

Research Article

A STUDY OF LIPID PROFILE IN HIV-POSITIVE PATIENTS ATTENDING GOVERNMENT GENERAL HOSPITAL, KURNOOL

Padma Vijayasree, A., *Amarnath Reddy, K., Sreelatha, D and Laxmi, P.

*Department of Biochemistry, Kurnool Medical College, Kurnool

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ABSTRACT

The present study assessed the lipid profile of HIV-infected patients attending the Government General Hospital, Kurnool during the period of 2009-2010. Fasting serum levels of total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C) and triglyceride (TG) were determined in sixty HIV-positive subjects and sixty age-matched HIV-negative controls. The CD4⁺ T-lymphocyte counts of the HIV-positive subjects were assessed and the HIV-positive subjects were grouped into three based on their CD4⁺ levels as follows: Group I (CD4⁺ count < 200 cells/ μ l); group II (CD4⁺ count 200- 499 cells/ μ l); group III (CD4⁺ count \geq 500 cells/ μ l). The total cholesterol, HDL-C, LDL-C, VLDL-C, TG and CD4⁺ T-lymphocyte for the HIV -positive subjects were 4.64 \pm 0.23 mmol/L, 2.02 \pm 0.17 mmol/L, 4.29 \pm 0.32 mmol/L, 0.34 \pm 0.02 mmol/L, 0.84 \pm 0.05 mmol/L and 350.68 \pm 49.25 cells/ μ l, respectively while the TC, HDL-C, LDL-C, VLDL-C and TG of HIV-negative controls were 3.90 \pm 0.20 mmol/L, 2.67 \pm 1.07 mmol/L, 2.85 \pm 1.09 mmol/L, 0.29 \pm 0.01 mmol/L and 0.71 \pm 0.03 mmol/L, respectively. A higher CD4⁺ count in the HIV- positive subjects was associated with higher HDL-C (p<0.05) while a lower CD4⁺ count was associated with higher TG and VLDL- C levels (p<0.05). Among the three groups classified based on the CD4⁺ T-lymphocyte count, higher (p<0.05) LDL-C, VLDL-C and TG with lower (p<0.05) HDL-C were observed among the HIV positive subjects having CD4⁺ T-lymphocytes counts of <200 cells/ μ l compared to the HIV- control. No difference (p>0.05) in total cholesterol was found between group I and the control group. The results revealed that lipid profile monitoring is necessary for CD4⁺ T-lymphocyte count monitoring for the well being of HIV patients in this locality.

INTRODUCTION

Acquired Immune Deficiency Syndrome (AIDS) has become the focus of much global concern and that is reaching epidemic proportions in some parts of the world (Benjamini and Leskowitz, 1993). In 2007, 2.1 million people died of AIDS worldwide and 33.2 million people are currently living with HIV/AIDS (WHO, 2007). In India, about 2.1 million people are living with HIV and the country ranks third in terms of the actual number of people infected with HIV after South Africa and Nigeria (<http://www.thehindu.com/sci-tech/health/india-has-3rdhighest-number-of-hivinfected-people-un/article6220483.ece>). AIDS is caused by a retrovirus, Human Immunodeficiency Virus (HIV). The virus attacks the immune system and leaves the body vulnerable to a Variety of infections and cancers (Khangte *et al.*, 2007). A variety of endocrinologic, metabolic and nutritional disturbances are common during the course of HIV infection. Most HIV-infected patients develop multiple metabolic

