

# PARAOXONASE AND ARYLESTERASE ACTIVITY AMONG HYPERCHOLESTEROLEMIC PATIENTS

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## ABSTRACT

Human serum paraoxonase (PON) associated with high density lipoprotein (HDL), has been postulated to have a role in protecting low-density lipoprotein (LDL) against oxidative damage, which has led to the proposal that PON is an anti-atherogenic and anti-inflammatory enzyme. It has genetic polymorphism at the 191 (R → Q) and two alloenzymes A and B and three phenotypes A, B, and AB. We examined the activity of arylesterase and paraoxonase among healthy individuals and in patients with hypercholesterolemia. Equal activity distribution was observed among males and females for both enzymes. Arylesterase activity displayed a normal distribution in the population under study. However, paraoxonase activity demonstrated a three modal activity. Population distribution of A, B and AB polymorphism were 46, 10 and 44 percent respectively. A significant direct correlation between paraoxonase activity and triglyceride levels was observed ( $p < 0.05$ ) whereas arylesterase activity was similar among hyperlipidemic and control groups. The lack of a significant relationship between lipids and lipoproteins and PON phenotypes, suggests that PON phenotypes do not influence the lipid profile.

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**Keywords:** Paraoxonase, Arylesterase, Lipids, Lipoproteins, Arteriosclerosis, Polymorphism.

## INTRODUCTION

The oxidative modification of low-density lipoprotein (LDL) in the artery wall is currently believed to be central to the pathogenesis of atherosclerosis.<sup>1</sup> Therefore mechanisms that prevent the oxidation of LDL have received special attention in recent years. One such mechanism is the prevention of LDL oxidation by high-density lipoprotein (HDL).<sup>2</sup> In this process, the potential involvement of some HDL-associated enzymes, including paraoxonase (PON) (E.C. 3.1.8.1) is considered to play an important role in preventing LDL oxidation.<sup>3</sup> PON is a Ca-dependent ester hy-

drolase enzyme, which contributes significantly to the detoxification of several organo-phosphorous compounds. The insecticides parathion, chlorpyrifos and diazinone are bioactivated to potent cholinesterase inhibitors by cytochrome P-450 systems.<sup>4</sup> The resulting oxon forms can be hydrolyzed by PON, which also hydrolyses the nerve agents soman and sarin.<sup>5</sup> PON (aryldialkylphosphatase) is so called because paraoxon is the substrate commonly used to measure enzyme activity; however its natural substrate is unknown.

Purified human serum PON has a molecular mass of 43-45kDa and contains three carbohydrate chains accounting for 15.8% of its weight.<sup>6</sup> It is exclusively bound to HDL in the human plasma.<sup>7,8</sup> The amino acid sequence is highly conserved among animal species, suggesting an important metabolic role for this enzyme.<sup>9</sup> There is a wide variation in

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serum PON activity within and between population groups. A common polymorphism due to an amino acid substitution {Gln(Q) → Arg(R)} at either codon 191 or 192 is considered to be a major determinant of variation in serum PON activity; individuals with Gln (A allele) have lower enzymatic activity than individuals with Arg (B allele).<sup>10</sup> The discrepancy in the numbering of the polymorphic amino acid position depends on the exclusion (codon 191) or inclusion (192) of the initiator methionine residue on the N-terminal.

There is now growing evidence that PON plays an important role in lipoprotein metabolism and thus may affect the risk of coronary artery disease (CAD) and atherosclerosis in the general population. First, PON is an important structural component of the apolipoprotein (apo) A-I-containing lipoprotein subpopulation of HDL particles, although the physiological relevance of this biochemical association has not been determined. Second, PON purified from human HDL decreases the oxidative modification of LDL *in vitro*.<sup>11</sup> Third, plasma PON activity is significantly related to variation in plasma concentrations of triglycerides (TG), LDL cholesterol (LDL-C), apoA-II, and apoB.<sup>12</sup> For these reasons, we were interested in identifying associations between the arylesterase and PON activity and fasting serum cholesterol (TC), TG and lipoprotein variables.

