Original Article

Study of plasma and erythocyte membrane lipids in HIV positive patients

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Abstract

Introduction: Phospholipids are the major lipids in all cell membrane and their composition and deposition within the cell are fixed. The alterations in erythrocyte membrane components have been described in several diseases, in which there are abnormalities in plasma lipoprotein compositions. Although the altered lipid metabolism in HIV infection is reported in the previous studies, but the present study has reconsidered the impairment of lipid metabolism in HIV patients by taking into account phospholipids from erythrocyte membrane lipids. **Methods:** Blood samples of 50 HIV positive patients (age 20-30 years), were collected from Microbiology department. Erythrocyte membrane cholesterol was estimated calorimetrically by the method of ZAK and phospholipids estimated by the method of Connerty. The individual phospholipids from erythrocyte membrane were separated by thin layer chromatography (TLC). Quantitative data were analyzed using unpaired t test with SPSS software, version 17. **Results:** The cholesterol and phospholipid concentration of erythrocytes was altered significantly in HIV patients as compared to controls. The cholesterol content was increased (6.59 ± 2.89), but the total phospholipids were decreased significantly (7.62 ± 1.73) in HIV patients with respect to control subjects (cholesterol- 7.94 ± 2.68, phospholipid- 9.50 ± 2.65). As a result, cholesterol to phospholipid ratio was significantly increased in HIV patients (1.31 ± 0.26 in cases and 0.83 ± 0.17 in controls). The individual phospholipid composition of the erythrocyte membrane fractions showed significant decreased [$p < 0.01$] levels in HIV patients as compared to the controls. **Conclusion:** The increased cholesterol and decreased phospholipid levels of erythrocytes in HIV patients suggest the altered erythrocyte morphology in these patients. The biochemical parameters used in this study may be used as diagnostic and prognostic markers for the wellness of HIV infected patients. **Abbreviations:** (RBC): Red cell membrane, (HIV): Human iimmunodeficiency virus, (TLC): Thin layer chromatography, (LCAT): lecithin: cholesterol acyltransferase.
Keywords: HIV, Red blood cell membrane, RBC lipids, Thin layer chromatography.

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INTRODUCTION

AIDS, the acquired immunodeficiency syndrome, is a fatal illness caused by a retrovirus known as the human immunodeficiency virus (HIV) which breaks down the body's immune system, leaving the patient vulnerable to a host of life-threatening opportunistic infections, neurological disorders, or unusual malignancies (Adewole *et al*, 2010) HIV infection is characterized by a number of nutritional, metabolic and endocrinological abnormalities. Patients with human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome (AIDS) frequently present alterations in lipid metabolism due to infection with HIV itself, including elevated serum concentrations of triglycerides and low levels of total cholesterol (Souza *et al*, 2013). Adewole *et al* reportedthat Low density lipoprotein (LDL) was significantly higher (2.26+ 0.9 mmol/l) in HIV patients compared to the control (0.96+0.39mmol/L).The High density lipoprotein (HDL) was also significantly lower (0.8+0.6mmol/L) which caused dyslipidaemia in the HIV positive group than the control. The alteration in serum lipids have influence of the lipid composition and thus

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functioning of biological membranes such as RBC and leukocytes membrane. Various physiochemical alterations such as deformability, aggregability, viscoelasticity and fluidity take place and eventually impair hemorheological system (Athanassiou *et al*, 2010). The hematological factors which are referred to as the main hemorheological parameters associated with the disease are increased erythrocyte aggregation, increased leukocyte rigidity and decreased erythrocyte deformability (Kim *et al*, 2006). In the literature the alterations in serum lipids composition have been reported but blood cell abnormalities and changes in their lipid compositions have been scarcely reportedin the HIV infected population. So the aim of present study was to study the RBC membrane lipid composition of HIV patients and compare it with healthy controls.

MATERIAL AND METHODS

This single centered, case control study was conducted in Biochemistry department, Seth G. S. Medical College, Mumbai, India. The study protocol was approval by institutional Ethics Committee prior of starting the study.

Study Population

Fifty HIV infected patients of either sex having CD4+ cell count in the range of 400 - 500 cubic millimetres of blood were recruited from the Microbiology department during the year of 2010 to 20111. Informed written consent was obtained from all study participants before blood sample collection. Fifty age and sex matched healthy subjects were also recruited as control group. None of the patients in our study group were suffering from any secondary infections or were on antiviral treatment. All the patients and control subjects were between the age ranges of 20 to 30 years.

