Original Research Article

The pattern of alpha-fetoprotein, CD4+ count, albumin, AST, ALT and ALP in HIV subjects on long term antiretroviral therapy in Nauth Nnewi, Anambra State, Nigeria

I P Ezeugwunne1,*, E C Ogbodo2, R A Analike3, N A Okwara3, J C Nnamdi3, J C Iwuji4, C N Obi-Ezeani5, A K Amah6, I O Odumodu2, C C Onyenekwe3

1 Dept. of Human Biochemistry, Faculty of Basic Medical Sciences, Nnamdi Azikiwe University, Awka, Nigeria
2 Dept. of Medical Laboratory Science, Nnamdi Azikiwe University, Awka, Nigeria
3 Dept. of Chemical Pathology, Faculty of Medicine, Nnamdi Azikiwe University, Awka, Nigeria
4 Dept. of Science Laboratory, Federal University of Technology, Owerri, Nigeria
5 Dept. of Chemical Pathology, Chukwuemeka Odumegwu Ojukwu University, Awka, Nigeria
6 Dept. of Physiology, College of Medicine, Imo State University, Owerri, Nigeria

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ABSTRACT

This study determined the pattern of alpha-fetoprotein, CD4+ count, albumin, AST, ALT and ALP in HIV positive subjects on long term antiretroviral therapy in NAUTH Nnewi, Anambra State, Nigeria. A total of ninety six (96) participants who were aged between 18 and 60 years attending the voluntary counseling and testing unit (VCT) and antiretroviral therapy unit (ART) of NnamdiAzikiwe University Teaching Hospital (NAUTH), Nnewi were randomly recruited for the study and grouped based on WHO criteria for HIV staging. Six milliliters (6mls) of blood sample were collected from each of the participants in each group and dispensed into EDTA and plain containers in appropriate volumes for the determination of the alpha fetoprotein (AFP), albumin (ALB), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and CD4+ count using standard laboratory methods. Results revealed no statistically significant differences in mean AFP levels, AST and ALP activities when compared between HIV positive participants on long term ART, short term ART, and HIV positive subjects not on ART and control groups respectively (p>0.05). Serum albumin concentration and ALT was not significantly different in HIV positive participants on long term ART than in those on short term ART (p>0.05) although serum albumin levels were significantly higher in HIV participants on long term ART and short term ART than in HIV positive subjects not on ART in each case (p<0.05) but was significantly lower in HIV positive participants not on ART compared to control subjects (p<0.05). CD4+ count was significantly higher in HIV positive participants on long term ART when compared with those o short term ART and HIV positive participants not on ART respectively (p<0.05). Therefore, this study has shown improved immune recovery with no hepatotoxicity in HIV positive persons on long term duration of antiretroviral therapy. Importantly, hypoalbuminemia existed among HIV positive subjects not on ART which is suggestive of further progression of the disease.

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*Corresponding author.
E-mail address: goodnessifeoma007@yahoo.com (I. P. Ezeugwunne).

1. Introduction

Human immune deficiency virus (HIV) which causes Acquired Immunodeficiency Syndrome (AIDS), which affects the cells of the immune system, and destroys or impairs their function has continued to be an issue of public
health discuss worldwide especially in the developing countries of Africa where its ravaging effects seems to be on the increase despite the efforts been made on tackling the disease. The human immunodeficiency virus (HIV) targets the immune system and weakens people’s defense against many infections and some types of cancer. As the virus destroys and impairs the function of immune cells, infected individuals gradually become immunodeficient (May et al., 1 2015). Immunodeficiency results in increased susceptibility to a wide range of infections, cancers and other diseases that people with healthy immune systems can fight off (World Health Organization (WHO), 2020). The impacts of HIV infection has been dramatic especially in the past until the discovery of the antiretroviral therapy (ART) which caused a drastic reduction in mortality rate among HIV positive persons and improved life expectancy among this group of people. Worldwide, survival of HIV-infected populations has improved with the increasing availability of antiretroviral therapy (Wandeler et al., 5 2016). The life expectancy of a person who carries the HIV virus is now approaching that of a person that tests negative for the virus, as long as they adhere to the combination of medication (Nnodim and Emefulu, 3 2011). Studies have shown that early ART initiation reduces the risk of serious clinical conditions, the development of AIDS, and death (Danel and Moh 4 2015; Lundgren and Babiker, 5 2015). However, this is not without some worries as several authorities have documented possibility of hepatotoxicity arising from the usage of antiretroviral therapy in HIV positive patients (Shiferaw et al., 6 2016; Qin et al., 7 2019). ART damages the liver cells by direct toxicity of the parent drugs or from its active metabolites (Tesfa et al., 8 2019). Although some other authors have shown no deleterious effects of ART on liver enzymes in HIV/AIDS patients on therapy (Atiba et al., 9 2021; Johnkennedy et al., 10 2020). Thus these conflicting results in literature leaves us wondering what the possible effect of ART in HIV positive persons would be like following long term administration of therapy and hence, we assessed the pattern of alpha-fetoprotein, CD4+ count, albumin, AST, ALT and ALP in HIV positive subjects on long term antiretroviral therapy in NAUTH Nnewi, Anambra State, Nigeria.