### MATERIAL AND METHODS

#### Study subjects

The study sample comprised 187 unrelated Iranian volunteers (82 men and 105 women) 30 to 80 years old. Volunteers were either staff of Kashan Central Laboratory or attending for a routine laboratory check. All subjects were free of ischemic heart disease, as defined by a lack of history of angina and no previous myocardial infarction. A detailed family history of peripheral vascular disease, diabetes mellitus, renal disease, hepatic disease and hypertension or receiving any medication were obtained and were excluded from the study. Those with cholesterol levels of less than 200mg/dL were selected as control group. At baseline, when blood was collected for lipid profile, none of the subjects were taking lipid-lowering medication.

#### Biochemical analysis

Venous blood was obtained from subjects between 9 and 10 a.m. after 12-hr fast. Sera were obtained by low-speed centrifugation. Serum was stored at -20°C before further analysis. Serum glucose, TG, and TC were measured by enzymatic methods (Technicon Co., Terry Haute New Jersey, USA). TG levels were quantified by an enzymatic colorimetric procedure using lipase, glycerol-kinase and glycerol-3-phosphate-oxidase. HDL-cholesterol (HDL-C) was measured as cholesterol after precipitation of apolipoprotein B-containing lipoproteins with phosphotungstic-Mg<sup>+2</sup> (Technicon Co. Terry Haute New Jersey, USA).<sup>13</sup> LDL-C

was calculated using the Friedewald formula.<sup>14</sup>

Arylesterase activity was measured with phenylacetate as a substrate as described previously,<sup>15</sup> except that the stock substrate solution (0.9mM Tris-HCl, pH 8.0, 0.9mM CaCl<sub>2</sub> and 1mM phenylacetate) was prepared fresh each hour, kept in tightly closed bottle, and mixed vigorously before use. The assay was initiated by the addition of a 1:1 dilution of serum with double distilled water to the reaction mixture. The absorbance was continuously monitored at 270nm at 25°C (Jenway Co.). The molar extinction coefficient of 1,367 was used for calculations of activity and unites were expressed as micromolar of phenylacetate hydrolyzed per minute.

PON activity was measured according to Wehner's method.<sup>16</sup> The reaction was initiated by adding serum to 1mL Tris-HCl buffer (100mM, pH 8.0) containing 2mM CaCl<sub>2</sub> and 5.5 mM paraoxon (0,0-diethyl-0-p-nitrophenylphosphate; Sigma Chemicals Co. St. Louis, USA). The rate of formation of p-nitrophenol was determined at 405nm, 25°C with the use of a continuously recording UV/VIS spectrophotometer (Jenway Co.) (CV<6%). A molar extinction coefficient of 18.05×10<sup>3</sup> was used for calculation of enzyme activity. One unit of PON activity is defined as 1nmol 4-nitrophenol formed per minute under the assay condition. PON phenotype was classified according to its activity to type A (homozygous A; 25-100U/L); type B (homozygous B; 300-500U/L) and type AB (heterozygous AB; 100-300U/L). Serum protein content was determined according to Bradford's method.<sup>17</sup>

#### Statistical analysis

The results are presented as mean ± S.E.M. Statistical analysis was conducted using the SPSS statistical package for Windows 95. Differences in parameters between genotypes variables was sought by Student's t-test for variables. Logarithmically transformed total cholesterol, LDL-C, and HDL-C have distributions that were not significantly different from normal. After triglyceride values were transformed as described,<sup>18-20</sup> their distribution was not significantly different from normal. The significance of the difference with respect to biochemical characteristics between the genotype groups was calculated using one-way analysis of variance (ANOVA). Chi-square test was used to compare genotype distribution and allele frequencies between the control subjects and hypercholesterolemic patients and to determine Hardy-Weinberg's equilibrium in the genotype distribution.

