Use of plasma albumin, hepatic lipase and lipoprotein lipase enzyme as predictive markers of treatment failure in HIV-1 infected individuals in federal medical center, Lokoja, Nigeria

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ABSTRACT

This was a cross-sectional study aimed to evaluate the use of albumin, hepatic lipase (HL) and lipoprotein lipase (LPL) enzyme as predictive markers of treatment failure in HIV-1 infected individuals. 154 participants (40 (group A), 35 (group B) on antiretroviral drugs (Test group) and 79 (group C) HIV naive participants (Control group)) aged 18 and 65 years were randomly recruited. Blood sample was collected from each test participant 6 months apart and once from control for determination of Albumin, HL, LPL, viral load (VL), CD4+ cells count. VL was significantly decreased while, Albumin, HL and LPL activities were significantly higher in test participants when compared with control (P < 0.05 respectively). Biochemical markers in test participants at 6 months of therapy were significantly lower compared with 12 months of therapy (P < 0.05). Albumin and VL correlated positively with CD4 count while, lamivudine, nevirapine, tenofovir, HL, LPL correlated strongly and negatively with VL (P < 0.05 respectively). The high sensitivities and positive predictive value of albumin showed their predictive superiority over CD4 count, HL, LPL and antiretroviral drug concentrations. The study thus, concludes that hypoalbuminemia with decreased HL and LPL activities were associated with unsuppressed viral load above 1000 copies/ml. This suggests that albumin; HL and LPL are good biochemical markers for prediction of treatment failure or success in participants on antiretroviral drugs.

INTRODUCTION

Human immunodeficiency virus infection has the ability to progressively shut down the host immune system due to the exponential growth of the virus in some infected individuals (Ifeanyichukwu et al., 2011). This may result in the manifestation of acquired immune deficiency syndrome (AIDS) if HIV progression in a host is not check meted (Ifeanyichukwu et al., 2011).

Despite vigorous researches and prophylactic mea
sures on HIV/AIDS, the infection and the disease conditions continue to threaten the lives of millions of people in Africa (Iheanyichukwu et al., 2011). The continued surge of HIV/AIDS in Africa can be attributed to the influence of so many other factors on the pathogenesis of the disease condition. Considering the fact that many infectious agents are endemic in Sub-Saharan Africa, many immune complexes are formed and the presence of circulating immune complexes (CICs) in HIV/AIDS individuals stimulate many immune-pathological conditions ranging from chronic inflammatory responses to organ tissue damage (Chukwudi et al., 2010). Antiretroviral therapy (ART) restores immune function and reduces HIV-related outcomes, but when treatment fails, increased morbidity and compromised quality of life in HIV patients are observed (NACA, 2014). HIV/AIDS has to lead to reduced life expectancy, particularly in developing countries that also doubles with the highest prevalence of the infection (World Health Organization, 2010).

There are different antiretroviral drugs combinations used in the treatment of HIV at first line therapy. These drugs combinations are being removed at intervals in order to get a better combination with good therapeutic effect and minimal toxicological effects. The purpose of the act was to suppress viral load and to improve the immune system (WHO 2014). The principal aim of antiretroviral therapy (ART) is the suppression of circulating plasma virus to undetectable levels, thereby prolonging the life span of infected individuals (Neogi et al., 2013). Expanding access to ART in resource-limited settings, along with close monitoring, is expected to yield successful treatment outcomes (Bryant et al., 2013). However, in resource-limited settings, a therapeutic outcome is evaluated on the basis of CD4+ T-cell count, viral load or clinical findings (Ceulemans et al., 2019), however in high income settings, this is achieved by performing quantitative viral load monitoring every 3 – 6 months (Bryant et al., 2013).

In resource-limited settings, viral load assays are based on the amplification of HIV-RNA that requires infrastructural facilities for molecular diagnosis, expensive equipment and skilled scientist which are often unavailable; an alternative to HIV-RNA load is to measure the viral p24 antigen and HIV-1 protease in an Enzyme Linked Immunosorbent Assay (ELISA) based format; HIV treatment monitoring using ELISA in resource-limited settings is easier than polymerase chain reaction (Balakrishnan et al., 2011).

Presently CD4+ T cells count is used in Nigeria to monitor disease progression and treatment success. However, it has been found to have its setbacks or limitations (Osuji et al., 2018). In the management of HIV-1 infected individuals, hepatic, lipoprotein lipases, plasma albumin, p24 antigen and protease enzyme activities are not commonly used for monitoring treatment success or disease progression, therefore developing alternative biomarkers that may indicate treatment failure or success in Nigerian patients will help in the proper management of HIV/AIDS patients.

MATERIALS AND METHODS

Study Design

This was a longitudinal cross-sectional study that recruited all participants enrolled for ART first-line regimens and those not yet on drugs at the time of the study. For HIV-1 infected participants on HAART, blood samples were collected at 2 different points: at 6 months into HAART and 12 months into HAART, while blood samples were collected at 1 point for HIV-1 infected HAART naive participants who were newly diagnosed as HIV-1 positive in the antiretroviral therapy clinic and served as HIV-1 positive control. The HIV status of the subjects was confirmed using Geenius HIV confirmatory system. The diagnosis of HIV and the criteria for the commencement of ART was based on Nigeria National guidelines for adult HIV and AIDS treatment and care (Idoko and Folayan, 2014).

Study Area

The study was carried out at Anti-Retroviral Therapy (ART) Clinic of Federal Medical Centre Lokoja. Federal Medical Centre Lokoja (FMCL) is located at number 1 Salihu Ibrahim way, Lokoja. FMCL was established on 9th November 1999. The ART Clinic of FMCL provides comprehensive HIV care services for the city of Lokoja, which is located in the Kogi Central Senatorial district in Lokoja Local Government Area of Kogi State as well as some neighbouring states such as Kwara, Benue and Ekiti State. Lokoja is situated at 7.8° North Latitude, 6.74° East Longitude and 55 meters elevation above the sea level. Lokoja is a town in Nigeria, the capital of Kogi State, having about 60,579 inhabitants.