2. Materials and Methods

2.1. Study design

This is a case controlled study carried out to assess the levels of alpha-fetoprotein, albumin and CD4+ , ALT, AST and ALP activities in HIV infected subjects on long term antiretroviral therapy in NAUTH Nnewi, Anambra State, Nigeria. A total of ninety-six (96) participants who were aged between 18 and 60 years attending the voluntary counseling and testing unit (VCT) and antiretroviral therapy unit (ART) of Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi were randomly recruited for the study. In line with World Health Organisation (WHO) criteria for staging HIV, the participants were grouped into:

1. Group A: HIV positive symptomatic subjects on long term ART for a period of more than five (> 5) years (n= 24).
2. Group B: HIV positive symptomatic subjects on short term ART for a period of 1-4 years (n= 21).
3. Group C: HIV positive symptomatic subjects NOT on ART (n= 26).
4. Group D: control subjects (n=25).

Lamivudine (150 mg twice daily), Stavudine (40mg twice daily) and Nevirapine (200 mg twice daily) were administered to the symptomatic HIV stage 11 subjects on ART.

2.2. Inclusion and Exclusion criteria

Participants on triple combination of Stavudine, Lamivudine and Nevirapine based on WHO first line of ART, were included in this study. Only participants who were aged between 18 and 60 years and fulfilled WHO criteria for HIV staging were included in the study.

Pregnant women, and subjects who has history of smoking, hypertension, tuberculosis, diabetes, heart and renal diseases and any other clinical condition apart from HIV infection were excluded from the study.

2.3. Sample collection

Six millilitres (6mls) of blood sample were collected from each of the participants in each group and dispensed into EDTA and plain containers in appropriate volumes for the determination of the said parameters. The serum samples were stored at -20°C until analyzed.

2.4. Methods

2.4.1. HIV determination

The participants were screened for HIV infection using Immunoassay and Immunochromatographic method. Antibodies to HIV-1 and HIV-2 in human plasma were determined using Abbott determine TM HIV -1 and HIV-2 kit, which is an in-vitro visually read immunoassay (Abbott Japan Co. Ltd. Tokyo, Japan) and HIV-1 and 2 STAT-PAK Assay kit, which is an Immunochromatographic test for the quantitative detection of antibodies to HIV-1 and HIV-2 in Human plasma (CHEMBIO Diagnostic system, Inc, New York, USA).

2.4.2. Determination of CD4+T cells counts

This was achieved by using Cyflow counting system described by Ezeugwunne et al., (2018).
2.5. Determination of serum albumin

The albumin concentration in the plasma was determined using the method of Doumas and Watson (1971).

2.6. Determination of L-Aspartate aminotransferase (AST) activity

Aspartate aminotransferase (AST) was estimated according to the method of Reitman and Frankel, (1957).

2.7. Determination of L-Alanine aminotransferase (ALT) activity

Alanine aminotransferase (ALT) was estimated according to the method of Reitman and Frankel, (1957).

2.8. Determination of alkaline phosphatase (ALP) activity

Alkaline phosphatase activity was assayed for using the method described by Bessey et al. (1946).

2.9. Evaluation of Alpha fetoprotein

The serum alpha fetoprotein was determined by enzyme linked immunosorbent assay (ELISA).

2.10. Informed consent and ethical approval

Informed consent of participants was properly sort and obtained. Ethical approval for the research was obtained from Ethical Committee, Nnamdi Azikiwe University Nnewi, Anambra State, Nigeria.

