



Lipids, lipoproteins & cd4 count of HIV-infected patients on anti-retroviral treatment

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ABSTRACT

Background: 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 has a prevalence of 4.3%. HIV is a retrovirus that primarily infects components of the human immune system such as CD4 T-cells, macrophages and dendritic cells. This study is therefore designed to evaluate the CD4+ T cell count & lipid profile of adult HIV seropositive subjects on Highly Active Antiretroviral Therapy (HAART) and those yet to be started on HAART as well as HIV seronegative control and determine the effect of antiretroviral therapy on the parameters. **Methodology:** Serum levels of CD4+ count were determined using flow cytometry while serum triglycerides, total cholesterol & HDL cholesterol were determined using enzymatic spectrophotometric endpoint method. All data were expressed as Mean \pm Standard Deviation (SD) and analysed with Analysis of Variance (ANOVA) while multiple comparisons were done using Post Hoc test. **Results:** The mean CD4 counts of the two groups are significantly decreased as compared with control. The mean serum TG, TC and Very Low Density Lipoprotein Cholesterol (VLDLC) is significantly decreased in the HAART naïve group as compared with controls. The mean serum HDL Cholesterol is significantly decreased in the HAART group as compared with the control group, with reduction in the mean LDL cholesterol of subjects in the HAART naïve group as compared with the HAART group. **Conclusion:** HIV infection itself may have an effect on the metabolism of lipids and probably worsened by antiretroviral therapy as dyslipidaemia was observed mainly in the group of subjects on HAART, which constitute a major risk for cardiovascular diseases.

KEY WORDS: HIV/AIDS; CD4 count; HAART; Lipid

INTRODUCTION

HIV/AIDS is an epidemic in sub-Saharan Africa, especially Nigeria. According to the 2012 National Reproductive Health Survey Plus report [1], Nigeria has a HIV/AIDS prevalence rate of 3.4% while Ondo State has 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). HIV, a retrovirus, directly or indirectly destroys CD4 T-cells. It primarily infects CD4 T-cells, macrophages and dendritic cells, which are components of the human immune system [2, 3, 4]. During the asymptomatic state, there are no major symptoms, although there may be few swollen glands. But, during the symptomatic stage, there are emergence of opportunistic infections and cancers such as pneumonia, Kaposi's sarcoma [5]. This study is therefore designed to evaluate the CD4+ T cell count, triglyceride (TG), total cholesterol (TC), very low density lipoprotein cholesterol (VLDLC), high density lipoprotein cholesterol (HDL) and low density lipoprotein cholesterol (LDL) in adult HIV seropositive patients on Highly Active Antiretroviral Therapy (HAART) and those yet to be started on HAART

as well as HIV seronegative control subjects. Also, the study is to determine the effect of antiretroviral therapy on the various parameters and compare the results.

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 total study size comprised of 210 subjects. The subjects were divided into three groups of 70 each: Group 1 (HAART group) included HIV-seropositive individuals who were already on Highly Active Antiretroviral Therapy (HAART) for at least 12 months. Group 2 (HAART naïve group) included HIV-seropositive individuals yet to be started on HAART. Group 3 (Control subject group) consisted of HIV-seronegative control individuals. Participation was voluntary. An 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 & authorities of the State Specialist Hospital Akure, as well as, the implementing partner of the HIV/AIDS care, treatment and control in the State, Equitable Health Access Initiative (EHAI), Akure & Lagos, Nigeria. All the participants were adults more than 18 years of age and included both males and females.

Sampling Technique / Method Of Recruitment Of Subjects

Patients positive to HIV were selected into the HAART and HAART-naïve groups while HIV negative subjects were selected into the control subject group. They were age and sex matched. CD4 count was done six months apart.

Inclusion And Exclusion Criteria

Inclusion criteria for the subjects were: HIV seropositive adult patients on HAART (first line regimen), HIV seropositive adult patients not on HAART, HIV seronegative adult control subjects with no disease and physically healthy, Adults more than 18 years (male and female individuals), non-smokers, occasional or non-alcohol consumers.

