

Research Article



SERUM PARAOXONASE (PON1) ACTIVITY AND ITS RELATIONSHIP WITH ATHEROGENIC INDEX OF PLASMA (AIP) IN PATIENTS WITH ISCHEMIC HEART DISEASE

Gurulingappa.A.Patil¹, Khaja Moinuddin², S.M Awanti²

1. Department of Anesthesiology, M.R Medical College, Gulbarga, India.

2. Department of Biochemistry, M.R Medical College, Gulbarga, India.

*Corresponding author's E-mail: drgurupatil@gmail.com

Accepted on: 25-01-2012; Finalized on: 20-03-2012.

ABSTRACT

Paraoxonase (PON1) is a High density lipoprotein (HDL-C) associated enzyme, is proposed to have anti-atherogenic effect by preventing LDL-C oxidation. Atherogenic index of plasma (AIP) is an index used to predict the risk of atherosclerosis. Aim of our present study was to investigate the relationship between AIP and paraoxonase activity in newly diagnosed ischemic heart disease (IHD) patients. Serum lipid profile and serum paraoxonase were estimated in 32 newly diagnosed ischemic heart disease patients and 32 healthy controls. Serum lipid profile was analyzed by Semi-autoanalyser and serum paraoxonase activity was determined spectrophotometrically using paraxon as substrate. AIP was calculated using formula $\log (TG/HDL-C)$. There was significant increase in Total cholesterol (TC), Triglyceride (TG), Low density lipoprotein (LDL-C) and AIP ($p < 0.01$) and significant decrease in High density lipoprotein (HDL-C), paraoxonase ($p < 0.001$) in cases compared to controls. Significant positive correlation was observed between AIP and TC/HDL-C. According to our study IHD patients are more prone to the development of atherosclerosis due to high TC/HDL-C ratio and AIP when compared to healthy controls. AIP can predict the size of LDL particle. Size and high LDL-C levels are important factors in the development of atherosclerosis. Decrease in HDL associated paraoxonase activity with increase in LDL-C along with high TC/HDL-C and AIP indicate increased susceptibility to accelerated atherogenesis leading to Ischemic heart disease.

Keywords: Paraoxonase, AIP (Atherogenic Index of plasma), HDL-C, LDL-C, Total Cholesterol (TC), Triglyceride (TG).

INTRODUCTION

Ischemic Heart disease (IHD) is widely prevalent both in the developed and developing countries and continues to be a leading cause of mortality despite recent advances in diagnostic facilities and treatment modalities.¹ It is a multifactorial disease where atherosclerosis and dyslipidaemia are the prominent causes involved. Low density lipoprotein cholesterol (LDL-C) is considered as the most important risk factor for IHD and its oxidation 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.² Oxidized LDL may also be involved in atherogenesis by inducing smooth muscle cell proliferation.³ 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 atherosclerosis. Serum paraoxonase (PON1) is an HDL associated calcium dependent esterase mainly synthesized in liver that hydrolyses organophosphates such as paraoxone, diazoxon, sarin and soman and also arylesters such as phenyl acetate.⁴ Although the natural substrates for paraoxonase are unknown, paraoxonase (PON1) decreases low density lipoprotein oxidation by its peroxidase activity and by preventing homocysteinylolation of ApoB-100.⁵ The biological role of HDL is attributed to the presence of paraoxonase associated with it.⁴ Serum

paraoxonase (PON1) is located in a subfraction of high density lipoprotein (HDL) that contains apoA-1 and clusterin (apoJ). It has been suggested that, this subfraction of HDL is principally responsible for the breakdown of lipid peroxides and that it consequently protects against lipoprotein oxidation.^{6,7} Low serum paraoxonase activity, independent of genotype, has been reported with diseases which are known to be associated with cardiovascular diseases such as Diabetes Mellitus, hypercholesterolemia and renal failure.⁸