### RESULTS

Arylesterase activity was determined in selected subjects (105 females and 82 males). The mean activity was 446.35 and 478.27nmol/min/mg protein for females and males respectively (Table I). No significant difference was observed in females and males with respect to the arylesterase activ-

ity. Logarithmic activity of PON was determined among females and males (0.322 and 0.307 for females and males respectively) and no significant difference was observed. In order to assess the effect of lipids and lipoproteins on the activity of arylesterase and PON, the individuals were divided into two groups of high TC level (>200mg/dL) and normal TC (<200mg/dL). On the basis of NCEP (National Cholesterol Education Program) guidelines, similar designation was defined for TG (200mg/dL), LDL-C (130mg/dL) and HDL-C (35mg/dL). No significant difference was observed in the arylesterase activity in the groups with normal and high TC, TG, LDL-C and HDL-C levels (Table II).

Similarly, the effects of various lipid and lipoprotein levels were compared to the PON activity. There was significant difference in PON activity between normal and high TC, LDL-C and HDL-C levels. A significant difference was observed between normal and high levels of TG and PON activity ( $p<0.018$ ). Comparison was made between arylesterase/paraoxonase ratio to the normal and high TC, TG, LDL-C and HDL-C levels. Significant difference was observed in the ratio of arylesterase/paraoxonase ratio and normal and high levels of TG ( $p<0.017$ ) (Table II).

Arylesterase activity distribution had a normal distribution in the population studied (Fig. 1). The activity distribution of PON enzyme in our study obeys Hardy-Weinberg model for two-allele system (three phenotypes) (Fig. 2). In

order to determine the relationship between PON phenotypes and lipid and lipoprotein levels, PON activities were classified into three groups; PON activity less than 100U/L (type A), above 300U/L (type B) and in 100-300U/L range (type AB). One-way analysis of variations of three PON phenotypes and lipid and lipoprotein levels displayed significant difference between PON phenotypes and TG levels. Individuals with type B had significant high levels of TG in their blood ( $p<0.05$ ) (Table II). Analysis variation of three phenotypes and the HDL-C among female patients showed a significant difference ( $p<0.05$ ).

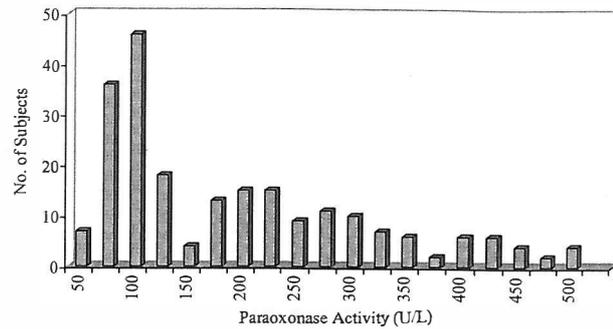


Fig. 1. Arylesterase activity distribution in Iranian subjects.

Table I: Mean values for lipids, lipoproteins, arylesterase and paraoxonase activity.

	Females	Males	Population
Number	105	82	187
Age	49.15+/-11.42	51.72+/-14.82	50.28+/-12.78
Total Cholesterol (mg/dL)	216.4+/-43.35	206.9+/-43.9	206.6+/-43.7
Triglyceride (mg/dL)	217.16+/-115.5	202.8+/-110.8	206.6+/-102.7
HDL-Cholesterol (mg/dL)	41.6+/-9.2	39.3+/-11.1	40.61+/-10.1
LDL-Cholesterol (mg/dL)	131.6+/-40.3	126.4+/-45.7	129.4+/-42.7
Arylesterase (U/L)	446.38+/-116.19	478.27+/-91.88	460.36+/-109.05
Paraoxonase (nmol/min/mg protein)	2.54+/-1.35	2.59+/-1.36	2.56+/-1.31
Paraoxonase U/L	159.9+/-82.7	162.3+/-82.7	160.9+/-79.9
Log Paraoxonase	0.31+/-0.27	0.32+/-0.28	0.316+/-0.271
Arylesterase / Paraoxonase	0.12+/-0.1	0.12+/-0.1	0.12+/-0.09

Table II: Arylesterase and paraoxonase activity relationship and lipid and lipoprotein profile.