Study Population

The study population consist of 154 HIV-1 infected individuals aged 18-64 years (37 + 0.74), which consist of 75 HIV-1 infected individuals that have been on drug combination (HAART) for a period of 6 – 12 months while the remaining 79 HIV-1 infected individuals have not commenced drug combination.
therapy (HAART naïve). For HIV-1 infected individuals on HAART, blood samples were collected at 2 different points: at exactly 6 months into HAART and exactly 12 months into HAART, while blood samples were collected at 1 point for HIV-1 infected HAART naïve individuals who were newly diagnosed as HIV-1 positive in the antiretroviral therapy clinic served as HIV-1 positive control. The HIV-1 infected subjects on drug combinations were divided into 2 groups based on the type of drug combinations. They were administered,

**Group A**

40 participants placed on a once daily fixed dose combination of tenofovir (300mg), Lamivudine (300mg) and efavirenz (600mg).

**Group B**

35 participants placed on a once daily fixed dose combination of zidovudine (300mg), Lamivudine (150mg) and nevirapine (200mg).

While the participants not yet on antiretroviral drugs that were newly diagnosed as HIV positive in the antiretroviral therapy clinic served as HIV-1 positive control (C).

Group A participants placed on Tenofovir, Lamivudine and efavirenz were those individuals with baseline investigations with hepatic impairment, while group B participants placed on zidovudine, Lamivudine and nevirapine were those individuals with baseline investigations with evidence of renal impairment and psychiatric manifestations and these served as the basis of segregation into group A and B.

**Regrouping of participants based on World Health Organization criteria**

Participants in each group were staged based on World Health Organization (WHO) criteria for HIV staging as follows: WHO clinical stage I (performance scale 1: asymptomatic, normal activity), WHO clinical stage II (performance scale 2: symptomatic, normal activity), WHO clinical stage III (performance scale 3: Bed-ridden <50% of the day during the past one month), WHO clinical stage IV (performance scale 4: bed-ridden >50% of the day during the last one month).

**Inclusion and Exclusion Criteria**

Participants aged between 18 and 64 (37+0.74) years on the two different drugs combination must have taken the drugs for six months before qualifying for participation. Any HIV infected individuals that had co-morbidity were excluded. Also, any HIV infected individuals on contraceptives, smokers and alcoholics were excluded.

**Informed Consent**

Informed consent was obtained from participants for the study following the guidelines of the Ethics Committee of Federal Medical Centre, Lokoja.

**Ethical Clearance**

Approval to carry out the study was obtained from the Ethics Committee of Federal Medical Centre, Lokoja, with reference number FMCL/MED/115/II/271.

**Specimen Collection**

Eight milliliters of venous blood was collected from each subject at the point of joining the research and six months after joining the research. 4ml of blood was dispensed into Ethylene diamine tetra-acetic acid (EDTA) bottle and was used for CD$_4^+$ count, Viral Load and antiretroviral drug concentrations; 3mls was dispensed into Lithium heparin bottle and was used for Hepatic Lipase, Lipoprotein Lipase and plasma albumin. Sample, when taken, were centrifuged immediately at 3000rpm for 3 minutes, plasma obtained were stored at -20°C before analysis. For CD$_4^+$ counts, whole blood samples were used to measure the CD$_4^+$ count immediately after samples were collected.

**Laboratory for Analysis**

CD$_4^+$ count analysis was carried out at Federal Medical Centre, Lokoja, Kogi State. The Viral Load was analyzed at Asokoro General Hospital, Abuja. Hepatic Lipase, Lipoprotein Lipase, ARVs concentrations were analyzed at Biotechnology Research Centre of Nnamdi Azikiwe University, Awka, Anambra State. Plasma Albumin was analyzed at the Federal Medical Centre, Lokoja, Kogi State, Nigeria.

**Methods**

Determination of HIV Viral Load was by Polymerase Chain Reaction (RT PCR using COBAS Ampliprep/COBAS Taqman) (Easley, 2006). CD$_4^+$ count estimation was by flow Cytometry using BD Facscount (Cossarizza et al., 2017). Hepatic Lipase Assay was done using Enzyme linked Immunoassay (Olivecrona and Olivecrona, 2010). While Lipoprotein Lipase assay using Enzyme Linked Immunoassay (Olivecrona and Olivecrona, 2010). Serum albumin estimation was analyzed using Dye Bromocresol green as described by (Doumas et al., 1971).

Determination of Sensitivity, specificity, positive predictive value and the negative predictive value was as follows,

Sensitivity is the ability of an assay to truly identify those who have the disease (Mallett et al., 2012).
Sensitivity = \frac{True \ positive}{True \ positive + false \ negative} \times 100

Specificity is the ability of an assay to truly identify those who do not have the disease (Cadogan et al., 2013).

Specificity = \frac{True \ negative}{True \ negative + false \ positive} \times 100

Positive predictive value is the probability that people with a positive screening test result indeed do have the condition of interest (Trevethan, 2017).

Positive Predictive Value (PPV) = \frac{True \ positive}{True \ positive + false \ positive} \times 100

Negative predictive value is the probability that people with negative screening test result indeed do not have the condition of interest (Trevethan, 2017).

Negative Predictive Value (NPV) = \frac{True \ negative}{True \ negative + false \ negative} \times 100

Statistical Analysis

Values obtained were expressed as mean + standard deviation (SD) using SPSS version 17.0. All numerical results were analyzed with one-way ANOVA with post hoc multiple comparisons tests, while paired student t-test was used to compare means from the same group at different times. Spearman’s correlation analysis between antigenic index, biochemical parameters and plasma antiretroviral drug concentrations was done within groups. The diagnostics performance of the parameters was assessed using sensitivity, specificity, positive predictive value and negative predictive value. P values below 0.05 were considered statistically significant.

