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ORIGINAL ARTICLE

Assessment of Alpha-Fetoprotein, Albumin, Cd4+ and Some Liver Enzymes in HIV Infected Adult on Art in Nauth Nnewi, South Eastern Nigeria

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ABSTRACT

This study was carried out to assess alpha-fetoprotein, albumin, CD4+ and some liver enzymes in HIV infected adult on ART in NAUTH Nnewi, South Eastern Nigeria.A total of 97 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 and grouped based on WHO criteria for HIV staging. 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 alpha fetoprotein (AFP), albumin (ALB), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and CD4+ count using standard laboratory methods. Results showed statistically significant increases in the mean serum albumin level and CD4+ count in HIV/AIDS participants on ART than in those not on ART but significantly lower in both HIV/AIDS subjects on ART and those not on ART compared with control subjects respectively ($p<0.05$). AFP, AST and ALP activities remained the same when compared between the groups ($p>0.05$). Also, ALT activity did not differ significant in HIV/AIDS participants on ART compared with HIV/AIDS participants not on ART ($p>0.05$) but were significantly higher than in control subjects respectively ($p<0.05$). Therefore, this study has shown no hepatocellular carcinoma or hepatotoxicity emanating from antiretroviral drugs use in HIV/AIDS patients rather it revealed an immune system recovery in HIV/AIDS subjects on ART.

KEY WORDS: *HIV, AIDS, ART, Liver, liver enzymes, alpha fetoprotein (AFP), albumin, CD4+ count, hepatotoxicity.*

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INTRODUCTION

Human Immunodeficiency Virus (HIV) continues to be a major global public health issue, having claimed almost 33 million lives so far with an estimated 38.0 million people still living with HIV at the end of 2019 [29]. HIV is a retrovirus that is principally known for its ravaging impacts on the human immune system.HIV breaks down the body's immune system, causing Acquired Immune Deficiency Syndrome (AIDS), which affects so many organs including the liver [9]. It is transmitted via sexual intercourse,

shared intravenous drug paraphernalia, and mother-to-child transmission (MTCT), which can occur during the birth process or during breastfeeding by means of contact with infected body fluids such as blood, breast milk, semen and vaginal secretions [29]. It is important to note that people with HIV who are taking ART and are virally suppressed do not transmit HIV to their sexual partners [29]. HIV is known to attack the host immune system by destroying the specific cells of the host immune system via the destruction of immune cells called CD4 cells or T-cells. Thus, the impact of HIV on the immune cells on a long term leaves the host immune system weak and vulnerable to all forms of infections otherwise called opportunistic infections. At this point, HIV leads to Acquired Immune Deficiency Syndrome (AIDS). AIDS is a condition in humans in which progressive failure of the immune system allows life-threatening infections and cancers to thrive [14].

With the advent and availability of antiretroviral therapy (ART), there has been a drastic decline in morbidity and mortality associated with HIV infection across the globe [26], although not without its own attendant problems especially with regards to the health of the liver. ART is a treatment regimen for HIV/AIDS which aims to increase life expectancy, minimize opportunistic infections but does not cure HIV/AIDS. ART is usually commenced when CD4 levels fall to 200 cells per microlitre of blood and this often coincide with the establishment of the disease. Globally, 36.4 million people living with HIV had access to ART by the end of 2020 [19]. In spite of the benefits of ART, adverse effect of which hepatotoxicity is a common finding has emerged and threatens patients' compliance to regime [27, 21] Liver-related disease has been estimated to account for 13%–18% of all-cause mortality in HIV-infected patients and is one of the leading causes of non-AIDS-related death [15, 25]. Numerous mechanisms leading to hepatotoxicity in HIV infected persons have been documented among which are ART-related toxicity, oxidative stress and direct injury to the liver by the HIV virus itself [10]. Thus, assessment of liver function status among HIV infected population becomes an issue of utmost importance in patients monitoring and management.

Human Alpha-fetoprotein (AFP) is a plasma protein produced by the embryonic yolk sac and the fetal liver. AFP content in fetal serum is high and gradually decreases to the level of adults after birth. An elevated serum alpha fetoprotein level serves as a tumor marker in identification of cancers especially those related to the liver [8]. Hepatic enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP), as well as serum total protein and albumin are commonly known Liver function tests which help to assess the status of the liver. Liver cells contain more AST than ALT, but ALT is confined to the cytoplasm, in which its concentration is higher than that of AST. An increase in ALT serum levels is, therefore, more specific for liver damage (Crook, 2012). The highest concentrations of ALP are found in bone, liver, spleen, intestine, placenta, and kidneys. ALP contains a number of isoenzymes, with bone, liver, and placenta types being the most extensively studied [13]. Elevated level of ALP is a sensitive marker of biliary cholestasis as increased synthesis of ALP in the affected ducts increases the activity of this enzyme in the plasma [6]. Several studies have documented elevated activities of liver enzymes in HIV infection persons [2, 6, 18, 20, 21] although some others report no effects on hepatic enzymes [20]. CD4+ cells have been shown to be lower in HIV infection [11]. Therefore, the present study assessed the levels of alpha-fetoprotein, albumin and $CD4^+$and some liver enzymes activities in HIV infected adult on ART in NAUTH Nnewi, South Eastern Nigeria.