	Arylesterase (nmol/min/mg protein)	log Paraoxonase (nmol/min/mg protein)	Arylesterase/Paraoxonase Ratio
Cholesterol >200mg/dL	440.2+/-10.3 (0.19)*	0.34+/-0.029 (0.149)	0.136+/-0.011 (0.075)
Cholesterol <200mg/dL	460.8+/-12.5	0.28+/-0.028	0.107+/-0.010
HDL-Cholesterol >35mg/dL	441.9+/-9.2 (0.18)	0.32+/-0.025 (0.913)	0.124+/-0.009 (0.895)
HDL-Cholesterol <35mg/dL	464.8+/-15.3	0.31+/-0.036	0.122+/-0.013
LDL-Cholesterol >130mg/dL	441.6+/-11.7 (0.62)	0.31+/-0.030 (0.925)	0.124+/-0.012 (0.968)
LDL-Cholesterol <130mg/dL	455.8+/-10.9	0.315+/-0.028	0.123+/-0.010
Triglyceride >200mg/dL	437.5+/-11.7 (0.190)	0.367+/-0.034 (0.018)	0.145+/-0.013 (0.018)
Triglyceride <200mg/dl	458.4+/-1.8	0.27+/-0.025	0.107+/-0.009

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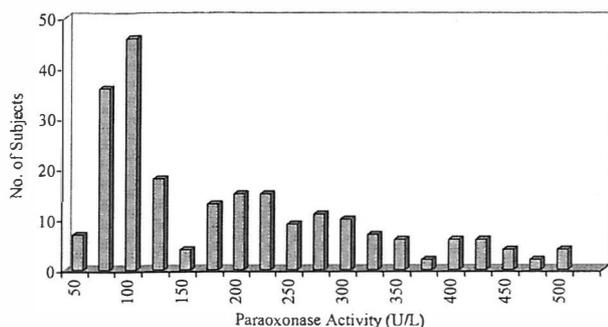


Fig. 2. Paraoxonase activity distribution in Iranian subjects.

### DISCUSSION

The present study was undertaken to establish a relationship between arylesterase and paraoxonase activity and lipid and lipoprotein profile. In a previous study there was an increasing levels of arylesterase activity associated with the cardiovascular diseases.<sup>21</sup> Arylesterase enzyme activity in hypercholesterolemia falls into a predicted pattern (bell shaped). There is no report of polymorphism associated with the arylesterase enzyme. The present study showed that arylesterase activity was not altered among patients with abnormal lipid and lipoprotein levels. In 1986, Diepgen and et al, performed PON activity among different ethnic groups.<sup>22</sup> However, there was no significant correlation between paraoxonase activity and ethnic group and sex. In the present study, no correlation between PON activity and sex was found.

In addition, t-student analysis of PON enzyme showed a significant correlation between PON specific activity and TG levels. Similar significant correlation was reached between arylesterase/paraoxonase activity ratio and TG levels. However no such correlation was reached between PON specific activity and paraoxonase/arylesterase ratio and other lipid and lipoprotein levels. It was shown patients with TG levels of higher than 200mg/dL had higher serum PON activity.

In a recent study<sup>23</sup> performed on two population groups in Belfast, Ireland and Toulouse France, a three-fold greater risk of coronary artery disease (CAD) in Belfast, was shown to be due to higher prevalence of R paraoxonase phenotype. Subjects from Belfast also had significantly higher serum TC, TG, LDL-C and apo B, and significantly lower HDL-C and apo A1, however these lipoprotein parameters were independent of the paraoxonase 191-polymorphism. Overall PON activity was significantly lower in Belfast than in Toulouse. Life style, environmental factors and genetic background may influence expression and activity of PON, as it is evident from a previous report.<sup>23</sup>

Although the effect of PON activity toward inhibition of LDL peroxidation through the hydrolysis of leukotriene-like compounds is well documented,<sup>24-27</sup> however the associa-

tion of high PON activity with increased risk of CAD is not clear. It seems that the ability of PON to hydrolyze paraoxon is inversely related to its capacity to hydrolyze lipid-peroxides, and thus to its antiatherogenic action.

