



HIV-1 INFECTION AND HIGHLY ACTIVE ANTI-RETROVIRAL THERAPY EFFECT ON LIPID METABOLISM IN GHANAIAN PATIENTS

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Abstract: To investigate the impact of HIV-1 infection and highly active anti-retroviral therapy on lipid metabolism in Ghanaian patients. A total of 60 HIV-1-infected patients on highly active anti-retroviral therapy and 18 HIV-naïve were age-matched with 45 sero-negative normal healthy volunteers. Participants demographic and clinical data were obtained from medical records and well-structured questionnaire after consented. Healthy individuals with lipid disorder were excluded from the study. Serum lipids were determined following standard protocols. The study was approved by the University of Ghana, School of Allied Health Sciences, Ethical and Protocol Review Committee. Most frequent combined therapy used was nucleoside analogue reverse transcriptase inhibitors. Married individuals (61.5%) were almost as twice as the singles receiving treatment. Body mass index, waist-to-hip ratio, diastolic and systolic blood pressures were significantly raised in patients on highly active anti-retroviral therapy than naïve and healthy volunteers. Total cholesterol and TG were significantly elevated in patients on highly active anti-retroviral therapy treatments than naïve group and volunteers ($p < 0.001$). Patients on treatment showed significantly raised LDL-cholesterol and VLDL-cholesterol with increased cardiovascular risk index than the naïve and NHV ($p < 0.01$). High density lipoprotein level decreased significantly in the patient on HAART than the volunteers ($p < 0.05$). Serum lipids were elevated in HIV-1 infected individuals but more pronounced in patients receiving highly active anti-retroviral therapy and may suggest an increased risk of cardiovascular disease in the patients.

Keywords: lipid, metabolism, anti-retroviral, inhibitors, infection, lipoproteins

INTRODUCTION

Chronic infection either by viruses or bacteria have been suggested to play important role in altering lipid metabolism [1-3]. HIV-1 virus infection is not an exception and has been strongly implicated in lipid metabolism abnormalities [4]. *In vitro* study showed that HIV infection-induced dyslipidemia is due to increased production and decreased clearance of lipoproteins [5]. Even though the molecular mechanism responsible for lipid metabolism abnormalities in HIV infected patients is not clear, it has been linked to factors that influence lipid homeostasis. Many infectious agents including HIV alter the physiological functions of adipocytes making lipids storage impossible leading to hyperlipidemia [5]. It has clearly been demonstrated that HIV replication in human T-cells without any environmental factors such as drug can affect the production of cellular key enzymes and proteins responsible for lipid synthesis and clearance [5]. Studies have shown that HIV infected naïve-patients have altered lipid metabolism [6-7]. Increased plasma triglyceride (TG) levels in naïve-patients have been attributed to decreased circulating lipoproteins. Reduced lipoprotein lipase activity and stimulated hepatic lipid synthesis coupled with increased re-esterification of fatty acid derived from lipolysis have been connected with the changes [8].

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Introduction of highly active anti-retroviral therapy (HAART) in the treatment of HIV/AIDS considerably reduced morbidity and mortality in patients [9]. However, several lines of evidence have implicated HAART in dyslipidemia aggravation in HIV/AIDS patients [9-10]. HAART-induced hypercholesterolemia, hypertriglyceridemia and raised low density lipoprotein cholesterol (LDL-cholesterol) as well as decreased high density lipoprotein cholesterol (HDL-cholesterol) have been reported [11]. Studies have demonstrated that the elevated circulatory lipids in HIV/AIDS patients are associated with both protease inhibitors (PIs) and non-protease highly active anti-therapy regimens [12]. The therapy has also been mentioned to increase the risk of cardiovascular disease [9-11, 13-15]. Elevated triglyceride and reduced high density lipoprotein were found to be the important independent risk factors for coronary artery disease [16-18]. There was a contrary report suggesting that the metabolic syndrome and associated outcomes were more closely linked to the traditional risk factors than HAART [19]. This divergent view makes it imperative to assess the lipid profile of different population on HAART. Such studies to elicit responses to HAART medication may contribute to the identification of individuals with high risk and improve



management. Currently, there is not much data on HAART medication and lipid metabolism in Ghana. Considering HIV/AIDS treatment in Ghana which is not different from elsewhere, it is important to intensify studies on the effect of HAART medication on lipid metabolism in HIV/AIDS patients. This study aims at adding to knowledge the effect of HIV-1 infection and management with HAART on lipid metabolism by comparing patients exposed to HAART, HIV-naïve and sero-negative apparent healthy volunteers in Ghana.

