluded from the study.

Similarly, 106 normal healthy adults (age, range: 30-70 years), who had been matched for gender, BMI, socio-economic status, and age within 10 years, were selected from the personnel of the Armed Forces Institute of Cardiology and Military Hospital, Rawalpindi. They were also assessed for the above mentioned risk factors. More stringent criteria were used for the selection of normal healthy control subjects. In addition to being matched for BMI, sex and socioeconomic background, they had no evidence of CAD or hypertension on the basis of their clinical history and physical examination. Informed consent was obtained from the participants and the study was approved by the Ethics Review Committee of the Aga Khan University.

Five ml venous blood was obtained from AMI patients as well as normal healthy subjects in vacutainer tubes. For separation of serum, tubes were kept at 25°C for 3 hours and then subjected to centrifugation at 4°C. Aliquots of serum were immediately transferred to a -70°C freezer. Analyses for glucose, total cholesterol and HDL-cholesterol were carried out within 3 months using colorimetric kit method (RANDOX UK). The concentration of LDL-cholesterol was calculated by using the Friedwald formula. Serum paraoxonase activity was determined spectrophotometrically as described by Furlong et al,<sup>8</sup> with the only difference that paraoxon was dissolved in water rather than acetone and 5µl of serum was used in each reaction. One unit of paraoxonase activity was defined as one nmole of paraoxon hydrolyzed per minute.

Values presented are means ± standard deviation (SD). Means were compared using Student's t-test. Multiple linear regressions were used to examine the relations of serum paraoxonase activity with HDL-cholesterol levels, controlling for covariates (age, sex, BMI). P<0.05 was considered significant.

## RESULTS

Table-I Show the demographic and clinical characteristics of the patients and normal healthy subjects. Male to female ratios in both normal healthy subjects and AMI patients were nearly the same. Mean age of AMI patients was higher compared to mean age in the normal healthy subjects ( $54\pm 10.7$  years vs  $44.1\pm 9.2$  years). Mean serum concentrations of HDL-cholesterol and LDL-cholesterol in AMI patients were not significantly different from mean values of these parameters in normal healthy subjects. As shown in Figure-1, mean values of serum paraoxonase activity in AMI patients and normal healthy subjects were not significantly different ( $66\pm 32$ U/ml vs  $69\pm 48$ U/ml).

Table-II shows serum paraoxonase activity in AMI patients and normal healthy subjects with low and normal HDL-cholesterol. In normal healthy subjects, paraoxonase activity values were significantly lower in subjects with low HDL-cholesterol (levels  $<40$ mg/dl) compared to those with normal levels of HDL-cholesterol ( $P=0.04$ ). In AMI patients, paraoxonase activity was lower in subjects with low HDL-cholesterol, however, the decrease was not statistically significant ( $P=0.06$ ).

Seventy three percent of normal healthy subjects and 65% of AMI patients had low HDL-cholesterol indicating a very high prevalence

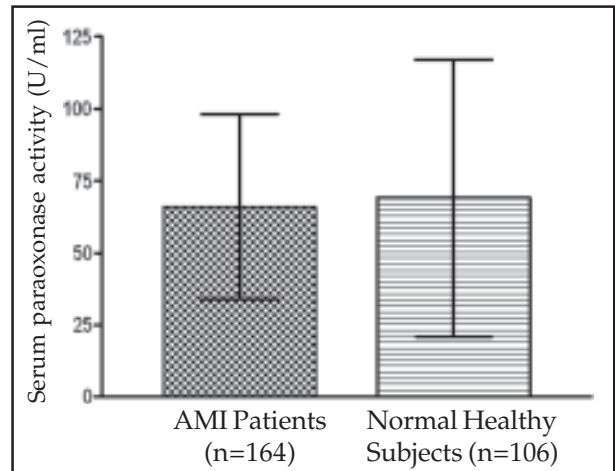


Fig-1: Serum paraoxonase activity in Pakistani AMI patients & normal healthy subjects. Comparison of the distribution of paraoxonase activity values in these two groups was carried out using Student's t-test.