abnormalities including insulin resistance, lipodystrophy and dyslipidaemia (Rasheed *et al.*, 2008). Insulin is known to inhibit lipolysis in adipose tissue by inhibiting hormone sensitive lipase. Thus, insulin resistance that occurs in HIV infection will lead to increased lipolysis in adipose tissue and consequently an increase in free fatty acid, triglycerides and cholesterol in plasma. Low CD4⁺ lymphocyte count in HIV infection has been associated with low insulin levels and evidence of insulin resistance (Gadd *et al.*, 2005). Metabolic disturbances in the HIV-infected patients are incriminated to be risk factors of accelerated atherosclerosis and cardiovascular diseases (Pynka *et al.*, 2004) and altered lipid metabolism is known to affect immune Processes (Ceraciolo *et al.*, 2002). Racial variations in serum lipid levels of HIV - infected patients have been observed by Gadd (Gadd *et al.*, 2005). Different studies on lipid profile carried out in different countries show variations in results. For example, a study by Crook (9) showed that HIV infection is normally associated with hypocholesterolaemia, hypertriglyceridaemia and low plasma HDL-cholesterol. Another study by (Pynka *et al.*, 2004) showed that there was no significant difference in total cholesterol and low density lipoprotein between HIV-infected and healthy women.

*Corresponding author: Dr. Amarnath Reddy,
Department of Biochemistry, Kurnool Medical College, Kurnool

MATERIALS AND METHODS

Reagents

All reagents that were used in this study were of analytical grade. Enzymatic colorimetric kits for total cholesterol determination, kits for quantitative determination of HDL-Cholesterol and kits for quantitative determination of triglycerides in serum were from AGAPPE Diagnostics, Kerala (HDL, TC) and RECKON Diagnostics, Baroda (TG). The screening of subjects to determine their HIV status were made by using ELISA (Enzyme linked immunosorbent assay) technique (Cheesbrough, 2001). The kit reagents were manufactured by SD – Biostandard Diagnostics, Gurgaon, Haryana. CD4+ assay was done by using Partec Cyflow 300 green, a product of Partec company, Germany.

Study Protocol

The procedure employed consisted of oral interview and subjects that gave their consent to participate in the study were enrolled in the study. Five milliliters of blood was collected from the antecubital fossa. Three milliliters of the blood was discharged into EDTA tube and the remaining two milliliters was discharge into a chemically clean plastic tube, allowed to clot, centrifuged for five minutes at 3000 rpm. The serum was separated and stored frozen. The samples were analyzed within two hours of melting to minimize any change due to instability of the analytes. The test group consisted of males and females who were HIV-positive. There were twenty one males and thirty nine females with a mean age of 32 years. Subjects who were on Antiretroviral Therapy were excluded from the study. The subjects were grouped into three based on their CD4 levels as follows: group I (CD4 count < 200 cells/μl); group II (CD4 count: 200 – 499 cells/μl); group III (CD4 count: ≥ 500 cells/μl).

Controls

The control group consisted of thirty one apparently healthy HIV-negative volunteers who gave consent to participate in the study. Thirteen subjects were males and eighteen were females. The mean age of the subjects was 32 years.

Materials and Equipment

The materials used in this study consisted of needle and syringe, specimen containers, test tube racks, pipettes. The equipment includes: Water bath, Centrifuge, Spectrophotometer, Partec cyflow 300 green.

Table 1. Lipid Profile of HIV-Positive Subjects and Control Subjects

	Age (years)	TC (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)	TG (mmol/L)	VLDL-C (mmol/L)	N
HIV-Positive	32.2±1.38 ^{NS}	4.64±0.23 ^{NS}	2.02±0.17*	4.29±0.32*	0.84±0.05*	0.34±0.02*	60
Control	33.48±1.84	3.90±0.20	2.67±1.07	2.85±1.09	0.71±0.03	0.29±0.01	31

Results are presented as mean± standard deviation; * = significantly different when compared with control (P<0.05), NS= Not Significant

Statistical Analysis

All results are expressed as mean ± standard deviation. Student’s t test was used to compare the mean values of lipid profiles of HIV positive subjects and control HIV-negative subjects. One-Way Analysis of Variance (ANOVA) was used to compare the mean values of lipid profiles of the three HIV

positive groups and control HIV- negative subjects.