RESULTS

Characteristics of the study population

The characteristics of the study population (mean age, sex and WHO clinical stage) are shown in Table 1. The mean values of age in group A subjects on TDF + 3TC + EFV was 37 + 0.73 years, while that of group B subjects on AZT + 3TC + NVP was 38 + 0.63 years were significantly higher (P<0.01) than similar value in the controls (36 + 0.71 years). However, women were the majority of subjects (71.43%). In total, 108/154 (70.13%) of subjects were living in rural areas.

The majority of subjects were classified on WHO clinical stage I 57/154 (37%) followed by WHO clinical stage II 47/154 (30.52%), with no remarkable differences between subjects on WHO clinical stage III and Stage IV (Table 1).

Serum Alb, HL, LPL, CD4+ T-cells count and VL concentrations in HIV patients on drug combinations (Group A and B) at 12 months and HIV-positive Controls (Group C)

The Hepatic lipase enzyme level was significantly different amongst the three groups (f=42.589, p=0.001). The difference was due to lower values of Hepatic lipase enzyme level in the HIV-1 participants not on antiretroviral drug. The lipoprotein lipase enzyme level was significantly different amongst the three groups (f=26.867, p=0.001). The difference was due to lower values of lipoprotein enzyme level in the HIV-1 participants not on antiretroviral drugs. The HIV-1 viral load was significantly different amongst the three groups (f=117.541, p=0.001). The difference was due to higher values of viral load in the HIV-1 participants not on antiretroviral drugs. The CD4+ cells count showed no significant difference amongst the three groups (f=0.218, p=0.804). The serum albumin level was significantly different amongst the three groups (f=12.268, p=0.007). The difference was due to lower values of plasma albumin in the HIV-1 participants not on antiretroviral drugs (Table 2).

Serum albumin, hepatic and lipoprotein lipases in HIV-1 infected participants after 6 months and 12 months of antiretroviral therapy at the different staging of HIV/AIDS in group A

The Hepatic Lipase showed no significant difference at 6 months of therapy amongst the 4 stages of HIV/AIDS (f=1.511, p=0.229). Similarly, the Hepatic lipase showed no significant difference at 12 months of therapy (f=0.148, p=0.932). Lipoprotein lipase showed no significant difference at 6 months of therapy amongst the 4 stages of HIV/AIDS (f=0.399, p=0.755). Similarly, Lipoprotein lipase showed no significant difference at 12 months of therapy amongst the 4 stages of HIV/AIDS (f=0.986). The serum albumin was significantly different at 12 months of therapy amongst the 4 stages of HIV/AIDS (f=5.072, p=0.013).

The difference was due to the low value of albumin at stage 4 of HIV/AIDS. The serum albumin showed no significant difference at 6 months of therapy (f=2.445, p=0.08) (Table 3).

Serum albumin, hepatic and lipoprotein lipases in HIV-1 infected participants after 6 months and 12 months of antiretroviral therapy at different staging
Table 1: Characteristics of the Study Population

| Variables                  | All Subjects N = 154 | Group A (TDF + 3TC + EFV) n = 40 | Group B (AZT + 3TC + NVP) n = 35 | Group C (those not on drugs as control) n = 79 | P values |
|----------------------------|----------------------|-----------------------------------|-----------------------------------|---------------------------------------------|---------|
| Mean age in years (+SD)    | 36.68 (+0.74)        | 37.3 (+0.73)                      | 38.11 (+0.63)                     | 35.86 (+0.71)                               | 0.005   |
| Male                       | 44 (28.57%)          | 11 (27.5%)                        | 26 (74.29%)                       | 55 (69.62%)                                 | 0.001   |
| Female                     | 110 (71.43%)         | 29 (72.5%)                        | 9 (25.71%)                        | 24 (30.38%)                                 |         |
| Residential Status         |                     |                                   |                                   |                                             |         |
| Semi-urban                 | 46 (29.87%)          | 13 (32.5%)                        | 12 (34.29%)                       | 21 (26.58%)                                 | 0.05    |
| Rural                      | 108 (70.13%)         | 27 (67.5%)                        | 23 (65.71%)                       | 58 (73.42%)                                 | 0.001   |
| WHO Clinical Stage         |                     |                                   |                                   |                                             |         |
| Stage I                    | 57 (37.01%)          | 14 (35%)                          | 10 (28.57%)                       | 33 (41.77%)                                 | 0.04    |
| Stage II                   | 47 (30.52%)          | 13 (32.5%)                        | 13 (37.14%)                       | 21 (26.58%)                                 | 0.04    |
| Stage III                  | 29 (18.83%)          | 9 (22.5%)                         | 4 (11.43%)                        | 16 (20.25%)                                 | 0.03    |
| Stage IV                   | 21 (13.64%)          | 4 (10%)                           | 8 (22.86%)                        | 9 (11.39%)                                  | 0.01    |

*Values differ significantly from controls (P<0.05) *Values differ significantly within stages (P<0.001).

Table 2: Serum Alb, HL, LPL, CD4+ T-cells count and VL concentrations in HIV patients on drug combinations (Group A and B) at 12months and HIV-positive Controls (Group C)

| Group | N  | HL (ng/ml)       | LPL (pg/ml) | VL (copies/ml) | CD4 (cell/µl) | Alb (g/l) |
|-------|----|------------------|-------------|----------------|---------------|-----------|
| A     | 40 | 0.33±0.08        | 1714.83±438.39 | 94±18          | 515±81        | 37.35     |
|       |     |                  |             |                |               | ±4.41     |
| B     | 35 | 0.32±0.09        | 2138.45±709.15 | 160±30         | 579±53        | 35.09     |
|       |     |                  |             |                |               | ±6.21     |
| C     | 79 | 0.11±0.06        | 1104.74±107.12 | 17429±1351     | 584±27        | 32.33     |
|       |     |                  |             |                |               | ±6.43     |

f-value 42.589 26.867 117.541 0.218 12.268
p-value 0.001* 0.001* 0.001* 0.804 0.007*
A vs B 1.000 0.001* 0.001* 1.000 0.646
A vs C 0.001* 0.001* 0.008* 1.000 0.002*
B vs C 0.001* 0.001* 0.001* 1.000 0.005*

*Significant, A = TDF + 3TC + EFV drug combination group, LPL: Lipoprotein Lipase, B = AZT + 3TC + NVP drug combination group, VL: Viral load, TDF = Tenofovir, 3TC = Lamivudine, EFV = Efavirenz, SD: Standard Deviation

staging of HIV/AIDS in group B.