Subjects physically unhealthy (males/females), regular alcohol drinkers, smokers, subjects on any other regimen apart from first line, those less than 18 years old & those on drugs especially that will interfere with parameters to be studied such as lipid-modifying medications including statins, resins, etc were all excluded.

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, that is, Nigerians currently living with HIV/AIDS is about 3.4% while Ondo State has a prevalence of 4.3% [1]. The formula for sample size is: $n = Z^2PQ/d^2$ [6].

$$n = Z^2PQ/d^2$$

Where:

n = minimum sample size, d = degree of precision (taken as 0.05),

Z = standard normal deviation at 95% confidence interval which is 1.96,

P = proportion of the target population (estimated at 4.3% which is $4.3/100 = 0.043$),

Q = alternate proportion (1-P) which is $1-0.043 = 0.957$

$$n = \frac{(1.96)^2 (0.043)(0.957)}{(0.05)^2} = 63$$

Sample Collection, Storage And Analysis

Venous blood was collected aseptically after an overnight fast through a clean vacutainer system venepuncture from each subject into plain vacutainer bottle for retroviral test re-screening, lipids & lipoproteins analysis and in an Ethylene Diamine Tetra Acetic (EDTA) containing tube for CD4+ lymphocytes count. Retroviral HIV-1/2 antigen/antibody test re-screening was done promptly to confirm the status of the subjects via rapid testing using the serial testing algorithm. Blood samples were centrifuged at 4000 Revolution per Minute (RPM) for 10 minutes and the serum of each sample was extracted into fresh plain bottle for immediate analysis while those not analysed immediately were stored at - 20 degree celsius until analysis few days later. The CD4+ lymphocytes count was carried out using a flow cytometry technique through a cyflow counter (Partec GmbH Görlitz Germany). Serum total cholesterol (TC), triglyceride (TG) and High Density Lipoprotein Cholesterol (HDL) were estimated by enzymatic spectrophotometric endpoint method using reagent kits procured from Randox Laboratories Limited, United Kingdom. Very Low Density Lipoprotein Cholesterol (VLDL) and Low Density Lipoprotein Cholesterol (LDL) were estimated by calculation. LDL was estimated by Friedwald's equation calculation.

Calculation

$$VLDL \text{ (mmol/L)} = \frac{\text{Triglyceride}}{2.2}$$

$$LDL \text{ (mmol/L)} =$$

$$\frac{[\text{Total cholesterol} - \text{Triglyceride} - \text{HDL}]}{2.2}$$

Statistical Analysis

Data was statistically analysed using Statistical Package for the Social Sciences (SPSS) for windows version 20.0 software (SPSS Inc., Chicago, IL, USA). All data were expressed as Mean ± 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 < 0.05 and highly significant if P < 0.01. Pearson's correlation coefficient was used for correlational analysis of the test.

RESULTS

Demographic Data, Physical & 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 subject group) had 51 females and 19 males.

Group 1 & 2 subjects all tested positive to retroviral test, while all subjects in group 3 tested negative. The average duration (in months) of Highly Active Antiretroviral Therapy (HAART) in the group 1 subjects is 25.63 ± 19.99 while the average duration (in months) of cotrimoxazole use for subjects in group 2 is 7.10 ± 4.89 . 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.

DISCUSSION

The outcome of this study shows a significant mean increased weight in the control subjects as compared with that of the other two groups. This is similar to the outcome of a 2010 study on lipid profile in HIV/AIDS patients in Abuja, Nigeria, which reported that the mean Body Mass Index (BMI) was significantly higher among the control group compared with the test group [7]. It is also similar to that of a research on the lipid profile of antiretroviral treatment naive HIV-infected patients in Jos, Nigeria, which reported that the HIV-infected patients had a significantly lower BMI [8]. It is however in contrast to the outcome of other researches that reported there was no statistically significant difference in BMI [9, 10]. Also,

