

# Atherogenic index of plasma in highly active antiretroviral therapy-naïve patients with human immunodeficiency virus infection in Southeast Nigeria

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## ABSTRACT

**Introduction:** Metabolic abnormalities are often common among human immunodeficiency virus (HIV) patients. The atherogenic index of plasma (AIP) is increasingly being used as a screening tool for dyslipidemia as it predicts the presence of small, dense, and highly atherogenic low density lipoprotein (LDL) and high density lipoprotein (HDL) particles. The aim of this study was to identify the pattern and predictors of an abnormal atherogenic index in highly active antiretroviral therapy (HAART)-naïve HIV patients. **Materials and Methods:** HAART-naïve patients with HIV infection were recruited for this cross-sectional study. Anthropometric indices, blood pressure, CD4 count, viral load, fasting blood glucose, and lipid profiles were determined. Total cholesterol (TCH)/HDL, triglyceride (TG)/HDL, and LDL/HDL ratios were calculated. The AIP was calculated as  $\log(TG/HDL)$ . The correlations between AIP and the other lipoprotein ratios and predictors of AIP were determined using stepwise multiple linear regression.  $P < 0.05$  was considered as significant. **Results:** A total of 353 patients with a mean age of 37.3 (9.6) years were recruited for this study. Low HDL level was the most common abnormality in 222 (62.9%) patients while elevated TCH was seen in 54 (15.3%) patients. Those with medium risk (AIP 0.1-0.24) and high risk category (AIP > 0.24) constituted up to 226 (64%) of the patients. There were significant correlations between AIP and CD4 count, body mass index, LDL, TCH/HDL, and LDL/HDL. Predictors of AIP were CD4 count, TCH/HDL, and LDL/HDL. **Conclusion:** Abnormal AIP is frequent in HAART-naïve HIV patients and is inversely related to their level of immunity. We recommend that AIP estimation should be part of baseline assessment of HIV patients before the commencement of therapy.

**Key words:** Atherogenic index, dyslipidemia, human immunodeficiency virus, lipids, plasma

## INTRODUCTION

In the majority of human immunodeficiency virus (HIV) infection patients, the disease has become chronic requiring that greater attention be paid to disease prevention

strategies.<sup>[1]</sup> Metabolic abnormalities are often common among these HIV patients, and include dyslipidemia, diabetes, insulin resistance, altered fat distribution and endothelial dysfunction.<sup>[2]</sup> Various lipid abnormalities have been recorded in both highly active antiretroviral therapy (HAART)-naïve HIV patients and those on HAART. Hypertriglyceridemia, low total cholesterol (TCH) and reduced low density lipoprotein (LDL) levels have been observed in HAART-naïve patients.<sup>[3]</sup> Low levels of high density lipoprotein (HDL) were also found to be common especially in those with low CD4 count.<sup>[4]</sup> In HIV infection, there is an increase in the activity of the cholesterol ester transfer protein which acts to transfer cholesterol from

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HDL to apo-B-containing proteins, hence resulting in a decrease in HDL.<sup>[5]</sup> Major epidemiological trials have emphasized on the role of elevated LDL and reduced HDL in the pathogenesis of atherosclerosis and the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) guidelines recommend LDL lowering as the initial goal of therapy.<sup>[6]</sup> Risk stratification using risk calculators is important in the management of dyslipidaemia and is the cornerstone of the American College of Cardiology/American Heart Association guidelines.<sup>[7]</sup> Recent meta-analyses of prospective studies indicate that elevated triglycerides (TGs) are also an independent risk factor for coronary heart disease (CHD).<sup>[8,9]</sup>

The NCEP ATP III panel thus has also advocated the treatment of nonHDL cholesterol in patients with elevated TGs with the nonHDL target calculated as LDL target plus 30 in mg/dl.<sup>[6]</sup> da Luz *et al.* demonstrated that a high ratio of TG/HDL showed the strongest association with coronary disease as compared to other lipid variables.<sup>[10]</sup> Other lipid ratios in use include the TC/HDL and the LDL/HDL ratios.<sup>[11]</sup> The ratio log (TG/HDL), which is called AIP correlates well with the size of HDL and LDL particles and with the fractional esterification rate of cholesterol by lecithin: Cholesterolacyl transferase in plasma.<sup>[12]</sup> This ratio accurately reflects the presence of atherogenic small LDL and HDL particles, is a sensitive predictor of coronary atherosclerosis and cardiovascular risk<sup>[12]</sup> and a useful surrogate for insulin resistance.<sup>[13]</sup> Other studies on lipid profile in HIV patients in Nigeria have not taken this index into consideration.<sup>[14,15]</sup> We hypothesize that HAART-naïve HIV patients in Nigeria have dyslipidaemia. The aim of this study was to determine the atherogenic index of plasma (AIP) among HAART-naïve patients in South-East Nigeria while comparing it with other lipid ratios. It also aimed to determine the predictors of the AIP.

