

ORIGINAL ARTICLE

To Study the Activity of Paraoxonase-1 and High Density Lipoprotein-cholesterol in Alcoholic Liver Cirrhosis

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Abstract:

Background: Alcoholic liver cirrhosis is the most common complication of ethanol abuse. Alcoholic fatty liver progresses to alcoholic hepatitis, cirrhosis and liver failure. Lipoproteins are synthesized by the liver and secreted into the circulation. Alcoholic liver cirrhosis causes alteration in lipoprotein metabolism producing liver steatosis and necrosis. Paraoxonase-1 (PON-1) is an enzyme synthesized in liver and has an esterase activity towards lipid peroxides and circulates in plasma bound to High-Density Lipoproteins - cholesterol (HDL-c). **Aim and Objectives:** To determine the activity of PON-1 and levels of HDL-c in alcoholic liver disease and to correlate PON-1 activity with HDL-c. **Materials and Methods:** A Cross sectional study done in Department of Biochemistry and Department of Medicine, Belagavi Institute of Medical Sciences, Belagavi, Karnataka, India, from 1st December 2014 to 31st January 2016 Study included 50 males (age range 25-55 years) with alcoholic liver cirrhosis and 50 healthy male participants (age range 25-55 years). PON-1 activity was estimated using spectrophotometric method by the hydrolysis of phenylacetate. HDL-c level was measured by cholesterol oxidase-peroxidase method. **Results:** The serum PON-1 activity and levels of HDL-c in patients with alcoholic liver cirrhosis were significantly reduced ($p < 0.001$) compared with controls. **Conclusion:** A significant decrease in PON-1 and HDL-c in alcoholic liver cirrhosis may contribute to the risk of atherosclerosis in alcoholic liver cirrhosis patients.

Keywords: Alcoholic liver cirrhosis, Paraoxonase-1, High density lipoprotein-cholesterol.

Introduction:

Chronic and excessive alcohol ingestion is one of the major causes of liver disease. Chronic liver disease is the tenth most common cause of death in adults, and alcoholic cirrhosis accounts for approximately 40% of deaths due to cirrhosis. Alcohol is metabolized in the liver by three different enzymes: Alcohol Dehydrogenase (ADH), Cytochrome P-4502E1 (CYP2E1) and mitochondrial catalase. About 90% to 100% of heavy drinkers have steatosis, 10% to 35% have alcoholic hepatitis and 8% to 20% have alcoholic cirrhosis. Alcoholic fatty liver progresses to alcoholic hepatitis, cirrhosis and then liver failure [1, 2]. Lipoproteins are synthesized by the liver and secreted into the circulation [3] Alcoholism produces alteration in the lipoprotein metabolism producing liver steatosis and necrosis [4].

Paraoxonase (PON)-1 (E.C- 3.1.8.1) is an enzyme synthesized in liver and has lactonase and esterase activity towards lipid peroxides and circulates in plasma bound to high-density lipoproteins. PON enzyme family comprises 3 members PON-1, PON-2 and PON-3. In human beings PON-1 and PON-3 are mainly found in the circulation bound to high-density lipoproteins. Alterations in circulating PON-1 levels have been reported in different diseases like cardiovascular, Alzheimer's, chronic renal failure, HIV-infection, metabolic syndrome and chronic liver impairment [5].

Serum PON-1 is associated with High Density Lipoprotein-cholesterol (HDL-c) and is a calcium-dependent esterase that is known to catalyze hydrolysis of organophosphates, and is widely distributed among tissues such as liver, kidney, intestine, and also serum [6, 7]. Although PON-1 can offer protection against the toxicity of some organophosphates, its physiological role is still not known; however, evidence exists for a protective effect of PON-1 against oxidative damage. PON-1 was suggested to contribute to the antioxidant protection conferred by HDL-c on Low Density Lipoprotein-cholesterol (LDL-c) oxidation [8, 9, 10, and 11]. The effect of HDL-c associated PON-1 or of purified PON-1 on the LDL-c oxidation process, including its initiation (conjugated dienes formation), propagation (peroxides formation), and decomposition (aldehyde formation) phases could be analyzed by using PON-1 inhibitors [11]. Oxidative modification of HDL-c has also been shown to impair the ability of the lipoprotein to promote cholesterol efflux [12]. Thus, inhibition of HDL-c oxidation by PON-1 may preserve the anti-atherogenic functions of HDL-c in reverse cholesterol transport, as well as its protection of LDL-c from oxidation. Thus, the current study was undertaken to determine the activity of PON-1 and levels of HDL-c in alcoholic liver disease and to correlate PON-1 activity with HDL-c.