Blood Samples and Clinical Tests

Blood sample from patients and controls (6 ml) was taken in heparin and plain evacuated tubes by venepuncture after 12 hour fasting and immediately processed. The samples were centrifuged for 10 minutes at 1500 rpm. The plasma was separated and the red blood cell settled at the bottom was used for used for estimation of various lipid parameters. Plasma cholesterol, triglycerides and HDL levels were measured using the enzymatic methods of Allian *et al*, 1974; Foster and Dunn, 1982 and Assam *et al*, 1980 respectively. RBC membrane lipid extraction was carried using method of Rose and Oklander, 1965. Aliquots of lipid extract were taken for RBC membrane cholesterol and total phospholipids estimation using the methods of Zlatkis *et al*, 1953 and Connerty *et al*, 1961 respectively. For individual phospholipid estimation, 1ml of lipid extract was taken in clean glass tube and evaporated till dryness. Few drops of chloroform: methanol mixture was added to make it concentrated in proportion of 2: 1. Then few drops of chloroform - isopropanol mixture was added tomake it concentrated in proportion of 2: 3 then the individual phospholipids were estimated by spotting on Thin Layer Chromatography (Rouser *et al*, 1966). The solvent system chloroform: methanol: water (65: 25: 4). RBC cholesterol, total phospholipids and individual phospholipids were expressed in the terms of mg per gram of Hemoglobin. Normal reference ranges for RBC membrane cholesterol and total phospholipids were 6-9 mg per gram of Hemoglobin and 9-12 mg per gram of Hemoglobin respectively. The hemoglobin concentration was measured by cyanmethaemoglobin method as given by Baker and Silverton, 1985 before the extraction procedure.

Statistical analysis

Data were analyzed using SPSS version 17. All the quantitative parameters were expressed as mean and Standard Deviation (SD) and qualitative parameters were presented as frequency (%). To compare a continuous variable between groups, the Student's unpaired t-test was performed. P value of <0.05 was considered statistically significant.

RESULTS

In our study 50 HIV patients and 50 controls were included. Out of 50 patients, 41 (82.0 %) were male and 9 (18.0 %) were female.

Plasma lipids parameters in control and HIV patients

The Table 1 shows plasma triglyceride and cholesterol levels in HIV infected and control subjects. The analyses of lipid values revealed significant differences between HIV seropositive and HIV seronegative subjects. As compare to controls, the plasma triglycerides levels were increased significantly [$p < 0.001$] but the cholesterol levels were observed to be declined [$p < 0.001$] in HIV patients. The decreased in cholesterol levels was proportionally greater than increased in the triglyceride levels in HIV patients. Thus the HIV patients suffer from hypocholesterolemia (cholesterol levels <150mg %) and hypertriglyceridemia in the early stages of infection. In additional to this the other major abnormalities in lipid profile were, decreased in HDL, HDL_2, HDL_3 and LDL cholesterol levels [$p < 0.001$] in HIV patients as compared to controls. As the total cholesterol decreased drastically the net effect was observed in HDL and its fraction total cholesterol levels.

Table 1: Plasma lipid composition of HIV positive and healthy control subjects

Parameters	Controls (N = 50)	HIV Positive Patients (N = 50))
Total plasma triglyceride (mg %)	134.52 ± 5.2	149.36 ± 7.22 **
Total plasma cholesterol (mg %)	156.62 ± 9.16	139.32 ± 6.28 **
HDL cholesterol (mg %)	45.21 ± 4.72	35.34 ± 3.62 **
HDL_2 cholesterol (mg %)	18.87 ± 2.32	13.91 ± 1.5 **
HDL_3 cholesterol (mg %)	26.34 ± 2.4	21.43 ± 2.12 **
LDL cholesterol (mg %)	111.62 ± 4.44	102.98 ± 2.96 **

*p < 0.01, **p < 0.001.

