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Original article

Paraoxonase-1 is a better indicator than HDL of Atherosclerosis – A pilot study in North Indian population

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ABSTRACT

Objectives: The present study aims to evaluate the levels of HDL and Paraoxonase-1 (PON1) and their correlation in atherosclerotic patients with and without diabetic mellitus (DM) as well as in control subjects in Northern Indian population.
Materials and methods: We analyzed lipid profiles and Serum PON1 levels by automated analyzer and ELISA, respectively. Study subjects (N = 150) were divided in three groups; Group I: Atherosclerotic patients without DM (N = 50), Group II: Atherosclerotic patients with DM (N = 50); Group III: Controls (N = 50).
Results: We found a significantly ($p < 0.0001$) low levels of HDL-C in Group I (32.2 ± 7.3) and Group II (36.9 ± 11.5) as compared to Group III (41.0 ± 7.1). PON-1 levels were also significantly lower in Group I (60.1 ± 10.5) and Group II (50.0 ± 13.9) when compared to Group III (95.0 ± 12.0). We observed a significant correlation ($r = 0.59$, $p < 0.001$) between the levels of PON1 and HDL-C in study subjects.
Conclusions: The reduced levels of HDL and PON-1 and their significant correlation in CAD patients may be associated with the pathogenesis of this disease. Considering HDL as a dependent variable, Paraoxonase-1 is the most important parameter contributing to the total variation in HDL in CAD.

1. Introduction

Cardiovascular disease (CVD) caused by the abnormalities in heart and blood vessels, leading to mortality worldwide, as well as in India. CVD accounts for 23% deaths before the age of 70 years in western populations, whereas 52% deaths have been reported in Indian population [1]. Atherosclerosis is a major cardiovascular disease, which cause the development of atheromatous plaques and further local thrombosis, leading to partial or total occlusion of the affected artery [2]. Atherosclerosis includes various clinical manifestations, such as peripheral arterial disease (PAD), Rheumatic heart disease (RHD), coronary artery disease (CAD), ischemic stroke and ischemic heart disease (IHD) etc. Recent Atherosclerosis Statistics reports suggested that overall prevalence is 13.8% among adults of 20 years and above, while 32% of deaths were account for atherosclerosis, worldwide [3]. Atherosclerosis is most prevalent CVD, affects nearly 1.8 million Indians in a total estimated population of 1.06 million individuals [4].

High-density lipoproteins (HDL), small in size and rich in proteins are one of the five main classes of lipoproteins, which bind to high-density cholesterols [5]. HDL plays a major role in cholesterol transport from the periphery to the liver through reverse cholesterol transport (RCT) mechanism. Previous studies reported that the levels of HDL-cholesterol are inversely associated with the risk of coronary artery disease (CAD) and its thrombotic complications [6], and important predictor of heart failure [7].

The most abundant HDL associated apolipoproteins A-I (apoA-I) and apoA-II and other proteins, including paraoxanase that co-transport with HDL in plasma, are well-known to have antioxidant properties [8,9]. Human Paraoxonase gene (PON) gene family located adjacent to chromosome 7 contains 355-amino acid, includes PON1, PON2, and PON3. Paraoxonase 1 (PON1) is primarily synthesized in the liver, and further it is secreted in association with HDL to the circulation [10]. The study reported that PON1 is associated in the hydrolysis of oxidized phospholipids in oxidized Low-density lipoproteins (LDL), while the complete mechanism is still unclear. [11]. Circulating paraoxonase-1 (PON1) is an important 'detoxification' protein, which is coupled with

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apolipoprotein A1 in the HDL, known for the antioxidant activities of HDL [12]. Meta analysis studies revealed that lower plasma PON1 levels were strongly associated with the increased risk of CAD [13,14]. A study in animal model suggested that PON1 inhibited the progression of atherosclerosis by reducing the oxidized LDL levels in plasma and in the plaque, through adenovirus-mediated gene transfer of Human PON1 [15]. The peroxidase-like activity of the HDL-associated PON1 inhibits oxidation of lipoproteins, stabilization of atherosclerotic plaques and stimulates cholesterol efflux, contributed towards anti-oxidative and anti-atherogenic role of HDL [16,17]. The total levels of HDL may not be as important as the functional capacity of HDL or its role in reducing oxidative stress via prevention of oxidation of LDL by PON1. The aim of the present study was to evaluate the levels of HDL and PON1 and their correlation in atherosclerotic patients with and without diabetic mellitus as well as in control subjects in Northern Indian population.