Material and Methods:

The study group comprised of 50 males with well diagnosed Alcoholic Liver Cirrhosis (ALC) patients in the age group of 25-55 years admitted in medicine wards of Belagavi Institute of Medical Sciences (BIMS) Hospital, Belagavi. The diagnosis of alcoholic liver cirrhosis was done by senior physician BIMS, Belagavi on basis of history of alcoholism with clinical, biochemical and ultrasonographic evidence of cirrhosis. Fifty males with age group of 25-55 years healthy participants were taken as control group.

Exclusion Criteria:

Known cases of diabetes mellitus, obesity, hypothyroidism, hyperthyroidism, renal diseases, Cardiovascular Diseases (CVD), Human Immunodeficiency Virus (HIV), metabolic syndrome and Alzheimer's disease were excluded from the study. Known cases of infective and drug induced hepatitis were also excluded.

The study was performed for a period from 1st December 2014 to 31st January 2016. Written informed consent was taken from all subjects involved in the study and the study was approved by Institutional Ethics Committee, Belagavi Institute of Medical Sciences, Belagavi. After obtaining written informed consent 5ml of 12hours fasting venous blood sample was collected by venipuncture with all aseptic precautions in a plain vacutainer and serum was used for estimation of HDL-c and activity of PON-1.

PON-1 was estimated spectrophotometrically by hydrolysis of phenyl acetate. Briefly, the assay mixture consists of Tris-HCl buffer (9mM, pH 8.00) containing 0.9mM CaCl₂ and 1.25 mM phenyl acetate. Pipette into a cuvette 500 µl serum and 2.0ml Tris-HCL buffer. Mix and read the absorbance at 270nm on spectrophotometer taken immediately at every minute for five minutes. First absorbance reading was taken as 0-minute reading and subsequent absorbance readings were taken as one-minute to four-minute readings. Mean absorbance was used to determine PON-1 activity. PON-1 activity was expressed in Units/millilitre of serum (nmol/mL/min), where 1U = 1 nanomole of p-nitrophenol formed per minute [13]. HDL-c level and total cholesterol was measured by Cholesterol Oxidase - Peroxidase (CHOD-POD) method [14]. Triacylglycerol estimation was done by Glycerol 3-Phosphate Oxidase Peroxidase (GPO-POD) method [14].

Very Low Density Lipoprotein-cholesterol (VLDL-c) and LDL-c was calculated by Friedwald's formula:

VLDL-c = Triglyceride / 5, LDL-c = Total cholesterol – VLDL-c – HDL-c [14].

Statistical analysis:

The values obtained were expressed as Mean \pm Standard Deviation. Significance of difference between mean was calculated with unpaired student's 't' test. Pearson's correlation coefficient was calculated to see the correlation between variables. $p < 0.05$ was considered for statistical significance. Statistical software Statistical Package for Social Sciences (SPSS) version 22 was employed for statistical analysis.

Results:

The activity of PON-1 in ALC (50.22 ± 17.17 U/mL) compared to controls (178.32 ± 30.30 U/mL) was significantly reduced with $p < 0.001$. Study found that the levels of HDL-c in ALC (27.57 ± 4.69 mg/dL) compared to controls (52.28 ± 9.41 mg/dL) were significantly reduced with $p < 0.001$. The levels of total cholesterol, LDL-c and VLDL-c were also reduced in ALC compared to control participants. Triacylglycerol (TAG) levels were significantly elevated in alcoholic cirrhosis patients. The results showed positive correlation between PON-1 activity and HDL-c in ALC which was negligible ($r = 0.26$) (Fig. 1).

Table 1: Baseline Characteristics of Patients and Control Groups

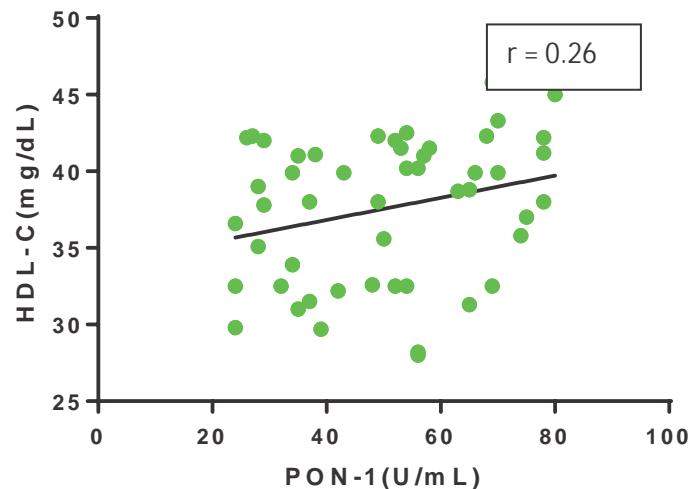
Characteristics	Control Group (n=50)	ALC Group(n=50)
Age (years)	39.00 \pm 7.55	40.10 \pm 9.63 (p=0.52)
History of Smoking (%)	0	0
History of Hypertension	0	0
History of Diabetes Mellitus	0	0

n = number of subjects, age expressed in Mean \pm SD,
No significant difference in the age between ALC and controls.