RBC membrane lipids parameters in control and HIV patients

Table 2 shows the comparison of RBC membrane lipid profile among the HIV positive patients group and control group. The mean total cholesterol and RBC membrane Cholesterol to phospholipid ratio was significantly higher while total phospholipid content of RBC membrane was significantly lower ($p < 0.01$) in patient group compared to the control group. When individual phospholipids were compared all 4 phospholipids (Sphingomyelin, Phosphatidylserine, Phosphatidycholine and Phosphatidylethanolamine) showed significant decrease ($p < 0.01$) in HIV patients group as compared to the healthy control group. (Table 2)

Table 2: RBC membrane lipids parameters in control and HIV positive patients

RBC membrane lipids	Controls (N = 50) (Mean ± SD)	HIV Positive Patients (N = 50) (Mean ± SD)
Total cholesterol (mg / gm Hb)	6.59 ± 2.89	7.94 ± 2.68 *
Total phospholipids (mg / gm Hb)	9.50 ± 2.65	7.62 ± 1.73 **
Cholesterol / Phospholipid	0.83 ± 0.17	1.31 ± 0.26 **
Sphingomyelin (mg / gm Hb)	2.35 ± 0.56	1.96 ± 0.42 **
Phosphatidylserine (mg / gm Hb)	1.52 ± 0.52	1.26 ± 0.30 **
Phosphatidycholine (mg / gm Hb)	2.91± 0.95	2.15 ± 0.53 **
Phosphatidylethanolamine (mg / gm Hb)	2.72 ± 0.62	2.25 ± 0.48 **

*p < 0.01, **p < 0.001

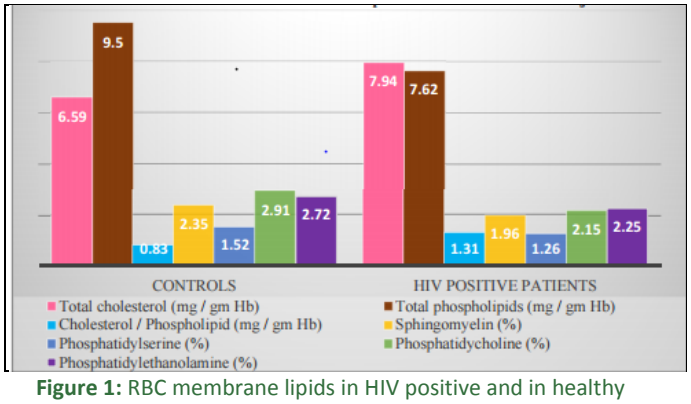

Figure 1: RBC membrane lipids in HIV positive and in healthy control subjects

DISCUSSION

In view of the increasing incidence of HIV infection throughout the world, the present study was undertaken to find out the changes in plasma and RBC membrane lipid profile during the HIV infection. The marked disturbances in lipid metabolism have been seen in patients with AIDS. The altered lipid metabolism may affect immune process so this study is characterized lipid profile of individual infected with HIV infection. The recent data have demonstrated a novel syndrome of fat redistribution and hyperlipidemia among the patients with human immunodeficiency syndrome (HIV) infection. The mechanism of responsible for the abnormal lipids metabolism in HIV patients is still not very clear, but may results from direct effect of HIV virus, cytokines, and various hormones on the major lipid i.e. Cholesterol, triglyceride, phospholipids (Grunfeld *et al*, 1992). HIV infection causes alterations of the immune functions along with the lipid metabolism mainly TG, cholesterol, and various lipoproteins lipids. This lipid metabolism alteration effect on the immune function may be a useful marker of disease progression. The previous studies have reported marked variations in plasma lipids during the infections. A few authors, who determined the levels of plasma triglycerides, total cholesterol and HDL cholesterol in HIV infected individuals by the level of immunological deficiency according to the $CD4^+$ T Lymphocytes count, also came to the same conclusion that, with an increase of immunological deficiency and clinical development of HIV infection, lipid profile disorders, indicated by an increase in triglyceride level and decreased concentrations of HDL cholesterol intensified as well (Ducobu *et al*, 2000). Within the present study we analyzed the serum lipid along with the erythrocytes lipids in HIV positive patients. The marked disturbance in lipid metabolism occurs during early stages of HIV infection. The total triglyceride and cholesterol levels along with the phospholipids and erythrocytes lipids are drastically altered in HIV patients as compare to the controls subjects. These findings are of particular interest in light of the effect that altered lipid metabolism may have on immune process. In the present study, the plasma lipids are reported to be altered drastically in HIV positive patients as compare to the controls samples. The level of total cholesterol is drastically decreased with simultaneous increased in triglyceride in HIV patients as compare to control samples. The alterations in red blood cell membrane lipids are reported in various infections and diseases. The maintenance of intact red cell membrane requires a continuing rapid exchange of cholesterol and other lipids with similar constituents in plasma, mainly lipoproteins. The cholesterol in RBC membrane normally comprises more than 99% of the

