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Original Research

Cluster of differentiation & renal assessment of reactive HIV subjects in Akure Nigeria

Saheed Opeyemi Usman[1], Ganiyu Babatunde Agboola[2], Patrick Olanrewaju Osho[3], Olusola John Fatunmbi[4], Nafisat Oladayo Akintayo-Usman[5]

[1]Department of Clinical Laboratory, Equitable Health Access Initiative, Akure, Nigeria.
[2]Department of Clinical Laboratory, Equitable Health Access Initiative, Lagos, Nigeria.
[3]Department of Haematology, Virology, State Specialist Hospital, Akure, Nigeria.
[4]Department of Laboratory Services, Union Diagnostics, Osogbo, Nigeria.
[5]Department of Medical-Surgical Nursing, Osun State School of Nursing, Osogbo, Nigeria.

Address for correspondence:
Saheed Opeyemi Usman,
Department of Clinical Laboratory,
Equitable Health Access Initiative,
Akure, Nigeria.
senatorhopsy@yahoo.com

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ABSTRACT

Background: HIV/AIDS is a rapidly growing epidemic in sub-Saharan Africa, Nigeria in particular. The report of the 2012 National Reproductive Health Survey 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%. HIV is a retrovirus that primarily infects components of the human immune system such as CD4 T-cells, macrophages and dendritic cells. **Objectives:** To evaluate the CD4+ T cell count, sodium, potassium, urea and creatinine in adult HIV seropositive subjects on Highly Active Antiretroviral Therapy (HAART) and those yet to be started on HAART as well as HIV seronegative control subjects. **Materials and Methods:** Serum levels of CD4+ count of adult HIV seropositive subjects on HAART, yet to start HAART and seronegative controls were determined using flow cytometry while their serum sodium, potassium, urea and creatinine were determined using enzymatic spectrophotometric method. All data were expressed as Mean ± Standard Deviation (SD) and analysed with Analysis of Variance (ANOVA) while multiple comparisons were done using Post Hoc test. **Results:** The serum sodium is significantly lower in the subjects in HAART group as compared with that of the other two groups. No significant difference in urea & creatinine was found in the three groups. Conclusion: Despite the fact that it appears the drugs did not have adverse effect on the kidneys, there is need for close monitoring of patients on antiretroviral medications as various drugs over time have adverse effects on various organs.

KEY WORDS: HIV/AIDS; HAART; Cluster of differentiation; Renal

INTRODUCTION

HIV/AIDS is a rapidly growing epidemic in sub-Saharan Africa, Nigeria in particular. The report of the 2012 National Reproductive Health Survey 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%. 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 is a retrovirus that primarily infects components of the human immune system such as CD4 T-cells, macrophages and dendritic cells. It directly and indirectly destroys CD4 T-cells [1 - 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, sodium, potassium, urea and creatinine 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 compare the results of the adult HIV seropositive patients both on HAART and not on HAART with adult seronegative 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, Ondo State capital city, Nigeria. The hospital is a secondary health care facility and 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.

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 were selected until the required number of willing participants is reached for each of the three groups.

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, as well as, those on drugs especially that will interfere with the parameters to be studied such as lipid-modifying medications including statins, nicotinic acid, resins, fibrates, among others, 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, electrolytes, urea & creatinine 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 sodium, potassium, urea and creatinine were estimated by enzymatic spectrophotometric method using reagent kits procured from Randox Laboratories Limited, United Kingdom.

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

Table 1. Comparison of age, weight & cd4 count results for the three groups

Groups	Parameters		
	Age (years)	Weight (kg)	CD4 count (cells/μL)
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/μL)	Latest CD4 count (cells/μL)	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 sodium (na), potassium (k), urea & creatinine results for the three groups

Groups	Parameters			
	Na (mmol/L)	K (mmol/L)	Urea (mmol/L)	Creatinine (μmol/L)
HAART group	136.44 ± 3.10	4.12 ± 0.42	4.23 ± 1.23	75.09 ± 13.08
HAART naïve group	139.53 ± 3.11	4.15 ± 0.52	4.06 ± 1.14	76.11 ± 16.00
Control subject group	139.67 ± 3.70	3.93 ± 0.43	4.04 ± 0.79	75.79 ± 13.62
F-value	21.184	4.775	0.659	0.092
P-value	0.001*	0.009*	0.519	0.912
POST HOC				
a/b	0.001*	1.000	1.000	1.000
a/c	0.001*	0.051	0.870	1.000
b/c	1.000	0.013*	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 (P < 0.05)

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. There was a positive correlation between urea and creatinine in all groups.

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 2013 study of antiretroviral treatment naive HIV-infected patients in Jos, Nigeria, which reported that the HIV-infected patients had a significantly lower BMI [7]. It is however in contrast to the outcome of other researches that reported there was no statistically significant difference in BMI [7]. Also, the mean CD4 count of the control subjects 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 [8], but differ from the 2014 research that revealed mean CD4 count of the control subjects was higher than that of HIV-infected subjects but was not significant [11].

The outcome of this study shows that serum sodium, though, within normal levels, is significantly lower in the subjects in HAART group as compared with that of the other two groups. The outcome of this study is similar to that of other studies which reported hyponatremia in patients on HAART [9, 10]. Also, slightly similar to the study that reported sodium levels are higher in the control subjects compared with the HIV positive subjects [11]. Serum potassium of the control subjects is significantly decreased compared with the HAART naïve group, while there was no significant difference in the serum potassium between the HAART naïve group and HAART group, as they all fall within the normal reference range. The outcome is in deviation from that of a 2008 study that reported hypokalaemia in the patients on HAART [9], as well as the 2014 study that revealed significantly lower potassium in patients on HAART than those of positive not on HAART [10]. Meanwhile, another research work showed potassium level did not differ significantly between HIV positive subjects and control subjects [11]. The serum levels of urea and creatinine are found to be within normal reference range and no significant difference was found in the three groups. The outcome is in agreement with a 2014 study that revealed urea and creatinine levels did not differ significantly between the groups [11]. This is however, in deviation from the report of a 2011 study which showed that serum creatinine was significantly higher in the control group than in the HIV infected subjects [12], as well as, another study that revealed mild increase in creatinine concentration in the patients on HAART [9]. Also, it differs from the outcome of another study which reported that the level of creatinine was reported to be significantly higher in patients on HAART than those of positive not on HAART or negative [10]. The electrolytes, urea and creatinine values were not suggestive of any renal disease indicating that the renal function is probably unaffected. This could be a result of the fact that majority of the seropositive subjects were placed on zidovudine-based regimens which are not usually linked with renal abnormalities. The implication is that the adverse effects of the drugs that form the majority part of the regimen have little or no effect on the kidneys. The insignificant difference in urea and creatinine of all groups is evident in the positive correlation between the two parameters.

CONCLUSION

In conclusion, despite the fact that it appears the drugs, which are largely zidovudine-based, did not have adverse effect on the kidneys, there is need for continuous and close monitoring of patients on antiretroviral medications as the various drugs over time have adverse effects on various organs.

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