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Original Article

Lipoprotein (a) profile in HIV-1 infected patients treated with highly active antiretroviral therapy (HAART)

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ABSTRACT: Lipoprotein (a) [Lp(a)] is recognized as an independent factor of arteriosclerosis. The aim of this study was to appreciate the profile of Lipoprotein (a) recognized as an independent factor of arteriosclerosis in the monitoring of HIV-infected patients receiving Nevirapine (NVP) regimens, an antiretroviral known to reduce cardiovascular disease risk. The study population (136 subjects) comprise of 106 HIV-infected subjects, and 30 HIV-negative individuals. The 106 HIV-infected subjects were divided into groups as follows. HAART-untreated (27), HIV-infected subjects that did not receive antiretroviral treatment; HAART-6M (36), HIV-infected subjects on antiretroviral treatment for six months; and HAART-12M (43), HIV-infected subjects on antiretroviral treatment for twelve months. All recruited patients had normal blood lipids values (Total cholesterol < 5.2 mmol/L, Triglycerides < 2 g/L, HDLc > 0.9 mmol/L). The Lp(a) levels were significantly higher in the HIV-infected group compared to the control (p = 0.0036). Within the HIV-infected subjects, Lp(a) level was found to be higher in HAART-treated group compared to HAART naive group (p=0.004). Infected subjects on the antiretroviral treatment for12 months had higher Lp(a) levels than those treated for 6 months (p=0.034). This study shows that adequate management of metabolic abnormalities of HAART-treated HIV-infected patients must include periodic measurement of Lp(a) levels.

KEYWORDS: HIV, HAART, dyslipidemia, Lipoprotein (a), Cardiovascular disease.

INTRODUCTION

HIV-infected patients are at increased risk of cardiovascular disease (CVD), a reflection of the interaction of risks associated with host, virus, and antiretroviral therapy factors (Currier, 1999). Dyslipidemia caused by the metabolic aspects of the cytopathogenicity of HIV is one of the causative factors of the increased susceptibility to CVD (Kanjanavanit *et al.*, 2011). Cohort studies showed associations between exposure to highly active antiretroviral therapy (HAART) and an increased risk of myocardial infarction (Friis-Moller *et al.*, 2003) Effects of HAART such as dyslipidemia and insulin resistance on increased coronary diseases in HIV are well established (Rossi *et al.*, 2009). Several studies have shown that HIV patients on Nevirapine (NVP) regimen had lower atherogenicity index (TC/HDLc, LDLc/HDLc), and hence reduced cardiovascular disease risk and insulin resistance than other regimens (Molina *et al.*, 2000; Moyle *et al.*, 2001; Ngoundi *et al.*, 2007; Nguemaim *et al.*, 2010). These observations are explained by the observation that HDLc increase is higher in patients on Nevirapine regimens, and that the increase in triglycerides, total cholesterol, LDLc and VLDL levels are smaller in patients taking Nevirapine than in those taking other combinations [8]. However, little is known on the levels of lipoprotein (a) in subjects treated with HAART. Indeed, recent published studies have provided increasing evidence that lipoprotein(a) may be a potential causal, genetic, independent risk factor for cardiovascular disease (CVD) (Riches and Porter, 2012; Tsimikas and Hall, 2012).

Table 1: Demographic and clinical characteristics of the population of study

Demographic and clinical characteristics	Variables	HIV-negative (n=30)	HIV-positive (n=106)	HAART-untreated (n=27)	HAART-6M (n=36)	HAART-12M (n=43)
Sex (n)	Men	14	48	12	16	20
	Women	16	58	15	20	23
	Sex Ratio	0.87	0.82	0.80	0.80	0.86
Age (years)	Men	34 ± 9	44.3 ± 7.2	43 ± 7	42 ± 10	45 ± 6
	Women	28 ± 7	35.8 ± 8.8	35 ± 10	37 ± 11	36 ± 5
	Total	31.0 ± 8.6	36.6 ± 9.8	36.8± 9.8	37.9± 10.4	41 ± 7.6
BMI	Total	24.5.±3.8	23.3±3.9	22.6±4	23.1± 2.2	23.5±3.8
CD4 (cell µL-^{1})	<200	-	14(13.2%)	5(18.5%)	4 (11.1%)	5 (11.6%)
	200-350	-	26(24.5%)	4(14.8%)	10 (27.7%)	12 (27.9%)
	> 350	-	66(62.2%)	18(66.6%)	22 (61.1%)	26 (60.4%)
Therapies	AZT/3TC/NVP	-		-	36(100%)	43 (100%)

AZT: Zidovudine, 3TC: Lamivudine, NVP: Nevirapine, HAART-6M: HIV-infected and on antiretroviral treatment for 6 months, HAART-12M: HIV-infected on antiretroviral treatment since 12 months

Lipoprotein (a) is synthesized and secreted by the liver and comprises a lipid core of LDL cholesterol and apolipoprotein B-100 surrounded by a unique glycoprotein apolipoprotein (a) that shares homology with plasminogen. Lipoprotein (a) may be athero-thrombotic through its low-density lipoprotein moiety, but also through apolipoprotein (a). Apolipoprotein (a) can be retained in the vessel wall and thereby mediate pro-inflammatory and proapoptotic effects including those potentiated by oxidized phospholipids [9]. Apolipoprotein (a) may also exert antifibrinolytic effects [9]. The aim of this study was to appreciate the profile of Lipoprotein (a) recognized as an independent factor of arteriosclerosis in the monitoring of HIV-infected patients receiving Nevirapine regimens, an antiretroviral known to reduce cardiovascular disease risk.