RESULTS AND DISCUSSION

In this study, the fasting total cholesterol (TC), High density lipoprotein cholesterol(HDL-C), low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C) and Triglyceride (TG) of aged matched HIV positive subjects and HIV-negative controls were evaluated. Also assessed were the CD4+ T-lymphocyte counts of all the HIV-positive subjects.

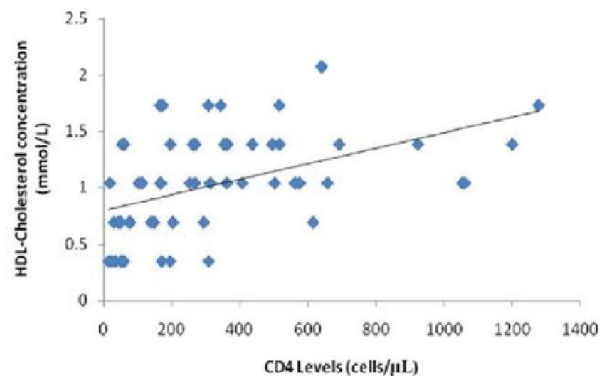


Fig. 1. Correlation plot between HDL-C and CD4 levels of HIV-positive subjects enrolled in the study (y=0.0007x+0.7966, R²=0.2175, r=0.4663, n=60, p<0.05)

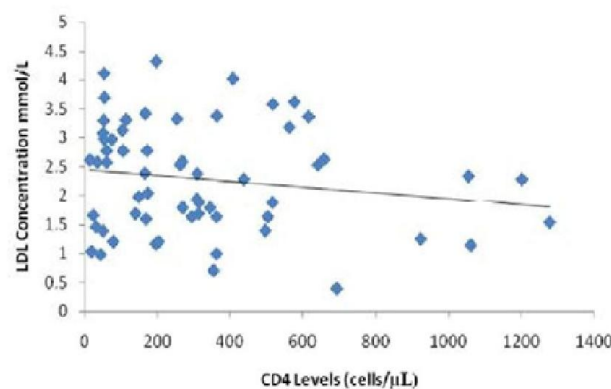


Fig. 2. Correlation plot between LDL-C and CD4 levels of HIV-positive subjects enrolled in the study (y=0.0005x+2.4466, R²=0.0272, r=0.1649, n=60, p<0.05)

This study showed that some lipid profiles were altered in HIV-positive subjects compared to the controls (Table 1).

The fasting total cholesterol did not differ significantly compared to the HIV-negative controls (P>0.05). While the VLDL-C, LDL-cholesterol and TG levels of HIV- positive subjects were significantly higher compared to the HIV-negative controls (P<0.05). Interestingly, the HDL-cholesterol levels of HIV-positive subjects were significantly lower than those of the HIV-negative controls (P<0.05). A significant positive correlation was

found between HDL-Cholesterol levels and the CD4⁺ T-lymphocyte levels of HIV-positive subjects (Figure 1). This is instructive in that as the infection progresses with drop in CD4⁺T lymphocytes count, there is an attendant drop in HDL-C levels of these subjects. A significant negative association was found between TG and CD4⁺T-lymphocyte levels of HIV positive subjects (Figure 3); a similar association also exists between VLDL-C levels and CD4⁺T-lymphocyte levels (Figure 4). These findings were not too surprising considering the fact that high levels of TG are found in VLDL. It therefore follows that as CD4⁺T-lymphocyte level continues to drop; there will be a corresponding increase in TG levels.

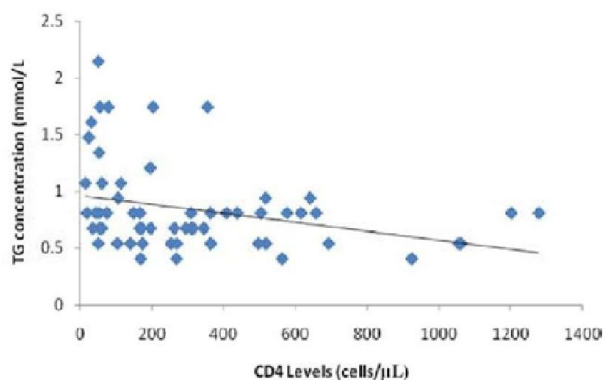


Fig. 3. Correlation plot between TG and CD4 levels of HIV-positive subjects enrolled in the study ($y=0.0004x+0.9855$, $R^2=0.0988$, $r=0.3108$, $n=60$, $p<0.05$)