The strong association between the risk of coronary artery diseases (CAD), high levels of LDL-C and low levels of HDL-C has been well established.^{9,10} High levels of triglycerides have been associated with increased incidence of CAD¹¹ and an increased population of small dense LDL-C particles¹². A lot of work has been done on the relationship between TG and HDL-C, and it has been shown that the ratio of TG to HDL-C was a strong predictor of myocardial infarction.¹³ Universally, atherogenic index of plasma (AIP) calculated as $\log (TG/HDL-C)$ has been used by some practitioners as a significant predictor of atherosclerosis.^{14,15} Though atherogenic index of plasma has been used as an index to predict risk of atherosclerosis, but there is paucity in literature on its correlation with (PON1) in ischemic heart disease patients. Hence the present study was undertaken with the objective of studying the PON1 activity in ischemic heart disease patients and to observe whether PON1 activity has any correlation with Atherogenic index of plasma (AIP) in this group of subjects.



MATERIALS AND METHODS

The study was conducted on 32 newly diagnosed Ischemic heart disease patients and 32 age matched controls. Under aseptic conditions blood samples (5 ml) were drawn into plain vacutainers from ante-cubital veins. The collected blood was allowed to clot for 30 minutes, and then centrifuged at 2000 g for 15 minutes for clear separation of serum. All assays were performed immediately after serum was separated. Concentrations of serum TC, TG, LDL-C, HDL-C, were determined on semiautomatic biochemical analyzer using enzymatic colorimetric kit (Agappe diagnostic kit). Special chemicals like paraoxone were obtained from Sigma chemicals, St Louis, MO, USA. All other reagents were of analytical grade. PON1 was estimated spectrophotometrically by method described elsewhere with minimal modification.¹⁶ Briefly the assay mixture consists of 500 μ L OF 2.2mM Paraoxone substrate in 0.1M tris-Hcl buffer, pH 8.0 containing 2mM CaCl_2 and 50 μ L of fresh serum specimen. The absorbance was monitored at 405 nm at 25°C. One unit (IU) of PON1 activity is defined as 1 μ mol of p-nitrophenol formed per min per liter at 25°C and activity was expressed as U/L of serum. AIP was calculated using formula $\log (\text{TG}/\text{HDL-C})$.

Statistical Analysis: All the values are expressed as mean \pm SEM. A p value less than 0.05 were considered as significant. Statistical analysis was done using SPSS (statistical package for social sciences, SPSS-17, Chicago, USA). Independent sample t test was used to compare mean values. Pearson's correlation was used to correlate between the parameters.

RESULTS

There was significant increase in Total cholesterol (TC), Triglyceride (TG), Low density lipoprotein (LDL-C) and AIP ($p < 0.01$) and significant decrease in High density lipoprotein (HDL-C), paraoxonase ($p < 0.001$) in cases compared to controls (Table 1). Significant positive correlation was observed between AIP and TC/HDL ($r = 0.129$, $p < 0.01$) (fig-1) and negatively with paraoxonase (PON1) ($r = -0.275$, $p < 0.01$) (fig-2).

Table 1: Serum lipid profile, Paraoxonase and Atherogenic index of plasma (AIP) in cases and controls

Parameters	Controls (n = 32)	Cases (n = 32)
Serum Total cholesterol (mg/dl)	168.09 \pm 12.10	183.84 \pm 13.6
Serum LDL-C (mg/dl)	79.88 \pm 7.9	116.82 \pm 13.7
Serum Triglycerides (mg/dl)	105.02 \pm 10.3	126.22 \pm 11.74
Serum HDL-C (mg/dl)	49.9 \pm 7.3	41.7 \pm 4.8
TC/HDL-C Ratio	3.45 \pm 0.29	4.46 \pm 0.6
AIP (Atherogenic index of plasma)	0.322 \pm 0.1	0.576 \pm 0.12

(Expressed in mean \pm SD). *P value < 0.01 compared to healthy controls.