2. Material and methods

2.1. Study population

A total of 150 study subjects were recruited for the present pilot study, were residents of North India. Study population is comprised of three groups:

Group I: Angiographically proven atherosclerotic CAD patients (N = 50) without diabetes mellitus (DM).

Group II: Included angiographically proven atherosclerotic CAD patients (N = 50) having DM.

Inclusion criteria: i) Angiographically proven patients [Age between 35 years to 70 years], who have 70% or more stenosis of at least one of the major coronary artery. ii) CAD patients without DM iii) CAD Patients with DM. Exclusion criteria: i) Patients with less than 70% stenosis, ii) Patient with abnormal liver function test and iii) Autoimmune diseases like Rheumatoid Arthritis

Patients were recruited from Department of Cardiology, G.B. Pant Hospital, Delhi, India in collaboration with Department of Biochemistry, Lady Hardinge Medical College, Delhi, India. All patients had stenosis (>50%) that was severe enough to require intervention, assessed by coronary angiography.

Group III: Included patients (N = 50) found to have no or insignificant atherosclerosis, considered as controls.

Inclusion criteria: No or insignificant atherosclerosis, when angiography was done.

Exclusion criteria: i) Patients with diabetes mellitus or autoimmune disease like rheumatoid Arthritis and ii) Patient with CAD.

Group I & II were considered as cases, whereas group III was considered as controls. Cases and controls were matched for age and sex. The study was conducted in accordance with the

guidelines of the Helsinki Declaration and written informed consent was obtained from all the participants recruited for the study. A detailed history of study subjects was obtained, followed by a complete clinical examination. Patients already undergoing angiography as advised by Cardiologist were included as study group. An approval of ethics committee of G.B. Pant Hospital, Delhi, India and Lady Hardinge Medical College, Delhi, India was obtained prior to the study.

2.2. Sample collection and processing

Two millilitres (2 ml) of blood were taken in Plain vials. Serum was used for HDL and PON1 estimation. The venous blood sample taken without anti-coagulant was allowed to clot, centrifuged and serum was separated. HDL estimation was done on same day. Serum samples were stored at −70 °C for estimation of PON1 levels.

2.3. Lipid profiles and paraoxonase-1 levels

Lipid profile such as total Cholesterol, Triglycerides, HDL-Cholesterol (HDL-C), LDL-Cholesterol (LDL-C), Very low-density lipoproteins-Cholesterol (VLDL-C) estimations were performed on CX-9 fully automated analyzer using multicalibrator (Lot no. M907396) and Randox Quality control (Lot no.14411). Para-oxonase-1 levels were estimated by ELISA using USCN Life science kit according to the manufacturer's protocol.

2.4. Statistical analysis

All statistical analysis was performed with the IBM SPSS statistical package 20. A one-way-ANOVA and post-hoc scheffe test was performed for the comparisons of continuous variables between the groups. Results were expressed as mean ± standard

Table 1
Lipid Profiles and Paraoxonase-1 levels in study subjects.

Parameters	CAD (Group I) (N = 50)	CAD with DM (Group II) (N = 50)	Controls (Group III) (N = 50)	*p-Value between groups
Total Cholesterol (mg/dl)	148.4 ± 54.1	149 ± 49.7	125.6 ± 22.3	I Vs II = 0.024 I Vs III = 0.002 II & III = 0.0001
Triglycerides (mg/dl)	143.4 ± 54.1	155.0 ± 72	102.4 ± 40.7	I Vs II = 0.024 I Vs III = 0.017 II Vs III = 0.0001
HDL-C (mg/dl)	32.2 ± 7.3	36.9 ± 11.5	41 ± 7.1	I Vs II = 0.035 I Vs III = 0.0001 II Vs III = 0.0001
VLDL-C (mg/dl)	28.6 ± 10.8	31.5 ± 14.6	20.4 ± 8.5	I Vs II = 0.476 I Vs III = 0.003 II Vs III = 0.0001
LDL-C (mg/dl)	87.4 ± 38.8	90.0 ± 49.3	63.9 ± 29.1	I Vs II = 0.722 I Vs III = 0.015 II Vs III = 0.013
PON1 (ng/ml)	60.1 ± 10.5	50.0 ± 13.9	95.0 ± 12.0	I Vs II = 0.0001 I Vs III = 0.0001 II Vs III = 0.0001

HDL-C, High density lipoprotein Cholesterol; LDL-C, Low density lipoprotein cholesterol; PON1, Paraoxonase-1; VLDL-C, Very low density lipoprotein cholesterol; mg/dl, milligram/deciliter; ng/ml, nanogram/milliliter.
Patients group were compared with controls with analysis of variance (ANOVA); *$p < 0.05$ is considered to be significant.