Castelli Risk Index, Atherogenic Index of Plasma, and Atherogenic Coefficient: Emerging Risk Predictors of Cardiovascular Disease in HIV-Treated Patients

6 authors, including:

Kamoru A. Adedokun
King Saud University Medical City
5 PUBLICATIONS   **1** CITATION

SEE PROFILE

Japhet Olisekodiaka
Nnamdi Azikiwe University, Awka
51 PUBLICATIONS   **101** CITATIONS

SEE PROFILE

Hammed Ojokuku
MeCure HealthCare Limited, lagos.
5 PUBLICATIONS   **0** CITATIONS

SEE PROFILE

Musa Abidemi Muhibi
Ladoke Akintola University of Technology Teaching Hospital, Osog...
40 PUBLICATIONS   **190** CITATIONS

SEE PROFILE

Some of the authors of this publication are also working on these related projects:

Project   WSVirology View project

Project   Ethics in Biomedical Research and Services View project

Castelli Risk Index, Atherogenic Index of Plasma, and Atherogenic Coefficient: Emerging Risk Predictors of Cardiovascular Disease in HIV-Treated Patients

Adedokun A. Kamoru[1,6], Olisekodiaka M. Japhet[1,4], Adeyeye D. Adetunji[2], Muhibi A. Musa[3], Ojokuku O. Hammed[1], Adepeju A. Akinlawon[2], Onifade A. Abdufatah[5], Adetoro A. Taofik[1], Ajibola A. Kabiru[1], Sheu M. Roji[7]

[1]Department of Biomedical Science, Ladoke Akintola University of Technology, Osogbo, Osun State. Nigeria.

[2]Department of Chemical Pathology, Ladoke Akintola University of Technology Teaching Hospital, Osogbo, Osun State. Nigeria.

[3]Department of Haematology, Ladoke Akintola University of Technology Teaching Hospital, Osogbo, Osun State. Nigeria .

[4]Department of Chemical Pathology, Nnamdi Azikwe University, Awka, Anambra State. Nigeria.

[5]Department of Chemical Pathology, College of Medicine, University of Ibadan, Ibadan, Oyo State. Nigeria.

[6]Department of Oral and Maxillofacial Pathology, DUH, King Saudi University, Riyadh, Saudi Arabia

[7]Department of Environmental Science, King Saud University, Riyadh, Saudi Arabia

Abstract: Atherogenic cardiovascular risk assessment is important in the management of dyslipidaemia associated with antiretroviral therapy (ART). Generally, Castelli's Risk Index (CRI), Atherogenic Index of Plasma (AIP) and Atherogenic coefficient (AC) are becoming useful indices as risk predictors for cardiovascular disease (CVD). We studied the importance of these indices in identifying at-risk HIV individuals receiving treatment. Eighty (80) HIV-patients comprising treatment (n=40) and treatment-naïve (n=40) groups with age- and sex-matched were enrolled. Traditional lipid parameters {Total cholesterol (TC), Triglycerol (TG), and HDLc} were analyzed using spectrophotometry. The LDLc was calculated using Friedewald equation. Non-HDLc, CRI-I (TC/HDLc ratio) and CRI-II (LDLc/HDLc ratio), AIP {Log_{10} (TG/HDL-c)} and AC {(Non-HDLc)/HDLc} were also calculated. The CD4+ cell count was determined using flow cytometry. The correlations between atherogenic indices and lipid profile were determined using Pearson's correlation. $P < 0.05$ was considered as significant. Risk estimation by stratification based on number of altered lipid profile parameter showed no significant increase ($P>0.05$) in any of the traditional lipid profile parameters except for HDLc with significant elevation ($P<0.001$) across the strata. Remarkably, all estimated atherogenic indices were found significantly high ($P<0.001$) across the strata. However, these indices showed positive significant correlations with LDLc and nonHDLc ($p<0.001$), but negative significant correlations with HDLc ($p<0.01$). In this present study, the estimated risk indices were suggestive of atherogenicity that helped identifying individuals with dyslipidemia at risk of CVD. Findings from this study also indicated that without markedly deranged lipid profile these estimated indices could serve as more sensitive risk predictors.

Keywords: Antiretroviral Therapy; Atherogenic Coefficient; Atherogenic Index of Plasma; Castelli's Risk Index; Cardiovascular Disease; Dyslipidemia.

INTRODUCTION

Our recent study shows that abnormal lipid profile is associated with antiretroviral therapy (ART) and there are significant positive associations of dyslipidemia as well as some lipid ratios (LDL-c /HDL-c and TC/HDL-c ratios) with ART in the management of human immunodeficiency virus (HIV) [1]. However, only few of these lipid ratios were laid to emphasis within the scope of the study. Currently, the emerging lipid ratios are Castelli's risk index I and II (CRI-I and CRI-II), atherogenic risk of plasma (AIP) and atherogenic coefficient (AC), respectively [2].

On a general note, recent data have shown that CRI-I and CRI-II (estimated as TC/HDL-c and LDL-c/HDL-c ratios) are more accurate predictors of cardiovascular risk than traditional lipid parameters such as serum total cholesterol (TC), serum triglycerol (TG), serum high density lipoprotein cholesterol fraction (HDL-c) and serum low density lipoprotein

cholesterol fraction (LDL-c). CRI-I, calculated from the ratio of two important parameters, TC and HDLc, both of which are reported independent risk factors for coronary artery disease (CAD) is similar with CRI-II in risk evaluation. CRI-II, also calculated as the ratio of LDLc and HDLc, has been reported to involve in predicting risk of CAD [2]. Indeed, changes in these ratios were equally reported to be better predictors of successful coronary heart disease (CHD) risk reduction when compared with changes in absolute levels of lipids or lipoproteins [3].