The Hepatic lipase was significantly different at 12 months of therapy amongst the 4 stages of HIV/AIDS (f=7.827, p=0.001). The difference was due to high values of Hepatic Lipase at stage 3 of HIV/AIDS. The Hepatic lipase was not significantly different at 6 months of therapy amongst the 4 stages of HIV/AIDS (f=0.053, p=0.985). The lipoprotein lipase was not significantly different 6 months of therapy amongst the 4 stages of HIV/AIDS (f=0.053, p=0.985). The lipoprotein lipase was not significantly different 6 months of therapy amongst the 4 stages of HIV/AIDS (0.558, p=0.647). Similarly, the lipoprotein lipase was not significantly different at 12 months of therapy amongst the 4 stages of HIV/AIDS (f=0.283, p=0.837). The plasma Albumin was significantly different at 6 months of therapy amongst the 4 stages of HIV/AIDS (f=15.36, p=0.004). Similarly, plasma albumin was significantly different at 12 months of therapy amongst the 4 stages of HIV/AIDS (f=14.59, p=0.001). The difference was due to the low value of albumin at stage 4.
### Table 3: Serum albumin, hepatic and lipoprotein lipases in HIV-1 infected participants after 6 months and 12 months of antiretroviral therapy at different staging of HIV/AIDS in group A

| Group (N=40) | A | N | HL (6 months) ng/ml | HL (12 months) ng/ml | LPL (6 months) pg/ml | LPL (12 months) pg/ml | Albumin (6 months) g/l | Albumin (12 months) g/l |
|--------------|---|---|---------------------|---------------------|---------------------|---------------------|-----------------------|-----------------------|
| Stage I (a)  | 14| 0.31| ±0.05 | 0.32| ±0.25 | 1778.64| ±375.29 | 1925.64| ±600.41 | 31.93 | ±6.24 | 37.50 | ±4.94 |
| Stage II (b) | 13| 0.33| ±0.10 | 0.34| ±0.10 | 1515.28| ±460.05 | 1894.54| ±521.95 | 36.38 | ±3.80 | 38.85 | ±2.48 |
| Stage III (c) | 9 | 0.30| ±0.10 | 0.31| ±0.18 | 1828.73| ±561.67 | 1840.44| ±542.14 | 31.22 | ±4.64 | 36.11 | ±5.44 |
| Stage IV (d) | 4 | 0.30| ±0.05 | 0.32| ±0.13 | 1871.00| ±344.89 | 1937.75| ±725.92 | 32.25 | ±5.56 | 34.25 | ±7.27 |

**f-value**: 1.511 0.148 0.399 0.048 2.445 5.072  
**p-value**: 0.229 0.932 0.755 0.986 0.08 0.013*  
*a vs b 0.317 1 1 1 0.182 1  
*a vs c 1 1 1 1 1 1  
*a vs d 1 1 1 1 1 1  
*b vs c 1 1 1 1 0.157 1  
*b vs d 1 1 1 1 1 1  
*c vs d 1 1 1 1 1 1  

*Significant, HL: Hepatic Lipase, TDF: Tenofovir, LPL: Lipoprotein Lipase, 3TC: Lamivudine, Group A: TDF + 3TC + EFV drugs combinations, EFV: Efavirenz

### Table 4: Serum level of albumin, hepatic and lipoprotein lipases in HIV-1 infected participants after 6 months and 12 months of antiretroviral therapy at different staging of HIV/AIDS in group B

| Group (N=35) | B | N | HL (6 months) ng/ml | HL (12months) ng/ml | LPL (6 months) pg/ml | LPL (12 months) pg/ml | Albumin (6 months) g/l | Albumin (12 months) g/l |
|--------------|---|---|---------------------|---------------------|---------------------|---------------------|-----------------------|-----------------------|
| Stage I (a)  | 10| 0.32| ±0.04 | 0.33| ±0.07 | 1885.67| ±672.69 | 2175.60| ±377.53 | 35.60 | ±4.48 | 39.60 | ±3.03 |
| Stage II (b) | 13| 0.31| ±0.13 | 0.32| ±0.10 | 1932.09| ±715.81 | 2122.00| ±5625.08 | 34.38 | ±5.66 | 42.00 | ±4.21 |
| Stage III (c) | 4 | 0.32| ±0.04 | 0.35| ±0.05 | 1924.50| ±461.74 | 2030.25| ±442.70 | 34.00 | ±6.38 | 42.00 | ±2.94 |
| Stage IV (d) | 8 | 0.32| ±0.07 | 0.33| ±0.15 | 1976.10| ±875.23 | 2084.13| ±438.10 | 29.00 | ±4.87 | 29.25 | ±4.83 |

**f-value**: 0.053 7.827 0.558 0.283 15.536 14.59  
**p-value**: 0.985 0.001* 0.647 0.837 0.004* 0.001*  
*a vs b 1 1 1 1 1 1  
*a vs c 1 1 1 1 1 1  
*a vs d 1 1 1 1 1 1  
*b vs c 1 0.231 1 1 0.01* 1  
*b vs d 1 0.00*2 1 1 0.805 1  
*c vs d 1 0.087 1 1 0.036 1  

