ORIGINAL RESEARCH PAPER

Biochemistry

ROLE OF LECITHIN: CHOLESTEROL ACYL TRANSFERASE, PARAOXONASE-1 AND APOLIPOPROTEIN A-I IN ALCOHOLIC LIVER CIRRHOSIS

KEY WORDS: Alcoholic liver cirrhosis, HDL-C, Apo A-I, LCAT, RCT, PON-1.

Dr Priya Patil Assistant Professor, Department Of Biochemistry, Belagavi Institute Of Medical Sciences, Belagavi, Karnataka 590001

Dr Padmaja Nikam Associate Professor, Department Of Biochemistry, Belagavi Institute Of Medical Sciences, Belagavi, Karnataka 590001

Dr Shashikant Nikam* Professor And HOD Department Of Biochemistry, Belagavi Institute Of Medical Sciences, Belagavi, Karnataka 590001. *Corresponding Author

ABSTRACT

BACKGROUND- Alcoholic liver cirrhosis and chronic liver disease are the 10th leading cause of death for men in united-states in 2001, killing about 27,000 people each year. Alcoholic liver cirrhosis is the most common complication of ethanol abuse. Alcoholic fatty liver progress to alcoholic hepatitis, cirrhosis and liver failure. Lipoproteins are synthesized by the liver and secreted into the circulation. Apolipoprotein A-I (apo A-I) is the most abundant protein of HDL-C and acts as a cofactor for lecithin: cholesterol acyl transferase (LCAT). LCAT promotes reverse cholesterol transport (RCT). The objective of the study was to determine the activity of LCAT and levels of apo A-I and to correlate the LCAT activity with levels of apo A-I and HDL-C I alcoholic liver cirrhosis.

MATERIALS AND METHODS- A cross sectional study done in Department of Biochemistry, Belagavi Institute of Medical Sciences, Belagavi from December 2014 to January 2016. Study included 50 males (age range 25- 55 years) with alcoholic liver cirrhosis and 50 healthy males (age range 25-55 years). LCAT activity was assessed by measuring the difference between esterified and free cholesterol. Determination of free and esterified cholesterol was done by using digitonin precipitation method. Apolipoprotein A-I was measured by immunoturbidimetric method using semi auto analyzer. Paraoxonase -1 (PON-1) activity was estimated using spectrophotometric method by the hydrolysis of phenylacetate. HDL-C level was measured by CHOD-POD method.

RESULTS- The serum LCAT activity and levels of apo-AI and HDL-C in patients with alcoholic liver cirrhosis were significantly reduced ($p<0.001$) compared with controls. A significant decrease in LCAT activity and levels of apo A-I and HDL-C in alcoholic cirrhosis may contribute to the risk of atherosclerosis in alcoholic liver cirrhosis patients.

CONCLUSION- The decreased LCAT activity, Apolipoprotein A-I levels, PON-1 activity and HDL-C may be associated with a reduction in RCT and contribute to the development of atherosclerosis in alcoholic liver cirrhosis patients.

INTRODUCTION

Alcoholic liver cirrhosis is a global health problem. Chronic and excessive alcohol ingestion is one of the major causes of liver diseases. Globally in 2010, 7.2 deaths per 100,000 people (4.6 deaths per 100,000 females and 9.7 deaths per 100,000 males) were caused by liver cirrhosis attributable to alcohol consumption.[1] Alcoholic liver disease represents a spectrum of clinical illness and morphological changes that range from fatty liver to hepatic inflammation and necrosis (alcoholic hepatitis) to progressive fibrosis (alcoholic cirrhosis).[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]

Human LCAT is a 416 amino acid glycoprotein circulating in plasma associated with lipids and apolipoproteins in the HDL fraction.[5] In human plasma LCAT plays a key role in cholesterol homeostasis by mediating the production of most of the cholesteryl esters. It has been suggested that LCAT plays an important role in RCT by creating a concentration gradient for the efflux of free cholesterol from peripheral cells to HDL particles and its conversion to cholesteryl esters[5].