## MATERIALS AND METHODS

### Ethical clearance

Approval for the study protocol was obtained from the ethics committee of the University of Nigeria Teaching Hospital Enugu. Only patients who gave informed written consent were recruited.

### Study setting

This was a cross-sectional study carried out at the adult HIV clinic of the University of Nigeria Teaching Hospital (UNTH), Enugu. The hospital is a tertiary health care facility located at Ituku-Ozalla, about 22 km from Enugu town. The UNTH is a major HIV treatment center in Nigeria and receives referrals from various states in South-East, South-South, and North-Central Nigeria. The

HIV treatment center at the UNTH is supported by the United States presidential emergency fund for AIDS relief in partnership with Harvard School of Public Health and AIDS Prevention Initiative in Nigeria since 2008.

The HIV clinic offers ambulatory services to about 7000 adults among whom over 4000 were receiving antiretroviral therapy (ART) at the time of data collection.

The study was conducted between June and December, 2012. Consecutive patients who were being enrolled into the HIV treatment program were recruited after meeting the study criteria and obtaining informed written consent. Patients who were enrolled into the clinic program but yet to commence HAART were recruited. Patients who were on lipid lowering drugs, known hypertensive and diabetic patients and those with evidence of kidney disease were excluded. Significant history of alcohol use, cigarette smoking and exposure to ART were also part of the exclusion criteria.

### Data collection

Demographic parameters were obtained from the study participants using a structured pretested interviewer administered questionnaire. A history of alcohol use and smoking was also obtained. The weight and height of the patients were measured using a standard weighing scale (H HHospitex) and a standard height scale (H HHospitex measuring stick). The body mass index (BMI) was then calculated using the formula: Weight (kg)/height (m<sup>2</sup>).

The blood pressure was measured with an Accoson sphygmomanometer and a standard sized cuff using the usual methods with the patient sitting quietly. The mean of two readings taken at least 5 min apart was recorded as the blood pressure.

The HIV status of the patients was confirmed by Western blot and the CD4 cell count was measured using flow cytometry (Partec, Germany). Fasting venous blood samples were collected from each participant. Fasting blood glucose was measured using glucose oxidase method.

Fasting lipid profile was assessed by measuring the TCH using a photometric assay, automated direct determination of LDL and HDL, while the Wahlefeld enzymatic method was used for TG measurement. These were done using a Hitachi® 902 auto analyzer (Mannheim, Germany).

### Data analysis

Data were entered and analyzed using Statistical Package for Social Sciences SPSS Version 17.0 (Chicago, IL, USA).

Continuous variables were presented using means and standard deviation or median and interquartile range (IQR) for skewed data, while categorical variables were recorded as frequencies and percentages. Chi-square test was used to test for the difference between categorical variables, whereas the difference between continuous variables was analyzed using the one-way ANOVA or Kruskal–Wallis test as appropriate. Pearson’s correlation coefficient was used to assess correlations between lipid ratios and clinical parameters. Multiple linear regression was used to determine the predictors of the AIP.  $P < 0.05$  was considered to be significant.