MATERIALS AND METHODS

Recruitment of Patients

The study population consists of 118 individuals made up of 60 HIV-infected patients on highly active antiretroviral therapy (HAART) and 18 HIV-naïve attending clinic at Ghana Police Hospital, Accra, Ghana. The patients were age-matched with 45 apparently healthy controls. Participants were recruited using well-structured questionnaire. Treatment and other relevant medical history were obtained by reviewing medical records of the patients after obtaining permission from the hospital authority. HIV-1 sero-negative control individuals with lipid disorder were excluded from the study. Patients were enrolled after the objectives of the study were clearly explained and written consent obtained. The research was approved by the University of Ghana, School of Allied Health Sciences, Ethical and Protocol Review Committee.

Assessment and measurements

Baseline demographics and clinical characteristics were measured following standard procedures. The body weight and height measured were used to calculate the body mass index (BMI). Mercury sphygmomanometer and stethoscope were used to measure the blood pressure after the patient had rested for at least 15 minutes. Overnight fasting venous blood (5ml) was taken into plain tube and centrifuge after clotting to obtain serum for triglyceride (TG), total cholesterol (T. chol) and high density lipoprotein cholesterol (HDL-chol). Low density lipoprotein cholesterol (LDL-chol), very low density lipoprotein cholesterol (VLDL-chol) and cardiovascular risk were calculated.

Laboratory procedure

Total cholesterol (T. chol), triglyceride (TG), and high density lipoprotein (HDL-chol) levels were determined using reagents from ELITECH Clinical Systems and Selectra Junior Auto-analyser (Vital Scientific B.V, version 04, Netherlands). The test was carried out strictly following the manufacturer's protocol.

Statistical method

Descriptive statistics were reported as mean values with standard deviation (Mean \pm SD) using Excel 2010. Graph Pad Prism 3.0 Bonferroni's Multiple Comparison Test was used to compare means of the groups and chi square was used for categorical variables.

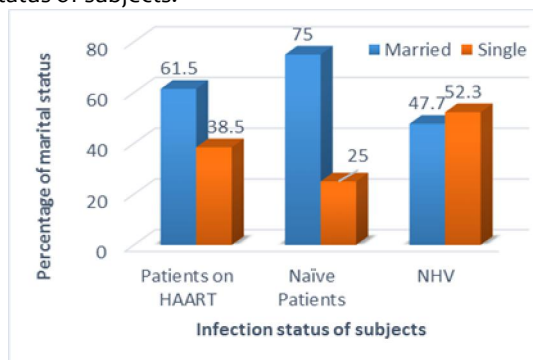
Differences between groups were considered significant at $p < 0.05$.

RESULTS

About 91% of the patients were on two nucleoside reverse transcriptase inhibitors (NRTIs) and a non-nucleoside reverse transcriptase inhibitor (NNRTI) and 9% on only nucleoside analog reverse-transcriptase inhibitors (NARTIs). About 3% of the total population was on at least one protease inhibitor (PI). None of the patients were either put on only non-nucleoside reverse transcriptase inhibitors or protease inhibitor. Percentage of married individuals among patients were significantly higher than the normal healthy volunteers (NHV) ($p < 0.05$) (Figure 1). Married individuals were almost as twice as the singles receiving treatment. Naïve married individuals were found to dominate the study as compared to patients on HAART ($\chi^2 = 3.96$, $p < 0.05$) and NHV ($\chi^2 = 15.95$, $p < 0.001$) (figure 1). In all, proportion of females in the study was significantly high ($p < 0.05$) (Figure 2).