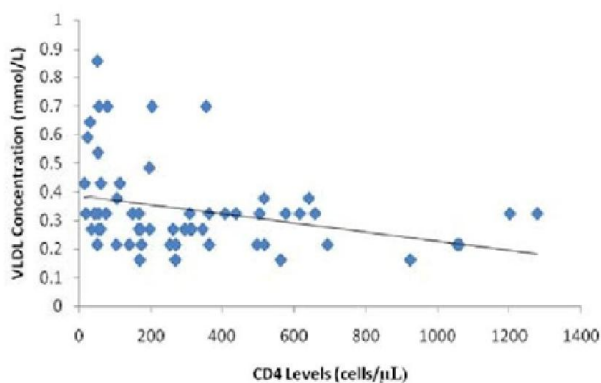


Fig. 4. Correlation plot between VLDL-C and CD4 levels of HIV-positive subjects enrolled in the study ($y=0.0002x+0.3861$, $R^2=0.0964$, $r=0.3105$, $n=60$, $p<0.05$)

was recorded in any of the groups compared to seronegative controls for the fasting to talcholesterol ($P>0.05$). This finding is consistent with the finding of Pynka *et al.*, (2004). Who also recorded no significant difference in the total cholesterol levels between HIV-positive women and apparently healthy seronegative controls.

Significantly lower HDL-C were found between group I and control; group I and Group III; group II and control; and between group III and control ($P<0.05$). These changes were proportional to lowering of CD4⁺ lymphocyte counts which reflects the severity of the infections. A study conducted by (Khangte *et al.*, 2007) showed that HDL-C level decreased as the disease progressed-signified by the decrease in CD4 count. HIV infection can lead to malnutrition (Noble, 2008). Various infections, which occur as a result of weakened immune system in HIV-infection, can affect appetite and ability to eat. Diarrhoea could lead to malabsorption of fat from food. HDL-C which is mainly supplied by fat from food will therefore be reduced as the disease progresses. Crook and Mir (Crook and Mir, 1999) reported significantly higher levels of LDL-Cholesterol in HIV-positive subjects compared to seronegative controls. This agrees with the finding of this study. A mean LDL-C level of 2.29 ± 0.12 mmol/L was recorded for HIV-positive subjects while a mean LDL-C level of 1.84 ± 0.08 was recorded for the seronegative controls ($t=2.407$, $P<0.05$). Amongst the groups, a significantly higher LDL-cholesterol level was found when the mean LDL-C of group I and seronegative controls were compared and also when group II and controls were evaluated. Proper assessment of Table 2 revealed that TG and VLDL-C levels of the subjects with CD4⁺-T lymphocytes counts of <200 cells / μ l were significantly higher than those of the seronegative controls. Also, the TG and VLDL-C levels of group I were found to be significantly higher than the mean TG and VLDL-C levels of group III (subjects with CD4⁺ >500 cells/ μ l) ($P < 0.05$). According to El-Sadir (El- Sadir, 2005), patients with lower CD4⁺ T lymphocyte counts of < 200 cells / μ l were associated with elevations in very low density lipoprotein (VLDL) cholesterol and triglyceride ($P < 0.05$). This observation agrees with the finding from this present study. VLDL cholesterol carries fats around the body and elevations can increase the risk of heart disease.