2.11. Statistical analysis

The values were expressed as mean ± standard deviation. The significant difference between the mean value of control and experimental group was determined by one way analysis of variance (ANOVA) with post hoc t-test. P<0.05 was considered as statistically significant.

3. Results

The mean serum levels of albumin (g/L) and CD4+ (cells/µl) count values in HIV/AIDS participants on long term ART, HIV/AIDS participants on short term ART, HIV/AIDS participants not on ART, and control group were significantly different when observed among the groups (p>0.05), while the AFP (ng/ml) level observed among the groups and between groups was not significantly different (p>0.05). Group comparison between HIV/AIDS participants on long term ART and HIV/AIDS participants on short term ART showed no significant difference in serum albumin and AFP levels when observed (p>0.05). Also, there were no significant differences in the mean serum levels of albumin compared between HIV/AIDS participants on long term ART and HIV/AIDS participants on short term ART (p>0.05), but the mean serum albumin levels were significantly higher in HIV/AIDS participants on long term ART and short term ART than in HIV/AIDS participants not on ART respectively (p=0.00), although there were significantly lower mean serum albumin levels in HIV/AIDS participants not on ART than in control subjects (3.73±6.11 Vs 39.33±3.71; p=0.00). However, the CD4+ count values were observed to be significantly higher in HIV/AIDS participants on long term ART than in HIV/AIDS participants on short term ART (842.92±338.56 Vs 357.00±140.88; p=0.00). Also, group comparison between HIV/AIDS participants on long term ART and HIV/AIDS participants not on ART showed significantly higher mean CD4+ count in participants on long term ART than in those not on ART (p=0.00) but was not significantly different when HIV/AIDS participants on long term ART were compared with the control subjects (p>0.05). However, there were significantly lower mean value of CD4+ count in HIV/AIDS participants on short term ART when compared with HIV/AIDS not on ART and control group respectively (p<0.05). There was significantly lower CD4+ count in HIV/AIDS participants not on ART compared with control subjects (390.27±177.15 Vs 901.16±110.55; p=0.00), (Table 1)

The mean serum activities of AST (IU/L) and ALP (IU/L) in HIV/AIDS participants on long term ART; HIV/AIDS participants on short term ART; HIV/AIDS participants not on ART and control group were not significantly different when observed among the groups, (p>0.05). However, the mean serum activity of ALT (IU/L) was observed to be significantly different among the groups (F=6.12; P=0.00). Group comparison between HIV/AIDS participants on long term ART and HIV/AIDS participants on short term ART; between HIV/AIDS participants on long term ART and HIV/AIDS participants not on ART; between HIV/AIDS participants on long term ART and control group; between HIV/AIDS participants on short term ART and HIV/AIDS participants not on ART; between HIV/AIDS participants on short term ART and control group; and between HIV/AIDS participants not on ART and control group showed no significant difference in the mean serum activities of AST and ALP when observed, (p>0.05). Meanwhile, serum ALT activity was observed to be significantly different when observed between HIV/AIDS participants on long term ART and control group; between HIV/AIDS participants on short term ART and HIV/AIDS participants not on ART; between HIV/AIDS participants on long term ART and control group respectively (p<0.05). Nevertheless, group comparison between HIV/AIDS participants on long term ART and HIV/AIDS participants on short term ART; and between HIV/AIDS participants on long term ART and
Table 1: Comparison of mean±SD levels of AFP (ng/ml), Albumin (g/l), and CD4+ (cell/μl) in HIV/AIDS subjects on long term ART (A), HIV/AIDS subjects on short term ART (B), HIV/AIDS subjects not on ART (C) and control (D)

<table>
<thead>
<tr>
<th>Variables</th>
<th>AFP (ng/ml)</th>
<th>Albumin (g/l)</th>
<th>CD4+ (cells/μl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV/AIDS on long term ART (A), (n=24; &gt;5 years)</td>
<td>5.63±4.79</td>
<td>39.27±4.08</td>
<td>842.92±338.56</td>
</tr>
<tr>
<td>HIV/AIDS on short term ART (B), (n=21; 1–4 years)</td>
<td>3.66±2.75</td>
<td>36.83±5.74</td>
<td>357.00±140.88</td>
</tr>
<tr>
<td>HIV/AIDS not on ART (C), (n=26)</td>
<td>2.91±7.70</td>
<td>33.73±6.11</td>
<td>390.27±177.15</td>
</tr>
<tr>
<td>Control (D), (n=25)</td>
<td>6.11±4.85</td>
<td>39.33±3.71</td>
<td>901.16±110.55</td>
</tr>
</tbody>
</table>