*Significant, HL: Hepatic Lipase, AZT: Zidovudine, LPL: Lipoprotein Lipase, 3TC: Lamivudine Group B: AZT + 3TC + NVP drugs combinations, NVP: Nevirapine
Table 5: Serum Alb, HL, LPL, CD4+ T-cells count and VL concentrations in HIV positive control (group C) at the different staging of HIV/AIDS

| Group (N=79) | C  | N  | HL (ng/ml) | LPL (pg/ml) | VL (copies/ml) | CD4 (cell/μl) | Alb (g/l) |
|-------------|----|----|------------|-------------|----------------|----------------|----------|
| Stage I (a) | 33 | 1.20 | 1130.073 | 12652.30 | 561.61 | 34.64 |
|             |    | +0.65 | +10699.67 | +89.47 | +217.31 | +6.08 |
| Stage II (b) | 21 | 0.10 | 1036.13 | 15337.29 | 545.52 | 31.95 |
|             |    | ±0.61 | ±11175.60 | ±130.42 | ±233.13 | ±6.80 |
| Stage III (c) | 16 | 0.12 | 1007.544 | 17831.00 | 456.56 | 30.63 |
|             |    | ±0.46 | ±7108.90 | ±9866.19 | ±259.04 | ±7.59 |
| Stage IV (d) | 9  | 0.09 | 1005.456 | 20112.69 | 438.67 | 27.78 |
|             |    | ±0.61 | ±11338.14 | ±14.77 | ±159.91 | ±6.18 |

F-value 4.306 2.183 13.847 1.533 3.141
p-value 0.007* 0.069 0.001* 0.213 0.030*
a vs b 0.001* 1.000 1.000 1.000 0.899
a vs c 0.013* 0.267 0.770 0.006* 0.300
a vs d 0.001* 0.689 0.001* 0.008* 0.444
b vs c 0.906 0.202 1.000 0.007* 1.000
b vs d 0.575 1.000 0.001* 0.006* 0.041*
c vs d 0.091 0.067 0.001* 1.000 0.043*

* Significant HL:Hepatic Lipase, ALB: Albumin, LPL: Lipoprotein Lipase, CD4+: Cluster of differentiation, VL: Viral load, n= Stage Sample Size, SD: Standard Deviation, N= Group Sample Size

Table 6: Diagnostic performance of albumin, HL, LPL and CD4+ count in various groups using HIV-1 viral load as gold standard

| Parameters     | Groups | Sensitivity (%) | Specificity (%) | PPV (%) | NPV (%) |
|----------------|--------|-----------------|-----------------|---------|---------|
| HL (ng/ml)     | A      | 10.2            | 100.0           | 44.1    | 30.0    |
|                | B      | 15.9            | 100.0           | 35.3    | 30.0    |
|                | C      | 17.8            | 100.0           | 45.0    | 9.1     |
| LPL (pg/ml)    | A      | 7.1             | 91.7            | 46.7    | 29.7    |
|                | B      | 42.9            | 66.7            | 46.2    | 43.6    |
|                | C      | 2.7             | 83.3            | 36.7    | 6.6     |
| CD4 (Cells/μl)| A      | 53.6            | 83.0            | 42.0    | 40.9    |
|                | B      | 42.9            | 80.1            | 40.0    | 60.0    |
|                | C      | 41.1            | 90.9            | 45.0    | 85.0    |
| TDF (μg/ml)    | A      | 59.3            | 68.0            | 40.6    | 66.7    |
|                | B      | 21.4            | 95.2            | 45.0    | 64.5    |
| 3TC (μg/ml)    | A      | 7.5             | 100             | 40.0    | 32.4    |
|                | B      | 8.5             | 100             | 43.3    | 60.0    |
| EFV (ng/ml)    | A      | 35.7            | 83.3            | 43.3    | 35.7    |
|                | B      | 42.9            | 85.7            | 46.7    | 69.2    |
| ALBUMIN (g/l)  | A      | 60.7            | 51.7            | 94.4    | 50.0    |
|                | B      | 71.4            | 56.2            | 66.7    | 80.0    |
|                | C      | 56.2            | 43.3            | 97.6    | 13.5    |

A = TDF + 3TC + EFV, VL: Viralload, TDF = Tenofovir, CD4: Cluster of differentiation, 3TC = Lamivudine, ALB: Albumin, EFV = Efavirenz, LPL: Lipoprotein Lipase, B = AZT + 3TC + NVP, HL=Hepatic Lipase, AZT= Zidovudine, NVP= Nevirapine, C: Those not on drugs, PPV:Positive Predictive value, NPV: Negative Predictive Value
Table 7: Association between plasma antiretroviral drugs concentrations and biochemical parameters in group A participants

| Parameter | Group | n | r-value | P-value |
|-----------|-------|---|---------|---------|
| VL vs CD4 | A     | 40 | -.691** | 0.000   |
| VL vs Alb | A     | 40 | -.685** | 0.000   |
| LPL vs CD4 | A | 40 | .705** | 0.007   |
| HL vs CD$ | A | 40 | .803** | 0.000   |
| Alb vs CD4 | A | 40 | .594** | 0.000   |
| TDF vs 3TC | B | 35 | .502** | 0.001   |
| TDF vs EFV | B | 35 | .478** | 0.002   |
| 3TC vs EFV | B | 35 | .374* | 0.017   |
| 3TC vs Alb | B | 35 | .977** | 0.005   |
| CD4 VS Alb | B | 35 | .549** | 0.000   |
| VL vs CD4 | B | 35 | -.339* | 0.046   |
| VL vs Alb | B | 35 | -.539** | 0.000   |
| CD4 vs VL | B | 35 | -.339* | 0.046   |
| 3TC vs Alb | B | 35 | .932** | 0.006   |
| HL vs CD4 | B | 35 | .785** | 0.006   |
| HL vs Alb | B | 35 | .788** | 0.008   |
| LPL vs CD4 | B | 35 | .592* | 0.041   |
| LPL vs Alb | B | 35 | .823** | 0.039   |
| HL vs LPL | C | 79 | .606** | 0.000   |
| HL vs CD4 | C | 79 | -.251* | 0.026   |
| LPL vs VL | C | 79 | .381** | 0.001   |
| VL vs HL | C | 79 | -.251* | 0.026   |
| VL vs Alb | C | 79 | .474** | 0.000   |
| CD4 vs Alb | C | 79 | -.229* | 0.043   |

