Paraoxonase (PON1) activity in north west Indian Punjabis with coronary artery disease & type 2 diabetes mellitus


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Background & objectives: Paraoxonase (PON1), an arylesterase is associated with high density lipoprotein cholesterol (HDL-C). PON1 prevents low density lipo-protein cholesterol (LDL-C) from peroxidation and can also hydrolyze lipid peroxides, thereby providing protection against atherosclerosis and coronary artery disease (CAD). The incidence of CAD is known to be high in north western Indian Punjabis. Though many factors may play a role in its pathogenesis, low PON1 activity could be an independent risk factor. We carried out this study to determine PON1 activity in north-west Indian Punjabi patients with CAD with and without type 2 diabetes mellitus and compared with healthy individuals.

Methods: A total of 120 patients with angiographically proven CAD (57 with and 63 without type II diabetes mellitus) and 19 healthy controls were studied for plasma PON1 activity and lipid variables. Comparison was undertaken between CAD patients and healthy controls and between CAD patients with and without type II DM.

Results: Significantly lower plasma PON1 activity ($P < 0.05$) along with lower HDL-C ($P < 0.001$) and higher LDL-C ($P < 0.05$) levels were observed in CAD patients as compared to healthy controls. On univariate analysis of variance after adjusting for age and sex, no significant difference could be observed between PON1 activity and age and sex. On discriminant analysis, no clear cut-off could be observed in PON1 activity between patients CAD and controls. Similarly between CAD with and without patients type II diabetes mellitus, there was no significant difference in PON1 activity and lipids.

Interpretation & conclusion: The low plasma PON1 activity irrespective of being diabetic may be an independent risk factor for CAD in north-western Indian Punjabi population. Similar studies involving larger samples in different ethnic groups in India need to be done to find out the role of PON1 activity in CAD.

Key words Coronary artery disease - north western Indian Punjabis - paraoxonase activity

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Coronary artery disease (CAD) has a multifactorial aetiology involving physiological, environmental and genetic factors. Knowledge about the link between paraoxonase (PON1) activity and atherosclerosis comes largely from the biological rather than epidemiological studies as there is evidence that peroxidation of low density lipoprotein cholesterol (LDL-C) is an important risk factor for atherosclerosis. High density lipoprotein cholesterol (HDL-C) is protective against CAD but the exact mechanisms underlying its protective effect are not understood. One plausible explanation is that HDL-C prevents peroxidation of LDL-C which has been implicated in the development of CAD as lipid peroxides are atherogenic. This antioxidant effect of HDL-C seems to be mediated through its enzymes in particular, PON1 which catalyzes the hydrolysis of lipid peroxides and also acts as a potent hydrolyser of other substrates including the active metabolites of organophosphates and certain drugs.

The PON1 activity is genetically determined and has marked racial and inter-individual variation. The PON1 gene has two common polymorphisms in the coding region and five in promoter region. There are conflicting data regarding an association between PON1 gene polymorphisms and CAD. Although some of these controversial results can be explained by the type of population studied, dietary habits, environmental differences, differences in study design could also be playing a role. In a recent study it has been suggested that PON1 status, i.e., activity and/or concentration, is more closely related to CAD than genotype alone. As PON1 activity plays a preventive role in atherosclerosis by protecting against lipid peroxidation and by hydrolyzing lipid peroxides and as north west Indian Punjabi’s have a high incidence of CAD and are generally low producers of PON1 as determined by dual substrate in our earlier study, the present study was undertaken to find PON1 activity in north-west Indian Punjabi patients with angiographically proven CAD with and without type 2 diabetes mellitus and compared it with that in healthy controls and also tried to find its correlation with lipid variables in them.

Material & Methods

One hundred and twenty consecutive north-west Indian Punjabi adults (men = 97, women = 23) with angiographically proven and stable CAD attending were studied between July 2001 and June 2002 at Postgraduate Institute of Medical Education & Research (PGIMER), Chandigarh. Any patient with acute coronary event 4 wk prior to angiography was excluded from the study. Of these, 57 had type II diabetes mellitus (men = 46; women = 11) and 63 were without type II diabetes mellitus (men = 51; women = 12). One hundred and ninety one healthy individuals (men = 146; women = 45), who did not have CAD and diabetes, and unrelated to patients, were included as healthy controls. The CAD in them was ruled out by history, clinical examination and electrocardiography whereas diabetes was ruled out by fasting blood sugar (≥ 126 mg/dl). They were either health workers or healthy attendants of patients admitted to the Nehru Hospital attached to PGIMER, Chandigarh. Any individual with history of ischaemic heart disease, diabetes mellitus, hypertension, tuberculosis, malnutrition, chronic obstructive pulmonary disease or HIV were excluded from this group.