Total cholesterol  $>5.2$  mmol/l, TG  $>1.7$  mmol/l, LDL  $>3.5$  mmol/l, HDL  $<0.9$  mmol/l in males, and  $<1.0$  mmol/l in females were regarded as abnormal using WHO criteria.<sup>[16]</sup> The ratios of TC/HDL, LDL/HDL, and log (TG/HDL) were calculated. TC/HDL  $>5$ , LDL/HDL  $>3.3$ , and TG/HDL  $>3.0$  were regarded as abnormal. The AIP that is log (TG/HDL) was classified thus;  $-0.3$  to  $0.1$ ; low risk,  $0.1-0.24$ ; medium risk, and  $>0.24$ ; high risk.<sup>[17]</sup>

## RESULTS

Three hundred and fifty three patients met the inclusion criteria of which 250 (70.8%) were females, while 103 (29.2%) were males. The mean (SD) age of the patients was 37.3 (9.6) years. Highest educational attainment was tertiary in 71 (20.1%), secondary in 152 (43.1%), and primary in 117 (33.1%). Only 13 (3.7%) lacked any form of formal education. Other clinical characteristics are as shown in Table 1.

The overall proportion of dyslipidemia with at least one lipid abnormality was 74.7%. Only 16 (4.5%) had elevated LDL, 125 (35.4%) had elevated TG, while elevated TCH was identified in 54 (15.3%) patients. Low HDL was the most common abnormality, being present in 222 (62.9%) patients. The median CD4 count in patients with dyslipidemia was significantly lower than those without (295.7 cells/ $\mu$ l vs. 540.7 cells/ $\mu$ l,  $P < 0.001$ ).

The AIP ranged from 1.0 to 1.9 with a median (IQR) of 0.3 (0.6). Patients with atherogenic index  $<0.1$  were classified as low risk and 127 (36%) were in this category. Those with medium risk (AIP 0.1-0.24) were 42 (11.9%) while 184 (52.1%) were in the high risk category (AIP  $>0.24$ ). More males were in the high risk category than females (Chi-square 16.1,  $P < 0.001$ ). The patients were compared according to their atherogenic index in terms of low risk, medium risk, and high risk indices. Their characteristics are as shown in Table 2.

There were differences in lipid parameters among each of the three groups. Further, *post-hoc* analyses were done with Tukey’s test. Significant differences were observed between all the lipid levels for low risk and high risk patients. Significant differences were observed between medium risk and high risk in their TG, HDL, LDL, and TC/HDL but not their TC levels. The differences in lipids between low risk and medium risk patients differences were only significant between their TG and HDL values.

Using Pearson’s correlation coefficient, significant correlations were noticed between AIP and BMI ( $P < 0.001$ ), CD4 count ( $P < 0.001$ ), TCH ( $P < 0.001$ ), LDL ( $P < 0.001$ ), HDL ( $P < 0.001$ ), TCH/HDL ratio ( $P < 0.001$ ), and LDL/HDL ratio ( $P < 0.001$ ). This is as shown in Table 3.

Stepwise multiple linear regression analysis was carried out to determine the significant predictors of AIP. Predictors

**Table 1: Clinical and laboratory characteristics of the study population**

Characteristic/parameter	No. (%)
Males	103 (29.2)
Females	250 (70.8)
Overweight or obese	67 (18.9)
Systolic BP	110.5 (16.9)*
Diastolic BP	66.1 (8.1)*
CD4 count	270 (92-398)*
Viral load	18,869 (528-111,589)*
FBG (mmol/l)	5.2 (1.7)**
TCH (mmol/l)	4.0 (1.2)**
LDL (mmol/l)	1.9 (1.1)**
HDL (mmol/l)	0.8 (0.7)**
TG (mmol/l)	1.5 (0.8)**
TCH/HDL $>5$	146 (41.4)
TG/HDL $>3$	119 (33.7)

\*Median (IQR), \*\*Mean (SD). SD: Standard deviation, BP: Blood pressure, FBG: Fasting blood glucose, TCH: Total cholesterol, LDL: Low density lipoprotein, HDL: High density lipoprotein, TG: Triglyceride, IQR: Interquartile range

**Table 2: Characteristics of patients according to groups of AIP**

	Low risk (AIP $<0.1$ )	Medium risk (AIP 0.1-0.24)	High risk (AIP $>0.24$ )	P value
	n=127	n=42	n=184	
Age (years)	36.3 (9.5)	38.7 (9.6)	37.8 (9.7)	0.26
FBS (mmol/l)	5.3 (1.9)	5.1 (1.1)	5.2 (1.6)	0.87
CD4 (cells/ $\mu$ l)**	214	220	137	$<0.001^*$
BMI (kg/m <sup>2</sup> )	22.6 (4.2)	22.2 (6.2)	21.7 (10.7)	0.66
TCH (mmol/l)	4.3 (1.1)	4.3 (1.2)	3.8 (1.2)	$<0.001^*$
HDL (mmol/l)	1.3 (0.9)	0.8 (0.2)	0.5 (0.3)	$<0.001^*$
LDL (mmol/l)	2.2 (0.8)	2.3 (1.0)	1.6 (1.1)	$<0.001^*$
LDL/HDL	1.9 (0.8)	2.8 (1.2)	3.8 (2.9)	$<0.001^*$
TC/HDL	4.1 (2.9)	5.2 (1.3)	12.3 (9.3)	$<0.001^*$

