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Predictors of Cardiovascular Disease Risk and Atherogenic Indices of HIV Seropositives on Highly Active Antiretrovirals in Western Nigeria

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Abstract

Cardiovascular risk factors places HIV-infected patients at increased risk for cardiovascular diseases (CVDs) due to complex interactions between traditional CVD risk factors, antiretroviral therapy (ART) and HIV infection itself. The report of the 2012 National Reproductive Health Survey Plus indicated that the prevalence of HIV/AIDS in Nigeria is about 3.4% while Ondo State had a prevalence of 4.3%. In this study, we evaluated the CD4⁺ cell count, atherogenic indices and risk score of adult HIV seropositives on HAART. HAART-naïve and HIV seronegative individuals were monitored as controls. CD4⁺ cell count of adult HIV seropositive subjects on HAART, HAART-naïve and seronegative controls were determined using flow cytometry while their atherogenic indices and Framingham risk score were extrapolated from serum lipids and lipoproteins. All data were expressed as mean \pm standard deviation and analysed with analysis of variance while multiple comparisons were done using Post-Hoc test. The mean serum cardiac risk ratio (CRR), atherogenic index of plasma (AIP), atherogenic coefficient (AC) and Framingham Risk Score (FRS) were significantly increased in the HAART group as compared with those of HAART-naïve and HIV seronegative controls. HIV in itself may have an effect on the metabolism of lipids and lipoproteins, with ultimate effect on the atherogenic indices and risk score. This is probably worsened by antiretroviral therapy as the increased levels of these indices were mainly seen in the HAART group, constituting a major risk for cardiovascular diseases.

Keywords: Cardiovascular Disease, Atherogenic Indices, HIV, Highly Active Antiretrovirals, Nigeria

Introduction

HIV is a retrovirus that primarily infects components of the human immune system such

as CD4 T-cells, macrophages and dendritic cells, is the cause of HIV/AIDS, a rapidly growing epidemic in sub-Saharan Africa especially Nigeria (Alexander *et al.*, 2010). Metabolic abnormalities are often common among human immunodeficiency virus (HIV) patients (Alexander *et al.*, 2010). The atherogenic indices are 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 (Alexander *et al.*, 2010). The report of the 2012 National Reproductive Health Survey Plus (NARHS, 2012) indicated that the prevalence of HIV/AIDS in Nigeria is about 3.4% while Ondo State has a prevalence of 4.3%. Human Immunodeficiency Virus infection and Acquired Immune Deficiency Syndrome (HIV/AIDS) is a spectrum of conditions caused by infection with Human Immunodeficiency virus (HIV). It directly and indirectly destroys CD4 T-cells (Wihelm, 2008; Alexander *et al.*, 2010; Sepkowitz, 2012).

Cardiovascular disease (CVD), a primary cause of death worldwide, is a group of disorders of the heart and blood vessels which can manifest as coronary heart disease, peripheral arterial disease, pulmonary embolism, among others (WHO, 2016; WHO, 2008). Atherosclerosis is the most common pathological process that leads to it, involving dyslipidaemia, chronic



inflammation and vascular endothelial dysfunction. The traditional risk factors for CVD recognised by the current National Cholesterol Education Project (NCEP) Adult Treatment Panel III (ATP III) guidelines include age (≥ 45 years for men and ≥ 55 years for women), cigarette smoking, dyslipidaemia, hypertension or use of anti-hypertension medications, diabetes mellitus, obesity, among others are all implicated in its aetiology (NCEP, 2001). The Framingham risk assessment tool is a coronary prediction algorithm that provides estimates or a scoring system used to determine an individual's chances of developing cardiovascular diseases over the course of 10 years. The factors used to estimate risk include: age, gender, total cholesterol (TC), High Density Lipoprotein Cholesterol (HDL-C), systolic blood pressure and antihypertensive medication, and cigarette smoking (D'Agostino *et al.*, 2008). The Framingham risk score has the ability to only predicts future coronary heart disease (CHD) events. Individuals with low risk are said to have 10% or less CHD risk at 10 years, with intermediate risk 10 – 20% and high risk 20% or more (D'Agostino *et al.*, 2008).