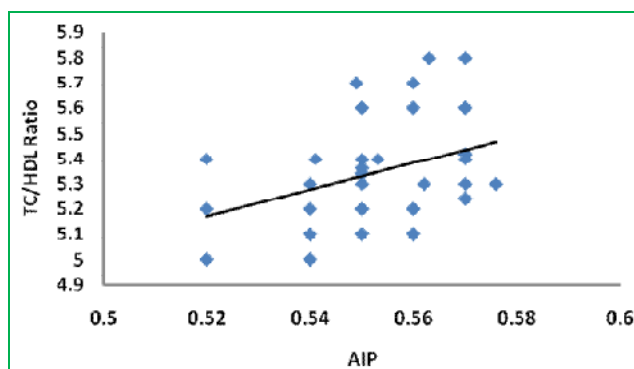


Figure 1: Correlation between TC/HDL ratio and atherogenic index of plasma (AIP)

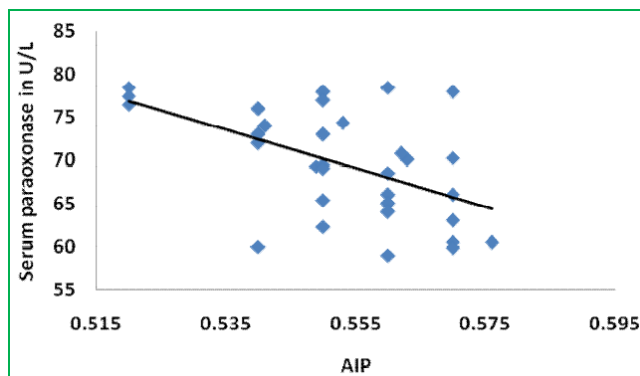


Figure 2: Correlation between Paraoxonase and atherogenic index of plasma (AIP)

DISCUSSION

In our study we found significant decrease in paraoxonase activity in Ischemic heart disease patients. This decrease in paraoxonase may be associated with decrease in levels of HDL-C. Decrease in HDL-C associated paraoxonase activity and increase in LDL-C in ischemic heart disease patients may be favorable for atherogenesis process, thus predisposing patients with IHD for premature coronary artery disease. The exact mechanism of anti-atherogenic function of HDL-C and its associated components is not clear but the role of paraoxonase activity in this process is increasingly stressed in recent times.¹⁷ Involvement of free radicals in the pathophysiology of inflammation, ischaemia and reperfusion causing damage in tissues and organs, have been reported earlier.¹⁸ Indirect evidence of free radical generation in AMI patients is substantiated by measuring PON1 activity in them,¹⁹ and lowering of PON1 activities in AMI patients have been reported earlier.^{20,21} The significant decrease in paraoxonase activity in our cases might be due to enhanced protection from free radical damage during the ischemic process. Due to increased production of toxic free radicals, the efficiency to hydrolyze lipid peroxides by paraoxonase is lowered, which further decreases its specific activity, and the same were observed in the present study. Epidemiological research conducted over the last 40 years has allowed the identification of markers of CHD risk. It is now well established that additional risk factors, such as diabetes, hypertension and smoking substantially increase risk of CHD for any given level of LDL-cholesterol.^{22,23}

Furthermore, it is now common practice to measure HDL-cholesterol levels²⁴⁻²⁷ and to compute the LDL-cholesterol: HDL cholesterol or the cholesterol: HDL-cholesterol ratios for a better assessment of CHD risk.^{22,28-30} In our present study also we found significant increase in TC/HDL-C ratio which correlated positively with atherogenic index of plasma.

CONCLUSION

We speculate that due to Overwhelming production of free radicals, there is a decreased activity of paraoxonase, and due to its incapability to scavenge free radicals, IHD patients become more susceptible to infarction. Hence decrease in HDL-associated paraoxonase activity with increase in LDL-C along with high TC/HDL-C and AIP indicate increased susceptibility of LDL-C molecules for oxidation, which is known to cause atherosclerosis leading to ischemic heart disease.

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