HIV infection has been shown to affect several key processes regulating the levels of lipids. Increased tumour necrosis factor

Table 2. Lipid Profile of the Three Groups HIV-Positive Subjects and HIV-Negative Controls

Groups	TC (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)	TG(mmol/L)	VLDL-C (mmol/L)	N
I (<200 cells/ μ l)	3.63 ± 0.20	$0.79\pm0.09^{*a,b}$	$2.46\pm0.18^*$	$0.96\pm0.09^{*a}$	$0.38\pm0.03^{*a}$	28
II (200-499 cells/ μ l)	3.54 ± 0.20	$1.17\pm0.09^*$	2.06 ± 0.21	0.77 ± 0.09	0.31 ± 0.04	18
III (=500 cells/ μ l)	3.79 ± 0.27	$1.28\pm1.10^*$	2.23 ± 0.69	0.69 ± 0.05	0.28 ± 0.02	14
Control	3.80 ± 0.10	1.67 ± 0.07	1.85 ± 0.09	0.71 ± 0.03	0.29 ± 0.01	31

Results are presented as mean \pm standard deviation. * = significantly different when compared with control ($P<0.05$), a = significantly different when

To assess the effect of immunological changes due to the HIV infection and its impact on lipid profiles, the HIV-positive subjects were grouped into three based on different CD4⁺ counts/ranges. Group 1 had subjects with CD4-lymphocyte counts of < 200 cells / μ l; group II, CD4 lymphocyte range of 200-499 cells / μ l and group III CD4 lymphocyte count of $=500$ cells / μ l. Using this criteria, as shown in Table 2, no asinificant alteration

(TNF) and other cytokines which occur during infection increases lypolysis and insulin resistance (Rasheed *et al.*, 2008). Insulin regulates the uptake of glucose into skeletal muscle tissue and other cells in the body. As insulin sensitivity decrease in HIV-infected subjects with reduction in CD4 counts, uptake of glucose into skeletal muscle tissue and other cells are reduced leading to increased free fatty acid in

circulation and reduced storage of triglycerides in adipose tissues. These free fatty acids return to the liver where they are sent back into circulation as triglycerides. Thus significantly higher triglyceride levels seen amongst Seroopositives compared to the seronegative controls was not due to chance occurrence. This finding is consistent with the report of Floris-Moore *et al.* (2006). HIV/AIDS is characterized by high prevalence of hypertriglyceridaemia and hypercholesterolaemia is usually associated with elevated levels of cytokines (Grunfeld *et al.*, 1991). Also, Grunfeld *et al.* (1991) observed that decreased cholesterol and cholesterol containing lipoprotein in both AIDS and HIV infection precede the appearance of hypertriglyceridaemia. It could be possible that with the development of AIDS, subsequent increase in interferon IFN may have contributed to increase in plasma TG levels by decreasing the clearance of plasma TG Grunfeld *et al.* (1991). Findings of Grunfeld *et al.* (1992) show that INF and interleukin/increased plasma TG levels by stimulating hepatic lipogenesis and that interferon (IFN) and interleukin-6 also increase hepatic lipogenesis. VLDLs are composed predominantly of triglycerides. This explains why VLDL is also elevated when the levels of triglycerides is increased among the HIV-positive subjects. Most LDL particles are derived from VLDL (Vasudevan and Sreekumari, 2007). This is seen in the concomitant increase in LDL-C in HIV-positive subjects as the levels of CD4 reduce.

In conclusion atherogenic lipids; LDL-C, VLDL-C and TG have been found to increase as the CD4+ T-lymphocyte count of HIV -positive subjects decreases. Levels of good cholesterol (HDL-C) reduce significantly as the disease progresses. Subjects with CD4+ T- lymphocyte count of < 200cells/ μ l were at the highest risk of coronary heart disease since this group showed the highest level of dyslipidaemia. Lipid profile can therefore be a good index of disease progression in HIV/AIDS patients. There is need for proper check on lipid levels as the CD4+ count reduces in HIV-infected patients in ART centre, Government General Hospital, Kurnool. This will help the doctors to decide on the type of antiretroviral therapy to administer to the patients as certain combinations of these drugs increase the levels of these atherogenic lipids.

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