

Paraoxonase activity in patients with atherosclerosis

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Abstract: Paraoxonase (PON), a high density lipoprotein (HDL) associated enzyme decreases the risk of vascular diseases like atherosclerosis by protecting against the oxidation stress. We have analyzed PON activity in the plasma from hypercholesterolemic atherosclerosis patients and compared the results with age and sex matched healthy individuals. Moreover, we have also estimated the total plasma proteins and cholesterol in the diseased and normal samples. An inverse relationship between PON activity and the plasma cholesterol concentration was observed i.e. a significant decrease in the PON activity (52.91 ± 9.50 Units/ml; $p < 0.001$) and a parallel increase in the plasma cholesterol concentration (266 ± 49.99 mg/dL; $p < 0.001$) in the atherosclerosis samples. In contrast to cholesterol, the total plasma protein was significantly decreased in the diseased samples (7.98 ± 0.80 mg/dL; $p < 0.00$) thereby establishing a direct relationship with the PON activity. Our results also predict that PON activity in correlation with plasma cholesterol concentration can be used as a preemptive marker of progression of atherosclerosis and associated cardiovascular diseases.

Keywords: Atherosclerosis, coronary artery diseases, oxidative stress, paraoxonase, total protein, cholesterol.

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INTRODUCTION

Atherosclerosis is a vascular disease which is characterized by deposition of cholesterol and other lipids and inflammatory cells in the arterial wall thereby resulting in plaque formation and blockade of vessels. Oxidation of low density lipoprotein (LDL) plays a major role in the progression of this disease. The high density lipoprotein (HDL) regulates innate and adaptive immune responses, thus clarifying its role as an anti-atherogenesis factor¹. The HDL associated proteins, especially paraoxonase (PON), reduces this inflammatory plaque formation and protects against macrophage mediated LDL oxidation². PON also stimulates cholesterol efflux by HDL bound to macrophages³⁻⁶, therefore the PON binding sites on macrophages may play a role in the protection from atherosclerosis. It has already been reported that the PON 192QQ genotype and decreased PON activity are associated with increased vascular oxidative stress and increased risk for cardiovascular diseases (CAD)^{7,9}. In addition to PON 192QQ genotype, PON R allele has also been shown to increase the risk of CAD secondary to age, cigarette smoking and diabetes mellitus¹⁰⁻¹².

A direct correlation exists between serum PON activity and concentration of HDL cholesterol. When the PON activity was measured in patients with CAD, it was found to be approximately half that of disease-free control subjects^{13,14}. Based on the studies that suggest the role of PON activity in CAD, we hypothesized that the same can be used as a strong predictor of atherosclerosis and associated coronary complications. In the present study we have linked decreased PON activity in the plasma of atherosclerotic patients with hypercholesterolemia.

MATERIALS AND METHODS

Collection of Samples

Blood samples were collected from 26 atherosclerotic patients and from 20 normal healthy subjects with their prior consent. Plasma was separated from the whole blood and was stored at -20°C until further processing.

Determination of total proteins in plasma and cholesterol

The total plasma protein was estimated by Lowry's Method¹⁵ and the cholesterol was estimated by using Randox (Randox Laboratories Ltd., UK) Kit.

Determination of PON activity

The activity of plasma PON was determined colorimetrically. Briefly, 1:5 or 1:10 diluted plasma sample was added to the reaction mixture containing 20 mM Tris-HCl buffer of pH-7.0, 1 mM CaCl_2 and 1mM of substrate phenyl acetate. The reaction was then monitored for 3 minutes at 25°C and then the change in absorbance was recorded. The activity is expressed in U/ml, based on the extinction coefficient of phenol at 270 nm. The non-enzymatic hydrolysis reaction was corrected by taking reagent blank samples containing water instead of plasma. The units of enzyme per ml were calculated by using the formula:

$$\text{Units of E/ml} = \frac{\text{Change in Abs/min} \times \text{Dilution factor}}{1.31}$$

Where 1.31 is the molar extinction coefficient

Statistical analysis

Student's t-test was used to determine statistically significant differences using the software Statistical Package for Social Sciences (SPSS).

RESULTS

Determination of total protein and cholesterol

The estimated concentration of plasma total protein and cholesterol is shown in figures 1 and 2. Our results indicated that the plasma total protein concentration in atherosclerotic patients was significantly lower i.e., 7.98 ± 0.80 mg/dL, as compared to the healthy individuals i.e., 9.60 ± 1.37 mg/dL ($p < 0.00$). In contrast, the plasma cholesterol level in atherosclerotic patients was found to be significantly higher ($p < 0.001$) i.e., 266 ± 49.99 mg/dL, as compared to healthy individuals, i.e., 147.15 ± 31.36 mg/dL.