MATERIAL AND METHODS

Study design and Subjects recruitment

This is a case controlled study carried out to assess the levels of alpha-fetoprotein, albumin and $CD4^+$ and some liver enzymes activities in HIV infected adult on ART in NAUTH Nnewi, South Eastern Nigeria. A total of 97 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 Organization (WHO) criteria for staging HIV, the participants were grouped into:

I) Group 1: HIV positive symptomatic subjects on ART (n= 45; male=31, female=14)
II) Group 2: HIV positive symptomatic subjects NOT on ART (n= 26; male=16, female=10)
III) Group 3: HIV negative subjects (control; n=26).

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.

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.

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.

Methods

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 andHIV-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).

Determination of CD4+T cells counts

This was achieved by using Cyflow counting system described by Ezeugwunne *et al*., [9].

Determination of serum albumin

The albumin concentration in the plasma was determined using the method of Doumas and Watson [7].

Determination of L-Aspartate aminotransferase (AST) activity

Aspartate aminotransferase (AST) was estimated according to the method of Reitman and Frankel, [22].

Determination of L-Alanine aminotransferase (ALT) activity

Alanine aminotransferase (ALT) was estimated according to the method of Reitman and Frankel, [22].

Determination of alkaline phosphatase (ALP) activity

Alkaline phosphatase activity was assayed for using*the method described by Bessey *et al.* [4].

Evaluation of Alpha fetoprotein

The Serum alpha feto protein was determined by enzyme linked immunosorbent assay (ELISA)**.**

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.

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.

RESULTS

The mean serum AFP (ng/ml) levels in HIV/AIDS participants on ART; HIV/AIDS participants not on ART and HIV negative participants (control group) were not significantly different when observed among the groups ($p>0.05$). Also, group comparison between HIV participants on ART and HIV participants not on ART; between HIV participants on ART and control group, and between HIV participants not on ART and control group showed that the mean serum levels of AFP was not significantly different when observed ($p>0.05$).

The mean serum albumin (g/l) concentrations in HIV/AIDS participants on ART; HIV/AIDS participants not on ART and HIV negative participants (control group) were significantly different when compared among the groups (F-value=9.13; p=0.00). Also, group comparison between HIV participants on ART and HIV participants not on ART showed that serum albumin concentration was significantly higher in participants on ART than those not on ART (38.13±5.01 Vs 33.73±6.11; p=0.00); comparison between HIV participants on ART and control group did not differ significantly (p=0.30). However, serum albumin concentration was significantly lower in HIV participants not on ART compared with control subjects(33.73±6.11 Vs 39.33±3.71; p=0.00).

The mean CD4+ (cells/µl) count values in HIV/AIDS participants on ART; HIV/AIDS participants not on ART and control group were significantly different across the groups (F=23.10; p=0.00). The mean CD4+ count value was significantly higher in HIV participants on ART than in those not on ART (616.16±359.22 Vs 390.27±117.15; p=0.00). However, the mean CD4+ count value was significantly lower in HIV participants on ART than in control group (616.16±359.22 Vs 901.16±110.55; p=0.00). Also, the mean CD4+ count value was significantly lower in HIV participants not on ART than in control group (390.27±117.15 Vs 901.16±110.55; p=0.00). See table 1.

Table 1: Comparison of mean (±SD) serum levels of AFP, Albumin and CD4+ in HIV/AIDS participants on ART, HIV/AIDS participants not on ART and control group

Variables	AFP (ng/ml)	Albumin (g/l)	CD4+ (cells/µl)
HIV/AIDS participants on ART (A)	4.71± 4.05	38.13±5.01	616.16±359.22
HIV/AIDS participants not on ART (B)	2.91±77.10	33.73±6.11	390.27±117.15
Control group (C)	6.11±4.85	39.33±3.71	901.16±110.55
F (p-value)	1.65 (0.20)	9.13 (0.00)*	23.10 (0.00)*
A Vs B:(p-value)	6.83 (0.14)	1.01(0.00)*	7.49(0.00)*
A Vs C:(p-value)	1.05 (0.20)	0.11(0.30)	15.15 (0.00)*
B Vs C:(p-value)	3.56(0.31)	1.71(0.00)*	4.43(0.00)*

for statistically significant result, p<0.05

The mean serum activities of AST and ALP in HIV/AIDS participants on ART; HIV/AIDS participants not on ART and HIV negative participants (control group) was not significantly different compared among the groups (p>0.05) whereas AIT activity differed significantly across the groups (F-value=9.08; p=0.00). Also, the mean serum AST and ALP activities were similar when compared between groups (p>0.05) respectively.