Apolipoprotein A-I is a single polypeptide with 243 amino acids. It is synthesized in liver. Apolipoprotein A-I is the most abundant protein in HDL, whose concentration is known to be inversely correlated with cardiovascular risk. HDL associated apo A-I play a crucial role in cholesterol homeostasis by regulating reverse cholesterol transport delivering it to the liver. Apolipoprotein A-I acts an activator for the enzyme LCAT[6].

HDL promotes cholesterol efflux from macrophages in the vessel wall. It has anti-inflammatory and anti apoptotic properties which prevents vascular endothelial dysfunction by stimulating nitric oxide synthase and also retards the oxidation of LDL[7]. Several protein components of HDL can inhibit the oxidation of LDL both in vitro and in vivo. These include apolipoprotein A-I (apo A-I), lecithin: cholesterol acyl transferase (LCAT), and paraoxonase-1 (PON-1)[8,9].

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. The effect of HDL associated PON-1 or of purified PON-1 on the LDL oxidation process, including its inhibition (conjugated dienes formation), propagation (peroxides formation) and decomposition (aldehyde formation) phases could be analyzed by using PON-1 inhibitors[10].

Hence the study planned to measure the activity of LCAT, Paraoxonase-1 and the levels of apo A-I and HDL-C in alcoholic liver cirrhosis to assess the risk of atherosclerosis in alcoholic liver cirrhosis patients.

MATERIALS AND METHODS:

The study group was comprised of 100 males with well diagnosed alcoholic liver cirrhosis patients in the age group of 25-55 years admitted in medicine wards 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. 100 males with age group of 25-55 years healthy participants were taken as control group. The study was performed for a period from December 2019 to January 2021. Written informed consent was taken from all subjects involved in the study and the study was approved by Institutional Ethical 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 LCAT activity, PON-1 activity and levels of apo A-I and HDL. LCAT activity was assessed by measuring the difference between esterified cholesterol and free cholesterol[11]. Determination of free cholesterol and ester cholesterol was done by using digitonin precipitation method.[11] Proteins are precipitated from serum by means of an ethanol-acetone mixture which also extracts the cholesterol and cholesterol esters. Cholesterol is then determined by means of the Liebermann-Burchard reaction after being precipitated with digitonin before and after saponification, thus giving free and total cholesterol respectively. Apolipoprotein A-I was measured by immunoturbidimetric method using semiautoanalyzer[12]. PON-1 was estimated spectrophotometrically by hydrolysis of phenyl acetate[13].HDL cholesterol 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-PAP) method[14], VLDL and LDL cholesterol was calculated by Friedewald formula VLDL= Triglyceride/ 5 LDL = Total cholesterol-VLDL-HDL.[14]

EXCLUSION CRITERIA:
Known cases diabetes mellitus, obesity, hypothyroidism and 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.

LIMITATIONS: Stages of severity of alcoholic liver cirrhosis was not known in the patients included in the study group.

STATISTICAL ANALYSIS: The results are expressed as mean±SD. The results are further subjected to unpaired t test, differences between means are considered significant at p<0.05. Correlation between LCAT activity and apo A-I, LCAT activity and HDL-C levels was done by Pearson's correlation coefficient.

RESULTS
The activity of LCAT in alcoholic liver cirrhosis (55.15 ± 3.19 IU/L) compared to controls (93.61± 5.96IU/L) was significantly reduced with p<0.001. The levels of apo A-I in alcoholic liver cirrhosis compared to controls (130± 8.47 mg/dL) were significantly reduced with p<0.001. 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. HDL-C in alcoholic liver cirrhosis (27.57± 4.69 mg/dL) on comparison with controls (52.28± 9.41 mg/dL) was significantly reduced with p<0.001. The levels of total cholesterol, LDL-C and VLDL were also reduced in alcoholic liver cirrhosis compared to control participants. TAG levels were significantly elevated in alcoholic cirrhosis patients on comparison with control subjects.

The results showed positive correlation between LCAT activity and apo A-I in alcoholic liver cirrhosis with r= 0.60 (Figure 1). There was also positive correlation between LCAT activity and HDL-C in alcoholic liver cirrhosis with r= 0.67 (Figure 2).

