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## Effect of Antiretroviral Therapy on Lipid Metabolism in HIV/AIDS Subjects in Cameroon

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This study sets out to investigate the effect of anti-retroviral therapy on lipid metabolism in HIV/AIDS patients in Cameroon. A descriptive and prospective study was carried out on 16 HIV/AIDS individuals with CD<sub>4</sub> counts of less than 200 cells mm<sup>-3</sup> and on antiretroviral drugs. The various drug regimens included Nevirapine-Lamivudine-Stavudine and Efavirenz-Lamivudine-Stavudine/or Zidovudine. No patients were on protease inhibitors. All patients were monitored at baseline and then monthly thereafter for 3 months. A complete clinical examination and blood samples were respectively collected for total cholesterol, LDL-cholesterol, HDL- cholesterol, triglycerides, Apolipoproteins A<sub>1</sub> (ApoA<sub>1</sub>) and B<sub>100</sub> (ApoB<sub>100</sub>). Total cholesterol and LDL cholesterol levels increased significantly ( $p < 0.05$ ) with administration of the antiretroviral therapy. HDL cholesterol as well as ApoA<sub>1</sub> levels was noted to rise significantly ( $p < 0.05$ ) with time, suggesting increased biosynthesis of HDL. On the other hand, triglycerides, ApoB<sub>100</sub> and the total cholesterol/ HDL cholesterol ratio remained unaltered. The increase of total and LDL-cholesterols may serve as indicators for the risk of cardiovascular disease in HIV/AIDS patients on antiretroviral drugs. The biosynthesis of HDL lipoprotein could also be associated with antiretroviral therapy.

**Key words:** HIV/AIDS, antiretroviral therapy, Apolipoproteins, cholesterol, triglycerides

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**INTRODUCTION**

The advent of antiretroviral therapy (ART) has made an impressive impact on the controlling of HIV infection and reducing the morbidity and mortality associated with AIDS. Highly Active Anti-Retroviral Therapy (HAART) that consists of Protease Inhibitors (PIs) such as Indinavir or Nucleoside analogues of Reverse Transcriptase Inhibitors (NNRTIs) such as Nevirapine in combination with Nucleoside Reverse Transcriptase Inhibitors (NRTIs) such as Lamivudine and Stavudine has led to drastic improvement in the prognosis of HIV infected/AIDS patients<sup>[1,2]</sup>. The suppression of viral replication and reconstitution of immunologic competence by ART have been associated with reduction of morbidity and mortality in HIV infected adults<sup>[3]</sup>. Since its introduction in Cameroon, ART has found favour with the prescribers, an attestation to its effectiveness in the treatment of HIV/AIDS.

The benefits of antiretroviral drugs are compromised by numerous undesirable side effects. Severe and potentially fatal toxicities associated with altered carbohydrate and lipid metabolism have recently been described. These are thought to be more profound in patients who receive PIs<sup>[4-6]</sup>. The frequent side effects may include hyper lipidaemia, insulin resistance, hypercholesterolaemia, changes of Apolipoproteins levels, elevated free fatty acids, impaired glucose tolerance, type 2 diabetes and lipodystrophy syndrome<sup>[2,4,7-9]</sup>.

Since the use of ART in Cameroon, their effect on lipid metabolism in Cameroonian HIV/AIDS subjects has not been clearly investigated. Thus, the present study was designed to investigate the effect of two regimens of ART used for routine care in Cameroon.

**MATERIALS AND METHODS**

**Patients:** This descriptive and prospective study took place from July-December 2002 in the Yaounde Central Hospital and Yaounde University Hospital. A total of 16 ART- naïve, consenting HIV/AIDS individuals aged 20-56 years were included in the study. The consent of each patient was obtained in accordance with the ethical guidelines of the medical ethical committees of both hospitals which approve the study. Precautions were taken to ensure that the participants were on no drugs known to influence glucose or lipid metabolism. Furthermore, all patients had CD<sub>4</sub> counts <200 cells mm<sup>-3</sup>. Patients were each seen 4 times during the duration of the study.

