



## Patterns of CD4+ T-lymphocytes and liver enzymes in adult HIV seropositive cases

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### ABSTRACT

**Objectives:** HIV/AIDS is a rapidly growing epidemic in sub-Saharan Africa. 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 and liver enzymes of adult HIV seropositive subjects receiving Highly Active Antiretroviral Therapy (HAART) and those yet to be started on HAART as well as HIV seronegative control subjects. **Methods:** Serum levels of CD4+ counts of subjects were determined using flow cytometry while their serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were determined using enzymatic spectrophotometric method. **Results:** Serum ALT was found to be significantly increased in the HAART group as compared with those of the two other groups. In addition, serum ALP was significantly increased in the HAART group as compared with the control subjects, while serum AST was higher in the HAART group as compared with the HAART naive group. **Conclusion:** HAART is associated with hepatotoxicity, thus, it is recommended that liver enzymes should be measured before and periodically after antiretroviral therapy is initiated and/or when HAART regimen is switched, as this will serve a good index for disease monitoring and/or progression.

**KEY WORDS:** AIDS, CD4, HAART, HIV, liver

### INTRODUCTION

'Human immunodeficiency virus infection' and 'acquired immune deficiency syndrome' (HIV/AIDS) are a rapidly growing epidemic in sub-Saharan Africa, in particular Nigeria. The report of the 2012 National Reproductive Health Survey Plus [1] 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; it directly and indirectly destroys CD4 T-cells [2-4].

The authors of an earlier study, conducted in Orlu, Nigeria, reported increased levels of liver enzymes alanine and aspartate aminotransferase (ALT and AST) and lowered mean CD4 counts among HIV positive individuals [5]. Another research on liver function of HIV positive patients receiving antiretroviral therapy (ART) revealed increase in the activities of ALT, AST and alkaline phosphatase (ALP) of HIV positive patients [6]. In Cameroon, increases in blood transaminases levels after highly active ART (HAART) initiation was reported with a conclusion that HAART is associated with low level hepatotoxicity [7]. Another

study on HIV/AIDS patients treated with antiretroviral drugs (ARV) showed significant increases in AST and ALT levels of HIV positive non-treated group compared to HIV negative control group; also, AST and ALT levels of HIV positive ARV-treated patients were significantly higher in comparison to HIV positive non-treated group. Meanwhile, the ALP activity was significantly lower in HIV positive treated compared non-treated group. Infection by HIV increases the activities of the three enzymes [8]. A Ghanaian study on HIV patients revealed that CD4 lymphocyte count of HIV-ART participants was higher than that of HIV-controls while ALP, ALT and AST values did not differ between those groups [9]. A Turkish research on hepatic adverse events during HAART containing nevirapine revealed elevated liver enzymes and concluded that close monitoring of liver enzymes during the first 12 weeks of nevirapine therapy is critical to prevent life threatening events [10]. A study conducted in Zimbabwe on cardiovascular disease risk profiles of patients attending an HIV treatment clinic in Harare revealed that there was no significant difference in mean body mass index (BMI) between the ART-naive and ART-experienced participants [11]. Another research on ART-naive HIV-infected patients

in Jos, Nigeria, reported that the mean BMI in both HIV-infected and negative control subjects were within normal limits [12].

This study is therefore designed to evaluate the CD4+ T cell count, ALT, AST and ALP in adult HIV seropositive patients undergoing HAART and those yet to be started on HAART as well as HIV seronegative control subjects. Also, the study is designed to compare the results of the adult HIV seropositive patients both receiving HAART and not receiving HAART with adult seronegative controls.

## MATERIALS AND METHODS

### Study design

This 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 center in Ondo State. The total study size comprised of 210 subjects. The subjects were divided into three groups of 70 each: (1) the HAART group included HIV-seropositive individuals who were already receiving HAART for at least 12 months; (2) the HAART naive group included HIV-seropositive individuals yet to be started on HAART; and (3) the control group consisted of HIV-seronegative control subjects. The study was conducted for a period of five months between May and September 2015. 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 and 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, inclusion and exclusion criteria