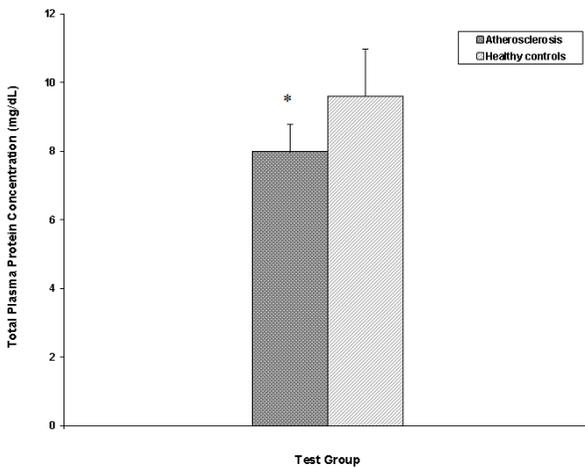


Figure 1: Total protein concentration in the plasma of the atherosclerosis patients and normal subjects. Each bar represents Mean \pm SD. of n = 26 for diseased samples and n = 20 for normal subjects. Where, * $p < 0.00$.

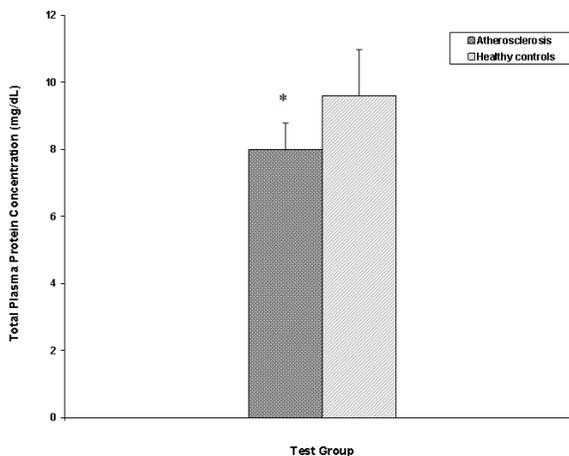


Figure 2: Cholesterol concentration in the plasma of diseased and normal samples. The values are represented as the Mean \pm S.D. of n = 26 for atherosclerosis samples and n = 20 for normal samples. * $p < 0.001$.

Plasma PON activity

PON activity in atherosclerotic patients was found to be significantly lower ($p < 0.001$) i.e., 52.91 ± 9.50 Units of Enzyme/ml, as compared to the normal age and sex matched healthy individuals from the general population, i.e., 74.86 ± 14.71 Units of Enzyme/ml (Figure 3).

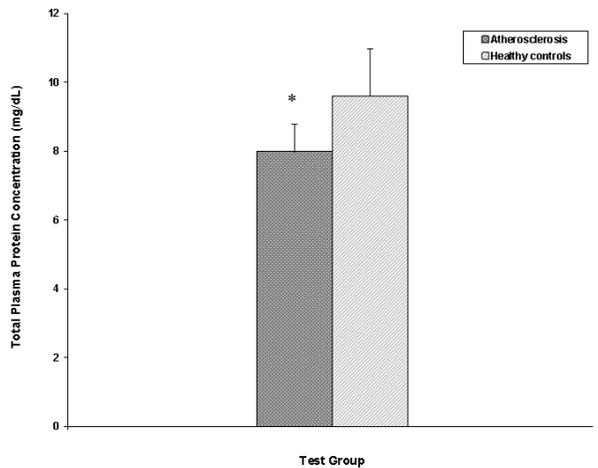


Figure 3: Paraoxonase activity in atherosclerosis patients and normal subjects. The PON activity was found to be significantly decreased (* $p < 0.001$) compared to the concurrent normal group. Results are represented as Mean \pm SD.

DISCUSSION

Oxidative stress in blood leads to oxidation of low density lipoproteins (LDL) that result in the deposition of cholesterol in the form of plaques on the vascular walls leading to atherosclerosis^{16,17}. There are few endogenous factors that aid in reducing the oxidation of LDL and protects from atherosclerosis. The HDL is amongst one of these important endogenous factors that reduces the risk of atherosclerosis. Several speculations have been made to explain the role of HDL as an anti-atherogenic factor^{18,19} and most of these focuses on the integral protein component of HDL i.e., paraoxonase enzyme. Paraoxonase is responsible for the oxidative and anti-inflammatory properties of HDL⁴. Various studies have shown that the marked reduction of paraoxonase activity can be a risk factor for atherosclerosis and CAD²⁰⁻²⁶. In light of these reports, we have confirmed that low paraoxonase activity is linked with the

atherosclerosis and this can be served as a preemptive marker for atherosclerosis.

In our study we have also estimated the plasma total protein and cholesterol in hypercholesterolemic atherosclerosis patients and in the plasma of normal age and sex matched healthy individuals. We have observed an inverse relationship between cholesterol oxidation and paraoxonase activity which confirms the previous studies which have shown that patients with a high risk of coronary heart disease have increased LDL oxidation^{27,28} and reduced PON activity. Moreover, we have also found a direct relationship between plasma protein concentration and enzyme activity of PON.

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