In addition, comparison of individual lipid ratio in subjects from the report of Framingham Study clearly indicates that lipid ratios are significantly more useful as predictors of cardiovascular diseases (CVD) than the individual levels of LDLc or HDLc [4]. Furthermore, earlier investigation, the Helsinki Study, reported that the lipid ratio defined as LDLC/HDL-C (known here as CRI-II) presented more prognostic value when compared with ordinary LDLc or HDLc fraction [5].

In a related but different term, another lipid ratio, AIP defined as log (TG / HDLc) was proposed as a marker of plasma atherogenicity [6]. Since then, series of reports have been emerging. Studies revealed that in circumstances where other atherogenic risk factors such as TG and HDLc are not altered significantly or show no changes, AIP is a useful diagnostic tool for possible replacement [7]. Again, findings have favourably proved that AIP is highly important in predicting CVD risk and for effective therapeutic monitoring [8].

In 2012, Ghuge and Zine reported that AIP was increased in people at higher risk of CAD thus a diagnostic alternative to assess the effect of risk factors related to CVD [9]. Currently, AIP is gradually receiving attention from some practitioners as a vital prognosticator or predictor of developing atherosclerosis in metabolic diseases. The suggestion of AIP values that fall within -0.3 to 0.1 are associated with low, while 0.1 to 0.24 and above 0.24 are associated with medium and high cardiovascular risk, respectively [10].

The fourth lipid ratio known as Atherogenic Coefficient (AC), estimated as {(non-HDLc)/HDL-C} and sometimes as {(TC-HDLc)/HDL-C}, represents total sums of cholesterol found in VLDLc, IDLc and LDLc lipoprotein fractions in relation to the protective value of HDLc fraction. This therefore mirrors the atherogenic tendency that entire lipoprotein fractions are bound to generate. Once more, in 2011, Hermans and colleagues reported that non-HDLc mimics the role of Apo-B in determining atherogenicity level as well as

lipoprotein burden and consequently termed a valid surrogate of Apo-B apolipoprotein [11].

On the other hand, traditional lipid parameters relating dyslipidemia with resultant CAD have been repeatedly reported, especially in HIV management using highly active antiretroviral therapy (HAART). The commonly estimated of such parameters are TC, TG, LDLc, and HDLc respectively. Dyslipidemia has been consistently recognized in HIV-patients receiving treatment with HAART as a notable risk for developing CAD often with elevated levels of TC, TG, and LDLc , reduced HDLc and typically hypertriglyceridemia in most severe cases cases [1, 12].

Several important trials have stressed on the role of raised LDLc and reduced HDLc in the pathogenesis of atherosclerosis [13]. Also, other but prospective meta-analysis studies pointed it out that high TGs are correspondingly an independent risk factor for developing CHD [14, 15].

However, there are scenarios where the usefulness of traditional risk factors is limited. Bhardwaj and co-workers [2] reported that calculating certain emerging lipid ratios using these common lipid parameters, particularly in such circumstances where LDLc levels are under the target range, brings about possibility of raising the chance of identifying at-risk individuals that could be missed if ordinary lipid profile is employed. In this same study, it was noted that no significant difference in TC and LDLc levels was observed between the two studied groups, conversely, further estimation using calculated ratio based on these reported parameters later uncovered a significant difference between the groups. This, therefore, suggests that application of lipid ratios provides further utility over ordinary individual lipid parameters [2]. In addition, studies have indicated that in situations where other common predictors of atherogenic risk such as TG and HDLc appear normal, further estimation with AIP indicates that this lipid ratio is a more sensitive tool for diagnosis [7].

Furthermore, considering the running cost and operationalization for some important CAD plasma markers such as apolipoproteins (for instance, apo-III and apo-B) in resource-limited centers, these lipid ratios can add significant value to the assessment of CAD risk without headache [2, 16].

Finally, applications of these lipids ratios are becoming more recognized on daily basis, however, up till present moment very few studies have investigated their benefits in the management of HIV, whereas there is accumulating evidence of associated risk of developing CVD using ART particularly in the Southwest Nigeria. Also, the use of more sensitive

diagnostic markers such as apolipoproteins is rarely in routine operation in most treatment centers in the developing countries due to financial implications, for this reason, obtainability of simple, more sensitive and specific, and at the same time of no-cost lipid ratios as alternatives (or adjuncts) and better predictors of CVD risk will contribute immensely to this assessment.

MATERIALS AND METHODS

The recruitment was conducted at the Institute of Human Virology of Nigeria (IHVN) clinic center and the analyses were carried out at the chemical pathology laboratory, both at Ladoke Akintola University of Technology Teaching Hospital, LAUTECH, Osogbo located in southwest of Nigeria.

Subject Selection and Sample Size

Eighty (80) voluntary participants with age range of 18-55 years were recruited for the study. These include;
(i) Fourty HIV-positive patients (n= 40) on HAART treatment for three months and above,
(ii) Fourty HIV-positive patient but treatment naïve (n=40).

The participants comprise both sexes and ages within the same bracket, all living in the same geographical area. The study was initiated after the proposal had been reviewed and successfully approved by LAUTECH Ethical Committee with the help of the laboratory scientist in-charge.

Inclusion and Exclusion criteria

Based on their clinical history and clinical records, subjects with the following manifestations were excluded; DM (Diabetes Mellitus), hypertensions, hepatitis, jaundice, hypothyroidism, and nephrotic syndrome. Similarly, participants placed on contraceptives and other corticosteroid therapies were equally excluded.