The mean serum ALT activity in HIV/AIDS participants on ART did not differ significant when compared with HIV/AIDS participants not on ART (p=0.15). The mean serum activity of ALT was significantly higher in HIV/AIDS participants on ART and HIV/AIDS participants not on ART respectively when compared with the control subjects (p<0.05). See table 2.

Table 2: Comparison of mean(±SD) serum activities of AST, ALT and ALP in HIV/AIDS participants on ART(A), HIV/AIDS participants not on ART (B) and control group (C)

Variables	AST (IU/L)	ALT(IU/L)	ALP(IU/L)
HIV/AIDS participants on ART (A)	9.34±6.64	5.82±4.81	40.50±14.14
HIV/AIDS participants not on ART (B)	10.06±7.39	7.59±5.30	42.34±21.45
Control group (C)	6.53±8.03	2.36±2.47	37.16±11.84
F (p-value)	1.76(0.18)	9.08(0.00)	0.69(0.50)
A Vs B:(p-value)	0.02(0.68)	0.92(0.15)	2.37(0.66)
A Vs C:(p-value)	0.88(0.12)	3.50(0.00)	1.33(0.32)
B Vs C:(p-value)	0.70(0.11)	8.94(0.00)	4.05(0.29)

**for statistically significant result, p<0.05*

DISCUSSION

Human Immunodeficiency Virus (HIV) continues to be a major global public health issue, having claimed almost 33 million lives so far with an estimated 38.0 million people still living with HIV at the end of 2019 [29]. The present study assessed the levels of alpha-fetoprotein, albumin and $CD4^+$ and some liver enzymes activities in HIV infected adult on ART in NAUTH Nnewi, South Eastern Nigeria.

AFP is a major plasma protein expressed at high concentrations during fetal development; however, concentrations decrease drastically after birth. When hepatocytes regenerate or proliferate, AFP concentrations may increase. AFP is commonly used as a tumor marker for hepatocellular carcinoma (HCC) [17]. An elevated serum alpha fetoprotein level serves as a tumor marker in identification of cancers especially those related to the liver [8]. In this study, it was observed that there were no significant differences in the mean serum levels of AFP when compared among and between the groups, although AFP level was insignificantly increased in HIV/AIDS participants on ART than in those not on ART. This agrees with the findings of Johnkennedy and Ukamaka [12] on the alterations of alpha fetoprotein and some liver enzymes, in HIV patients undergoing antiretroviral therapy at Federal Medical Centre, Owerri that documented insignificant increase in AFP levels in HIV patients on ART than in those not receiving therapy.

In the present study, the mean serum albumin levels were significantly higher in HIV/AIDS participants on ART compared with HIV/AIDS participants not on ART. Serum albumin is synthesized in the liver and remains a major fraction of the group of proteins found in the human body. It is a vital biomarker for the assessment of the synthetic function of the liver. This result may be due to an immune recovery by subjects on antiretroviral therapy which further enhanced the synthetic functionality of the liver. Serum albumin has been documented in literature as a useful prognostic biomarker for assessment of HIV disease progression [23, 24]. This is in keeping with the report of previous studies [5]. Interestingly, there was no significant difference observed in the mean serum albumin concentrations in HIV/AIDS patients

on antiretroviral therapy when compared with apparently healthy seronegative or control subjects. This is a possible pointer to the recovery potential of antiretroviral therapy on the HIV disease. Expectedly, the mean serum albumin level was significantly lower in HIV/AIDS participants not on ART compared with control subjects. This may be due to an immunosuppressive effect of the HIV infection. Recent studies have suggested that low albumin levels in HIV infected patients are associated with rapid progression to AIDS and may account for increased mortality [3, 16].

