Role of LCAT and Apo A-I in Newly Diagnosed HIV Patients

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Abstract Human immunodeficiency virus (HIV) infection is a growing concern for health workers. The two major components of dyslipidemia in HIV infected patients are hypertriglyceridemia and decreased levels of high density lipoprotein (HDL) which contribute to increased atherosclerotic risk. The study included 150 newly diagnosed HIV patients and 150 healthy controls. In all these cases Lecithin cholesterol acyl transferase (LCAT) activity was assessed by measuring the difference between esterified and free cholesterol by digitonin precipitation method and levels of Apolipoprotein A-I (apo A-I) were measured by immunoturbidimetric method. There was a significantly decreased LCAT activity ($p < 0.05$) as well as reduced levels of apo A-I ($p < 0.05$) in newly diagnosed HIV patients (54.98 ± 3.44 IU/L and 131.85 ± 8.49 mg/dl) when compared with healthy controls (88.17 ± 14.36 IU/L and 187.05 ± 35.25 mg/dL). A significant positive correlation ($r = 0.7406$) was found between LCAT activity and apo A-I levels. In conclusion decreased LCAT activity and decreased levels of apo A-I reduce the reverse cholesterol transport in newly diagnosed HIV patients which may lead to atherosclerosis in the future.

Keywords HDL · HIV · apo A-I · LCAT

Introduction

HIV infection is a growing concern for healthcare workers and physicians with 36.9 million people living with HIV infection worldwide [1]. India has 2.39 million HIV positive individuals with adult prevalence rate of 0.31% [2]. There is an increasing incidence of HIV infection and also greatly improved long term survival with Highly active antiretroviral therapy (HAART) in these patients [3]. The clinical and pathological consequences of HIV associated dyslipidemia are not completely known, but several studies reported an increased risk of coronary artery disease in HIV positive individuals [4]. The two major components of dyslipidemia in HIV infected patients are hypertriglyceridemia and decreased levels of high density lipoprotein which contribute to increased atherosclerotic risk [5].

HDL plays a central role in Reverse Cholesterol transport (RCT) because it not only promotes the efflux of cholesterol from peripheral tissues but is also the major site for the esterification of cholesterol by LCAT. Human LCAT is a glycoprotein made up of 416 amino acids circulating in plasma associated with lipids and apolipoproteins in the HDL [6]. LCAT is the enzyme that generates cholesterol esters in plasma. It plays a key role in reverse cholesterol transport and is activated by the Apolipoprotein A-I in HDL [7]. It promotes reverse cholesterol transport by maintaining a free cholesterol gradient between HDL and peripheral tissues [8].

Current knowledge regarding the etiology of altered lipid metabolism and the mechanism of development of atherosclerosis in newly diagnosed HIV patients has not shown a clear picture. There are few studies on LCAT activity and levels of apo A-I in HIV patients. Thus, the aim of the study was to study the cardioprotective role of

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LCAT activity and levels of apo A-I in the development of atherosclerosis in newly diagnosed HIV patients.

Materials and Methods

The study group included 150 (75 males; 75 females) newly diagnosed HIV patients between 20 and 45 years visiting Integrated Counseling and Testing Centre (ICTC), BIMS Hospital, Belagavi. The diagnosis was done by Senior Medical Officer ART centre Belagavi Institute of Medical Sciences and Hospital, Belagavi by COOMBAIDS (HIV 1 + 2 immunodot test kit), HIV-1/2 Triline card test and Retrocheck HIV-WB (Rapid immunochromatographic test for HIV 1/2 antibodies). 150 (75 males; 75 females) age and sex matched healthy controls were selected from the general population. Brief clinical history, personal history regarding consumption of alcohol, cigarette smoking and tobacco chewing were taken. Clinical examination with measurement of BP and BMI was done. The study was a cross sectional study conducted in Department of Biochemistry Belagavi Institute of Medical Sciences, Belagavi. All the participants gave written informed consent and the study was approved by Institutional Ethical Clearance Committee. All procedures performed in this study were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

HIV patients on antiretroviral treatment, known cases of hypertension, diabetes mellitus, thyroid dysfunction, renal and hepatic diseases, cardiac disorders were excluded by taking clinical history, clinical examination and clinical investigations against these disorders and history of consumption of drugs against these disorders. Participants whose BMI > 30 kg/m² were excluded from the study.