Laboratory Methods

Following an overnight fast, ten milliliters (10mls) of venous blood was collected from the cubital vein using a sterile needle and syringe into EDTA bottle. The blood was centrifuged and the plasma was then separated after 10 minutes at 4000 rpm. The plasma was kept in plain bottle and stored at -20°C before the time of analyses.

Lipid profile

The plasma concentrations of TC, TG, and HDLc were estimated using spectrophotometry method.
➢ Plasma TC was estimated by an enzymatic method [17]. Cholesterol level was determined following an enzymatic hydrolysis and oxidation processes. The mixture was incubated for ten (10) minutes at room temperature, and then the absorbance was finally read at wavelength of 500nm.
➢ Plasma TG concentration was estimated by an enzymatic method [18]. TG level was determined following enzymatic hydrolysis process with lipase enzymes. The mixture was incubated for 10 minutes at room temperature following thorough mixing, and then the absorbance was finally read at wavelength of 500nm.
➢ HDLc was estimated by precipitated method [19]. LDLc, VLDLc and chylomicrons were precipitated by centrifugation for 10 minutes in the presence of phosphotungstic acid and magnesium chloride, at 4000 rpm. The top supernatant was extracted immediately while the cholesterol concentration was determined. Here, centrifugation was left with only the HDLc in the supernatant. The HDLc fraction in the supernatant was removed as sample containing HDLc fraction which was in turn estimated by cholesterol assay method.

Calculations of risk indices:

1. LDLc: After the estimation of TC, TG and HDLc as stated above, LDLc was calculated using Friedewald formula as follows; LDLc = Total cholesterol – (TG/5 +HDLc).

This was scientifically applicable since the combined concentration of both cholesterol and TG was less than 400mg/dl [20].

2. NonHDLc: NonHDL cholesterol is estimated as total cholesterol minus HDLc Mathematically, it is estimated as; NonHDLc = TC-HDLc.

3. Castelli's Risk Index (CIR): Castelli's Risk Index is based on three important lipid profile parameters i.e. TC, LDLc and HDLc and it is categorized into two; CIR-1 and CIR-11 [21].

CRI-1: CRI-I is established on the ratio of two important parameters of lipid profile, TC and HDLc. Mathematically, it is estimated as;

CRI-I= TC/HDLc ratio

CRI-11: CRI-II is calculated as the ratio of LDLc to HDLc. Mathematically, it is estimated as;

CRI-II = LDLc/HDLc ratio

4. Atherogenic Index of Plasma (AIP) = Atherogenic Index of Plasma was proposed by Dobiasova and Frohlich in 2001. AIP is a logarithmically transformed molar ratio of TG to HDLc [8].

AIP= $\log_{10}$ (TG/HDLc) ratio

5. Atherogenic Coefficient (AC) = Atherogenic coefficient is an indirect measure of cholesterol in VLDLc, IDLc, and LDLc lipoprotein fractions in

reference with HDLc fraction. Mathematically, it is expressed as;

$$AC = \{(TC - HDLc)/HDLc\} \text{ or } \{(Non-HDLc)/HDLc\} \text{ ratio}$$

CD4+ cell count

CD 4+ T-cell count was determined using Partec flow cytometer (TUVRheinland) by method of flow cytomery. Flow cytometry was a very useful method of estimating CD4+ cells through which cell or micro particles in liquid suspension were differentiated and determined according to their cell sizes, fluorescence emission as well as their internal structures [22].

Statistical analysis

The study data were statistically analyzed using the Statistical Package for Social Science version program (SPSS program-version 10.0 – SPSS Inc., Chicago, IL. USA). The data were expressed as means (± SEM) using student's t-test and Analysis of Variance (ANOVA). Means were also analyzed using Post Hoc test to compare the significant variables. Pearson's correlation coefficient (r) was used to determine the association between and across means of the variables. Results were regarded as significant at P<0.05.

RESULTS AND INTERPRETATION

Table 1: Independent Sample T-Test Showing Means± SEM of Group Characteristics, Lipid Profile and Atherogenic Indices

Parameter	Treatment-naïve Group[a] (n=40)	Treatment Group[b] (n=40)	P-Value
Characteristics			
Age (years)	37.22 ± 1.39	33.35 ± 1.33	0.066
Sex			0.388
Male	16 (40%)	20 (50%)	
Female	24 (60%)	20 (50%)	
Traditional lipid profile/parameters			
TC (mmol/L)	2.43 ± 0.04	3.93 ±0.07	0.001*
TG (mmol/L)	1.97 ± 0.02	1.83 ± 0.03	0.013*
HDLc (mmol/L)	0.85 ± 0.02	1.06 ± 0.03	0.004*
LDLc (mmol/L)	0.60 ± 0.03	2.03 ± 0.08	0.001*
Other biochemical parameters			
nonHDLc (mmol/L)	1.59 ± 0.05	2.87 ± 0.07	0.001*
CD4 Count (cells/µ)l	183.55 ± 21.79	478.65 ± 21.38	0.001*
Atherogenic indices			
CRI-I	2.90 ± 0.09	3.78 ± 0.11	0.000*
CRI-II	0.73 ± 0.05	1.98 ± 0.09	0.000*
AIP	0.37 ± 0.01	0.24 ± 0.01	0.005*
AC	2.06 ± 0.12	2.78 ± 0.11	0.001*

P-value ≤ 0.05 is considered statistically significant; All values are expressed as Mean ± S.E.M (Standard Error of Mean); CRI: Castelli's Risk Index (I&II); AIP: Atherogenic Index of Plasma; AC: Atherogenic Coefficient; a: Group1; b: Group 2

A total number of 80 patients infected with HIV/AIDS disease met overall inclusion criteria and were randomly selected into two groups; treatment-naïve group (n=40) and another age- and sex-matched group already on steady treatment for not less than three months (treatment group, n=40), all living within the same geographical area. The need for matching a treatment-naïve group was necessary to assess the effect of the drugs on both lipid profile and atherogenic indices before further evaluations.