F (p value) 1.04(0.38) 7.08(0.00)* 44.65(0.00)*
A Vs B (p value) 4.53(0.10) 0.70(0.11) 11.31(0.00)*
A Vs C (p value) 3.44(0.31) 1.65(0.00)* 8.67(0.00)*
A Vs D (p value) 0.01(0.73) 0.01(0.95) 19.34(0.42)
B Vs C (p value) 3.56(0.31) 1.71(0.00)* 4.43(0.00)*
B Vs D (p value) 4.88(0.05) 0.70(0.08) 1.89(0.00)*
C Vs D (p value) 3.56(0.31) 1.71(0.00)* 4.43(0.00)*

*for statistically significant result, p<0.05

HIV/AIDS participants not on ART showed no significant difference in the serum activity of ALT when observed, (p>0.05) respectively. (Table 2)

4. Discussion

HIV/AIDS disease as impacted negatively on millions of lives worldwide and HIV continues to infect more people across the globe with African countries having greater HIV disease burden. However, with increasing access to effective HIV prevention, diagnosis, treatment and care, including for opportunistic infections, HIV infection has become a manageable chronic health condition, enabling people living with HIV to lead long and healthy lives (WHO, 2020). Importantly, access to antiretroviral therapy and its availability is pivotal in the management of HIV infected patients. This is study assessed the levels of alpha-fetoprotein, albumin and CD4+, ALT, AST and ALP activities in HIV infected subjects on long term antiretroviral therapy in Nnamdi Azikiwe University Teaching Hospital (NAUTH) Nnewi, Anambra State, Nigeria.

In this study, there were no statistically significant differences observed in the mean alpha fetoprotein (AFP) levels when compared between HIV/AIDS participants on long term ART and those on short term ART; between HIV/AIDS participants on long term ART and HIV/AIDS participants not on ART, between HIV/AIDS participants on short term ART and control subjects, between HIV/AIDS participants on short term ART and those not on ART, between HIV/AIDS participants on short term ART and control subjects and between HIV/AIDS participants not on ART and control subjects respectively. This could mean that there was no hepatotoxicity resulting from the usage of antiretroviral therapy or drugs in the studied subjects. However, the statistically insignificant increase in the mean AFP level witnessed in this study in HIV/AIDS participants on long term ART compared with HIV/AIDS participants on short term as well as in HIV/AIDS participants not on ART may not be totally overlooked owing to the smallness of our sample size which may have contributed to the current finding. Therefore, it is suffice to say that hepatotoxicity leading to hepatocellular carcinoma may not be totally ruled out in HIV/AIDS subjects on antiretroviral therapy over a long term and hence, it is vital to monitor therapy over time in these persons. Clinically, AFP serves as a serum biomarker for adult liver diseases such as hepatocellular carcinoma, alcoholic cirrhosis, necrosis, viral hepatitis, and hepatoblastoma (Mizejewski, 2009). This result is in keeping with the report of Johnkennedy et al., (2020).

The present study showed that the mean serum albumin levels were significantly higher in HIV/AIDS participants on long term ART than in those on short term ART; and significantly higher in HIV/AIDS participants on short term ART than in HIV/AIDS participants not on ART, although there were significantly lower mean serum albumin levels in HIV/AIDS participants not on ART than in control subjects. This shows an improvement in the synthetic capacity of the liver in HIV/AIDS subjects on antiretroviral therapy in preference to HIV/AIDS subjects not receiving antiretroviral therapy in both short and long term respectively. However, HIV/AIDS subjects who were not receiving antiretroviral therapy suffered hypoalbuminemia (33.73±6.11 g/l) which may have resulted from the effects of HIV on the liver. Hypoalbuminemia refers to serum albumin levels <35.0 g/L and this is associated with more rapid progression of HIV disease. During acute-phase responses resulting from HIV infection, there are wide ranges of pathophysiological responses including the reduction in serum albumin levels. Reductions in serum albumin levels are attributed to increasing levels...
of inflammation in this subjects which are responsible for reductions in hepatic albumin synthesis and increases in albumin leakage to the extravascular space, and this enhances the degradation of albumin in HIV/AIDS patients not on ART. Hypoalbuminemia have been documented in HIV patients in recent time (Leal et al., 2018). Previous studies have shown that serum albumin concentrations increase significantly after the initiation of therapy (Chong et al., 2015).