of low HDL-cholesterol in Pakistani population. In a multiple linear regression model, age, gender and BMI had no significant effect on serum paraoxonase activity in both AMI patients and normal healthy subjects. Similarly, age, gender and BMI also had no significant effect on serum levels of HDL-cholesterol in both patients and normal healthy subjects. Correlation analyses revealed that serum HDL-cholesterol and paraoxonase activity were moderately correlated in both AMI patients and normal healthy subjects (Pearson's  $r = 0.225$ ,  $P < 0.01$  for AMI patients and  $r = 0.281$ ,  $P < 0.01$  for normal healthy controls).

Table-I: Demographic and clinical characteristics of normal healthy subjects and AMI patients

Variable	Normal healthy subjects(n=106)		Patients (n=164)	
	Value (mean±SD)	Frequency (%)	Value (mean±SD)	Frequency (%)
Age (Years)	44.1±9.2 (30-70)		54.2±10.7 (30-74)	
Gender				
	Male	85 (80.2)		128(78.0)
	Female	21(19.8)		36(22.0)
BMI (kg/m <sup>2</sup> )	23.2±3.9		25.7±4.4	
Hypertensive	Yes	0		78(47.6)
	No	106(100)		86(52.4)
Glucose (mg/dl)	84±24		127±79	
Cholesterol (mg/dl)	159±42		179±62	
HDL (mg/dl)	36.1±10.2		39.8±31	
LDL (mg/dl)	95±42		102±45	

Table-II: Serum paraoxonase activity in AMI patients and normal healthy subjects with low HDL-cholesterol (&lt;40 mg/dl) and normal levels of HDL-cholesterol

Group	Status of HDL-cholesterol(mg/dl)	Number (n)	Mean±SD	
			Paraoxonase activity(U/ml)	p-value*
AMI patients	<40	108	63±31	0.06
	≥40	56	73±32	
Normal healthy subjects	<40	76	61±35	0.04
	≥40	30	90±70	

\* p-value compares the two values of paraoxonase activity in each group (AMI patients or normal healthy subjects) by Student's t-test.

## DISCUSSION

This case-control study was designed to investigate any relationship between human serum paraoxonase activity and CAD in a Pakistani population. Our results showed no significant difference in serum paraoxonase activity in normal healthy subjects and AMI patients. However, the mean enzyme activity values of serum paraoxonase in our population (both normal subjects and AMI patients) were significantly less than most values reported in the West.<sup>5,9</sup> Mackness et al. have shown that serum paraoxonase concentration in Belfast population (which has 3-fold greater rate of CHD than the Toulouse population) is significantly lower than levels in Toulouse.<sup>5</sup> In a study by Carro-Ciampi et al., Canadian Indians (with high prevalence of CHD) were also found to have lower paraoxonase activity compared to Caucasians.<sup>10</sup> This indicated that populations differed in terms of their serum paraoxonase activity/concentration, hence susceptibility to developing CHD.

Pakistani people belong to a South Asian population that has the highest known rate of CAD.<sup>11</sup> Low HDL-cholesterol and hypertriglyceridemia appear to be the major lipid abnormalities in Pakistani normal healthy subjects as well as AMI patients.<sup>12,13</sup>