Random sampling was used to 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 criteria for the subjects were: HIV seropositive adult patients on HAART (first line regimen), HIV seropositive adult patients not on HAART, HIV seronegative adult physically healthy control subjects; and, adults more than 18 years (male or female), non-smokers, occasional or non-alcohol consumers. Physically unhealthy subjects, 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 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:

$$n = Z^2PQ/d^2 \text{ [13]}$$

$n$  = 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), and  $Q$  = alternate proportion (1-P) which is 1-0.043 = 0.957. So, the final calculation was:

$$n = (1.96)^2 \times (0.043) \times (0.957) / (0.05)^2 = 63$$

### Sample storage and analysis

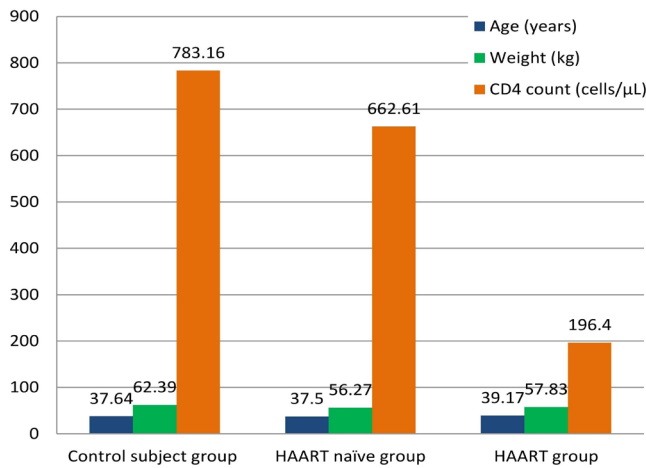
Venous blood was collected aseptically after an overnight fast through a clean vacutainer system. Venipuncture from each subject into plain vacutainer bottle for retroviral test re-screening, lipids and lipoproteins analysis and in an ethylenediaminetetraacetic acid (EDTA) containing tube for CD4+ lymphocytes 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 rpm for 10 min and the serum of each sample was extracted into fresh plain bottle for immediate analysis while those not analyzed immediately were stored at  $-20^{\circ}\text{C}$  until analysis few days later. The CD4+ lymphocytes count was carried out using a flow cytometry technique through a cyflow counter (Partec GmbH; Gorlitz, Germany). ALT, AST and ALP were estimated by enzymatic spectrophotometric method using reagent kits procured from Randox Laboratories Limited, United Kingdom.

### Statistical analysis

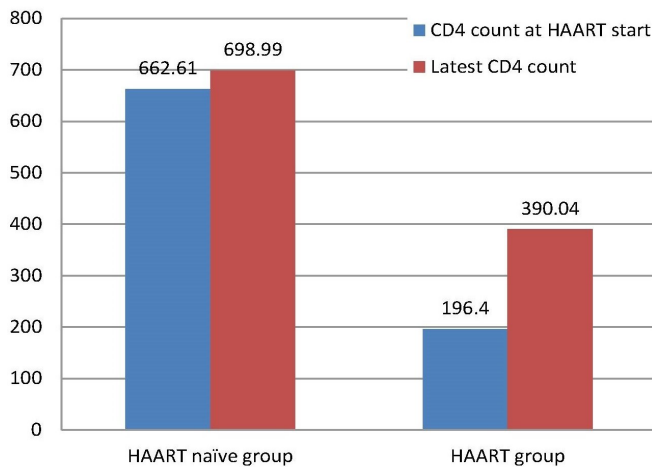
Data was statistically analysed using Statistical Package for the Social Sciences (SPSS) version 20.0 software (SPSS Inc; Chicago, IL, USA). All data were expressed as mean  $\pm$  standard deviation (SD). Statistical analysis of the data was performed using t-test and analysis of variance (ANOVA) while multiple comparisons was done using post hoc Bonferroni test. Comparison of results of CD4 count done before drug commencement and latest CD4 count was done using t-test. Significance was fixed at  $P < 0.05$  and highly significant if  $P < 0.01$ .

## RESULTS

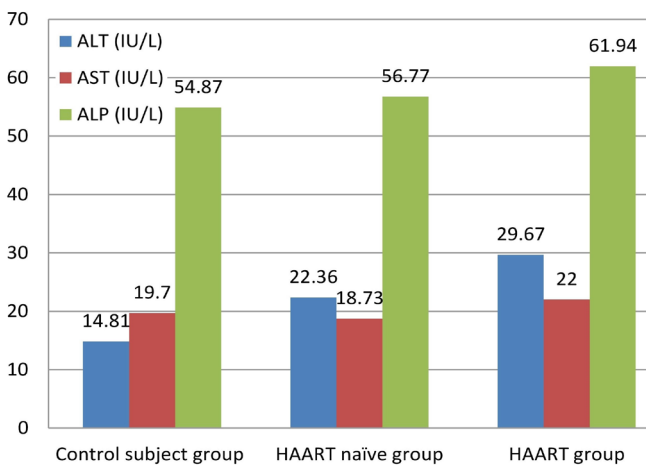
Figures and Tables 1-3 summarize the main outcome of the study. A total of 210 subjects participated in the study. The HAART group consisted of 51 females and 19 males, the HAART naive group contained 49 females and 21 males, and the control group had 51 females and 19 males. The HAART and HAART naive group subjects all tested positive to retroviral test, while all subjects of the control group tested negative. The average duration of HAART in the HAART group subjects was  $25.63 \pm 19.99$  months, while the average duration of cotrimoxazole use for subjects in the HAART naive group 2 was  $7.10 \pm 4.89$  months.