In this study, the CD4+ count values were observed to be significantly higher in HIV/AIDS participants on long term ART than in HIV/AIDS participants on short term ART. Also, group comparison between HIV/AIDS participants on long term ART and HIV/AIDS participants not on ART showed significantly higher mean CD4+ count in participants on long term ART than in those not on ART but was not significantly different when HIV/AIDS participants on long term ART were compared with the control subjects (p>0.05). However, there were significantly lower mean values of CD4+ count in HIV/AIDS participants on short term ART when compared with HIV/AIDS not on ART and control group respectively. There was significantly lower CD4+ count in HIV/AIDS participants not on ART compared with control subjects. This implies that administration of antiretroviral drugs in HIV positive persons is accompanied by an improved immune recovery. This result further shows that there is a much more robust immune recovery in HIV positive subjects receiving antiretroviral therapy on a long term basis in preference to those on a short term antiretroviral therapy. Thus in order to achieve a better and robust immunity in HIV patients required to fight off invading pathogens, compliance to antiretroviral drug regime over a long period of time is important. On the other hand, HIV positive subjects who are not receiving antiretroviral therapy suffer a decline in their CD4+ count and thus experiences immuno-suppression resulting from cell death as a result of HIV infection. The CD4+ T cell counts are the primary target of HIV infection because of the affinity of the virus to the CD4 surface marker. Infection with HIV leads to a progressive impairment of cellular functions, which is characterized by a gradual decline in peripheral blood CD4+ T cell counts levels (Ezeugwunne et al., 2018). According to Ifeanyichukwu et al., (2011), the lowered CD4+T cell count suggests possible quantitative destruction of cellular immune cells (mainly Th1 cells). CD4+ count remains the best measurement of a patient’s immune and clinical status, the risk of opportunistic infections, and supports diagnostic decision-making, particularly for patients with advanced HIV disease (Ford et al., 2017). Our finding is in consonance with previous studies (Lok et al., 2010; Osakunor et al., 2015; Gezie, 2016).

Interestingly, there were no statistically significant differences in the mean serum activities of aspartate aminotransferase (AST) and alkaline phosphatase (ALP) compared amongst the groups and between the groups in each case. This suggests that the duration of antiretroviral therapy in HIV positive persons does not affect these enzymes. Aspartate aminotransferase is a marker of hepatocellular injury and is present in cytosolic and mitochondrial isoenzymes and is found in the liver, cardiac muscle, skeletal muscle, kidneys, brain, pancreas, lungs, leucocytes, and red cells. It participates in gluconeogenesis by catalysing the transfer of amino groups from aspartic acid to ketoglutaric acid to produce oxaloacetic acid. This enzyme leaks out of their membranes into circulation following damage to the liver and thus becomes elevated in such condition (Lala et al., 2020). However, due to its wide spread sources in human body, it is less sensitive and specific for liver damage (Lala et al., 2020). On the other hand, alkaline phosphatase is part of a family zinc metalloenzymes that are highly concentrated in the

### Table 2: Comparison of mean±SD serum activities of AST (IU/L), ALT (IU/L) and ALP (IU/L) in HIV/AIDS subjects on long term ART (A), HIV/AIDS subjects on short term ART (B), HIV/AIDS subjects not on ART (C) and control (D)