Data illustrated in table (1) show the effects of ART in matching group of treatment-naïve with another group of HIV-patients on ART treatment. There were significant differences (P ≤ 0.05) in the mean values of traditional lipid parameters (TC, TG, LDLc and HDLc), nonHDL cholesterol, CD4 cell count and atherogenic indices (CRI-1 and II, AIP and AC) when comparisons between the two groups were carried out. In other words, there were significant increases (P≤0.05) in all these estimated parameters except the plasma triglyceride (TG) level and atherogenic index of plasma (AIP) where significant reductions (P ≤ 0.05) were observed.

Table 2: Percentage Frequency of Plasma Lipids, Lipoparticle Fractions and Atherogenic Index of Plasma along with Risk Stratification in Treatment Group

Variable	Category	Frequency (n)	Percentage (%)
TC (mmol/l)	Normal (<5.17mmol/l)	40	100.0
	Elevated	0	0.0
TG (mmol/l)	Normal (<1.7mmol/l)	7	17.5
	Elevated	33	82.5
HDLc (mmol/l)	Normal (≥0.9 mmol/l)	31	77.5
	Reduced	09	22.5
LDLc (mmol/l)	Normal (<3.5mmol/l)	40	100
	Elevated	0	0.0
AIP	Low-risk (<0.1)	1	2.5
	Intermediate risk (0.1-0.24)	17	42.5
	High risk (>0.24)	22	55.0
Abnormal lipid profile			
	0	2	5
	1	17	42.5
	2	21	52.5
	3	0	0.0
	4	0	0.0

TC: Total cholesterol; TG: Triglyceride; HDLc: High density lipoprotein; LDLc: Low density lipoprotein; AIP: Atherogenic index of plasma

Table (2) shows the results of percentage frequency of referenced lipid profile along with risk stratifications using AIP and abnormal lipid profile in ART-experienced patients infected with HIV/AIDS. .

Reflecting the interpretations from routine clinical assessment and perspectives, plasma total cholesterol (TC) and low density lipoprotein cholesterol fraction (LDLc) were all normal and less than 5.17mmol/l and 3.5mmol/l respectively in the entire treatment group {n= 40; 100%).

However, plasma TG level indicated that only 17.5% of this group showed normal value less than 1.7mmol/l and majority of about 82.5% of the group showed elevated value, while plasma high density lipoprotein cholesterol fraction (HDLc) were normal with ≥ 0.9 mmol/l value in (77.5%) of the group. Surprisingly, stratification of this group of ART-experienced patients that accorded predominantly normal values of lipid/lipoprotein levels under traditional lipid profile showed various risk strata using AIP.

AIP in predicting cardiovascular risk revealed that only 2.5% proportion of the group was within low risk (<0.1), 42.5% were in intermediate risk level (0.1-0.24) while majority in the group, 55% posed with high risk (>0.24) respectively. Again, the group was further stratified into number of abnormal lipid/lipoprotein present. Patients proportion with all lipid/lipoprotein normal, that is, "0" abnormal lipid constituted 5% the proportion with "1" abnormal lipid/lipoprotein constituted 42.5% while the proportion with "2" abnormal lipid/lipoprotein was 52.5% respectively. In addition, none of the patients consisted of abnormal lipid/lipoprotein values beyond "2" of the four lipid profile parameters.

Table 3: Analysis of Variance Showing Mean± SEM of Age, Traditional Lipid Parameters, and Atherogenic Indices across the Stratified Risk Levels in Treatment Group

Variable	Risk Level			F-value	P-value
	0 Abnormal lipid profile (n=2)	1 Abnormal lipid profile (n=17)	2 Abnormal lipid profile (n=21)		
Age	34.00±7.07	33.29±9.12	34.05±7.39	0.041	0.960
Lipid parameters					
Cholesterol	3.55±0.80	4.02±0.33	3.94±0.44	1.189	0.316
TG	1.64±0.04	1.85±0.20	1.92±0.21	1.991	0.151
HDLc	1.17±0.04	1.16±0.19	0.83±0.21^{ab}	13.467	0.000*
LDLc	1.65±0.82	2.02±0.42	2.24±0.48	2.088	0.138
nonHDLc	2.39±0.83	2.87±0.35	3.12±0.50^{a}	3.169	0.054
Atherogenic indices					
CRI-I	3.06±0.78	3.55±0.55	5.22±2.03^{ab}	6.350	0.004*
CRI-II	1.43±0.75	1.81±0.53	3.05±1.53^{ab}	5.881	0.006*
AC	2.06±0.78	2.55±0.55	4.22±2.03^{ab}	6.335	0.004*
AIP	0.15±0.02	0.21±0.05	0.38±0.16^{ab}	11.344	0.000*

a= significantly different from group"0" lipid profile alteration (Post Hoc)
b= significantly different from group"1" lipid profile alteration (Post Hoc)
F: value for ANOVA test; *= statistically significant (p ≤ 0.05)

Table (3) illustrates analysis of variance for the mean (±SEM) values of age, traditional lipid parameters and atherogenic indices across the stratified risk levels based on burden of abnormal lipid profile in ART-experienced group AIDS.