Table 1: Comparison of various serum parameters in alcoholic liver cirrhosis and control participants.

Sl. No	Parameters	Alcoholic liver cirrhosis (n=100)	Control Subjects (n=100)
1	Apolipoprotein A-I (mg/dL)	130.4±8.47*	190.64±18.80
2	LCAT activity (IU/L)	55.15±3.19*	93.61±5.96
3	Total cholesterol (mg/dL)	173.63±25.03*	198.04±13.98
4	HDL (mg/dL)	27.57±4.69*	52.28±9.41
5	LDL (mg/dL)	105.65±17.63*	115.45±18.12
6	VLDL (mg/dL)	24.71±2.47*	29.85±4.26
7	Triglycerides (mg/dL)	190.1±11.83*	145.90±17.20
8	PON-1(U/mL) (nmol/mL/min)	50.22±17.17*	178.32±30.30

P<0.001=significant, n=number of subjects, all values are expressed as Mean ± Standard deviation

Figure 1: Levels of Apo A-I in Alcoholic Liver Cirrhosis cases and control participants [Apo A-I levels were significantly decreased p<0.001 in alcoholic liver cirrhosis cases 130.4±8.47 when compared with control participants 190.64 ± 18.80]

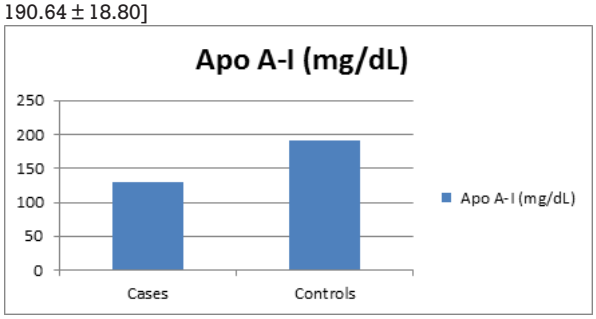

Figure 2: LCAT activity in Alcoholic Liver Cirrhosis cases and control participants [LCAT activity was significantly decreased p< 0.001 in alcoholic liver cirrhosis cases 55.15± 3.19 when compared with control participants 93.61± 5.96]

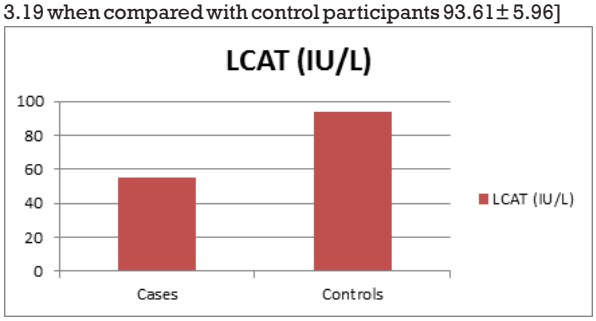

Figure 3: PON-1 activity in Alcoholic Liver Cirrhosis cases and control participants [PON-1 activity was significantly decreased p<0.001 in alcoholic liver cirrhosis cases 50.22±17.17 when compared with control participants 178.32± 30.30]

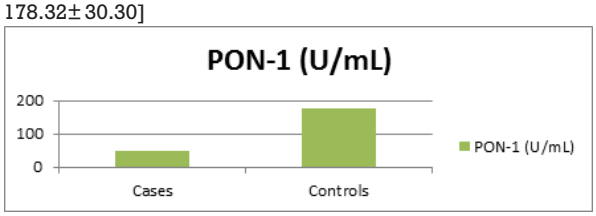

Table 2: Correlation between LCAT activity with levels of Apo A-I and HDL-C in Alcoholic Liver Cirrhosis [Positive correlation between LCAT activity and Apo A-I (r=0.60) and LCAT activity and HDL-C (r=0.67)]

r-value	LCAT activity
ApoA-I	0.60
HDL-C	0.67

DISCUSSION:
Liver is the most important organ for the metabolism of lipids, lipoproteins and apolipoproteins[15]. Apolipoprotein A-I (apo A-I) is produced mainly by hepatocytes and its level varies according to the degree of liver fibrosis[16,17]. The liver is the major source of plasma lipoproteins and of lecithin-cholesterol acyltransferase (LCAT), a key enzyme involved in lipoprotein metabolism, liver injury should be accompanied by abnormalities of lipoprotein biosynthesis and metabolism[18].

