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The Long-term Effect of Different Combination Therapies on Glucose Metabolism in HIV/Aids Subjects in Cameroon

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The use of antiretroviral drugs is associated with an increase of metabolic abnormalities such as impaired glucose metabolism and insulin resistance. This study was designed to investigate the long-term effect of anti-retroviral combinations therapy on glucose metabolism in HIV/AIDS patients in Cameroon. A descriptive and prospective study was carried out on 58 patients on HAART and 80 pre-HAART patients. The various drugs regimens comprised Lamivudin-Zidovudin associated with Efavirenz (Therapy I, n = 9) or Nevirapin (Therapy II, n = 13) and Stavudin-Lamivudin associated with Nevirapin (Therapy III, n = 30) or Efavirenz (Therapy IV, n = 16). All patients were monitored at baseline and then 12 months after. Blood glucose levels increased significantly in both pre-HAART (34.08±9.93%) and HAART patients (45.56±7.86%) The mean blood glucose levels increased by 78.19% ($p<0.001$), 61.50% ($p<0.0001$), 69.96% ($p<0.0001$) and 16.92% ($p<0.01$) with therapy I, II, IV and III, respectively. The increase was associated with efavirenz or zidovudine use. It is possible that efavirenz or zidovudine use in a combination therapy may exaggerate an underlying tendency to develop mitochondrial toxicity or insulin resistance.

Key words: HIV infection, long-term effect, glucose metabolism, antiretroviral therapy

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INTRODUCTION

Infection with HIV and consequent AIDS is a major public health problem affecting more than 40 million people worldwide. Successful combination therapy based on two reverse transcriptase inhibitors and one non-nucleoside analogs are most prescribed with the goal of complete suppression of viral replication and immune restoration. In Cameroon, among nucleoside reverse transcriptase inhibitors, Lamivudine is always prescribed with zidovudine or stavudine. Nevirapine or efavirenz are prescribed as non-nucleoside reverse transcriptase inhibitors. The use of these combinations therapy also called highly active antiretroviral therapy (HAART), has improved the prognosis of patients to the point that long-term complications of treatment which are metabolic have been termed the HIV-lipodystrophy syndrome. Highly active anti-retroviral therapy (HAART) is associated with a number of metabolic abnormalities including dyslipidemia, impaired glucose metabolism and insulin resistance (Carr *et al*., 2001). Previous studies demonstrated an increase of blood glucose disorders among HIV positive patients on HAART. The prevalence of diabetes mellitus is estimated to 7%, impaired glucose tolerance rate is 46% and pathologic insulin sensitivity rate is 61% (Dube *et al*., 2001). Observational study found elevated fasting glucose above 120 mg dL^{-1} in 14% of patients tested (Hadigan *et al*., 2001). Since the introduction of effective HAART, It is a general belief that protease inhibitors are associated with disturbance of lipid and glucose metabolism comparatively to non-nucleoside Reverse Transcriptase Inhibitors (NNRTI) and Nucleoside Reverse Transcriptase Inhibitors (NRTI). Hadigan *et al*. (2001) reported that 40% of the patients initiated on a PI-containing regimen have developed impaired glucose tolerance.

Although the proportion of HIV infected individuals being on HAART is increasing in Cameroon, data on long-term effect of these drugs on glucose metabolism are lacking. The objective of this research is to present the effect of HAART on glucose metabolism in HIV-infected patients after 12 months of treatment.

MATERIALS AND METHODS

Patients were eligible if they had confirmed HIV infection, they were above 18 years and had not taken antiretroviral drugs before (Naive) or being on Highly Active Antiviral Therapy (HAART). HIV positive out patients (138) were recruited from the Yaounde Central Hospital in Cameroon in 2005. All patients were monitored at baseline and after 12 months. Patients were classified according to their sex, immune status such as the CD4 + count (cells/mm^3). Among them, 80 patients were not on any form of antiviral therapy (pre-HAART patients), while 58 were on one of the different therapies outlined below (HAART patients). These individuals showed no serological evidence for HBV and or HCV-infection. All subjects gave their informed consent before participating in the study and the local ethics committee approved the project. Precautions were taken to ensure that the participants were on no drugs known to influence glucose or lipid metabolism. Fasting venous blood was collected from subjects and distributed into a heparinized containing tubes. After centrifugation at 3000 g for 10 min at 4°C, plasma was aliquoted and set aside for analysis of blood glucose which was determined by glucose oxidase method (Trinder, 1969).