The use of atherogenic index of plasma (AIP) in a previous report involving highly active antiretroviral therapy-naïve patients with human immunodeficiency virus infection in Southeast Nigeria revealed that abnormal AIP is frequent in HAART-naïve HIV patients and is inversely related to their level of immunity (Onyedum *et al.*, 2014). Another study on lipid profile of HIV positive patients on antiretroviral therapy revealed that there was a significant increase in LDLC/HDL ratio in all the groups, except those on therapy for 7-12 months compared to control group (Abduljalil *et al.*, 2015). It was also reported that there were significantly higher lipids and lipoproteins in HAART group when compared to pre-HAART group in another research in Southern Ethiopia (Agete *et al.*, 2012). Researches on dyslipidaemia and cardiovascular disease risk profiles showed that the use of first-line antiretroviral therapy regimens that contain efavirenz and nevirapine were associated with raised total cholesterol (TC), LDL cholesterol and triglycerides (TG)

(Zhou *et al.*, 2015). A previous report on HIV-infected patients on antiretroviral treatment showed that serum TG, TC and Very Low-Density Lipoprotein Cholesterol (VLDLC) are significantly decreased in the HAART-naïve and control groups as compared with the HAART group, with a conclusion that HIV itself may have an effect on the metabolism of lipids (Agboola *et al.*, 2016). This study was therefore designed to evaluate the CD4+ T cell count, atherogenic indices and Framingham risk score of adult HIV seropositive subjects on antiretrovirals. HAART-naïve and HIV seronegative participants were monitored as controls.

Materials and Methods

Study Site/ Subject Selection/Study Design

The study is a case-control study carried out at the State Specialist Hospital Akure, the capital city of Ondo State, Nigeria. The hospital is a secondary health care facility. It is a major HIV treatment centre in Ondo State. The study included 210 subjects. The subjects were divided into three groups of 70 each: Group 1 (HAART group) included HIV-seropositive individuals who were already on HAART for at least 12 months. Group 2 (HAART-naïve group) included HIV-seropositive individuals yet to be started on HAART. Group 3 (control group) consisted of HIV-seronegative control individuals. Participation was voluntary and written informed consent was obtained from all participants and confidentiality of all information gathered was strictly ensured. Ethical approval for the study was obtained from the Ondo State Government Ministry of Health.

Sampling Technique / Method of Recruitment of Subjects

Random sampling was used to randomly select the required number of patients/subjects (in each of the three groups). The subjects who met the inclusion criteria were consecutively recruited until the required number of willing participants was reached for each of the three groups.

Inclusion and Exclusion Criteria

Inclusion criteria included: HIV seropositive, age (≥ 18 years), HAART competent for ≥ 12 months (first line HAART regimen), non-use of lipid-modifying medications (statins, nicotinic acid, resins, fibrates) and willingness to offer a written informed consent after counselling.

Individuals who did not meet the inclusion criteria [non-adults (< 18 years), HAART competent for < 12 months patients not on first line HAART regimen), use of lipid-modifying medications (stations, nicotinic acid, resins, fibrates) and those that refused to offer a written informed consent after counselling] were excluded from participation in the study.

Sample Size

Sample size calculation was done using 95% confidence interval, 0.05 precision and prevalence rate. The report of the 2012 National Reproductive Health Survey Plus (NARHS-Plus) indicated that the prevalence of HIV/AIDS in Nigeria is 3.4% while Ondo State has a prevalence of 4.3% (NARHS, 2012). The formula for sample size is: $n = Z^2PQ/d^2$ as previously reported (Daniel, 2013).

Sample Collection, Storage and Analysis

Venous blood was collected aseptically after an overnight fast through a clean vacutainer system from each subject into plain vacutainer bottle for retroviral confirmation, lipids and lipoproteins analysis and in an Ethylene Diamine Tetra Acetic (EDTA) containing tube for CD4+ lymphocytes count. Retroviral HIV-1/2 antigen/antibody test was carried out to confirm the status of the subjects using the serial testing algorithm. Blood samples were centrifuged at 4000 Revolution Per Minute (RPM) for 10 minutes and the serum from each subject was extracted into fresh plain bottle for immediate analysis while those not analysed immediately were stored at -20 -degree Celsius until analysis. The CD4+ lymphocytes count was carried out using a flow cytometry technique through a Cyflow counter (Partec GmbH Görlitz, Germany). Atherogenic indices were determined from enzymatic spectrophotometrically determined lipids and

lipoproteins and the Framingham risk score (FRS) was determined from enzymatic spectrophotometrically determined total cholesterol and high-density lipoprotein cholesterol and systolic blood pressure (SBP).