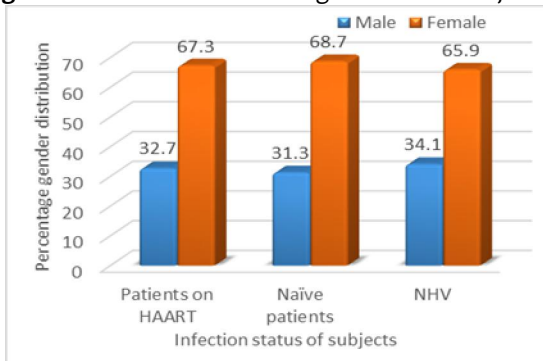
The mean age differences between patients on HAART (40.10 ± 8.16), naïve (38.63 ± 8.46) and control (38.93 ± 7.25) years were not statistically significant ($p > 0.05$). Body mass index (BMI) and waist-to-hip ratios (WHR) were significantly raised in patients on HAART than naïve and controls groups ($p < 0.05$) (Table 1). Blood pressures were significantly elevated in the patients on treatment than the naïve and health volunteers ($p < 0.05$). Total cholesterol was significantly raised in patients on HAART than the naïve and control groups ($p < 0.05$). There was a slight change in triglyceride levels among the HIV-1 patients but significantly raised in patients on HAART as compared with the control group ($p < 0.001$) (table 2). High density lipoprotein (HDL-chol) level was significantly lowered in the patients on treatment than the control ($p < 0.05$), while low density lipoprotein (LDL-chol) and VLDL-chol levels were elevated in patients on HAART. Calculated cardiovascular risk was higher in HIV infected individuals ($p < 0.01$) and patients on HAART ($p < 0.001$) as compared to the controls (table 2).

Fig.1: Percentage distribution of marital and infection status of subjects.



NHV is normal healthy volunteers. Married individuals were high among the patients than NHV ($p < 0.05$). Naïve patients tend to dominate the married subjects as compared to patients on HAART ($\chi^2 = 3.69$, $p < 0.05$) and NHV ($\chi^2 = 15.95$, $p < 0.001$).

Fig.2: Gender distribution among the studied subjects.



NHV is normal healthy volunteers. Females dominate each group but showed no significant difference when groups were compared ($p > 0.05$)

Table 1: Socio-demographic and clinical characteristics of the study participants

Variables	HIV/AIDS patients on HAART (n = 60)	Naïve Patients (n=18)	NHV (n = 45)
Age /years	40.10 ± 8.16	38.63 ± 8.46	38.93 ± 7.25
BMI (Kg/m ²)	25.23 ± 4.17 ^{††}	23.41 ± 3.28	23.30 ± 0.66
WHR	0.94 ± 0.15 ^{††}	0.86 ± 0.07	0.87 ± 0.06
DBP (mmHg)	74.95 ± 11.47 ^{††}	68.75 ± 7.17	68.00 ± 7.86
SBP (mmHg)	121.75 ± 9.20 [†]	117.08 ± 10.55	116.00 ± 11.51

Normal healthy volunteers (NHV). Naïve patients are HIV-infected patients but not on HAART. Body Mass Index (BMI), Waist-to-Hip Ratio (WHR), Systolic Blood Pressure (SBP) and Diastolic Blood Pressure (DBP). The values are presented as mean ± standard deviation (SD). Independent t-test was used to compare patients on HAART and Naïve with NHV. ^{††} $p < 0.01$, [†] $p < 0.05$. Mean values without sign show no significant mean difference, $p > 0.05$.

Table 2: Biochemical parameters of the study population

Variables	Patients on HAART (n = 60)	Naïve Patients (n = 18)	NHV (n = 45)
T. chol (mmol/l)	4.56 ± 1.02 [†]	4.11 ± 0.79	4.12 ± 0.67
TG (mmol/l)	1.21 ± 0.59 ^{†††}	1.15 ± 0.32 [†]	0.78 ± 0.27
HDL-chol (mmol/l)	1.11 ± 0.35 [†]	1.09 ± 0.32	1.30 ± 0.42
LDL-chol (mmol/l)	2.82 ± 0.88 [†]	2.52 ± 0.98	2.40 ± 0.66
VLDL-chol (mmol/l)	0.61 ± 0.29 ^{†††}	0.58 ± 0.16 [†]	0.39 ± 0.14
Cardiovascular Risk	4.34 ± 1.21 ^{†††}	3.78 ± 2.44	3.03 ± 2.00

Normal healthy volunteers (NHV). Naïve patients were HIV patients not on HAART, Total cholesterol (T. chol), Triglyceride (TG), High density lipoprotein cholesterol (HDL-chol), Low density lipoprotein cholesterol (LDL-chol), Very low density lipoprotein cholesterol (VLDL-chol), Cardiovascular risk (T. chol/HDL-chol). The values are presented as mean ± standard deviation (SD). Independent t-test was used to compare patients on HAART and Naïve with NHV. ^{†††} $p < 0.001$, ^{††} $p < 0.01$, [†] $p < 0.05$. Mean values without sign show no significant mean difference, $p > 0.05$.