There was no significant difference (P>0.05) both across and between the groups when the ages of patient subgroups in the age was compared. Considering traditional lipid parameters, only plasma HDLc level showed significant changes (P<0.05) both across and between the groups of the three categories of

abnormal lipid profile. Similarly, there was significant difference (P<0.05) in plasma nonHDLc level between subgroup of "2" abnormal lipid profile against "0" abnormal lipid profile, although no significant difference (P>0.05) was observed across the subgroups based on number of abnormal lipid profile.

On the contrary, all indices of atherogenicity; Castelli's risk index I &II (CRI-1 & CRI-II), atherogenic coefficient (AC) and AIP showed significant differences (P<0.05) both across and between the subgroups of abnormal lipid profile.

Table 4: Relationship between Castelli Indices, Atherogenic Index of Plasma, Atherogenic Coefficient and Traditional Lipid Profile in Antiretroviral Therapy

Parameters	CRI-1		CRI-II		AIP		AC	
	r-value	P-value	r-value	P-value	r-value	P-value	r-value	P-value
TC (mmol/l)	0.017	0.919	0.191	0.238	0.119	0.461	0.454	0.003**
TG (mmol/l)	-0.024	0.885	-0.034	0.833	0.282	0.078	0.114	0.484
HDLc (mmol/l)	-0.903	0.000**	-0.787	0.000**	-0.917	0.000**	-0.553	0.000**
LDLc (mmol/l)	0.771	0.000**	0.915	0.000**	0.684	0.000**	0.497	0.001**
nonHDLc (mmol/l	0.388	0.013*	0.494	0.001**	0.484	0.002**	0.630	0.000**
CD4+ (cells/µl)	-0.307	0.054	-0.223	0.166	-0.409	0.009**	-0.325	0.041*
Age (yrs)	0.116	0.477	-0.148	0.363	0.148	0.363	0.141	0.384
CRI-I	-----	1	0.780	0.000**	0.817	0.000**	0.517	0.001**
CRI-II	0.780	0.000**	-----	1	0.733	0.000**	0.498	0.001**
AIP	0.817	0.000**	0.733	0.000**	-----	1	0.586	0.000**
AC	0.517	0.001**	0.498	0.001**	0.586	0.000**	-----	1

*: Correlation is significant at the 0.05 level (2-tailed)
**: Correlation is significant at the 0.01 level (2-tailed)
TC: Total cholesterol; TG: Triglyceride; HDLc: High density lipoprotein; LDLc: Low density lipoprotein; CD: Cluster differentiation; CRI: Castelli's risk index; AIP: Atherogenic index of plasma; AC: Atherogenic coefficient

Table (4) shows relationship between traditional lipid parameters and atherogenic indices in a

group of ART-experienced patients infected with HIV/AIDS.

Plasma TC level showed significant positive association with AC (P<0.01; r=0.454) in ART-treatment group. Meanwhile, no significant association (P>0.05) was observed between TG and any index of atherogenicity. However, plasma HDLc level showed significant negative associations with CRI-I (P<0.01; r= -0.903), CRI-II (P<0.01; r= -0.787), AIP (P<0.01; r= -0.917) and AC (P<0.01; r= -0.553) correspondingly. Conversely, plasma LDLc level showed significant positive associations with CRI-I (P<0.01; r= 0.771), CRI-II (P< 0.01; r= 0.915), AIP (P<0.01; r= 0.684) and AC (P<0.01; r= 0.497) respectively. Similarly, plasma nonHDLc level showed significant positive association with CRI-I (P<0.05; r= 0.388), CRI-II (P<0.001; r= 0.494), AIP (P<0.01; r= 0.484) and AC (P<0.01; r= 0.630), while CD4 cell counts showed significant negative associations with AIP (P<0.01; r= -0.409) and AC (P<0.05; r= -0.325) respectively. Individually, all indices of atherogenicity show significant positive associations (P<0.01) with one another; CRI-I vs CRI-II (P<0.01; r= 0.780), CRI-1 vs AIP (P<0.01; r= 0.817), CRI-I vs AC (P<0.01; r= 0.517), CRI-II vs AIP (P<0.01; r= 0.733), CRI-II vs AC (P<0.01; r= 0.498) and AIP vs AC (P<0.01; r= 0.586) respectively.

DISCUSSION

Dyslipidemia has been recognized as an important risk factor associated with coronary artery disease (CAD) [23]. A number of lipid parameters have been employed in predicting risk of coronary atherosclerosis and cardiovascular diseases (CVD). These chiefly comprise plasma concentrations of lipids and lipoproteins, while occasionally involves plasma apolipoproteins. Of more recent, the use of lipid ratios as risk indices involving the lipids/lipoproteins has been noteworthy [1, 24, 25]. Many reports show the relevance of evolving lipid ratios above traditional lipid parameters [2, 7].

In our study, we assessed atherogenic status of HIV-individuals at risk of CVD during the treatment, by first comparing a group of HIV-treated patients against another without prior exposure to treatment, indicative of drug effect. The outcomes greatly indicated lipid alteration reflected on both lipid ratios and traditional lipid profile. However, when classified by plasma total cholesterol (TC), LDL-cholesterol (LDLc), and nonHDL-cholesterol (nonHDLc) levels along with Castelli's risk indexes I and II (CRI-1 & II) and atherogenic coefficient (AC), CVD risk seemed to be more likely in HIV-treated patients. Whereas, when considering HDL-cholesterol (HDLc) and triglyceride (TG) levels in the company of atherogenic index of plasma (AIP), there seemed to be high risk of CVD in antiretroviral therapy (ART) treatment-naïve group of the patients.