**Blood collection:** A 5 mL blood sample was collected from all patients following an overnight fast (~12 h) prior to

starting ART regimens (day 0). Subsequently, samples were respectively collected one month (day 30), 2 months (day 60) and 3 months (day 90) after the start of treatment. Samples were always centrifuged at 3000 rpm for 15 min and the sera obtained was used for measuring total cholesterol, High Density Lipoprotein (HDL) cholesterol, triglycerides, Low Density Lipoprotein (LDL) cholesterol levels. Enzymatic methods were used for measuring total cholesterol, HDL cholesterol and triglycerides levels whereas the Friedewald formula<sup>[10]</sup> was used for LDL cholesterol levels. Enzyme Linked Immuno Sorbent Assays (ELISA) determined ApoA<sub>1</sub> and B<sub>100</sub> levels.

**Statistical analysis:** The results obtained were presented as mean±SD. Statistical analysis of experimental results was done using the Analysis of Variance (ANOVA). The statistical package SPSS version 8.0 was employed. Data were considered significantly different when p<0.05.

**RESULTS**

Of the 116 subjects recruited initially (day 0) into the study only 16 (13.8%) eventually constituted the study group on day 90. On day 30 of the study only 52 patients were examined, with 64 absent from various reasons including compliance to treatment, side effects, financial constraints and death. By day 60 of the study, the number further dropped by 18 patients and by day 90 only 16 patients were left for final analysis.

The mean age of the study group was 40±12 years (range 20-56 years) there were 5 males (31.25%) and 11 females (69.75%). The mean CD<sub>4</sub> count was 130.63±61.75 cells mm<sup>-3</sup> (range 68.9-192.4).

The 16 patients were on two major ART regimens as shown in Table 1. The various clinical findings are shown in Table 2.

Table 1: Patients characteristics and therapeutic protocol

	Sex	Age (years)	CD <sub>4</sub> (cells mm <sup>-3</sup> )	Therapeutic protocol
Female	11(69%)	-	-	-
Male	5(31%)	-	-	-
20-40 years	-	9(56%)	-	-
40-56 years	-	7(44%)	-	-
≤200 (cells mm <sup>-3</sup> )	-	-	16(100%)	-
NVP+3TC+d <sub>4</sub> T	-	-	-	10(62.5%)
EFV+3TC+d <sub>4</sub> T	-	-	-	6(37.5%)

Table 2: Clinical presentation of the patients

Clinical signs and symptoms	No. of patients (%)
Fever	10(62.5%)
Weight loss>10% (kg)	9(56.3%)
Cutaneous disorders	8(50.0%)
Diarrhoea/dehydration	6(37.5%)
Bronchopneumonia	5(31.3%)
Herpes Zoster	3(18.5%)
Tuberculosis	2(12.5%)
Cerebral toxoplasmosis	2(12.5%)

**Table 3: Lipid profile during 3 months treatment with ART**

Biochemical results	Duration of treatment				p-value
	0 day	30 days	60 days	90 days	
Total cholesterol (mmol L <sup>-1</sup> )	3.44±1.40	4.89±1.66	6.06±2.07	7.35±2.38	0.00*
LDL cholesterol (mmol L <sup>-1</sup> )	2.25±1.08	3.18±1.65	4.40±2.27	5.12±2.38	0.00*
HDL cholesterol (mmol L <sup>-1</sup> )	0.80±0.41	1.32±0.59	1.13±0.51	1.63±0.62	0.01*
Triglycerides (mmol L <sup>-1</sup> )	0.78±0.24	0.75±0.48	0.80±0.42	1.29±0.95	0.56
Total cholesterol/HDL cholesterol ratio	4.29	3.70	5.31	4.50	0.74

**Table 4: Evolution of Apolipoprotein A<sub>1</sub> and B<sub>100</sub> levels during 3 months treatment**

Biochemical results	Duration of treatment				p-value
	0 day	30 days	60 days	90 days	
Apolipoprotein A <sub>1</sub> (g L <sup>-1</sup> )	2.01±1.11	2.40±1.92	3.46±2.86	9.1±6.34	0.000*
Apolipoprotein B <sub>100</sub> (g L <sup>-1</sup> )	1.08±0.67	1.14±0.86	1.03±0.70	1.12±0.95	0.99

\*Significant p<0.005

The mean total cholesterol and LDL cholesterol levels significantly (p<0.05) increased during the use of ART (Table 3). HDL cholesterol showed inconsistent alterations; with a significant decrease noted on day 60 of ART (Table 3). The total cholesterol/HDL cholesterol ratio remained unaltered. Triglyceride levels did not rise significantly during the study period.