**Figure 1.** Comparison of age, weight and CD4 counts of the study groups



**Figure 2.** Comparison of CD4 count done before and after (latest) drug commencement



**Figure 3.** Comparison of ALT, AST and ALP activities

In the HAART group, HAART regimen taken at the initiation or commencement of therapy shows that 4 subjects (5.7%) 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 subjects (47.1%) 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 (NRTI) class of antiretroviral drugs in combination with a drug in the non-nucleoside reverse transcriptase inhibitors (NNRTI) class.

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 increased weight in the control subjects as compared with that of the other two groups. This is similar to the outcome of a previous study of antiretroviral treatment on naïve HIV-infected patients in Jos, Nigeria, which reported that the HIV-infected patients had a significantly lower BMI [13]. It is, however, in contrast to the outcome of other researches that reported there was no statistically significant difference in BMI [12]. 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 former study that reported lowered mean CD4 counts in HIV positive individuals [6].

The outcome of the present study is slightly similar to that of other studies, which reported significantly raised levels of liver enzymes (ALT and AST) in HIV positive individuals [6]. Also, significantly higher activities of the liver enzymes (AST, ALT & ALP) of HIV positive patients compared with controls were reported [7], as well as increases in blood transaminases levels after HAART initiation in another research [8] and elevated liver enzymes in patients on NVP treatment [11]. It is also related to another studies outcome that reported significant increases in AST and ALT levels of HIV-positive non-treated group individuals compared to HIV-negative control group, with AST and ALT levels of HIV-positive patients treated with ARVs significantly higher in comparison to HIV-positive non-treated group [9]. The outcome is in deviation from that of certain other studies that reported the ALP activity significantly lower in HIV-positive treated group compared to non-treated group; also, mean values of ALP, ALT and AST did not differ between the HIV-positive subjects and controls [10].

**Table 1.** Comparison of age, weight and CD4 count of study groups

Groups and comparisons	Age (years)	Weight (kg)	CD4 count (cells/μl)
HAART	39.17 ± 10.08	57.83 ± 13.78	196.4 ± 109.58
HAART naive	37.5 ± 7.52	56.27 ± 6.85	662.61 ± 158.86
Control	37.64 ± 6.5	62.39 ± 6.38	783.16 ± 237.07
F-value	0.9	7.642	215.808
P-value	0.408	0.001*	0.001*
HAART vs HAART naive	0.683	1	0.001*
HAART vs control	0.809	0.017*	0.001*
HAART naive vs control	1	0.001*	0.001*

\*Results compared are significantly different

**Table 2.** Comparison of CD4 count done before and after (latest) drug commencement

Groups	CD4 count at start (cells/μl)	Latest CD4 count (cells/μl)	T-value	P-value
HAART	196.4 ± 109.58	390.04 ± 232.07	39.852	0.001*
HAART naive	662.61 ± 158.86	698.99 ± 161.42	1.805	0.181

\*Significantly different

**Table 3.** Comparison of ALT, AST and ALP activities for the study groups

Groups and comparisons	ALT (IU/l)	AST (IU/l)	ALP (IU/l)
HAART	29.67 ± 17.05	22 ± 7.26	61.94 ± 20.61
HAART naive	23.36 ± 9.56	18.73 ± 5.63	56.27 ± 14.59
Control	14.81 ± 7.42	19.7 ± 6.51	54.87 ± 12.61
F-value	26.511	4.675	3.58
P-value	0.001*	0.01*	0.03*
HAART vs HAART naive	0.001*	1	1
HAART vs control	0.001*	0.113	0.031*
HAART naive vs control	0.001*	0.01*	0.18

\*Results compared are significantly different

The increases in ALT, AST and ALP in the subjects of the HAART group most especially though still within the reference range, could be that HIV alters the liver enzymes by direct or indirect mechanism or due to hepatotoxicity, a major side effect or risk factor of most antiretroviral drugs especially EFV and NVP, with liver cell injury or damage leading to slight leakage of these enzymes.

In conclusion, HAART is associated with hepatotoxicity, particularly those containing EFV and NVP; thus, it is recommended that liver function tests should be done before and periodically after antiretroviral therapy is initiated and/or when HAART regimen is switched, as this will serve a good index for disease monitoring and/or progression.

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**Conflict of Interest:** None declared