Statistical Analysis

Data was statistically analysed using Statistical Package for the Social Sciences (SPSS) for windows version 23.0 software (SPSS Inc., Chicago, IL, USA). All data were expressed as Mean \pm Standard Deviation (SD). Statistical analysis of the data was performed by Analysis of Variance (ANOVA) while multiple comparisons was done using Post Hoc Bonferroni test. Significance was fixed at $p \leq 0.05$. Pearson's correlation coefficient was used for correlational analysis of the test.

Results

Demographic Data, Physical and Biochemical Parameters

A total of 210 subjects participated in the study. Group 1 (HAART group) contained 51 females and 19 males. Group 2 (HAART naïve group) had 49 females and 21 males. Group 3 (control group) had 51 females and 19 males.

Group 1 and 2 subjects all tested positive for HIV, while all subjects in group 3 participants tested negative. The average duration (in months) of Highly Active Antiretroviral Therapy (HAART) in the group 1 subjects was 25.63 ± 19.99 years while the average duration (in months) of cotrimoxazole use for subjects in group 2 was 7.10 ± 4.89 years. In group 1, the HAART regimen taken at the initiation or commencement of therapy shows that 4 (5.7%) subjects were placed on first line regimen 1A, 41 (58.6%) placed on regimen 1B, 13 (18.6%) placed on regimen 1C, 5 (7.1%) placed on regimen 1D, 6 (8.6%) placed on regimen 1E and 1 (1.4%) placed on regimen 1F. The current regimen used by the subjects shows that 33 (47.1%) subjects were placed on first line regimen 1B, 16 (22.9%) placed on regimen 1C, with 21 (30.0%) placed on regimen 1E. All are first line regimen, which consist of the combination of two drugs in the



nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs) class of antiretroviral drugs in combination with a drug in the non-nucleoside reverse transcriptase inhibitors (NNRTIs) class of antiretroviral drugs. The Nigerian guidelines recommended preferred first line regimen is a combination of zidovudine (ZDV) or tenofovir (TDF) plus

lamivudine (3TC) or emtricitabine (FTC) plus efavirenz (EFV) or nevirapine (NVP). Thus, first line regimen 1A contains ZDV + 3TC + EFV, 1B contains ZDV + 3TC + NVP, 1C contains TDF + FTC + EFV, 1D contains TDF + FTC + NVP, 1E contains TDF + 3TC + EFV while 1F contains TDF + 3TC + NVP.

Table 1: Comparison of Age, Weight and CD4 Count Results for the Three Groups

Group	Age (years)	Weight (kg)	CD4 count (cells/ μ L)
HAART group	39.17 \pm 10.08	57.83 \pm 13.78	196.40 \pm 109.58
HAART naïve group	37.50 \pm 7.52	56.27 \pm 6.85	662.61 \pm 158.86
Control group	37.64 \pm 6.50	62.39 \pm 6.38	783.16 \pm 237.07
F-value	0.900	7.642	215.808
p-value	0.408	0.001*	0.001*
Post -Hoc			
a/b	0.683	1.000	0.001*
a/c	0.809	0.017*	0.001*
b/c	1.000	0.001*	0.001*

Key:

a – HAART group *b* – HAART naïve group *c* – Control subject group

* = Results compared are significantly different at p -value \leq 0.05.

Table 2: Comparison of Results of CD4 Count Done Before Drug Commencement and Latest CD4 Count

Group	CD4 count at baseline (cells/ μ L)	Latest CD4 count (cells/ μ L)	F-value	p-value
HAART group	196.40 \pm 109.58	390.04 \pm 232.07	39.852	0.001*
HAART naïve group	662.61 \pm 158.86	698.99 \pm 161.42	1.805	0.181

Key:

* = Results compared are significantly different at p -value \leq 0.05.