Diagnostic performance of albumin, HL, LPL and CD4+ count in various groups using HIV-1 viral load as the gold standard

Table 6 showed the sensitivity, specificity, PPV and NPV of parameters in various groups. The sensitivity, specificity, PPV and NPV of Hepatic lipase in group A were 10.2%, 100%, 44.1%, and 30.0% respectively, that of group B were 15.9%, 100%, 35.3% and 30% respectively while that of group C were 17.8%, 100%, 45.0% and 9.1% respectively. This results showed that Hepatic lipase lacks sensitivity and predictive values but with good specificity.

The sensitivity, specificity PPV and NPV of LPL in group A were 7.1%, 91.7%, 46.7% and 29.7% respectively, that of group B were 42.9%, 66.7%, 46.2% and 43.6% respectively while that of group C were 2.7%, 83.3%, 36.7% and 6.6% respectively. LPL lacks sensitivity similar to HL. LPL also lacks predictive values but has good specificity.

The sensitivity, specificity, PPV and NPV of CD4+ cells count in group A were 53.6%, 83.0%, 423% and 40.9% respectively, that of group B were 42.9%
80.1%, 40.0% and 60% respectively while that of group C were 41.1%, 90.9%, 45% and 85% respectively. CD \(_4^+\) count in group A showed low sensitivity with strong specificity and low positive predictive value but strong negative predictive value. CD \(_4^+\) lacks sensitivity in group B and also lacks PPV in group C, CD \(_4^+\) count lacks sensitivity but strong NPV and has good specificity but poor PPV.

The sensitivity, specificity, PPV and NPV of TDF in group A were 59.3%, 68.0%, 40.6% and 66.7% respectively. This showed that TDF has good sensitivity and PPV with moderate specificity and NPV.

The sensitivity, specificity, PPV and NPV of AZT in group B were 21.4%, 95.2% 45.0% and 64.5% respectively. This showed that AZT lacks sensitivity but good specificity and predictive values.

The sensitivity, specificity, PPV and NPV of 3TC in group A were 7.5%, 100%, 40% and 32.4%, respectively. This showed 3TC lacks sensitivity and predictive values but has good specificity. Those of group B were 8.5%, 100%, 43.3% and 60% respectively. This showed 3TC in group B lacks sensitivity and PPV but good specificity and NPV.

The sensitivity, specificity, PPV and NPV of EFV in group A were 35.7%, 83.3%, 43.3% and 35.7%, respectively. This showed good specificity but lacked sensitivity and predictive values.

The sensitivity, specificity, PPV and NPV of NVP in group B were 42.9%, 85.7%, 46.7% and 69.2%, respectively. This showed NVP has good specificity and NPV but lacks sensitivity and PPV.

The sensitivity, specificity, PPV and NPV of albumin in group A were 60.7%, 51.7%, 94.4% and 50.0% respectively that of group B were 71.4%, 56.2%, 66.7% and 80.0% respectively while that of group C were 56.2%, 43.3%, 97.6% and 13.5% respectively. From albumin results in group A, it has good sensitivity, poor specificity and strong PPV with moderate NPV. In group B, albumin demonstrated good sensitivity, and good predictive values, while in group C, albumin showed moderately good sensitivity, poor specificity and strong PPV with poor NPV.

Correlation analysis between antigenic index, plasma antiretroviral drugs concentrations and biochemical parameters in group A, B and C participants

There was significant association between plasma antiretroviral drugs concentration, antigenic index and biochemical parameters in group A participants as follows: VL and CD \(_4\) (r = 0.691, p = 0.000), VL and albumin (r = 0.685, p = 0.000), HDL and CD \(_4\) (r = 0.803, p = 0.008), LPL and CD \(_4\) (r = 0.705, p = 0.007), CD \(_4\) and albumin (r = 0.594, p = 0.000). All were found significant (p < 0.05). There were significant correlation between TDF and 3TC (r = 0.502, p = 0.001), TDF and EFV (r = 0.478, p = 0.002), 3TC and EFV (r = 0.374, p = 0.17), 3TC and alb (r = 0.977, p = 0.005), CD \(_4\) and albumin (r = 0.549, p = 0.000). All were found significant (p < 0.05).

The association between plasma antiretroviral drug concentrations, antigenic indices and biochemical parameters in group B participants: There was a significant correlation between 3TC and alb (r = 0.923, p = 0.006), HL and CD \(_4\) (r = 0.785, p = 0.008), HL and alb (r = 0.788, p = 0.008), LPL and CD \(_4\) (r = 0.592, p = 0.041), LPL and Alb (r = 0.823, p = 0.039). All were found significant (p < 0.05) (Table 7).