Table 1. Comparison of age, weight & CD4 count results for the three groups

Groups	Parameters		
	Age (years)	Weight (kg)	CD4 count (cells/ μ L)
HAART group	39.17 ± 10.08	57.83 ± 13.78	196.40 ± 109.58
HAART naïve group	37.50 ± 7.52	56.27 ± 6.85	662.61 ± 158.86
Control subject group	37.64 ± 6.50	62.39 ± 6.38	783.16 ± 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 < 0.05 (P < 0.05)

Table 2. Comparison of results of cd4 count done before drug commencement & latest cd4 count

Groups	Parameters			
	CD4 count @ start (cells/ μ L)	Latest CD4 count (cells/ μ L)	F-value	P-value
HAART group	196.40 ± 109.58	390.04 ± 232.07	39.852	0.001*
HAART naïve group	662.61 ± 158.86	698.99 ± 161.42	1.805	0.181
KEY:				

* = Results compared are significantly different at P-value < 0.05 (P < 0.05)

Table 3. Comparison of TG, TC, HDLC, LDLC & VLDLC results for the three groups

Groups	Parameters				
	TG mmol/L mmol/L	TC	HDLC mmol/L	LDLC mmol/L	VLDLC mmol/L
HAART group 1.41	2.46 ± 0.62	5.76 ±	2.06 ± 0.26	2.53 ± 1.28	1.12 ± 0.28
HAART naïve group 0.92	1.13 ± 0.54	4.58 ±	2.08 ± 0.22	1.98 ± 0.80	0.52 ± 0.24
Control subject group 0.54	2.14 ± 0.23	5.08 ±	2.16 ± 0.17	1.94 ± 0.50	0.97 ± 0.10
F-value	139.130	23.637	3.375	9.187	139.562
P-value	0.001*	0.001*	0.036*	0.001*	0.001*
POST HOC					
a/b	0.001*	0.001*	1.000	0.001*	0.001*
a/c	0.001*	0.001*	0.042*	0.001*	0.001*
b/c	0.001*	0.013*	0.167	1.000	0.001*
KEY:	a – HAART group	b – HAART naïve group		c – Control subject group	

* = Results compared are significantly different at P-value < 0.05 (P < 0.05)

the mean CD4 count of the control subjects is significantly increased as compared with that of the other two groups, while that of the HAART naïve group is also significantly increased as compared with those in the HAART group. There was a significant increase in the mean latest CD4 count of the subjects in the HAART group as compared with the mean CD4 count done prior to commencement of the drug. This is on one hand in agreement with a Nigerian study that said the CD4 count of controls was significantly higher than that of both groups of patients while it is in contrast to the part that stated that there was a statistically significant higher CD4 count in patients on ARV therapy than ARV-naïve patients [9]. The outcome showed that the patients' adherence level is high as they remain on the first line regimen.

The increases in the TG, TC & VLDLC in the subjects in HAART group most especially are not within the reference range, possibly pointing to cardiovascular risk. The increase in TG might be attributed to decreases in the activities of lipoprotein lipase (LPL), which is a key enzyme for the breakdown of triglyceride-rich lipoproteins. Increase in HDL, mostly observed in the control group, is often associated with a significant decrease in mortality from coronary heart disease. The decrease in LPL activity suggests that reduced lipolysis of triglyceride-rich lipoproteins may be an initial step in lower serum levels of HDLC. The HDLC helps in transporting cholesterol from peripheral tissues to the liver for degradation. The outcome of this study is slightly similar to that of other studies, which reported that significant difference was obtained in the serum High Density Lipoprotein (HDL) of the HIV positive subjects as compared with the controls, while the mean Low density Lipoprotein (LDL) was higher among the HIV positive subjects compared with control,