Different HAARTs used: Patients on antiviral therapy followed one of the following daily therapies:

Therapy I (9): Efavirenz associated with a generic fixed-dose combination of zidovudin and Lamivudin (3TC+AZT+EFV)

Therapy II (13): Nevirapin associated with a generic fixed-dose combination of zidovudin and Lamivudin (3TC+AZT+NEV)

Therapy III (16): Efevirenz associated stavudin and Lamivudin (d4T+3TC+EFV)

Therapy IV (30): Nevirapin associated with stavudin and lamivudin (d4T+3TC+NEV)

Statistical analysis: The analysis of variance (ANOVA) was used to determine quantitative variables with normal distribution, followed by the Bonferonni ltiple comparisons test to compare the means between groups. Kruskal-Wallis test was used to compare the non-parametric distribution. Probability levels of 0.05 or less were considered significant.

RESULTS

One hundred and thirty eight subjects constituted the study group, the mean age was 36.05±7.09 years, there were 80 women and 58 men. The mean CD4 count was 209.23±29.14 (cells mm^3) (Table 1).

After 12 months of follow up, the weight gained (10.45%) by HAART patients was higher than that of pre-HAART patients (1.42%). Therapy I (10.92%) and II (2.47%), induced a weight lost while therapy III and IV

Table 1: Demographic and clinical characteristics of patients

Characteristic	
Number of patients (n)	138
Women/men	80/58
Age (years)	36.05±7.09
CDC clinical stage	
A	87 (63.04%)
B	32 (23.18%)
C	19 (13.78%)
Baseline CD4 count (cells mm^{-3})	209.23±29.14
Hepatitis serology	
HBsAg positivity	0 (0%)
Anti HCV positivity	0 (0%)

Data are mean±standard error of mean or number of patients (%)

Table 2: Body mass index and blood glucose variation after 12 months in pre-HAART and HAART patients

	BMI (kg m^{-2})			Blood glucose (mg dL^{-1})		
	Baseline	After 12 months	Change (%)	Baseline	After 12 months	Change (%)
Pre HAART patients	23.03±0.85	23.23±0.58	1.42±0.90	88.42±2.16	$109.70±7.21^{++}$	34.08±9.93
HAART patients	22.80±0.48	24.13±0.39	10.45±2.63	80.25±1.44	118.85±5.81	45.56±7.86
3TC+AZT+EFV (n = 9)	26.03±2.24	$24.75±0.62^{++}$	-10.90±3.36	83.50±4.42	$148.79±15.36^{++}$	78.19±22.53**
3TC+AZT +NEV (n = 13)	25.66±0.71	24.44±0.96	-2.70±1.90	82.00±0.74	$142.00±3.19^{+}$	61.50±4.93*
3TC+d4T+NEV (n = 30)	20.50±0.63	23.85±0.60	$18.20±3.38^{bc}$	78.30±3.16	$89.04±7.38^{++}$	16.92±12.43
3TC+d4T+EFV (n = 16)	20.78±0.66	24.08±0.92	$18.38±6.19^{ac}$	78.85±1.71	$134.02±14.46^{+++}$	69.96±19.58*

$^{+}p<0.05$; $^{++}p<0.001$; $^{+++}p<0.0001$ are by comparison with initial BMI or blood glucose level, *p<0.02; **p<0.005 are by comparison with the HIV positive patients on therapy III (3TC+d4T+NEV), $^{a}p<0.001$; $^{b}p<0.0001$; are by comparison with the HIV positive patients on therapy I (3TC+AZT+EFV), $^{c}p<0.001$ are by comparison with the HIV positive patients on therapy II (3TC+AZT+NEV)

induced a weight gain of 18% (Table 2). Fasting blood glucose levels increased from 88.42 to 109.70 mg dL^{-1} (34.08±9.93%) in pre-HAART and from 80.25 to 118.85 mg dL^{-1} (45.56±7.86%) in HAART patients (Table 2).