DISCUSSION

The present study focused on some index that could be used to monitor the management of HIV/AIDS infected participants, such as hepatic lipase, Lipoprotein lipase and albumin. In this study, activities of hepatic and lipoprotein lipases were enhanced in Nevirapine and Efavirenz based regimens, but this was severely reduced in HIV-1 infected individuals without HAART therapy. The study observed that the activities of hepatic and lipoprotein lipases in participants on antiretroviral therapy were significantly higher when compared with HAART naive participants. The study also reveals that increased hepatic and lipoprotein lipases were associated with undetectable viral load for participants on HAART, whereas reduced hepatic and lipoprotein lipases were associated with detectable viral load for participants without HAART. This is an indication of the effectiveness of Nevirapine and Efavirenz based regimens on the activities of hepatic and lipoprotein lipases. This finding concur with the study done by Cunha et al. (2015) that activities of hepatic and lipoprotein lipases are reduced in HAART naive HIV-1 infected participants and also reduced for HIV-1 infected participants on protease inhibitors but enhanced for participants on nucleoside and non-nucleoside reverse transcriptase inhibitors. The possibility of HIV-1 virus causing reduction in hepatic lipase is due to disruption and reduction of high density lipoprotein (HDL) and apoprotein A \(_1\) (AP \(_{A1}\)), which occurs as a result of activation of the immune system in HIV-1 infection, this promotes an increase in pro-inflammatory cytokines and an...
increase in lipid peroxidation. This process promotes an imbalance in the antioxidant system, a decrease in the production of anti-inflammatory cytokines and an elevation of pro-inflammatory cytokines, which increases the chance of developing atherosclerosis (Osuji et al., 2018). These inflammatory processes initiated by viral infection leads to stimulation of endothelial lipase and phosphohopse A2 which in turn causes a reduction in HDL concentrations. Since High Density Lipoprotein (HDL) is responsible for the release and activation of hepatic lipase, these processes of immune activation that leads to HDL reduction affects the activation and activities of hepatic Lipase (Osuji et al., 2018).

Similarly, the reduced activity of lipoprotein lipase enzyme in HAART naive participants was due to lack of its activation by apoprotein CII (APoCII) (Fueir and Gafencu, 2019). This is because the lipoprotein lipase enzyme is inactive until it becomes bound to its cofactor, apoprotein CII (APoCII). And activation of the immune system in HIV-1 infection promotes pro-inflammatory cytokines synthesis and release that leads to the poor synthesis of apoprotein CII from the liver and, in turn, affects the activation of lipoprotein lipase (Ifeanyichukwu et al., 2011).

From another study, it has been reported that immune system activation in HIV-1 infection causes a knockdown of APoA-1 and APoCII expression due to elevations of interferon-gamma (IFNγ) and tumor necrosis factor-α (TNFα) that promotes lipids peroxidation and disturbances in the metabolism of lipids (Cunha et al., 2015); these lead to lipodystrophy, myositis and hepatomegaly. The report in another study by Boothby et al. (2008) has shown that the clearance rate of lipoproteins is reduced in HAART naive HIV-1 infected individuals and reduced in patients on protease inhibitors, whereas the lipoprotein clearance rate is normal for participants on nucleoside and non-nucleoside reverse transcriptase inhibitors. This is an indication that nucleoside and non-nucleoside reverse transcriptase inhibitors may likely improve the activities of hepatic and lipoprotein lipase enzymes. From all these findings, it could be hypothesized that reduced activities of hepatic and lipoprotein lipases are predominant in HAART naïve participants due to pro-inflammatory cytokines due to immune system activation in HIV-1 infection.

In this study, the HIV-1 viral load in participants on antiretroviral drugs was significantly lower when compared with HAART naïve participants. The low viral load at 12 months of therapy indicates the efficacy and suitability of the regimen for the individual client. In this study, viral load was predominantly undetectable in participants on efavirenz and nevirapine based regimens. This result is similar to that predicted by Cain et al. (2012).

The study observed no statistical difference in CD4+ cells counts at 12 months for participants on antiretroviral drugs when compared with treatment naive participants. Since the CD4+ T cell count was the same between those on drugs and those not on the drug but some biochemical parameters are not the same, this is an indication of CD4+ count limitation in the monitoring of patients on antiretroviral therapy. This finding is supported by the work of Chukwudi et al. (2010) that CD4+ count depletion is not bizarre to HIV-1 infection but retention of circulating immune complexes, which is a great burden to HIV/AIDS individuals. (Chukwudi et al., 2010) research findings further revealed that one would expect from 70 participants who do not have HIV, up to 99% of them should have CD4+ count above 500cells/mm³. However, only 55.7% have CD4+ count above 500cells/mm³. There may be other factors other than HIV viral infection, adversely affecting CD4+ count. It was hypothesized that malaria reduces the CD4+ count more than HIV infection (Jegede et al., 2017). In a similar development, Chukwudi et al. (2010) reported that after antimalarial treatment, the median CD4+ count at day 28 of follow-up increased from 498 to 811cells/μl in HIV-1 negative and from 297 to 447cells/μl in HIV-positive patients and that after successful treatment, the proportion of patients with CD4+ count <200/μl at day 45 decreased from 9.6% to 0% in HIV-1 negative and from 28.7% to 13.2% in HIV-1 positive malaria patients. Hence CD4+ T-lymphocytopenia is caused majorly by circulating immune complexes due to malaria parasite, Salmonella species and, other microbial agents than HIV-1 infection (Chukwudi et al., 2010).

In this study, the plasma albumin value in participants on antiretroviral drugs was significantly higher at 12 months when compared with HAART naïve participants. The results of this study suggest that plasma albumin level has the potential to be used as a surrogate prognostic marker for HIV-1 disease monitoring because of a significant increase in plasma albumin level at 12 months of therapy. This finding is supported by Ifeanyichukwu et al. (2011); Sharma et al. (2016) that low plasma albumin in HAART naïve participants may be due to acute-phase responses in HIV-1 infection leading to a wide range of pathophysiological reactions that resulted in increased levels of inflammatory biomarkers such as tumor necrosis factor (TNF) and interleukin-6 (IL-6), which are responsible for the reduction in hepatic albumin synthesis and increase in albumin leakage to the extravascular space while enhanc-
ing the degradation of albumin. In a similar study by Ifeanyichukwu et al. (2011), it was shown that HIV-1 infection is accompanied by a robust plasma pro-inflammatory and anti-inflammatory cytokine response in HAART naive individuals. The high pro-inflammatory cytokines were probably produced by monocytes and macrophages to initiate inflammation which is essential for the development of innate immunity, while the increased anti-inflammatory cytokines were stimulated probably to deactivate the activities of the pro-inflammatory cytokines. The above observation is supported by the fact that there was a strong significant correlation between viral load and pro-inflammatory cytokines in HAART naive HIV-1 infected patients suggesting these cytokines were induced in response to HIV-1 replication in the absence of HAART (Osuji et al., 2018). All these inflammatory responses cause leakage of albumin to extravascular space leading to albumin breakdown. In another study by Mazzaferro et al. (2002), albumin has the ability to bind toxic substances generated during the inflammatory process due to HIV infection, the release of free radical species and lipid peroxidation during inflammation is thought to be a major cause of tissue damage. Plasma albumin is used to scavenge these free radical species as well as bacterial toxins that end up destroying albumin molecules (Mazzaferro et al., 2002).