\*Significant difference, \*\*Mean rank using Kruskal–Wallis test. AIP: Atherogenic index of plasma, FBS: Fasting blood sugar, BMI: Body mass index, TCH: Total cholesterol, LDL: Low density lipoprotein, HDL: High density lipoprotein, TC: Total cholesterol

in the model were CD4 cell count, TCH, TCH/HDL, and LDL/HDL as shown in Table 4.

## DISCUSSION

This study assessed the AIP in HAART-naïve HIV patients in South-East Nigeria. The most common pattern of dyslipidaemia was low HDL while only a small proportion of them had elevated LDL. More than half of the patients had AIP levels in the high risk category. The AIP correlated with BMI, CD4 count and other lipoprotein ratios such as TCH/HDL and LDL/HDL. The significant predictors of AIP were CD4 count, TCH, TCH/HDL, and LDL/HDL.

The female preponderance in our study is similar to other clinic based studies in patients with HIV in our environment.<sup>[14,15]</sup> Majority of the patients in the study had a normal BMI and only few were obese. This is likely because of the usual association of HIV with weight loss. The low median CD4 count of the patients recruited also demonstrated that they already had low immunity and a high disease burden, which was also seen in their high median viral load. The mean TCH, HDL, and LDL levels in this study were similar to the values obtained in a previous study on HAART-naïve patients in Northern Nigeria<sup>[14]</sup> and also as reported in a study in Cameroun.<sup>[17]</sup> Low TCH and HDL values as seen in this study have been reported in other earlier studies on lipid profile in HAART-naïve HIV patients.<sup>[14,15,18]</sup> Our patients had low LDL levels similar to the findings of some other previous

studies.<sup>[18,19]</sup> However, some studies have reported high LDL in their patients.<sup>[20]</sup>

The patients had relatively normal mean TG levels unlike in other studies where the mean TG was found to be high in similar patients.<sup>[20]</sup> It is postulated that in HIV, high levels of TG occur due to the presence of inflammation, resulting in elevation of interferon- $\alpha$  which interferes with TG clearance.<sup>[21]</sup> In addition, elevated tumor necrosis factor- $\alpha$ , another cytokine interferes with free fatty acid metabolism and lipid oxidation also contributing to abnormalities in the lipid profile.<sup>[21]</sup> The relatively normal TG in our patients may be due to the fact that they had a fair immune status as reflected by a median CD4 cell count of 270 cell/ $\mu$ l. This is because increased TG tends to occur with profound immunosuppression.<sup>[3]</sup> However, hypertriglyceridemia is known to be uncommon in blacks even in the presence of insulin resistance, type 2 diabetes mellitus and cardiovascular disease.<sup>[22]</sup> It has been postulated that the low TG levels in blacks is due to increased activity of lipoprotein lipase in them leading to increased TG clearance. The decrease in HDL in patients with HIV infection was proposed by Mujawar *et al.* who demonstrated that HIV patients have a reduction in cholesterol efflux from macrophages due to deleterious effects on the ATP-binding cassette transporter A1, which mediates transport of cholesterol to apo-A1 to form HDL.<sup>[23]</sup>