Table 3: Comparison of CRR, AIP & AC Results for The Three Groups

Group	CRR	AIP	AC
HAART group	2.89 ± 0.99	0.09 ± 0.11	1.88 ± 1.01
HAART naïve group	2.22 ± 0.53	0.02 ± 0.04	1.19 ± 0.47
Control group	2.38 ± 0.35	0.03 ± 0.04	1.38 ± 0.35
F-value	18.296	21.112	19.656
p-value	0.001*	0.001*	0.001*
Post -Hoc			
a/b	0.001*	0.001*	0.001*
a/c	0.001*	0.001*	0.001*
b/c	0.552	1.000	0.308

Key:

a – HAART group *b* – HAART naïve group *c* – Control subject group

* = Results compared are significantly different at *p*-value ≤ 0.05.

Table 4: Comparison of SBP and FRS Results for The Three Groups

Group	SBP (mmHg)	FRS (%)
HAART group	120.23 ± 8.42	1.00 ± 0.07
HAART naïve group	119.73 ± 7.51	0.16 ± 0.27
Control group	118.90 ± 5.21	0.34 ± 0.62
F-value	0.626	8.613
P-value	0.536	0.001*
Post- Hoc		
a/b	1.000	0.001*
a/c	0.808	0.007*
b/c	1.000	1.000

Key:

a – HAART group *b* – HAART naïve group *c* – Control subject group

* = Results compared are significantly different at *p*-value ≤ 0.05.

Discussion

The outcome of this study shows a significant increase in the weight of controls as compared with that of the other two groups. This finding is consistent with the outcome of a 2013 study of antiretroviral treatment naïve HIV-infected patients in Jos, Nigeria, which reported that the HIV-infected patients had a significantly lower BMI (Danilyam and Iroezindu, 2013). It is however in contrast to the outcome of other researches that reported there was no statistically significant difference in BMI (Zhou *et al.*, 2015). Also, the mean CD4 count of the controls is significantly increased as compared with that of the other two groups. This is in

agreement with a 2010 study that reported lowered mean CD4 counts were seen in HIV positive individuals (Lucien *et al.*, 2010).

The outcome of this study also showed that there is a significant increase in the Framingham Risk Score (FRS) and atherogenic indices, that is, the cardiac risk ratio (CRR), atherogenic index of plasma (AIP) and atherogenic coefficient (AC) in the HAART group as compared with the HAART naïve group and control subject group. The atherogenic indices outcome indicate that participants in all three groups have low risk of cardiovascular diseases or coronary heart disease since their average AIP is < 0.11, CRR



is < 3.8 and AC is < 2.40 but the HAART group had about 10 – 20% intermediate to high risk cardiovascular diseases subjects based on the classification using atherogenic indices reference ranges. This observation could be due to the effect of certain components of their antiretroviral regimen (Martirosyan *et al.*, 2007; Brehm *et al.*, 2004; Dobiasova, 2004 and Dobiasova and Frolich, 2001). Our finding is at variance with previous report which indicated an abnormal AIP in HAART-naïve HIV patients (Onyedum *et al.*, 2014). However, our finding is in agreement with a previous report which indicated a significant increase in LDL/HDL-C ratio (Atherogenic Index) of HAART naïve group and HAART group for 1 - 6 months compared to control group (Abduljalil *et al.*, 2015) and significantly higher lipids and lipoproteins in patients on antiretroviral compared to other groups (Agboola *et al.*, 2016; Zhou *et al.*, 2015; Agete *et al.*, 2012). The Framingham Risk Score (FRS) generally is used as a risk assessment tool for estimating the ten-year risk of an individual having heart attack or coronary heart disease. This study has indicated a low coronary heart disease (CHD) risk due to the fact that a vast majority of the subjects in all groups including the HAART group had 10% or less FRS, which is an indication for less CHD risk at ten years (D'Agostino *et al.*, 2008). This could be as a result of the fact that subjects in all groups were non-smokers, occasional or non-alcohol consumers and not on anti-hypertensive medications that often influence the risk score.

Conclusion

HIV in itself may have an effect on the metabolism of lipids and lipoproteins, with ultimate effect on the atherogenic indices and risk score. This is probably worsened by antiretroviral therapy as the increased levels of these indices were mainly seen in the HAART group, constituting a major risk for cardiovascular diseases. Thus, it is recommended that these indices and risk score be done periodically after antiretroviral therapy is initiated, as this could serve a good index for disease monitoring and/or progression.

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