Meanwhile, attention has been drawn to different lipid abnormalities in both treatment-naïve HIV patients and ART-experienced [1] and that corroborates our study. More so, our present study is also in accordance with earlier study, where high risk factors such as significant increases in TC and LDLc levels along with increased HDLc level (cardinal marker of improved dyslipidemic risk) were reported in ART treatment [26].

On the other hand, in ART-naïve patients, hypertriglyceridemia, reduced TC and LDL levels have been observed [12]. Rose and colleagues [27] also reported reduced HDLc level, indicating an increase in the activity of the cholesterol ester transfer protein (CETP) which acts in transfer of cholesterol from HDLc to apo-B lipoproteins, hence consequential reduction in HDLc [27]. More interestingly, a study conducted on newly diagnosed HIV-positive individuals revealed that ART resulted in an improved dyslipidemic profile that was earlier observed before initiating treatment [28].

On the contrary, in one closely related study from the same study location with our study, Oduola *et al*. [29] followed a group of HIV-infected urban dwellers, and the lipid profile was monitored for 15 months in categorized groups of HIV-patients on ART and treatment-naive who were hitherto asymptomatic. When finally matched with another set of apparently healthy HIV-negative participants, TG, TC, HDLc, and LDLc levels in the ART-treated patients were insignificantly different, while the TC and HDLc levels significantly reduced in the HIV-treatment naïve against ART-treated group [29].

Nevertheless, consistent with our findings, some studies reported significant increases in TC, HDLc, and nonHDLc levels after ART treatment of HIV positive patients [30, 31]. Although, our study expanded beyond the scope of the existing literature with annexation of probable risk predictors such as CRI-I & II indexes, AC and AIP. It is bit difficult to make succinct comparison due to different study design. However, Kalkinami *et al*. [32] reported CRI index in a separate but retrospective cohort study on HIV-monoinfection group of their study, where apart from significant increases in TC, HDLc and nonHDLc levels, CRI index also showed elevation but not statistically significant.

On these conflicting reports, we were therefore obliged to further assessment of the ART-experienced individuals in our study to understand interrelationship between the common lipid profile parameters and the evolving lipid ratios on ART therapy, succinctly. In this regard, advanced stratifications of the ART-experienced group clearly refined individual risk levels using AIP

index and burden of abnormal lipid and lipoproteins. Interestingly, we did not observe significant alterations in TC, TG, LDLc and nonHDLc levels in this group considering the risk factors by means of abnormal lipid profile, although HDLc showed significant reduction, whereas the CRI-indexes, AIP and AC all indicated significant increased risk levels across the group strata.

The reduction in HDLc levels has been linked with increased rate of catabolism. Again, alterations in lipid metabolism involving reduced HDLc has been associated with CAD [33], while reduced level of HDLc is described an independent risk factor for enhanced susceptibility to CAD [34]. Our study outcome is very typical of dyslipidemia observed in angiographically confirmed patients in Indians where the absolute values of lipid profile parameters such as TC, LDLc, HDLc and nonHDLc levels were not markedly deranged but showed significant elevations in CRI-I, CRI-II, AIP and AC indices [2] that have been repeatedly reported as probable predictors of CVD in the management of HIV most especially when lipid profile appears to be normal [2, 8].

Besides, studies have revealed that in circumstances where other atherogenic risk parameters such as TG seem normal, AIP may possibly serve as the diagnostic alternative [7] while da Luz and coworkers established that a high ratio of TG/HDL strongly indicated correlation with coronary disease than lipid variables [35]. Furthermore, studies have indicated the role of AIP in predicting CVD risk and effectiveness of therapy [8]. In our study, we could not observe significant changes in TC, TG, LDLc and nonHDLc levels in our ART-experience group whereas, AIP along with other lipid ratios (CRI-I, CRI-II, and AC indices) showed significant increases. This clearly suggests the relevance of the risk predictors over individual lipid parameters.

In a related study, studies have revealed the association between TC/HDLc ratio (here known as CRI-I) and coronary plaques development [36], this is supported by the Canadian study group that TC/HDLc is a more sensitive, specific and CVD risk-targeting index than ordinary TC [37].

AIP correlated well with HDLc, LDLc and nonHDLc fractions in our study in the same way with CRI-I, CRI-II, and AC indices. According to Grover's model, either the ratio of LDLc/HDLc or TC/HDLc (that is, CRI-I & II) is the best lipid related predictive risk calculators of future cardiovascular events [38].

Similarly, Dobiásová and Frohlich demonstrated correlations of AIP with HDL size and LDL particles and with the fractional esterification rate of cholesterol catalyzed by lecithin-cholesterolacyl transferase (LCAT) in plasma. By indication, index ratio perfectly reveals the presence of atherogenic small LDLc and HDLc particles, and therefore an accepted sensitive predictor of atherosclerotic cardiovascular risk [6]. Likewise, CRI-I and II are predictive of AIP [24] as well as AC [2] and therefore are likely to be related in their ability to pinpoint patients with risk of CVD.

CONCLUSION

The findings in this study were in conformity with mammoth of evidence showing that calculated lipid ratios- Castelli's risk indexes, atherogenic index of plasma, and atherogenic coefficient could serve as more sensitive risk predictors for atherosclerotic cardiovascular disease. Many of ART-experienced patients might be wrongly captured within low risk status using traditional lipid profile in desirable range consequently escape right medical decision. Meanwhile, sudden coronary death is the first manifestation of atherosclerotic disease in large proportion of the cases [39]; further estimation of lipid ratios beyond ordinary lipid profile could avert sudden death in HIV-patients well receiving treatment.