The mean CD4+ count value was significantly higher in HIV participants on ART than in those not on ART but the mean CD4+ count value was significantly lower in HIV participants on ART than in control group . Also, the mean CD4+ count value was significantly lower in HIV participants not on ART than in control group. CD4 count has been used widely as the important prognostic marker of HIV disease progression [3]. The disease progression in HIV is defined on the basis of clinical features, CD4 cell count and HIV virus levels estimated by RNA or DNA PCR [1]. The pathogenesis of HIV infection is largely attributable to the decrease in the number of T cells (a specific type of lymphocyte) that bear the CD4 receptor (CD4+). Progressive depletion of CD4+ T cells is associated with progression of HIV disease. It can be inferred that the significant increase in CD4+ count in HIV subjects on ART is an indication of the recovery effect of the antiretroviral drugs on the immune system, otherwise simple put it that ART has an immunity boosting effect that helps in achieving viral load suppression in HIV positive subjects. Thus, HIV positive persons who are not on antiretroviral therapy suffer immuno-suppression due to the effect of the virus on the host immune system. This is evident in the present study in which there was a significantly lower CD4+ count in HIV/AIDS participants that were not on antiretroviral therapy.

The mean serum activities of AST and ALP in HIV/AIDS participants on ART; HIV/AIDS participants not on ART and HIV negative participants (control group) was not significantly different compared among the groups whereas ALT activity differed significantly across the groups. Also, the mean serum AST and ALP activities were similar when compared HIV/AIDS participants on ART, those not on ART and control groups respectively. The mean serum ALT activity in HIV/AIDS participants on ART did not differ significant when compared with HIV/AIDS participants not on ART. The mean serum activity of ALT was significantly higher in HIV/AIDS participants on ART and HIV/AIDS participants not on ART respectively when compared with the control subjects. Hepatic otherwise called liver enzymes are an important component of a group of tests called liver function tests which are utilized in the assessment of the functionality of the liver viz-a-viz its metabolic, synthetic and excretory functions. These enzymes include alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT).The highest concentrations of ALP are found in bone, liver, spleen, intestine, placenta, and kidneys although the most commonly evaluated ones are those of the bone, liver and placenta [13]. An elevated level of ALP is a sensitive marker of biliary cholestasis as increased synthesis of ALP in the affected ducts increases the activity of this enzyme in the plasma. On the other hand, liver cell damage is characterized by the release of enzymes (AST and ALT) from damaged hepatocytes. Aminotransferases such as AST and ALT are present in high concentrations in the liver. AST is also found diffusely represented in the heart; skeletal muscle, kidneys, brain and red blood cells, and ALT have low concentrations in skeletal muscle and kidney. A rise in plasma aminotransferase activities is a sensitive indicator of damage to cytoplasmic and/ or mitochondrial membranes. Plasma enzyme activities rise when the membranes of only very few cells are damaged. Liver cells contain more AST than ALT, but ALT is confined to the cytoplasm, in which its concentration is higher than that of AST. An increase in ALT serum levels is, therefore, more specific for liver damage [6]. This study has shown no significant differences in the mean serum activities of the hepatic enzymes; ALP, AST and ALT in HIV/AIDS participants on ART when compared with HIV/AIDS subjects who are not on ART respectively. This implies that the liver function in these subjects is not impaired and that there is no hepatotoxicity induced by the antiretroviral drugs on the participants. This result agrees with the findings of several studies [20], although it differ also with some previous studies which documented varying degrees of elevations in hepatic enzymes in HIV patients receiving antiretroviral therapy [2, 18, 21, 12]. Furthermore, this study showed significantly higher values of ALT activities in both HIV/AIDS patients on ART and those not on ART when compared with control subjects. This is a possible indication that HIV infection causes an elevation in hepatic enzymes following its effect on the liver in absence or presence of other co-morbidity.

CONCLUSION

This study showed no significant differences in the mean serum levels of AFP, AST and ALT activities amongst the groups but serum albumin, CD4+ count and ALT activity differed significantly amongst the group studied. The mean serum level of AFP did not differ significantly in HIV/AIDS participants on ART when compared with those not on ART and control subjects respectively. There were statistical

significant increases in the mean serum albumin level and CD4+ count in HIV/AIDS participants on ART than in those not on ART but significantly lower in both HIV/AIDS subjects on ART and those not on ART compared with control subjects respectively. AST and ALP activities remained the same when compared between the groups. Also, ALT activity did not differ significant in HIV/AIDS participants on ART compared with HIV/AIDS participants not on ART but were significantly higher than in control subjects respectively. Therefore, this study has shown no hepatocellular carcinoma or hepatotoxicity emanating from antiretroviral drugs use in HIV/AIDS patients rather it revealed an immune system recovery in HIV/AIDS subjects on ART and an immune suppression in those not on antiretroviral therapies, thus suggesting that ART improves the immune system in these patients which will help in minimizing various opportunistic infections that comes with HIV/AIDS infection.

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