Table 2: Serum Parameters in ALC Patients and Control Groups

Parameters	Control Group(n = 50)	ALC Group(n = 50)
PON-1 (U/mL)(nmol/mL/min)	178.32 \pm 30.30*	50.22 \pm 17.17*
Total Cholesterol (mg /dL)	198.04 \pm 13.98*	173.63 \pm 25.03*
HDL-c (mg/dL)	52.28 \pm 9.41*	27.57 \pm 4.69*
LDL-c (mg/dL)	115.45 \pm 18.12*	105.65 \pm 17.63*
VLDL-c (mg/dL)	29.85 \pm 4.26*	24.71 \pm 2.47*
Triacylglycerol (mg/dL)	145.90 \pm 17.20*	190.1 \pm 11.83*

* $p < 0.001$ = highly significant, *n* = number of subjects, All values are expressed in Mean \pm SD



Positive correlation between PON-1 activity and levels of HDL-c ($r = 0.26$)

Fig. 1: Correlation between PON-1 activity and HDL-c in ALC cases:

Discussion:

The liver plays a key role in the synthesis of PON-1 [15]. Alterations in circulating PON-1 levels have been reported in variety of diseases which involves oxidative stress [16, 17]. Chronic liver diseases are associated with increased oxidative stress, Monocyte Chemoattractant Protein 1 (MCP-1) synthesis, and inflammation [18, 19]. Levels of HDL-c was significantly ($p < 0.001$) reduced in ALC when compared with healthy control participants (Table 2). Phukan *et al.* (2013) [20] also showed reduced HDL-c in ALC compared to controls. Recent studies Ghadir *et al.* (2010) [21] and Cicognani *et al.* (1997) [22] on cirrhosis of liver showed that serum HDL-c, LDL-c and total cholesterol values were significantly diminished. HDL-c is a substrate for Lecithin Cholesterol Acyl Transferase (LCAT) enzyme. Apolipoprotein A-I is an activator of LCAT enzyme [23]. In ALC there is reduced LCAT activity [24], due to this, undesirable structural change occurs in HDL-c. These undesirable structural changes may reduce the levels of HDL-c

in ALC patients. A study by Ferré *et al.* (2002) [24] stated that decrease in PON-1 activity in patients with chronic liver diseases such as chronic hepatitis and cirrhosis, was related to degree of liver damage. Keskin *et al.* (2009) [25] also reported reduced baseline and stimulated PON-1 activity in patients with chronic liver disease.

The finding of present study was similar to the above author's findings. The activity of PON-1 was significantly ($p < 0.001$) reduced in ALC when compared with healthy control participants (Table 2). In ALC the capacity of the liver to synthesize protein will be reduced, this may affect the synthesis of PON-1 in alcoholic liver cirrhosis, which might be responsible for reduced activity of PON-1 in ALC patients.

PON-1 degrades oxidized phospholipids in lipoproteins and plays an important role as an antioxidant [26, 27], and prevent the oxidation of LDL-c both *in vitro* and *in vivo* [28-30]. The levels of TAG and LDL-c were significantly ($p < 0.001$) increased and significantly ($p < 0.001$) reduced in

ALC compared with healthy control participants respectively (Table 2). Alteration in LCAT and PON-1 may alter the HDL-c in alcoholic liver cirrhosis. The present study found a positive correlation between PON-1 and HDL-c (0.26) (Fig.1) in ALC patients. Thus reduced activity of PON-1 in ALC may increase the oxidised LDL-c. Hence the reduced PON-1 can alter the oxidised LDL-c and oxidative stress. Thus the present study concluded that decreased activity of PON-1 and HDL-c levels in ALC may contribute to risk of atherosclerosis via alteration in oxidised LDL-c and oxidative stress.

Conclusion:

Reduced PON-1 activity and HDL-c may be associated with increased oxidised LDL-c and oxidative stress which may contribute to the risk of atherosclerosis in alcoholic liver cirrhosis.

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