LIMITATIONS

Our study was not without limitations, one of which was the sample size. Further studies will require large sample size and better still of multicenter design to better validate this conclusion. More so, since information concerning certain other potential risk factors for cardiovascular dieases such as- diet, physical activity, classes of drug, genetic factors and other demographic and anthropometric variables was not collected during the recruitment for our study, this may limit the validity of our outcomes to populations similar to our study alone.

ACKNOWLEDGMENTS

REFERENCES

1. Kamoru, A. A., Japhet, O. M., Adetunji, A. D., Akinlawon, A. A., Musa, M. A., Onifade, A. A., Jelili, O. A., Taofik, A. A., Prince, U. L., & Oyenike, M. A. (2017). CD4+ Cell Count, Lipid And Lipoprotein Levels In Hiv Patients On Drug Treatment. *Int. J. AIDS Res, 4*, 140-146.
2. Bhardwaj, S., Bhattacharjee, J., Bhatnagar, M. K., & Tyagi, S. (2013). Atherogenic index of plasma, castelli risk index and atherogenic coefficient new parameters in assessing cardiovascular risk. *Int. J. Pharm. Biol. Sci, 3,* 359–364.

3. Millán, J., Pintó, X., Muñoz, A., Zúñiga, M., Rubiés-Prat, J., Pallardo, L. F., Masana, L., Mangas, A., Hernández-Mijares, A., González-Santos, P., Ascaso, J. F., & Pedro-Botet, J. (2009). Lipoprotein ratios: Physiological significance and clinical usefulness in cardiovascular prevention. *Vasc. Health Risk Manag, 5,* 757–765.

4. Kannel, W. B. (2005). Risk stratification of dyslipidemia: insightsfrom the Framingham Study. Curr. Med. Chem. Cardiovasc. Heamatol. Agents, 3,187-193

5. Manninen, V., Tenkanen, L., Koskinen, P., Huttunen, J. K., Mantarri, M., Heinomen, O. P., & Frick, M. H. (1992). Joint effects of serum triglyceride and LDL cholesterol and HDL concentrations on coronary heart disease risk in the Helsinki Heart Study. Implications for treatment. *Circulation, 85*, 37-45.

6. Dobiasova, M., & Frohlich, J. (2001). The plasma parameter log (TG/HDL-C) as an atherogenic index: correlation with lipoprotein particle size and esterification rate in apoB-lipoprotein-depleted plasma (FERHDL). *Clin. Biochem, 34,* 583–588

7. Nwagha, U. I., Ikekpeazu, E. J., Ejezie, F. E., Neboh, E. E., & Maduka, I. C. (2010). Atherogenic index of plasma as useful predictor of cardiovascular risk among postmenopausal women in Enugu, Nigeria. *Afri. Health Sci, 10*, 248–252.

8. Dobiášová, M., Frohlich, J., Šedová, M., Cheung, M. C., & Brown, B. G. (2011). Cholesterol esterification and atherogenic index of plasma correlate with lipoprotein size and findings on coronary angiography. *J. Lipid Res, 52,* 566–571.

9. Ghuge, G. D., & Zine, R. (2012). Atherogenic index of plasma in myocardial infarction in rural population of Marathwada region. *J. Evol. Med. Dent. Sci, 1,* 237-240

10. Dobiasova, M. (2006). AIP-atherogenic index of plasma as a significant predictor of cardiovascular risk: from research to practice. *Vnitr. Lek, 52*, 64-71.

11. Hermans, M. P., Sacks, F. M., Ahn, S. A., & Rousseau, M. F. (2011). Non-HDL-cholesterol as valid surrogate to apolipoprotein B100 measurement in diabetes: Discriminant Ratio and unbiased equivalence. *Cardiovasc. Diabetol, 28,* 20.

12. El-Sadr, W. M., Mullin, C. M., Carr, A., Gibert, C., Rappoport, C., Visnegarwala, F., Gunfeld, C., & Raqhavan, S. S. (2005). Effects of HIV disease on lipid, glucose and insulin levels: Results from a large antiretroviral-naive cohort. *HIV Med, 6,* 114-121.

13. National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. 2002. *Circulation, 106*, 3143–3421

14. Patel, A., Barzi, F., Jamrozik, K., Lam, T. H., Ueshima, H., Whitlock, G., & Woodward, M. (2004). Serum triglycerides as a risk factor for cardiovascular diseases in the Asia-Pacific region. *Circulation, 110*, 2678-2686.

15. Sarwar, N., Danesh, J., Eiriksdottir, G., Sigurdsson, G., Wareham, N., Bingham, S., Boekholdt, S.M., Khaw, K., & Gundnason, V. (2007). Triglycerides and the risk of coronary heart disease: 10,158 incident cases among 262,525 participants in 29 Western prospective studies. *Circulation, 115*, 450-458.

16. Gotto, A., Whitney, E., Stein, E. A., Shapiro, D. R., Clearfield, M., Weis, S., Jou, J. Y., Langgendorfher, A., Beere, P. A., Watson, D. J., Downs, J. R., & de Cani, J. S. (2000). Relation between baseline and on-treatment lipid parameters and first acute major coronary event in the Air Force/Texas Coronary Atherosclerosis Prevention Study (AFCAPS/TexCAPS). *Circulation, 101,* 477–484.