<table>
<thead>
<tr>
<th>Variables</th>
<th>AST (IU/L)</th>
<th>ALT (IU/L)</th>
<th>ALP (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV/AIDS on long term ART (A), (n=24; &gt;5 years)</td>
<td>9.80±7.34</td>
<td>5.45±4.84</td>
<td>43.40±16.31</td>
</tr>
<tr>
<td>HIV/AIDS on short term ART (B), (n=21; 1-4 years)</td>
<td>8.82±5.86</td>
<td>6.23±4.85</td>
<td>37.18±10.60</td>
</tr>
<tr>
<td>HIV/AIDS not on ART (C), (n=26)</td>
<td>10.06±7.39</td>
<td>7.59±5.30</td>
<td>42.34±21.45</td>
</tr>
<tr>
<td>Control (D), (n=25)</td>
<td>6.53±8.03</td>
<td>2.36±2.47</td>
<td>37.16±11.84</td>
</tr>
<tr>
<td>F (p value)</td>
<td>1.23(0.30)</td>
<td>6.12(0.00)*</td>
<td>1.04(0.38)</td>
</tr>
<tr>
<td>A Vs B (p value)</td>
<td>0.37(0.63)</td>
<td>0.04(0.59)</td>
<td>3.05(0.14)</td>
</tr>
<tr>
<td>A Vs C (p value)</td>
<td>0.01(0.90)</td>
<td>0.61(0.14)</td>
<td>0.39(0.85)</td>
</tr>
<tr>
<td>A Vs D (p value)</td>
<td>0.84(0.14)</td>
<td>3.58(0.01)*</td>
<td>3.33(0.13)</td>
</tr>
<tr>
<td>B Vs C (p value)</td>
<td>0.70(0.11)</td>
<td>8.94(0.00)*</td>
<td>4.05(0.29)</td>
</tr>
<tr>
<td>B Vs D (p value)</td>
<td>0.22(0.28)</td>
<td>2.33(0.00)*</td>
<td>0.07(1.00)</td>
</tr>
<tr>
<td>C Vs D (p value)</td>
<td>0.70(0.11)</td>
<td>8.94(0.00)*</td>
<td>4.05(0.29)</td>
</tr>
</tbody>
</table>

*for statistically significant result, p<0.05
microvilli of the bile canaliculus as well as several other tissues (e.g., bone, intestines, and placenta). Elevation in ALP is an important marker of biliary disease in the absence of some other factors such as pregnancy, and bone disorder (Ujjawal et al., 2014).

Surprisingly, there was no significant difference in the mean serum alanine transferase (ALT) activity in HIV/AIDS participants on long term antiretroviral therapy compared with those on short term antiretroviral therapy as well as in those not on antiretroviral therapy. This implies that the duration of antiretroviral therapy does not induce hepatic damage in the HIV subjects. However, serum ALT activity was significantly higher in both HIV subjects on long term and short term ART when compared with the control group. This may be as a result of the continuous debilitating effect of the virus on the liver cell irrespective of the length of antiretroviral therapy. ALT participates in gluconeogenesis by catalysing the transfer of amino groups from alanine to ketoglutaric acid to produce pyruvic acid. It is a cytosolic enzyme that is found in high concentrations in the liver and is more specific to the liver (Pratt and Kaplan, 2003). Hepatocellular injury and not necessarily cell death is the trigger for the release of these enzymes into the circulation. This result agrees with the documented report of Atiba and colleagues that showed no statistically significant differences in plasma AST, ALT and ALP in patients on antiretroviral therapy linked to the duration of treatment (Atiba et al., 2021). However, this is not in keeping the report of Qin et al. (2019) stating that liver damage always exists among HIV infected patients on ART with normal baseline liver function and without HBV/HCV infection (Qin et al., 2019), although our result agrees with the fact that cumulative ART duration does not increase the risk of liver damage (Qin et al., 2019).

5. Conclusion

This study revealed no statistically significant differences in mean AFP levels, AST and ALP activities when compared between HIV positive participants on long term ART, short term ART, and HIV positive subjects not on ART and control groups respectively. Serum albumin concentration and ALT was not significantly different in HIV positive participants on long term ART than in those on short term ART although serum albumin levels were significantly higher in HIV participants on long term ART and short term ART than in HIV positive subjects not on ART in each case but was significantly lower in HIV positive participants not on ART compared to control subjects. CD4+ count was significantly higher in HIV positive participants on long term ART when compared with those on short term ART and HIV positive participants not on ART respectively. Therefore, this study has shown improved immune recovery with no hepatotoxicity in HIV positive persons on long term and short term antiretroviral therapy. Interestingly, we also reported hypoalbuminemia among HIV positive subjects not ART which is suggestive of further progression of the disease. Thus, ART in a long term enhanced immune recovery and posed no harmful effect on the liver following long term administration in HIV positive subjects.

6. Source of Funding

None.

7. Conflict of Interest

The authors declare that there is no conflict of interest.

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