A mean increase of 78.19% (p<0.001), 61.50% (p<0.0001), 16.92% (p<0.01) and 69.96% (p<0.0001) was observed with Therapy I, II, III and IV, respectively. The increase observed with therapy I, II, IV was significantly higher than that of therapy III (Table 2).

DISCUSSION

The IAS-USA's recommendations on the management of metabolic complications advise that fasting glucose should be assessed before and during treatment (prior to starting ART, 3-6 months after starting and annually thereafter) with a regimen containing one or more protease inhibitor (Schembelan *et al*., 2002). This recommendation is extended to all subjects initiating anti retroviral regimens. This study was designed to assess the effect HIV infection and HAART on glucose metabolism after a-12 months period. Four therapeutic protocols: 3TC+AZT+EFV, 3TC+AZT+NEV, 3TC+d4T+EFV, 3TC+d4T+NEV were prescribed. We observed that, the use of stavudine and zidovudine as NRTIs in a combination therapy induced a significant weight loss while Stavudine and lamivudine induced a weight gain after 12 months of treatment. Fat loss and accumulation have also been reported in PI-naïve patients treated with NRTIs alone. Lamivudine (3TC) (Gervasoni *et al*., 1999) and stavudine (d4T) (Saint-Marc *et al*., 1999; Mallal *et al*., 2000) as well as various combinations of NRTI (Chene *et al*., 2002) have been implicated in causing body fat redistribution. Carr *et al*. (2000) suggested that the features of body fat loss in 14 patients treated with NRTI alone were indistinguishable from those of PI-induced lipodystrophy. It is possible, therefore, that the fat loss and fat accumulation in patients treated with NRTIs alone represent a different disorder than the lipodystrophy syndrome in patients treated with PI-containing HAART and could be complicated by organ toxicity or failure and AIDS wasting in many of them. Whether a particular NRTI causes more fat loss than others is not yet clear. A long duration of NRTI therapy may be required to observe significant fat loss, as no significant body fat changes were noted in 151 patients participating in a randomized, controlled trial of combination NRTI therapy for a 6-month period (Molina *et al*., 1999). It is speculated that NRTIs may cause slow fat (Mallal *et al*., 2000).

We also noticed that blood glucose level increases with HIV infection and its treatment and this could be due to pancreatic B-cell function, or insulin secretion depletion. A decrease in B-cell responsiveness to glucose is a necessary component of the cascade of events that culminate in the development of diabetes mellitus (Kahn and Porte, 1997). Therefore, in order for patients infected with HIV to develop glucose intolerance and diabetes, there presumably must also be some component of either