Under all aseptic precautions, 5 mL fasting venous blood sample was drawn, from newly diagnosed HIV patients and healthy participants. Serum was separated after centrifugation and used for estimation of various parameters. The difference between esterified and free cholesterol gives the activity of the enzyme LCAT in IU/L. Determination of free and esterified cholesterol was done by using digitonin precipitation method [9]. Free cholesterol was extracted from serum by acetone ethanol mixture. The free cholesterol digitonide was precipitated with 2.5 mL digitonin (0.5% digitonin solution in 50% ethanol) and kept for overnight incubation in dark and colour was developed with 2 mL glacial acetic acid and ferric chloride and sulphuric acid in the ratio of 20:1 and read at 620 nm with Systronics UV–VIS Spectrophotometer 117. The levels of apo A-I were estimated by end point immunoturbidimetric method using polyclonal goat antihuman immunoglobulin supplied by the ERBA kit in semiauto

analyser. HDL [10] and total cholesterol were estimated by CHOD-POD method [10]. Triglyceride estimation was done by GPO-PAP method [10], VLDL and LDL were calculated by Friedwald's formula.

Statistical analysis was done by expressing all the values as mean $\pm$ SD. Pearson's correlation coefficient was used to find out the association between the variables. Students Unpaired 't' test was employed to know the significance between both variables.

Limitations of the study—The LCAT activity was indirectly measured as the difference between esterified cholesterol and free cholesterol.

Results

The results showed significant decrease in apo A-I levels (Fig. 1) and LCAT activity (Fig. 2) in newly diagnosed HIV patients compared with the control participants. A positive correlation is found between LCAT activity and apo A-I levels in newly diagnosed HIV patients (Fig. 3).

Discussion

Increased risk of atherosclerosis and coronary artery disease (CAD) is a recognized clinical problem in HIV-infected patients [11–15]. The features of HIV associated dyslipidemia are decreased plasma concentrations of total cholesterol and HDL with elevated LDL and plasma TG [16–18].

In this study, total cholesterol and LDL (Table 1) were significantly high in newly diagnosed HIV patients when compared with control participants. Howard et al. [19] also showed that LDL levels were increased in HIV patients when compared with control group. Crook et al. [20] showed that HIV infection is normally associated with

Fig. 1 Levels of apo A-I in newly diagnosed HIV patients and control participants: (apo A-I levels were significantly ($p < 0.05$) reduced in newly diagnosed HIV patients compared to the control participants)

Fig. 2 Activity of LCAT in newly diagnosed HIV patients and control participants: (LCAT activity was significantly ($p < 0.05$) reduced in newly diagnosed HIV patients compared to the control participants)

Fig. 3 Correlation between LCAT activity and apo A-I in newly diagnosed HIV patients: (Positive correlation between LCAT activity and apo A-I levels ($r = 0.7406$), Two tailed p value < 0.0001)

hypcholesterolaemia, hypertriglyceridaemia and low plasma HDL.

Present study found significantly decreased levels of HDL and apo A-I ($p < 0.05$) in newly diagnosed HIV patients when compared with the control participants. The study also found that in newly diagnosed HIV patients the

activity of LCAT was significantly reduced ($p < 0.05$) when compared with control participants (Table 1) which were similar to findings of Honor Rosea et al. [18].

HDL plays a central role in RCT because it not only promotes the efflux of cholesterol from peripheral tissues but it is also the major site for the esterification of cholesterol by LCAT. LCAT plays a key role in RCT and it is activated by the apo A-I in HDL [7]. It promotes RCT by maintaining a free cholesterol gradient between HDL and peripheral tissues [8].