Ten ml of blood was withdrawn from the antecubital vein, of which 5 ml was heparanized, put into centrifuge tubes and the plasma was separated. The plasma and sera were then stored at -20°C till assays for enzymes and lipids were carried out within 2 wk of collection of samples. The paraoxonase activity was estimated by a modification of Eckerson’s method using paraoxon as substrate. The total serum cholesterol (TC), HDL-C and triglycerides (TG) were estimated by enzymatic methods using autozyme kits manufactured by Accurex Biomedical Limited, (Mumbai) and LDL-C was determined by Friedwald’s formula.

The paraoxon was obtained from Sigma Chemicals Co, USA, other chemicals were of analytical grade (obtained from E Merck, India). The statistical analysis of data was done by Student t-test, Mann Whitney test, Pearson and Spearman correlation, univariate analysis of variance and discriminant analysis. The significance was defined at P < 0.05. The protocol was approved by institute’s ethics committee.

Results

The mean age of healthy controls was 48.5 ± 9.7 yr (range 28-70 yr) with men 47.3 ± 9.99 and women 52.1 ± 7.68 yr, respectively. In patients with CAD with type II diabetes mellitus, it was 57.4 ± 6.0 (range 36-65 yr, men 54±8.0 and women 54 ± 7.0 yr). In CAD patients without type II diabetes mellitus it was 56 ± 6.0 (men 59 ± 6.0 and women 59 ± 3.0 yr). The plasma PON1 activity was significantly lower in patients with CAD as compared to healthy controls (P < 0.05) and this was accompanied by significantly higher LDL-C (P < 0.05) and significantly lower HDL-C (P < 0.001) (Table I). However, no correlation could be observed between lipid...
On univariate analysis of variance after adjusting for age and sex, no significant difference could be observed between plasma PON1 activity and age and sex. On discriminant analysis, no clear cut-off could be observed for PON1 activity which predisposes to CAD, between healthy controls and CAD patients. On classifying data on the basis of plasma PON1 activity derived from average of mean values for two populations as 61.5 n mol/min/ml plasma showed sensitivity of 63.33 per cent and specificity of 46.6 per cent with a total predictivity of 57.2 per cent, and when average of median for two populations was undertaken i.e., 56.0 n mol/ml/min the values were 58.33, 55.0, and 56.3 per cent, respectively.

Comparison of these variables between 57 patients with CAD and type II diabetes mellitus and 63 CAD patients without type II diabetes, revealed no significant difference in plasma PON1 activity and LDL-C and HDL-C levels (Table II).

### Discussion

The paraoxonase gene family has at least three members PON1, PON2, PON3 of which the PON1 plays an important role as its product paraoxonase is exclusively bound to HDL. Its activity varies widely in population and this variation has been attributed to glutamine (Gln) arginine (Arg) polymorphism at amino acid position 192 and leucine/methionine polymorphism at position 55 of PON1 protein. A strong association between polymorphism in the PON1 gene and PON1 activity has been found. In Chinese Han population, the PON1-162G/A and R 160- G polymorphisms were found to be independently associated with CAD rather than PON1 192 polymorphism. Though there have been many case control studies to test the hypothesis that the 192 R allele of the PON1 gene is associated with CAD, in a recent metaanalysis, it was found that the PON1-192R allele though was significantly related to the presence of CAD, there was evidence of publication bias and it has been suggested that the PON1 Q 192 R polymorphism is not associated with CAD risk in Caucasian women or men.