While a significantly steady increase was observed in Apolipoprotein A<sub>1</sub> levels, alterations in Apolipoprotein B<sub>100</sub> levels were not significant (Table 4).

### DISCUSSION

The present study was designed to report on the effect of antiretroviral therapy on the lipids and Apolipoproteins metabolism of HIV/AIDS patients in Cameroon. Two therapeutic protocols: NVP +3TC+d<sub>4</sub>T and EFV+3TC+d<sub>4</sub>T were used by the study group. A third regimen, IDV+3TC+AZT was prescribed to some patients but they dropped out of the study eventually. There was a high drop out rate (86.2%) because many patients who were willing to be part of the study did not have a steady source of income. Death, drug side effects, lack of compliance and drop out of patients from the study reduced the number of patients from 116 to 16 by the end of the study. Many patients were put on the NVP+3TC+d<sub>4</sub>T and EFV+3TC+d<sub>4</sub>T regimens, these being the less expensive and most frequently prescribed in Cameroon.

Of the 16 patients 11 (69%) were female and 5 (31%) male (Table 1). One probable explanation is that the prevalence of HIV is higher in the females (10 to 15%) than in the males (4.3-6.5%) as previously observed<sup>[11,12]</sup>. Another reason may be that women present themselves more often to the hospital for consultation and can be easily tested for HIV/AIDS, unlike men who may only consult the medical doctor when their situation gets worse.

The epidemic of HIV has now moved to the middle-aged population who are already at great risk for cardiovascular disease<sup>[13]</sup>. Onusida<sup>[1]</sup> reported that of the HIV/AIDS population in Cameroon, 11.8% are adults (14-49 years). This was confirmed in our study in which the study population was mainly of 40±10 years old.

Cardiovascular complications are frequently encountered in the HIV-infected population which has been linked to disorders of lipid metabolism<sup>[13,14]</sup>. Plasma triglycerides and free fatty acids have been shown to increase in HIV/AIDS, while plasma total cholesterol, ApoA<sub>1</sub>, LDL-cholesterol and ApoB<sub>100</sub> level decrease<sup>[15,16]</sup>. Apolipoproteins are the protein moiety of the molecule responsible for the transportation of lipids in the blood. ApoA<sub>1</sub> is situated principally in HDL while the ApoB<sub>100</sub> and ApoB<sub>48</sub> are situated mainly in LDL (Low Density Lipoprotein) and VLDL (Very Low Density Lipoprotein), respectively. Thus, an increase in ApoB<sub>100</sub> will result in an increase risk of cardiovascular disorders.

The introduction of Highly Active Antiretroviral Therapy (HAART) and in particular Protease Inhibitors (PIs) suppresses the breakdown of the nuclear form of Sterol Regulatory Element Binding Proteins (nSREBP) in the liver and adipose tissues, resulting in increased cholesterol and fatty acid biosynthesis in the liver and lipodystrophy as well as reduction of leptin expression and the promotion of insulin resistance in adipose tissue<sup>[9]</sup>. Protease Inhibitors also inhibit proteasomal degradation of nascent ApoB, inhibit their secretion, thus resulting in the over production of VLDL which are triglycerides rich lipoprotein resulting in hyperlipidemia<sup>[9,17]</sup>.