DISCUSSION

Lipid metabolism abnormalities have been widely reported in HIV/AIDS patients either on highly active anti-retroviral therapy (HAART) or naïve to HAART treatment. This study confirmed the effect of either the disease or disease treatment with HAART on lipid metabolism in Ghanaian patients. Patients on HAART were found to have increased serum total

cholesterol (T. chol), triglyceride (TG), low density lipoprotein cholesterol (LDL-chol) and decreased high density lipoprotein cholesterol (HDL-chol) levels as compared to the naïve and sero-negative control groups. It has been indicated that the suppression of HIV viral replication by HAART is usually accompanied by elevated levels of T. chol and LDL-chol [20]. The increased lipid parameters put the patients on HAART at higher cardiovascular risk (CVR) than the naïve and sero-negative groups. Several studies have implicated HAART, especially combined with PIs as major risk factor in dyslipidemia [21-22]. Protease inhibitors combination therapies have been reported to maintain HDL-chol at appreciable low levels [23] and rather cause worse form of hypertriglyceridemia in patients [22], resulting in a markedly atherogenic lipid profile. In contrast, NNRTI based HAART regimens have been shown to raise T. chol, HDL-chol, and LDL-chol and moderately elevate TG as compared to the effect of PIs HAART regimen [24]. The mechanism of NNRTI and NNRTI based HAART regimens-induced dyslipidemia is not clearly understood but PIs effect has been proposed to inhibit the protease enzyme involve in the lipid metabolism. There is a structural similarity between the catalytic region of HIV-1 protease and that of human cytoplasmic retinoic acid-binding protein type -1 (CRABP-1) and low density lipoprotein-receptor-related protein (LRP) [25]. Binding of PIs to CRABP-1 eventually diminishes adipocyte proliferation and increases apoptosis leading to loss of adipocyte and consequence hyperlipidemia. Even though, most frequently used drug were NNRTI and NNRTI based HAART regimens, a contrary result was obtained for TG and HDL-chol in Ghanaian patients. This may suggest a genetic or environmental influence in the NNRTI-altered lipid metabolism of the patients.

Naïve HIV-infected individuals have been found to show different patterns of lipid metabolism. In this study, naïve patients were found to have unchanged T. chol, HDL-chol and LDL-chol with elevated TG and VLDL-chol. Elevated TG and VLDL-chol levels in naïve patients was contrary to earlier report [26]. In another study lower levels of T. chol and HDL-chol and higher level of LDL-chol were reported while TG level remained unchanged as compared to controls [27]. A recent publication on lipid metabolism in naïve Ghanaian patients in other part of the country revealed elevated TG and lowered T. chol, LDL-chol and HDL-chol [28]. Low level of HDL-chol and elevated TG have also been observed in a population in the US [29].

In addition, irregular elevations in serum T. chol, LDL-chol and TG were reported in HIV-infected patients [30]. These findings demonstrate the dynamic nature of serum lipids of naïve HIV patients. Apart from the viral manipulation of genetic machinery leading to

loss of lipid homeostasis, available data suggest other possible factors that need investigation.

Both systolic and diastolic blood pressures were elevated in patients on HAART than the naïve and sero-negative individuals. Patients on HAART were also found to have increased BMI, waist-to-hip ratio (WHR) than naïve and sero-negative control. Antiretroviral combination therapy has been reported to increase the level of oxidative stress in patients and is implicated in hypertension. Increased levels of oxidative stress and lipid peroxidation result in endothelial dysfunction, a precursor of high blood pressure [31-32]. The naïve compared to sero-negative individuals showed no clinical parameter differences. The study also confirmed earlier report that married and women were highly infected with HIV [27] but the reason is not immediately known.

In conclusion, serum lipids were elevated in HIV-1 infected individuals but pronounced in patients receiving highly active anti-retroviral therapy. The current study suggests further investigations into the variation in serum lipids of patients in different populations.

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