Many case-control studies have found that serum PON1 activity was more closely related to risk of CAD than genotype alone. Diminished protection of LDL against oxidation by HDL from patients with coronary atherosclerosis was reported. Serum PON1 activity decreased with in 2 h of the onset of symptoms of acute myocardial infarction and remained low subsequently suggesting that the decreased activity may have preceded the event. Furthermore, the lower activity in patients with myocardial infarction compared with controls was substantially greater than could be accounted by PON1 polymorphism. Two large case-control studies in which PON1 activity and genotype were measured in patients with angiographically proven CAD and controls concluded that serum PON1 activity was more closely

### Table I. Plasma paraoxonase activity and serum lipid variables in healthy controls and patients with CAD

<table>
<thead>
<tr>
<th>Variable</th>
<th>Controls (N=191)</th>
<th>CAD (N=120)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PON1</td>
<td>64.92±32.92</td>
<td>57.19±29.62*</td>
</tr>
<tr>
<td>TC (mg/dl)</td>
<td>155.38±42.99</td>
<td>160.81±47.36</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>41.82±9.11</td>
<td>36.92±8.15*</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>84.77±41.71</td>
<td>97.59±43.54**</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>150.40±105.69</td>
<td>131.45±58.54</td>
</tr>
</tbody>
</table>

PON1, Paraoxonase activity (n mol/ min/ml plasma); TC, total cholesterol; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; TG, triglycerides

\*<0.05, **<0.01, *<0.001 compared to controls

### Table II. Plasma paraoxonase activity and serum lipid variables in CAD patients with and without type II diabetes

<table>
<thead>
<tr>
<th>Variable</th>
<th>CAD without type II DM (N=63)</th>
<th>CAD with type II DM (N=57)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PON1</td>
<td>60.66±32.35</td>
<td>58.36±26.03</td>
</tr>
<tr>
<td>TC (mg/dl)</td>
<td>160.50±44.89</td>
<td>161.15±50.36</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>36.52±7.09</td>
<td>39.37±9.23</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>97.99±41.72</td>
<td>97.14±45.84</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>129.87±59.08</td>
<td>133.19±58.43</td>
</tr>
</tbody>
</table>

PON1, paraoxonase activity (n mol/ min/ml plasma); TC, total cholesterol; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; TG, triglycerides
related to the presence of CAD than the PON1-192 genotype. A prospective study i.e., Caerphilly prospective study involving cohort of men aged 49 to 65 yr observed for CAD event over 15 yr revealed that PON1 activity was 20% lower in men who had coronary event and low PON1 activity was considered as independent risk factor. In a study from Antalya region of Turkey involving 108 patients with CAD and 64 controls, decreased serum PON1 activity and increased lipid peroxidation indicators were observed. However, these case-control studies can give misleading results because of survivor effects, bias in case selection and effect of the disease and its treatment.

We earlier found that PON phenotype did not significantly influence the lipid profile. However, a significant negative correlation between the PON1 activity and total cholesterol (TC) and LDL-C suggested that low PON1 activity could be a risk factor for atherosclerosis. In our present study we found that PON1 activity was lower in angiographically proven CAD north west Indian Punjabis as compared to healthy controls and this was associated with higher LDL and low HDL similar to our previous study. However, between diabetic and non diabetics with CAD, no difference in activity or lipids could be observed. In a study by Mackness et al., significantly lower PON1 activity was seen in patients with both familial hyperlipidaemia and insulin dependant diabetes mellitus as compared to healthy controls. In another study, the same group observed lower PON1 activity in 252 patients with non-insulin dependant diabetes as compared to 282 non diabetic controls. In other two studies, association between low PON1 activity and impaired glucose metabolism and type II diabetes mellitus has not been observed.

Ruiz et al., in 434 French patients with non insulin dependant diabetes mellitus (171 having CAD and 231 no CAD) found BB genotype of PON1 polymorphism to be an independent risk factor for CAD. Odowara et al. in 164 Japanese patients with CAD and type 2 showed a similar association. However, this association was not observed in Chinese patients by Ko et al. Sanghera et al. observed this association in Singapore Asian Indians but not in Chinese patients with CAD. It has been suggested that high serum activity of PON1 may reflect a tendency to oxidative stress which together with modified capacity to eliminate lipid peroxides in diabetics could place them at higher risk for CAD. However, we could not find any difference in PON1 activity in CAD patients with and without diabetes. It is quite possible that ethnic differences play a role in this. The mean activity of PON1 in our healthy controls was much lower compared to healthy Belfast and Toulouse populations. In addition to ethnicity, dietary factors could also be playing a role in our population as diet in north-west Indian Punjabi’s is very different from that in Western population. However, there is a need for longitudinal prospective studies as to rule out the survival bias.

In conclusion, our study shows low PON1 activity in north-west Indian patients with CAD as compared to healthy controls. There is a need for more studies involving larger number of patients along with PON1 concentration and genotyping in other ethnic groups in India as well to determine the role of PON1 activity, and gene polymorphisms in pathogenesis of CAD.

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References


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