The use of the AIP as an index of dyslipidaemia showed that more than half of the patients are at high risk of atherosclerosis and CHD despite their low levels of TCH and LDL. The AIP was also found to be very closely related to the TC/HDL ratio, which has been shown to be a more useful marker of cardiovascular risk than the use of lipid parameters alone such as LDL and TCH levels.<sup>[11]</sup> The TC/HDL ratio has also been noticed to have a strong relationship with TG levels.<sup>[11]</sup> The AIP is a useful marker in patients even when LDL levels appear to be within normal range. This is because it has been shown to correlate more closely with the small LDL and HDL particles which are most atherogenic.<sup>[12]</sup> Indeed when other lipid markers appear normal, AIP may be the diagnostic alternative.<sup>[24]</sup> In patients on protease inhibitors who have a high risk of dyslipidemia, the AIP is likely a more useful index of atherogenicity and cardiovascular risk than single LDL and TCH measurements as it correlates more closely to the small dense LDL particles,<sup>[12]</sup> which are not estimated by conventional LDL assays. In a cohort of hypertensive menopausal women in South Eastern Nigeria, the AIP value approached high risk levels despite the apparently normal lipid markers in them.<sup>[25]</sup>

Our study also showed that the AIP was also closely related with CD4 cell count. Those who had lower

**Table 3: Correlation between AIP and clinical and laboratory parameters**

Parameter	R	P value
Age (years)	0.001	0.98
BMI (kg/m <sup>2</sup> )	-0.11	0.04
FBS (mmol/l)	0.032	0.56
CD4 (cells/ $\mu$ l)	-0.39	<0.001
Viral load (copies/ml)	0.21	0.004
TCH (mmol/l)	-0.32	<0.001
LDL mmol/l	-0.46	<0.001
HDL (mmol/l)	-0.67	<0.001
TCH/HDL	0.81	<0.001

AIP: Atherogenic index of plasma, FBS: Fasting blood sugar, BMI: Body mass index, TCH: Total cholesterol, LDL: Low density lipoprotein, HDL: High density lipoprotein

**Table 4: Predictors of AIP**

	Beta coefficient	95% CI	P
CD4 (cells/ $\mu$ l)	-0.078	0.000, 0.000	0.007
HDL (mmol/l)	-0.299	-0.24, -0.16	<0.001
LDL (mmol/l)	-0.156	-0.102, -0.036	<0.001
TCH/HDL	0.54	0.028, 0.037	<0.001
LDL/HDL	0.085	0.002, 0.032	0.028

LDL: Low density lipoprotein, HDL: High density lipoprotein, AIP: Atherogenic index of plasma, CI: Confidence interval



CD4 (hence lower immunity) had a higher atherogenic risk profile. The TC/HDL and LDL/HDL ratios in our study were predictive of the AIP and are thus likely to be similar in their ability to identify patients with high cardiovascular risk. The AIP is a simple and practical ratio which can be easily estimated in an out-patient setting to further stratify atherogenic risk in patients who may have an apparently normal lipid profile using other parameters. This is especially useful in HIV patients who have altered lipid metabolism due to inflammation.

This study however was not without limitations; it was a cross-sectional single center study and would have been better if the patients were followed up after the commencement of HAART especially in large multi-center studies. Lack of HIV negative control subjects may have affected some of the conclusions drawn from this study.

In addition, in practice, calculation of the AIP on a regular basis in a busy may be cumbersome.

The sample size was small, hence it is recommended that further studies on larger populations may be needed to draw further conclusions.

Despite these limitations, to the best of our knowledge, this is the first study that looked at AIP among HAART-naïve HIV patients in our region and will no doubt stimulate more research in this novel area. There is a need to follow up these patients after the commencement of HAART to see how the antiretroviral medications affect the dyslipidaemia indices and also follow them up on long term bases to identify those that will develop diseases associated with dyslipidaemia.

## CONCLUSION

Dyslipidemia is common in HAART-naïve HIV patients in our environment. The AIP is more useful than single lipid parameters such as LDL or TCH in identifying patients with atherogenic risk and is closely related to other lipid ratios such as the TCH/HDL ratio and the LDL/HDL ratio. In addition, reduced immunity is associated with increased atherogenicity. It is recommended that lipid assessment using lipid ratios such as the AIP should be carried out prior to initiation of therapy in patients with HIV infection.

## AUTHORS CONTRIBUTIONS

CCO and UIN conceived the idea and designed the study protocol and were involved in the data collection. EEY, MOI, and CJC were involved in data collection and analysis of data. EEY wrote the first draft of the manuscript. All

the authors were involved in the writing of the final draft and approved the final draft of the manuscript.

## ETHICS COMMITTEE APPROVAL

Granted by the Ethics Committee of University of Nigeria Teaching hospital Enugu, Nigeria.

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