Present study found that LCAT activity was significantly

(p<0.001) reduced in alcoholic liver cirrhosis when compared with healthy control participants (table 1). Seymour MS et al[18] also showed reduced LCAT activity in alcoholic liver cirrhosis compared to controls.

Levels of HDL-C was significantly (P<0.001) reduced in alcoholic liver cirrhosis when compared with healthy control participants (table 1). Phukan JP et al[19] also showed reduced HDL-C in alcoholic liver cirrhosis compared to controls. Recent studies by Ghadir MR et al[20] and Cicognani C et al[21] on cirrhosis of liver showed that serum HDL-C, LDL-C and total cholesterol values were significantly diminished.

In Present study the levels of apo A-I which was significantly (p<0.001) reduced in alcoholic liver cirrhosis when compared with healthy control participants. (table 1). Abraham Lemberg et al[22] also showed reduced LCAT activity and Apolipoprotein synthesis in alcoholic liver cirrhosis compared to controls. Recent studies[23,24] have documented low plasma LCAT activity in patients with liver disease and have shown that this decrease is associated with impaired plasma cholesterol esterification.

The activity of PON-1 was significantly (p<0.001) reduced in alcoholic liver cirrhosis when compared with healthy control participants (table 1). In alcoholic liver cirrhosis the capacity of 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 alcoholic liver cirrhosis patients.

In alcoholic liver cirrhosis there is defective biosynthesis of proteins resulting in reduced apo A-I, PON -1 and LCAT enzymes. HDL is a substrate for LCAT enzyme. Apo A-I is an activator of LCAT enzyme[22], in alcoholic liver cirrhosis there is decreased apo A-I levels. These reduced apo A-I levels may decrease the LCAT activity. In alcoholic liver cirrhosis there is reduced LCAT activity[25] due to this, undesirable structural changes may reduce the levels of HDL-C in alcoholic liver cirrhosis patients.

HDL plays a role in RCT because it not only promotes the efflux of cholesterol from peripheral tissues but is also the major site for esterification of cholesterol by LCAT[26]. LCAT plays a role in intravascular HDL metabolism and in RCT. A defective synthesis of LCAT results in defective LCAT function, which is thought to increase the risk of atherosclerosis by interfering with above processes[27].

It has been shown that Apo A-I, LCAT and PON-1 act in concert to prolong the protective effect of HDL against the oxidative modification of LDL[28]. APO A-I, LCAT and PON-1 have all been shown previously, to individually have antioxidant activity in preventing the oxidation of LDL both in vitro and in vivo and are therefore believed to retard the development of atherosclerosis by this mechanism[29,30]. PON-1 is component of HDL cholesterol. Reduced activity of PON-1 may be responsible for increase the risk of atherosclerosis by interfering the function of HDL cholesterol.

The levels triacylglycerol was significantly (p<0.001) increased in alcoholic liver cirrhosis compared with healthy control participants (table 1). The levels LDL-C were significantly (p<0.001) decreased in alcoholic liver cirrhosis compared with healthy control participants (table 1).

The present study found a positive correlation between LCAT and apo A-I (r=0.60) (figure 1) and LCAT and HDL-C (r=0.67) (figure 2) in alcoholic liver cirrhosis patients.

Thus reduced apo A-I in alcoholic liver cirrhosis may decrease LCAT activity. Reduced LCAT activity and PON-1may cause structural change in HDL-C leading to reduced functional capacity of HDL-C leading to reduced RCT. Hence risk of atherosclerosis is increasing in alcoholic liver cirrhosis patients.

CONCLUSION:

The decreased LCAT and PON-1 activity, Apolipoprotein A-I levels, and HDL-C may be associated with a reduction in reverse cholesterol transport and contribute to the development of atherosclerosis in alcoholic liver cirrhosis.

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