17. Trinder, P., & Barham, D. (1972). Estimation of LDL-cholesterol in plasma. *Clin. Chem, 78*, 19-22.

18. Buccolo, G., and David, H. (1973). Quantitative determination of serum triglyceride by the use of enzymes. *Clin. Chem, 19,* 476-482.

19. Benzie, A. (1979). High density lipoprotein cholesterol precipitation. *Med. lab. Sci, 36*, 289-291.

20. Friedwald, W. T., Levy, I. R., & Friedrickson, D. S. (1972). Estimation of the concentration of low density lipoprotein cholesterol in plasma without use of the preparative ultracentrifugation. *Clin. Chem, 18*, 499-502.

21. Castelli, W. P., Abbott, R. D., & McNamara, P. M. (1983). Summary estimates of cholesterol used to predict coronary heart disease. *Circulation, 67,* 730-734.

22. Greeve, B. (2003). "A new No-lyse, No-Wash Flow-Cytometric Method for the determination of CD4 T Cells in Blood Samples*". Transfus. Med. Hemoth, 30*, 8-13.

23. Yusuf, S., Hawken, S., Ounpuu, S., Dans, T., Avezum, A., Lanas, F., McQueen, M., Budaj, A., Pais, P., Varigos, J., & Lisheng, J. (2004). "Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study". *Lancet, 364*, 937-952.

24. Onyedum, C. C., Young, E. E., Iroezindu, M. O., Chukwuka, C. J., & Nwagha, U. I. (2014). Atherogenic index of plasma in highly active antiretroviral therapynaïve patients with human

immunodeficiency virus infection in Southeast Nigeria. *Indian J. Endocr. Metab, 18*, 631-636.

25. Kim, T., Park, A.Y., Baek, Y., & Cha, S. (2017). Genome-wide association study reveals four loci for lipid ratios in Korean population and the constitutional group. Constitutional Subgroup. *PLOS ONE, 12* (1), e0168137

26. Zephy, D. (2015). Lipid profile among Art treated and untreated patients in HIV positive cases. Arch. Med, 8, 2.

27. Rose, H., Hoy, J., Woolley, I., Tchoua, U., Bukrinsky, M., Dart, A., & Sviridov, D. (2008). HIV infection and high density lipoprotein metabolism. *Atherosclerosis, 199*, 79-86.

28. Fourie, C. M. T., Van Rooyen, J. M., & Schutte, A. E. (2011). HIV infection and cardiovascular risk in black South Africans. *Cardiovasc. J. Afr, 22*, 117–119.

29. Oduola, T., Akinbolade, A. A., Oladokun, L. O., Adeosun, O. G., Bello, I. S., & Ipadeola, T. I. (2009). Lipid profiles in people living with HIV/AIDS on ARV therapy in an urban area of Osun State, Nigeria. *World. J. Med. Sci, 4*, 18–21.

30. Levy, A. R., McCandless, L., Harrigan, P. R., Hogg, R., Bondy, G., Iloeje, U. H., Mukherjee, J., & Montaner, J. S. (2005). Changes in lipids over twelve months after initiating protease inhibitor therapy among persons treated for HIV/AIDS. *Lipids Health Dis, 4,* 4.

31. Asztalos, B. F., Schaefer, E. J., Horvath, K. V., Cox, C. E., Skinner, S., Gerrior, J., Gorbach, S. W., & Wanke, C. (2006). Protease inhibitor- based HAART, HDL, and CHD-risk in HIV-infected patients. *Atherosclerosis, 184,* 72–77.

32. Kakinami, L., Adams, J. M., Block, R. C., Cohn, S. E., Maliakkal, B., & Fisher, S. G. (2012). Short Communication. Risk of Elevated Total Cholesterol/High-Density Lipoprotein Cholesterol Ratio After Antiretroviral Therapy in HIV/Hepatitis C Virus Patients. *AIDS Res. Hum. Retrov, 28*, 1552-1556.

33. Brunzell, J. D. (2007). Hypertriglyceridemia. *N. Engl. J. Med, 357,* 1009-1017.

34. Von Eckardstein, A., Nofer, J. R., & Assmann, G. (2001). High density lipoproteins and arteriosclerosis: Role of cholesterol efflux and reverse cholesterol transport. *Arterioscler. Thromb. Vasc. Biol, 21*, 13–27.

35. da Luz, P. L., Favarato, D., Faria-Neto, J. R. Jr, Lemos, P., & Chagas, A. C. (2008). High ratio of triglycerides to HDL-cholesterol predicts extensive coronary disease. *Clinics (Sao Paulo), 63*, 427-432.

36. Nair, D., Carrigan, T. P., Curtin, R. J., Popovic, Z. B., Kuzmiak, S., Schoenhagen, P., Flamm, S. D., & Desai, M. Y. (2009). Association of total cholesterol/ high-density lipoprotein cholesterol ratio with proximal coronary atherosclerosis detected by multislice computed tomography. *Prev. Cardiol, 12*, 19-26.

37. Genest, J., Frohlich, J., Fodor, G., & McPherson, R. (2003). The Working Group on Hypercholesterolemia and Other Dyslipidemias. Recommendations for the management of dyslipidemia and the prevention of cardiovascular disease: 2003 update. *CMAJ, 169*, 921–924.

38. Grover, S. A., Levington, C., & Paquet, S. (1999). Identifying adults at low risk for significant hyperlipidemia: a validated clinical index. *J. Clin. Epidemiol, 52*, 49–55.

39. World Health Organization (1990). Prevention in childhood and youth of adult cardiovascular diseases: time for action: report of a WHO Expert Committee, 11.