The initial plasma step of RCT, the conversion of pre β 1-HDL to α -HDL and further maturation of HDL is most likely enhanced in HIV infected patients. However, the combination of hypertriglyceridemia and high activity of CETP diverts the latter steps of RCT from delivery of cholesterol directly to the liver towards delivery of cholesterol to atherogenic VLDL and LDL. Although the liver through LDL receptors will take up the majority of transferred cholesterol, the amount of cholesterol available for modification and uptake by extrahepatic tissues will also increase proportionally. This is likely to result in more cholesterol being delivered to extrahepatic cells. If this situation persists over long period of time, it may contribute to the increased risk of atherosclerosis [21].

Changes in HDL remodeling also lead to the alteration of HDL composition. Although the ability of HDL particles from HIV-infected patients to promote cholesterol efflux remained intact, the increased triglyceride content of HDL may impact on binding and uptake of these particles by liver scavenger receptors, impairing cholesterol delivery through these receptors and making HDL particles less protective against atherosclerosis [22]. Reduced levels of apo A-I and impairment of RCT are more likely to be caused by HIV infection. In HIV infection there is decrease in protein synthesis [23]. This may be responsible for reduction of apo A-I levels in newly diagnosed HIV patients. Apo A-I is an activator of LCAT enzyme so decreased levels of apo A-I leads to reduction of activation

Table 1 Comparison of various serum parameters in newly diagnosed HIV patients and control participants

Parameters	Newly diagnosed HIV patients (n = 150)	Control participants (n = 150)
Age	34.24 $\pm$ 5.98*	31.1 $\pm$ 7.86*
BMI	23.51 $\pm$ 2.98	23.17 $\pm$ 1.92
apo A-I	131.85 $\pm$ 8.49*	187.05 $\pm$ 35.25*
LCAT activity (IU/L)	54.98 $\pm$ 3.44*	88.17 $\pm$ 14.36*
HDL (mg/dL)	28.83 $\pm$ 2.17*	50.66 $\pm$ 10.31*
Total Cholesterol (mg/dL)	200.63 $\pm$ 15.25*	169.92 $\pm$ 33.99*
Triglycerides (mg/dL)	175.58 $\pm$ 91.29	160.75 $\pm$ 109.22
VLDL (mg/dL)	35.17 $\pm$ 18.18	31.32 $\pm$ 20.12
LDL (mg/dL)	136.43 $\pm$ 21.16*	87.99 $\pm$ 34.99*

* $p < 0.05$ = Significant, n number of subjects, all values are expressed as Mean $\pm$ SD

of LCAT. Thus there is decreased LCAT activity. Reduced LCAT activity reduces the functional capacity of HDL. This leads to reduction of esterified cholesterol.

A key element of atherosclerotic plaque is formation of foam cells, the majority of which are macrophages overloaded with cholesterol. Foam cells undergo apoptosis, necrosis, and calcification. The cholesterol released from foam cells forms the lipid-rich core of the plaque. The HIV viral proteins exert effects on the adipocytes resulting in mitochondrial dysfunction, reactive oxygen species (ROS) production, insulin resistance and decreased adiponectin. These factors may increase the levels of LDL. The viral proteins also induce a proinflammatory response in peripheral tissues and decrease plasma HDL by impairing the cholesterol dependent efflux transporter ATP-binding cassette protein A1 (ABCA1) in human macrophages, a condition that is highly atherogenic [24]. One of the main causes of foam cell formation and accumulation of cholesterol in the vessel wall may be dyslipidemia, especially high levels of VLDL and LDL, as well as low levels of HDL [25], and impairment of intracellular cholesterol metabolism.

Thus, this study shall help in predicting the risk of atherosclerosis in newly diagnosed HIV positive patients by the measurement of LCAT activity and estimation of levels of apo A-I which tells us about functional HDL along with other lipid parameters. Thus early interventions targeted to reduce the atherogenic lipid components and to increase the atheroprotective lipid components in newly diagnosed HIV patients will reduce the morbidity and mortality by decreasing risk of atherosclerosis in future.

Conclusion

The decreased LCAT activity and decreased apo A-I may be associated with a reduction in RCT and contribute to the development of atherosclerosis in newly diagnosed HIV patients in future.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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