neutral lipid in RBC membrane, has been found to exchange freely between both the inner and outer layers of the bilayer and is in equilibrium with non-esterified (free) cholesterol attached to the plasma lipoproteins. Thus in number of pathological states, alterations in RBC membrane lipid composition is associated with abnormalities in plasma and lipoprotein lipids. According to Kim *et al*.2006, increased aggregation and decreased deformability is associated with the HIV infected patients. Similarly, Martins Silva *et al*, 2006, showed the alterations in erythrocyte membrane properties in terms of membrane fluidity, intercellular calcium and calcium signalling. The binding of HIV virus to erythrocytes was used as a measure of the severity of the infection. In the present study the red blood cell membrane lipids, cholesterol, and phospholipids was estimated in HIV patients. The present study has revealed for the first time the drastically altered erythrocyte lipid composition in HIV patients, as described in the Table No. 1. The RBC membrane cholesterol in present study is reported to be increased (6.59 ± 2.89 mg / gm Hb) with simultaneous decreased in total phospholipids levels (9.50± 2.65 mg / gm Hb) in HIV patients as compare to controls. Besides the erythrocyte membrane total phospholipids levels, the four major individual phospholipid constituents of erythrocyte membrane i.e. sphingomyelin, phosphatidylserine, phosphatidycholine, phosphatidylethanolamine were found to be decreased significantly among the HIV patients as compare to the controls subjects ($p < 0.001$) in present study. The enzyme lecithin: cholesterol acyltransferase (LCAT) plays an important role in the metabolism of unesterified cholesterol and lecithin in serum lipoproteins. A relationship between LCAT activity and the red cell content of cholesterol has been suggested in previous studies. The various studies reveal the increased cholesterol contents of red cell membrane among the patients having low activity of LCAT enzyme. The previous studies revealed that HIV patients have decreased levels of various lipolytic enzymes (Rogowska-Szadkowska *et al*, 1999; Ducobu *et al*, 200 and Khiangte *et al*, 2007). It is unknown whether LCAT enzyme deficiency plays an etiological role in the genesis of cholesterol-rich red cell membranes; however it may be at least one factor, which leads to the accumulation of membrane cholesterol in HIV patients. In the present study the increased in cholesterol land decreased in phospholipids content of erythrocyte membrane among the HIV patients was accompanied by a significant increase in the cholesterol to phospholipid ratio. The increased RBC membrane cholesterol / phospholipids ratio in HIV patients demonstrated in this study may be due to the increase in the concentration of RBCs membrane cholesterol which, attributes to the increase rate of exchange between plasma cholesterol and RBC membrane cholesterol and hence, may cause lowering of the concentration of plasma cholesterol by in HIV patients. This proposed the increase in the rate of exchange between plasma and RBC membrane cholesterol may be explained by the loss of RBC membrane phospholipids asymmetry, allowing more plasma cholesterol to be incorporated in the RBC membrane possibly to retain some of the loss in RBC membrane integrity. There is close association between the HDL levels and erythrocyte membranes lipids contents. The previous studies stated the alteration in HDL levels during the infections affects their lipid exchange with the erythrocytes membrane. The HIV patients are reported to have decreased levels of HDL in the early stages of infection. Thus the present study may conclude that the altered erythrocyte membrane lipid composition of HIV patients may be associated with the low levels of HDL particles. Thus, the biochemical parameters used in this study may be used as diagnostic markers in assessment of HIV infected patients.

CONCLUSION

The erythrocyte lipid levels are altered significantly in HIV patients during initial stages of infection. The increased cholesterol and decreased phospholipid levels of erythrocytes in HIV patients suggest the altered erythrocyte morphology and increased membrane cholesterol to phospholipid ratio in HIV patients affects the membrane fluidity and represents a factor involved in the pathophysiology of this condition and a possible biochemical marker of the disease.

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