heritable or acquired B-cell dysfunction. There are few published data on B-cell function in patients infected with HIV. Dubé *et al.* (2001) noted decreased sensitivity of the pancreas to hyperglycemic stimuli in HIV-infected patients initiating therapy. HAART exacerbates the effect of HIV infection on glucose metabolism. In this study, we observed that the use of Stavudine and Zidovudine in combination with either efavirenz or nevirapine lead to a dramatic weight loss and a significant increase in blood glucose level after 12 months. This might be due to direct effects of Stavudine and Zidovudine and the indirect consequences of fat loss. These drugs are known to have an inhibitory effect on mitochondrial DNA (mtDNA) replication. Because mt DNA encodes many of the oxidative phosphorylation chains proteins, a decrease in mtDNA content could theoretically hinder aerobic respiration and other mitochondrial functions. The use of Stavudine and lamivudine in combination therapy with either nevirapine or efavirenz induced a weight gain but a different effect on glucose metabolism was noticed. When efavirenz was used as NNRTI, elevation in blood glucose level was 4 times higher than the effect observed with nevirapine as NNRTI. In both cases, there might be a development of insulin resistance and impaired cellular glucose uptake due to inhibition of both the glucose transporter (glut 4) and glucose phosphorylation (Behrens *et al.*, 2002). Reduced insulin sensitivity may also result of weight gain mediated by the elevated blood levels of Free Fatty Acids (FFAs) induced by fat redistribution or fat loss. Elevation of FFAs may interfere with cellular glucose transport through a reduction in the phosphorylation of insulin receptor substrate-1 (IRS-1) associated phosphatidylinositol 3-kinase resulting in impaired intracellular signaling and insulin resistance (Dresner *et al.*, 1999). An observational study of insulin sensitivity comparing HIV seronegative controls, HIV positive individuals receiving ART containing a Protease Inhibitor (PI) and HIV positive individuals receiving ART not including a PI, aknowledged that insulin insensitivity was 55% lower in patients receiving PI than in controls (Mulligan *et al.*, 2000). Insulin insensitivity was also 10% lower in those receiving ART without PI compared to controls. Data are inconsistent concerning the effects of NRTIs on glucose and lipid metabolism. Although some investigators have found dyslipidemia (Saint-Marc *et al.*, 1999) in patients treated with NRTIs alone, no relationship between metabolic abnormalities characteristic of insulin resistance and NRTI therapy has been reported by others (Carr *et al.*, 2000). In fact, patients with NRTI-associated body fat redistribution had either normal blood lipids, glucose and insulin levels or lower values compared with those in PI-related lipodystrophy HIV patients and values similar to those in patients not receiving any antiretroviral treatment (Carr *et al.*, 2000; Saint-Marc *et al.*, 1999). Yarasheski *et al.* (2001) reported multiple abnormalities in glucose and insulin metabolism, including decreased insulin secretion, in HIV-infected patients with glucose intolerance compared with those with just insulin resistance. Before PIs were available, reported abnormalities in glucose metabolism were limited primarily to the pancreatic effects of drugs such as pentamidine (Fisch *et al.*, 1990; Uzzan *et al.*, 1995) and didanosine (Bouvet *et al.*, 1990; Kilby and Tabereaux, 1998), Recent studies showed an increase in insulin sensitivity in comparison with that in healthy control subjects (Hommes *et al.*, 1991), as measured by the highly sensitive and reproducible euglycemic, hyperinsulinemic clamp technique. However, more recent cross-sectional data suggest that HIV infection itself and perhaps NRTI therapy may be associated with truncal obesity and insulin resistance (Hadigan *et al.*, 1999, 2000). In women infected with HIV there was increased truncal adiposity and fasting hyperinsulinemia that was independent of drug use (Hadigan *et al.*, 1999). These same authors also reported increased fasting insulin levels and insulin resistance, as measured by HOMA-IR, in wasting NRTI-treated male subjects in comparison with controlled subjects (Hadigan *et al.*, 2000). These data suggest that other factor can be involved but should be viewed as provocative rather than conclusive. In a study, Yarasheski *et al.* (2002) studies assess whether antiretroviral agents can accelerate the development of diabetes on Zucker-diabetic rat model, an animal model of obesity using rats that have been genetically modified to reproducibly develop pancreatic beta-cell exhaustion and type-2 diabetes. Four groups of animals were studied: placebo treatment, Nucleoside Reverse Transcriptase Inhibitors (NRTIs), indinavir and NRTIs plus indinavir. The NRTIs used were stavudine, didanosine, or zidovudine, each combined with lamivudine. They found that, both indinavir groups developed hyperglycemia earlier than it occurred in untreated or NRTI-treated animals. The occurrence of diabetes in the animals could be due to mitochondrial dysfunction, the changes were seen both in the presence and absence of NRTIs. Therefore, it is possible that efavirenz or zidovudine use in a combination therapy may exaggerate an underlying tendency to develop insulin resistance.

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