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Lipid Profile of HIV-Positive Patients Attending University of Calabar Teaching Hospital, Calabar - Nigeria

[1]T.S. Iffen, [2]H. Efobi, [1]C.A.O. Usoro and [3]N.E. Udonwa

[1]Department of Medical Laboratory Science (Chemical Pathology Unit),
College of Medical Sciences, University of Calabar, P.M.B. 1115, Calabar, Nigeria
[2]Department of Chemical Pathology, University of Calabar Teaching Hospital, Calabar, Nigeria
[3] Department of Family Medicine, College of Medical Sciences,
University of Calabar, P.M.B. 1115, Calabar, Nigeria

Abstract: The present study assessed the lipid profile of HIV-infected patients attending the University of Calabar Teaching Hospital. 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/µl); group II ($CD4^+$ count 200-499 cells/µl); group III ($CD4^+$ count = 500 cells/µl). The total cholesterol, HDL-C, LDL-C, VLDL-C, TG and $CD4^+$ T-lymphocyte for the HIV-positive subjects were 3.64±0.13 mmol/L, 1.02±0.07 mmol/L, 2.29±0.12 mmol/L, 0.34±0.02 mmol/L, 0.84±0.05 mmol/L and 320.68±39.25 cells/µl, respectively while the TC, HDL-C, LDL-C, VLDL-C and TG of HIV-negative controls were 3.80±0.10 mmol/L, 1.67±0.07 mmol/L, 1.85± 0.09 mmol/L, 0.29±0.01 mmol/L and 0.71±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/ µl 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 as necessary as $CD4^+$ T-lymphocyte count monitoring for the well being of HIV patients in this locality.

Key words: Lipid profile · HIV patients · $CD4^+$ T-lymphocytes · Calabar

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 [1]. In 2007, 2.1 million people died of AIDS worldwide and 33.2 million people are currently living with HIV/AIDS [2]. In Nigeria, about 3.86 million people are living with HIV and the country ranks third in terms of the actual number of people infected with HIV after India and South Africa [3].

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 [4]. 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 [5].

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 CD_4^+ lymphocyte count in HIV infection has been associated with low insulin levels and evidence of insulin resistance [6].

Corresponding Author: Theresa S. Iffen, Department of Chemical Pathology, College of Medical Sciences, University of Calabar, G.P.O.Box 3385, Calabar (540001), Nigeria, Tel: +2348032930027.

Metabolic disturbances in the HIV-infected patients are incriminated to be risk factors of accelerated atherosclerosis and cardiovascular diseases [7] and altered lipid metabolism is known to affect immune processes [8].

Racial variations in serum lipid levels of HIV-infected patients have been observed by Gadd [6]. 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*. [7] showed that there was no significant difference in total cholesterol and low density lipoprotein between HIV-infected and healthy women.

This study was conducted to determine the nature of dyslipidaemia in HIV-infected patients in Calabar Metropolis and to asses if any association exists between CD_4^+ levels and lipid levels in these patients.

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 GIESSE diagnostics, United Kingdom.

The screening of subjects to determine their HIV status were made using Determine test-strips (Inverness Medical Company Ltd, USA) and HIV I/2 Stat-Pak from CHEMBIO Diagnostics, Japan. $CD4^+$ assay was done 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. Six milliliters of blood was collected from the antecubital fossa. Three milliliters of the blood was discharged into EDTA tube and the remaining three 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 33 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.

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. Turkey's Test was used for Post Hoc Analysis on mean lipid levels that differed significantly among the groups.

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.

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}	3.64±0.13^{NS}	1.02±0.07*	2.29±0.12*	0.84±0.05*	0.34±0.02*	60
Control	33.48±1.84	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± standard deviation; * = significantly different when compared with control (P<0.05), NS= Not Significant

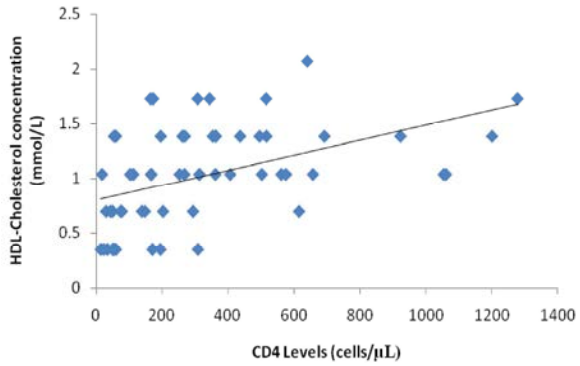

Fig. 1: Correlation plot between HDL-C and CD4 levels of HIV- positive subjects enrolled in the study (y=0.0007x+0.7966, R^2=0.2175, r=0.4663, n=60, p<0.05)