The role of PON may be complicated by the fact that concentration of serum PON and its specific activity is not similar in different populations.<sup>22</sup> In a study performed on healthy subjects from Geneva and Manchester, serum concentration of PON from subjects from Geneva was significantly lower than those from Manchester.<sup>28</sup> Similarly, PON specific activity was lower among subjects from Geneva. The variation of PON concentration and its specific activity may reflect differences in HDL particle composition in various population groups.

In our study, analysis of variance between PON phenotypes and HDL-C showed significant differences in female subjects. However no such difference was observed in the male subjects and in the population as a whole. There was a significant difference between PON phenotype and TG levels in male subjects. These results show that subjects with high PON activity have higher serum TG levels. Similar conclusion was reached by others working on Chinese and Canadian healthy subjects.<sup>12,19</sup> However, some studies have showed that PON activity does not have any correlation with lipid and lipoprotein levels.<sup>29,31</sup> In addition, Saha et al.<sup>12</sup> showed that subjects with low paraoxonase activity (phenotype A) have significantly elevated serum LDL-C levels. However, Hegele et al,<sup>19</sup> reached an opposite conclusion. We did not observe any significant correlation between PON phenotypes and LDL-C levels.

Various reports have shown that reduced arylesterase activity was associated with CAD.<sup>21</sup> In a similar study, carried out on Iranian CAD patients, reduced arylesterase activity was observed (personal communication). However, present study did not show any relationship between arylesterase activity and the lipid profile. Therefore according to the present data arylesterase activity is not a suitable indicator of CAD. Arylesterase activity in our population showed a normal distribution (Fig. 1). Others<sup>15,32</sup> reported similar results.

The activity distribution of PON in our study obeys Hardy-Weinberg model for two-allele system (three phenotypes). Others<sup>33-35</sup> have reached similar conclusions. Comparing the PON activity distribution with that of Japanese, Nigerian, Indian and European origin, it seems that PON activity distribution in our study is similar to the European population.

There are at least two possible interpretations of the significant associations between variation in PON polymorphism and variation in plasma lipoproteins. First, the genetic variations may affect some function in vivo and this in turn affects lipoprotein metabolism and plasma lipoprotein concentrations. For example, polymorphism of PON might have an impact on the function of the enzyme; such a func-

tional defect might affect either oxidation of, lipid transfer between, or uptake of lipoprotein particles. Although PON polymorphism has direct effect on its enzyme activity, however polymorphism is not the sole modulator of PON activity. On the HDL particles, there are six other proteins; LCAT, GSH peroxidase, apo AI, apo AII, apo J and PAF-AH.<sup>36</sup> It is possible that protein and nonprotein components of HDL may influence PON activity.

There is evidence that PON activity is an important factor in the prevention of oxidative damage that leads to atherogenic processes. This was demonstrated by a PON knockout mouse, which revealed increased susceptibility to development of atherosclerosis.<sup>35</sup>

Recent studies have investigated the role of R mutation with regard to development CAD. Whereas smaller studies reported increased coronary risk and 191 R carriers in American whites,<sup>37</sup> Asian-Indians,<sup>38</sup> Japanese<sup>39,40</sup> and diabetic French,<sup>41</sup> more extensive studies involving Middle Europeans,<sup>30,42-45</sup> Japanese<sup>46</sup> and Chinese<sup>47</sup> failed to show association with excess coronary risk. From the theoretical standpoint, however, it could be expected that a mutated allele leading to higher enzyme activity would afford protection from lipid oxidation. It remains an unresolved paradox as to why this potentially protective variant was found to be overexpressed in CAD patients in some smaller studies. One possible explanation for the results obtained in this report for the negative association between major lipid and lipoprotein abnormality and PON activity (except for triglyceride), may be due to the functional differences of PON variants. Degradation processes of phospholipid peroxides are too small to be resolved by such a complex clinical endpoint as development of lipoprotein metabolic abnormalities or CAD.

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#### EMR Index Medicus

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