concluding that abnormality of serum lipids is common among treatment naïve HIV patients [7]. Also, significantly higher serum triglycerides but lower serum HDL was reported in the HIV-infected patients as compared with the control subjects [8]. Also, a high prevalence of dyslipidaemia was reported in HIV-patients receiving first-line ART [13]. It was also reported that there were significantly higher lipids & lipoproteins in HAART group when compared to pre-HAART group [12]. The outcome is in deviation from that of certain other studies that reported there were no significant differences observed in lipid profiles in all groups including patients on first line regimen antiretroviral drugs, second line regimen antiretroviral drugs, positive control and negative control [11]. All the subjects in the study were on first line antiretroviral drugs, mostly zidovudine-based or tenofovir-based and these drugs possibly contribute to the dyslipidaemia. This is similar to the report that the use of first-line antiretroviral therapy regimens that contain efavirenz and nevirapine were associated with raised total cholesterol, LDL cholesterol and triglycerides [10, 12]. The decrease in LDLC and increase in HDLC confirms the inverse correlation that was observed between HDLC and LDLC.

CONCLUSION

In conclusion, HIV infection itself may have an effect on the metabolism of lipids and probably worsened by antiretroviral therapy as dyslipidaemia was observed mainly in the group of subjects on highly active antiretroviral therapy (HAART), which constitute a major risk for cardiovascular diseases. It is thus very essential to measure fasting lipid profile before and periodically after antiretroviral therapy is initiated and/or when HAART regimen is switched, as this will serve a good index for disease progression and/or monitoring.

REFERENCES

1. National HIV/AIDS and Reproductive Health Survey Plus (NARHS-Plus). Current status of HIV and other sexual and reproductive health situations in Nigeria and the expected interventions for the community leaders. 2012.
2. Alexander K, Mirjam K, Klaus K. Modern infectious disease epidemiology concepts, methods, mathematical models, and public health. New York: Springer, 2010; p. 88. ISBN 9780387938356.
3. Wihelm K. Encyclopaedia of public health. New York: Springer, 2008; p. 676 – 677. ISBN 9781402056130.
4. Sepkowitz KA. AIDS – The first 20 years. *N Eng J Med*, 2012; 344 (23): 1764 – 72.
5. World Health Organisation (WHO). WHO case definitions of HIV for surveillance and revised clinical staging and immunological classification of HIV-related disease in adults and children. 2006.
6. Daniel WW. Biostatistics: A Foundation for Analysis in the Health Sciences, 10th ed. 2013.
7. Adewole OO, Eze S, Betiku YE, Anteyi E, Wada I, Ajuwon Z, Erhabor G. Lipid profile in HIV/AIDS patients in Nigeria. *Afr Health Sci*. 2010 Jun; 10(2): 144–149.
8. Daniyam CA & Iroezindu. MO. Lipid Profile of Anti-Retroviral Treatment-Naïve HIV-Infected Patients in Jos, Nigeria. *Ann Med Health Sci Res*. 2013; 3(1): 26 – 30.
9. Yusuf R, Sambo AI, Mohammed MH, Abdulaziz H. Lipid profile of HIV/AIDS patients attending antiretroviral clinic in Zaria, North-Western Nigeria. *Sub-Saharan Afr J Med* 2014; 1: 31 – 35.
10. Zhou DT, Kodogo V, Chokuona KF, Gomo E, Oektedalen O, Stray-Pedersen B. Dyslipidemia and cardiovascular disease risk profiles of patients attending an HIV treatment clinic in Harare, Zimbabwe. *Research & Palliative Care*. 2015; 7: 145 – 155.
11. Ebuehi OAT, Awolola A, Akanmu AS. Changes in Serum Cortisol, Thyroid Hormones and Lipid Profiles in Nigerian Men and Women on 1st and 2nd Line Antiretroviral Therapy for 52 Weeks. *International Journal of Virology and Molecular Biology* 2015; 4 (1): 12-18.
12. Agete T, Zalalem A, Henock, Sandip B. Prevalence of dyslipidaemia among HIV-infected patients using first-line highly active antiretroviral therapy in Southern Ethiopia: a cross-sectional comparative group study. *AIDS Research and Therapy* 2012, 9:31.
13. Bekolo CE, Nguena MB, Ewane L, Sylvestre B & Kollo B. The lipid profile of HIV-infected patients receiving antiretroviral therapy in a rural Cameroonian population. *BMC Public Health* 2014, 14:236.

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