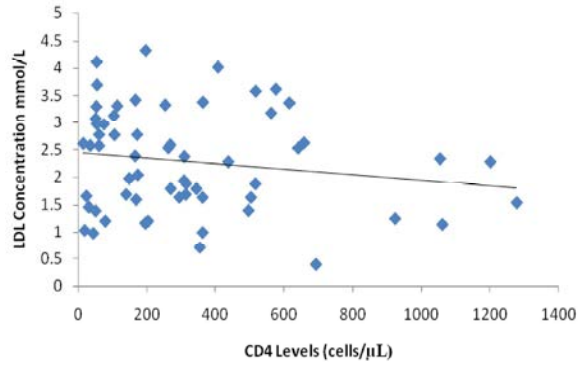

Fig. 2: Correlation plot between LDL-C and CD4 levels of HIV- positive subjects enrolled in the study (y=0.0005x+2.4466, R^2=0.0272, r=0.1649, n=60, p<0.05)

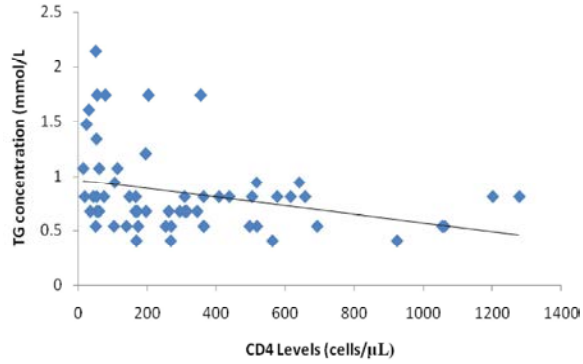

Fig. 3: Correlation plot between TG and CD4 levels of HIV- positive subjects enrolled in the study (y=0.0004x+0.9855, R^2=0.0988, r=0.3108, n=60, p<0.05)

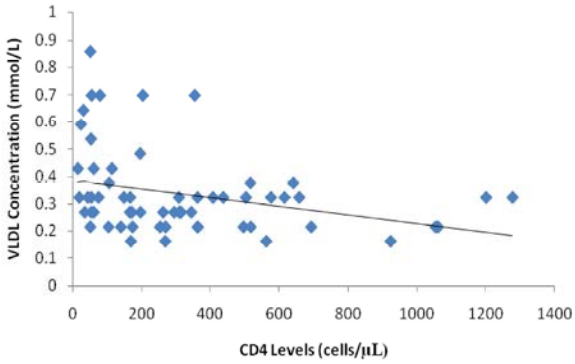

Fig. 4: Correlation plot between VLDL-C and CD4 levels of HIV- positive subjects enrolled in the study (y=0.0002x+0.3861, R^2=0.0964, r=0.3105, n=60, p<0.05)

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.

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 /µl; group II, CD4 lymphocyte range of 200-499 cells /µl and group III CD4 lymphocyte count of =500 cells /µl.

Using this criteria, as shown in Table 2, no significant alteration was recorded in any of the groups compared to seronegative controls for the fasting total cholesterol (P >0.05). This finding is consistent with the finding of Pynka *et al* [7],

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/µl)	3.63±0.20	0.79±0.09*[a,b]	2.46±0.18*	0.96±0.09*[a]	0.38±0.03*[a]	28
II (200-499 cells/µl)	3.54±0.20	1.17±0.09*	2.06±0.21	0.77±0.09	0.31±0.04	18
III (=500 cells/µl)	3.79±0.27	1.28±1.10*	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± standard deviation. * = significantly different when compared with control (P<0.05), a= significantly different when compared with group III (P<0.05), b = significantly different when compared with group II.

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 [4] showed that HDL-C level decreased as the disease progressed-signified by the decrease in CD4 count. HIV infection can lead to malnutrition [10]. 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 [11] 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±0.12mmol/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 /µl 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/µl) (P < 0.05). According to El-Sadir [12], patients with lower $CD4^+$ T lymphocyte counts of < 200cells /µl 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 (TNF) and other cytokines which occur during infection increases lypolysis and insulin resistance [5]. 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 Sereopostives compared to the seronegative controls was not due to chance occurrence. This finding is consistent with the report of Floris-Moore *et al*. [13].

HIV/AIDS is characterized by high prevalence of hypertriglyceridaemia and hypercholesterolaemia is usually associated with elevated levels of cytokines [14]. Also, Grunfeld *et al*. [14] 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 [14]. Findings of Grunfeld *et al*. [15] 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 [16]. 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 cholesterols (HDL-C) reduce significantly as the disease progresses. Subjects with CD4+ T-

lymphocyte count of < 200cells/µl 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 Calabar. 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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