Since serum paraoxonase is an HDL-associated enzyme, individuals with low HDL-cholesterol would be expected to have compromised serum paraoxonase activity. Therefore, in order to investigate any relationship between serum paraoxonase activity and serum HDL-cholesterol, we divided cases and controls into

two groups: those with low HDL-cholesterol (levels < 40mg/dl) and those with normal levels of HDL-cholesterol. Analysis of the data revealed that in both AMI patients and normal healthy subjects, individuals with low HDL-cholesterol had lower levels of paraoxonase activity compared to individuals with normal levels of HDL-cholesterol. This was suggestive of an association between serum paraoxonase activity and HDL-cholesterol. Correlation analyses revealed that serum HDL-cholesterol and paraoxonase activity were moderately associated in both AMI patients and normal healthy subjects (Pearson's  $r=0.225$ ,  $P<0.01$  for AMI patients and  $r=0.281$ ,  $P<0.01$  for normal healthy subjects). As there was no difference in mean serum levels of HDL-cholesterol between AMI patients and normal healthy subjects, serum paraoxonase activities were also not expected to be different in the two groups. Our results indicating that paraoxonase activities in AMI patients and normal healthy subjects are not significantly different conform well to those reported by Rahmani et al. and Wang et al., in Iranians and Chinese, respectively.<sup>14,15</sup> It has been mentioned above that compared to most Caucasian populations (exception being a Spanish population having low paraoxonase activity in plasma,  $70.23\pm 16.2U/ml$ ),<sup>16</sup> Pakistani population has lower serum paraoxonase activity. In a study on urban Indians by Singh et al., northwestern Indians also have been shown to have low paraoxonase activity ( $70.43\pm 30.41U/ml$ ) in plasma.<sup>17</sup> This observation is in line with the results of several studies reporting high

prevalence of low HDL-cholesterol and CHD among urban Indians.<sup>18</sup> Therefore, low HDL-cholesterol and compromised serum paraoxonase activity could be contributing to the development of atherosclerosis in Pakistani individuals – apparently healthy as well as AMI patients.

The definition of low HDL-cholesterol has undergone a change over the past 10 years. Initially, levels of HDL-cholesterol below 35mg/dl in men were considered falling into the category of “low HDL-cholesterol”. However, according to the Third Report of the National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATPIII), serum levels less than 40mg/dl in men were considered below normal.<sup>19</sup> Since then, most studies report low HDL-cholesterol to be serum levels <40mg/dl in men.<sup>20,21</sup> Therefore, in this study, we define “low HDL-cholesterol” as serum levels below 40mg/dl. With this definition of low HDL-cholesterol, 73% of apparently normal healthy adults and 65% of AMI patients in this study could be classified as having low HDL-cholesterol. Keeping in view results of some of the studies carried out on Western populations, these values for the prevalence of low HDL-cholesterol in a population are very high.<sup>22</sup> The difference between the mean age values in cases and controls is a limitation of this study, however, our analyses also show that age, gender and BMI had no significant effect on serum paraoxonase activity in both AMI patients and normal healthy subjects.

On the basis of our data, compared to several populations elsewhere, low serum paraoxonase activity and high prevalence of low HDL-cholesterol occur in Pakistan. These deviations from global norms could be contributing to the high rates of CHD in Pakistan. Drugs, such as simvastatin, omega 3-polyunsaturated fatty acids and aspirin which increase serum levels of paraoxonase would be of value in controlling the development of atherosclerosis in such populations.<sup>23-25</sup>

Further studies, especially at the community level, would be required to ascertain the anti-atherogenic role of paraoxonase activity in

Pakistani population, preferably focusing on subpopulations/ethnic groups which have known high rates of cardiovascular disease.

## CONCLUSION

Pakistani population has the highest known rate of CAD. There is high prevalence of low HDL-cholesterol in this population. Moderate association between serum paraoxonase activity and HDL-cholesterol is suggestive that low paraoxonase activity in this population (compared to most Western populations) could be contributing to the early development of atherosclerosis in Pakistanis.

## ACKNOWLEDGEMENTS

We gratefully acknowledge the help of Brig. Dr. Hamid Shafiq, Armed Forces Institute of Cardiology, Rawalpindi and Mr. Majid Shafiq for providing the clinical and normal samples. We also thank Professor John D Connor for his valuable suggestions. The study was supported by a grant from the Pakistan Academy of Sciences/Higher Education Commission, Pakistan to Dr. M. Perwaiz Iqbal.

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