The study also observed that the biochemical markers of participants on antiretroviral drugs were significantly higher at 12 months of therapy when compared with biochemical markers at 6 months of therapy. These increase in biochemical markers at follow-up in this study showed that they are good markers for the monitoring of treatment success or failure in antiretroviral therapy. This is because the increased biochemical markers were associated with decreased antigenic markers (virologic suppression), as observed in this study. However, there was no significant difference for an immunologic marker at 6 months and 12 months of antiretroviral therapy. This is the sign of limitation of CD4⁺ cells count in the monitoring of patients on antiretroviral therapy, as observed in this study.

The study also observed a remarkable correlation between antiretroviral drugs and biochemical markers. These results support that adequate therapeutical level of antiretroviral drug concentrations was associated with normal biochemical markers. It could also mean that sub-therapeutic and supra-therapeutic level of antiretroviral drugs concentrations may be associated with poor biochemical response. However, no correlation could be demonstrated between some antigenic markers and biochemical markers. This finding is supported by the work done by Boothby et al. (2008) that the clearance rate of lipoprotein is reduced in HAART naive HIV-1 infected individuals, whereas the lipoprotein clearance rate is normal for participants on nucleoside and non-nucleoside reverse transcriptase inhibitors. This is an indication that nucleoside and non-nucleoside reverse transcriptase inhibitors may likely improve the activities of hepatic and lipoprotein lipase activities in the metabolism of lipoprotein (Boothby et al., 2008).

However, no significant difference was observed in CD4⁺ cells count between 6 months and 12 months of therapy and HAART naive HIV-1 infected individuals. This may confer limitation of CD4⁺ cells count in the monitoring of response to antiretroviral therapy. This finding is supported by the work done by Onyenekwe et al., 2008. This may be due to higher T-cell activation in HIV-1 infection, which is associated with decreasing CD4 cells during inflammatory processes (Onyenekwe et al., 2008). Plasma albumin level was significantly lower at stage 4 of the HIV/AIDS disease when compared with other stages of the disease. These results showed that plasma albumin would be a very useful marker for predicting the severity of HIV infection and clinical monitoring of response to antiretroviral therapy. This finding is supported by reports by Onyenekwe et al. (2008) in which decreased serum albumin was associated with disease progression, AIDS associated mortality in people living with HIV (PLHIV) is independent of CD4 cell counts and HIV RNA load. Hence, serum albumin could be a useful, cheap and easily available surrogate test for predicting the severity of HIV infection, monitoring response to antiretroviral therapy and a predictor of survival (Onyenekwe et al., 2008). The study also observed significant improvement in albumin level between 6 months and 12 months of antiretroviral therapy. This finding is supported by (Ifeanyichukwu et al., 2011). The significant difference in mean serum albumin concentration, which was lowest in HAART naive HIV-1 infected participants and highest amongst participants on antiretroviral therapy, suggests that HAART may help to reverse synthetic capacity to produce albumin in the infected host (Onyenekwe et al., 2008). This evidence of reduced albumin in HIV seropositive participants indicates the impact of viral infection on the nutritional stability of the host (Ifeanyichukwu et al., 2011). Previous studies have also shown that albumin is a good index to monitor recovery and predict prognosis in HIV-1 infected participants (Jose et al., 2018; Olawumi and Olatunji, 2006).
However, Lipoprotein lipase did not show any significant difference amongst the stages, but hepatic lipase was significantly different amongst the stages of HIV/AIDS disease at 12 months of therapy. The normal hepatic and lipoprotein lipase enzymes activities at all stages of HIV/AIDS in this study showed that the activities of the lipases were not impaired by the nucleoside or non-nucleoside reverse transcriptase inhibitors. However, it has been hypothesized that hepatic and lipoprotein lipase activities are impaired under the condition of HIV/ART dyslipidemia in which there was impaired lipoprotein clearance by plasma lipases and or hepatic receptors (Gillard et al., 2013).

High sensitivities and a high positive predictive value of plasma albumin were observed in this study. It has been reported from previous studies by Ifeanyichukwu et al. (2011); Onyenekwe et al. (2008) that plasma albumin can be used in the place of CD4+ cell count in the monitoring of patients on antiretroviral therapy, especially in limited resource settings. Therefore, the use of this marker in the monitoring of patients on antiretroviral therapy may be recommended.

CONCLUSIONS

The present study thus concludes that hypoalbuminemia with decreased hepatic lipase and lipoprotein lipase activities were associated with unsuppressed viral load above 1000copies/ml. This suggests that albumin, hepatic and lipoprotein lipases are good biochemical markers for the prediction of treatment failure or success in participants on antiretroviral drugs. The study observed enhanced activities of hepatic lipase and lipoprotein lipases in efavirenz and nevirapine based regimen but hepatic and lipoprotein lipases were severely reduced in HIV-1 infected participants, not on antiretroviral drugs. From these findings, it is an indication that nucleoside and non-nucleoside reverse transcriptase inhibitors may likely improve the activities of hepatic and lipoprotein lipases and also confers albumin hepatic synthetic capacity. Further longitudinal studies should be done to establish alternative, appropriate, easy and simpler indices and methods suitable for monitoring treatment response in HIV infected patients on HAART in resource-limited settings.

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Conflict of interest

The authors declare no conflict of interest.

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