Subjects and Treatment Groups

A case-control study was carried out from September 2010 to May 2011 in Ouagadougou, the Capital city of Burkina Faso (West Africa). HIV-infected subjects were recruited at Pissy Medical Center of Ouagadougou and HIV-negative subjects (Control group) at the National Center of Blood Transfusion, Ouagadougou. Biological analyses were performed at the laboratory of Philadelphie Private Clinic, Ouagadougou.

The study population comprise of 136 subjects (106 HIV-infected and 30 HIV-negative) with an average age of 31±8.6 and 36.6± 9.8 years respectively. The 106 HIV-infected subjects (48 men and 58 women, sex ratio: 0.82) were grouped as follows: Twenty seven (27) HIV-infected without antiretroviral treatment (HAART-untreated), 36 HIV-infected on antiretroviral treatment for six months (HAART-6M), and 43 HIV-infected on antiretroviral treatment for twelve months (HAART-12M). The control group comprised 30 healthy subjects (14 men and 16 women, sex ratio: 0.87) who showed no serological evidence for HIV and/or HBV, HCV (HIV negative). All subjects were recruited in accordance with the Helsinki Declaration and the National Ethics Committee approved the protocol. Each of the subjects completed a consent form before their participation in the study. All recruited patients had normal blood lipids values (Total cholesterol < 5.2 mmol/L, Triglycerids < 2 g/L, HDLc > 0.9 mmol/L). In addition, the participating subjects were not enlisted in another clinical study during the period of this investigation. The following criteria of exclusion were defined and imposed: diabetes, coronaropathy (cardiac events), treatment with any lipid-modifying medication (statins, nicotinic acid, fibrate, resins), smokers and alcohol drinkers. The subjects that failed to adhere to the treatment, and patients with incomplete data were excluded from the final analysis.

Methods

After an overnight fast, venous blood was collected on a dry tube for biochemical analyses and in an EDTA-containing tube for CD4 lymphocytes count. Serum was separated by centrifugation at 3000 g for 10 minutes at 4 °C, stored at -80 °C and analyzed within a week. The CD4 lymphocytes count

Table 2: Lp(a) profile in HIV-positive patients

	HIV-negative (n=30)	HIV-Positive (n=106)	HAART-untreated (n=27)	HAART-treated (n=79)	HAART-6M (n=36)	HAART-12M (n=43)
Lp(a) (mg/L)	316 ± 151	488 ± 308	452 ± 239	483 ± 269	399 ± 256	548 ± 342
p	0.0036		0.004		0.034	

Table 3: Comparison of Lp (a) according to age

	HIV-positive		HIV-negative		
	n	Lp (a) mg/L	n	Lp (a) mg/L	p
17-35 years	56	415 ± 268	5	302 ± 99	0.35
35-50 years	38	587 ± 335	17	315 ± 165	0.049
≥ 50 years	12	515 ± 323	8	326 ± 160	0.14
p	0.025		0.96		

was carried out with a flow cytometer BD-Fascount (California, USA). Serum lipoprotein a (Lpa) was determined with an automated KONELAB 20i analyzer (Thermo Electron Corporation, Vantaa, Finland) by immunoturbidimetric method (Thermo kit 981915). The accuracy and precision of the measurements during the study were within the acceptable criteria of literature (Nascetti *et al.*, 1996).

Statistical analysis

The quantitative variables were expressed as means ± standard deviation and the qualitative variables in percentages. Analysis of Variance (ANOVA) was used to determine quantitative variables with normal distribution, followed by the Bonferonni multiple comparisons test to compare the means between groups. The statistical analysis was performed using the statistical software PASW, version 18.0 for Windows (SPSS CPSC., Chicago, USA). Probability levels of 0.05 or less were considered significant.

RESULTS

The demographic and clinical characteristics presented in Table 1 showed that the study population was heterogeneous in age (31±8.6 in HIV-negative group versus 36.6 ± 9.8 in HIV-infected group). The infected men were 44.3 ± 7.2 years old against 35.8 ± 8.8 years old in women ($p < 0.001$). The average body mass index (BMI) was homogeneous (23.3 ± 3.9 kg/m² in HIV-infected group versus 24.5 ± 3.8 in the control group). The CD4 lymphocyte count data show that 14 subjects (13.2%) had CD4 lymphocytes lower than 200 cells/μL, 26 subjects (24.5%) between 200-350 cells/μL, and 66 subjects (62.2%) with counts up to 350 cells/μL. Subjects in the two groups that received the HAART treatments were given the therapeutic antiretroviral protocol with Nevirapine combining treatment (Zidovudine + Lamivudine + Nevirapine).