In the present study we observed a significant increase in the levels of serum total cholesterol (p=0.00) and LDL-cholesterol (p=0.000) during the use of ARV drugs (Table 3). The increase was progressive with the duration of the ARV drugs administration. Such increase may lead to cardiovascular disorders. HDL-cholesterol

also increased significantly ( $p=0.01$ ) but the level of triglycerides was not altered ( $p= 0.56$ ) by ARV therapy. Constans *et al.*<sup>[16]</sup> had earlier reported similar increases in LDL-cholesterol. Lainka *et al.*<sup>[18]</sup> also reported an increase in total cholesterol, LDL cholesterol, triglycerides and ApoB during administration of Protease Inhibitors and no pathological deviations when Non-Protease Inhibitors (NPIs) were used.

ApoA<sub>1</sub> (major protein component of HDL) and HDL-cholesterol levels were not different in PIs and NPIs therapy<sup>[18]</sup>. In this study we noticed increased levels of ApoA<sub>1</sub> and HDL Cholesterol with the prolonged use of the two NPIs regimens. The moderate increase of the levels of HDL cholesterol and the high concentrations of ApoA<sub>1</sub> may be related to HDL lipoprotein biosynthesis. HDL lipoprotein plays an important role in cellular immune response against HIV through the production of IL-2<sup>[19]</sup> or by its anti-inflammatory and antioxidant properties that alter the course of HIV infection in human<sup>[20]</sup>. It is also known that the p17 region homologous to Apolipoprotein A<sub>1</sub> contains amino acids which suppress the infectivity of HIV-1<sup>[21]</sup>. It was shown by Panin and Kostina<sup>[22]</sup> that there is an interaction between human Apolipoprotein A<sub>1</sub> and recombinant soluble CD<sub>4</sub> receptor (RSCD<sub>4</sub>). Owens *et al.*<sup>[23]</sup> observed that Apolipoprotein A<sub>1</sub> and its amphipathic helix peptide analogue to that of the HIV glycoprotein envelope inhibit cell fusion in HIV-1 infected T cell and by this way inhibit the infectivity of HIV-1. This homology could also lead to competitive relationships impeding the involvement of human ApoA<sub>1</sub> in gene expression regulation<sup>[24]</sup> including activation of the biosynthesis of ApoA<sub>1</sub> to protect cell infection.

Ono and Freed<sup>[25]</sup> thought that there is better response to treatment of patients with higher HDL levels because of greater cell cholesterol depletion by higher levels of ApoA<sub>1</sub>. It could possibly be that ApoA<sub>1</sub> interaction with p17 together with cholesterol raft depletion combine in lowering HIV-1 particle production or in producing less infective viral particles. Unfortunately, the poor income of our patients did not permit us to either measure their viral loads before treatment or their CD<sub>4</sub> counts and the viral load 3 months after the beginning of the treatment, to confirm the Alonso *et al.*<sup>[26]</sup> hypothesis which demonstrated that high levels of HDL cholesterol are related to better viral response in treated HIV patients. This relationship may be due to the structural homology between ApoA<sub>1</sub> and p17.

The immunochemical homology of envelope HIV proteins and human ApoA<sub>1</sub> can be detected by Elisa<sup>[24]</sup>. Immunochemical cross reactions between ApoA<sub>1</sub> and HIV envelope protein during *in vitro* measurement of ApoA<sub>1</sub> might be possible. Therefore high levels of

ApoA<sub>1</sub> in association with moderate increase of HDL cholesterol would refer to the HDL biosynthesis to protect cell against HIV infection. But cross reactivity during *in vitro* measurement of ApoA<sub>1</sub> by ELISA might give some false positive response which also increases the level of ApoA<sub>1</sub>.

However it would have been interesting to know viral load levels and CD<sub>4</sub> counts throughout the study. This was not possible because of the poor income of the patients. Nevertheless future studies will include these as well as increase number of patients and the duration of the study.

The use of Non Protease Inhibitors to treat Cameroonian HIV/AIDS patients may increase the risk of cardiovascular disease as indicated by the increased LDL level. The moderate increase in HDL cholesterol levels as well as high concentrations of ApoA<sub>1</sub> obtained in this study reflects the response of patients to treatment with ART HIV therapy.

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