The Lp(a) level (Table 2) was significantly higher in HIV-infected group (488 ± 308 mg/L) compared to 316 ± 151 mg/L in the HIV-negative control group (p=0.0036). Among the infected subjects Lp(a) levels were higher in HAART-treated group compared to the group that were not treated (p=0.004). Infected subjects on the antiretroviral treatment for12 months had higher Lp(a) levels than those treated for 6 months (p=0.034). There was no correlation between the age of the subjects and Lp(a) level in the HIV-negative group, but in HIV-infected group an increase of Lp(a) with age (p=0.025) was found particularly in the group of 35 to 50 years (587±335mg/L) (Table 3).

The sex of the patient had no correlation with Lp(a) levels in both HIV-negative (p=0.64) and HIV-infected groups (p=0.34) (Table 4). Table 5 shows that there was a significant increase of Lp(a) was observed in subjects with CD4 lymphocytes counts between 200-350 cells/μL compared to control group (p=0.03).

Table 4: Comparison of Lp (a) according to sex

	HIV-positive		HIV-negative		
	n	Lp (a) mg/L	n	Lp (a)mg/L	p
Women	78	480 ± 304	7	364± 124	0.18
Male	28	511 ± 322	23	301 ± 158	
p	0.37		0.34		0.089

Table 5: Comparison of Lp (a) according to CD4 count

	HAART-untreated		HAART-treated		
	n	Lp (a) mg/L	n	Lp (a) mg/L	p
< 200	5	317± 241	9	406 ± 124	0.38
200-350	4	686 ± 304	22	384 ± 240	0.03
> 350	18	446 ± 264	48	566 ± 355	0.20
p	0.13		0.051		

DISCUSSION

In this study, we observed a significant increase in the Lipoprotein (a) levels in HIV-infected subjects when compared to control individuals that were HIV-negative (p=0.0036). Current evidence presented in the literature is that the most common lipid profile in HIV patients is an elevated triglycerides level, decreased HDL cholesterol and increase in Lp(a) level (Ngoundi *et al*., 2007; Sposito *et al*., 1997; Koppel *et al*., 2000; Shahmanesh et al, 2001; Velasquez and Glancy, 2003; Crook, 2007). Plasma Lp(a) levels are reportedly elevated in patients suffering from inflammatory diseases such as Crohn's disease and in the microvasculature of inflammatory lesions in gall bladder, heart, and lymph nodes (Riches and Porter, 2012). Expression of Lp(a) is increased by the proinflammatory cytokine interleukin-6 (IL-6), through binding to multiple sites in the *apo(a)* promoter, prompting speculation that it may act as an acute phase reactant (Riches and Porter, 2012). A chronic inflammatory process could explain the rise of Lp(a) in HIV infected patients by the same mechanism.

The results from this study shows that those treated with HAART had higher levels of Lp(a), and the increase was significantly higher in the group that received the treatment for 12 months in comparison with those who had it for six months (p=0.034). The observation that the level of Lp(a) in HAART-treated group was higher compared with those that did not receive the antiretroviral treatment is consistent with the report of Koppel (Koppel *et al*., 2000). The effect of antiretroviral therapies on Lp(a) level observed in our study also agrees with the findings of Shahmanesh *et al.* (2001) who reported that Lp(a) level higher than 300 mg/L was observed in 38% of subjects receiving Protease Inhibitor (PI), 41% receiving Non Nucleoside Reverse Transcriptase Inhibitor (NNRTI) and 33% of retroviral-negative patients. The lipids abnormalities increases including Lp(a) are usually found to be severe with protease inhibitors (PI) (Purnell *et al*., 2000; Acosta, 2002, Lainka *et al*., 2002; Walmsley *et al*., 2002; Hicks *et al*., 2006; Haubrich *et al*., 2009). There was no age correlation with Lp(a) in the control HIV-negative group, but in HIV-infected group an increase of Lp(a) with age (p=0.025) was found particularly in the group of 35 to 50 years. Sex dependency was not observed in HIV negative group (p=0.64) and HIV infected group (p=0.34). Earlier studies reported that there were no age and sex correlation with increase in Lp(a) levels [23,24].

There was no significant correlation between of Lp(a) levels and CD4 count in HAART-treated and untreated groups. However, significant increase of Lp(a) was observed in subjects with CD4 lymphocytes between 200-350 cells/μL compared to control group (p=0.03). Sposito *et al.* (1997) reported an increase in Lp(a) even in those patients with CD4 lymphocyte counts above 400 cells/mm^3. In the study of Constans *et al.* (1994) the patients below 200 CD4 cells/mm^3, had significantly lower total cholesterol than the controls and the patients below 400 CD4 lymphocyte/mm^3, had significantly higher triglycerides and Lp(a) but lower LDL cholesterol than the controls.

Conclusion

This study showed an increase of Lipoprotein (a) in patients treated with the Nevirapine regimen without showing any of the classic lipids abnormalities. This result shows that Lipoprotein (a) could be an independent factor of arteriosclerosis. In the monitoring of HIV infected patients treated with HAART, adequate management of metabolic abnormalities must include periodic measurement of Lipoprotein (a).

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