

Changes in Liver Function Enzymes of HIV/AIDS Patients Treated with Antiretroviral Drugs (ARVs) in Specialist Hospital, Sokoto, Nigeria

*¹M.G. Abubakar, ¹M.M. Abduljalil, ²Y.I. Nasiru

¹ Department of Biochemistry, Usmanu Danfodiyo University, Sokoto, Nigeria

² Sokoto State Agency for the Control of AIDS, Tuberculosis and Leprosy (SOSACAT)

[*Corresponding author: Email magusau@hotmail.com, ☎: +2348035052912]

ABSTRACT: This study assessed the effect of Human Immunodeficiency Virus and Antiretroviral Drugs (ARVs) on liver enzyme markers (Aspartate aminotransferase, Alanine aminotransferase and Alkaline phosphatase) and CD4 T-cells. A total of Seventy Five (75) individuals were enrolled into the study, which comprised Twenty Five (25) HIV negative (control), Twenty Five (25) HIV positive non-treated with ARVs and Twenty Five (25) HIV positive treated with antiretroviral drugs (ARVs). Females were found to be the majority of HIV infected patients and most patients were at the middle age of 20-39 years. AST and ALT were assessed according to the Reitman and Frankel's (1957) method, while ALP was based on King Armstrong's (1980) method and CD4 T-cells using a method assayed of Cassens *et al.*, (2004). The result show a significant increase ($p < 0.05$) in AST and ALT levels of HIV positive non treated group compared to HIV negative group (control). AST and ALT levels of HIV positive treated with ARVs is significantly higher in comparison to HIV positive non-treated group. But the ALP activity was significantly lower ($p < 0.05$) in HIV positive treated group compared to non-treated group. Infection by HIV increases the activities of the three enzymes, which may be due to liver cells apoptosis caused by HIV infection, intact immune response to HIV replication which subsequently leads to hepatocellular necrosis and inflammation. But at the commencement of antiretroviral therapy the activities of the three afore-mentioned enzymes decreases which may be as result of decrease in the negative effect of the virus to the liver enzymes by ARVs. For clinical significance, it is necessary to investigate the activities of liver enzymes in HIV positive patients in order to monitor the diagnosis and advanced infection of the liver cells by HIV.

Keywords: Aspartate aminotransferase, Alanine aminotransferase, Alkaline phosphatase, Human immunodeficiency virus and antiretroviral drugs.

INTRODUCTION

Human immunodeficiency virus belongs to a class of retroviruses known as lentiviruses, which are characterized by a long period of persistence and replication before any onset of disease. HIV virus leads to acquired immunodeficiency syndrome (AIDS) a condition in which the immune system is compromised, leading to life threatening opportunistic infections (Coffin *et al.*, 1986).

In 2005 alone, AIDS claimed an estimated of 2.5 to 3.3 million lives, of which more than 570,000 were children (Greener, 2002). Greener (2002) also reported that one-third of the deaths were in sub-saharan Africa. Before the introduction of antiretroviral therapy (ART), about 15 to 20% of the children born to HIV infected mothers became HIV positive due to vertical transmission (Thorne *et al.*, 1998).

Nigeria has a HIV prevalence rate of 5.8% of the adult population in 2001, which decreases to 4.4% in 2005

but still it was ranked as second in Africa and fourth in the world with about 3 million people infected (Oladebo *et al.*, 2008).

Antiretroviral drugs are useful in prolonging life and postponing complications of AIDS or AIDS related complexes (ARC), but do not cure the infection (DHHS, 2005). Current HAART options are combinations (or cocktails) consisting of at least three drugs belonging to at least two types/classes of antiretroviral agents.

The widespread of HIV infection as well as the extensive use of these drugs (ARDs) in management of HIV infection and speculations of their effects such as nausea, vomiting, rash, abdominal pain, skin rashes, peripheral neuropathy, pancreatitis, diarrhea, indirect hyperbilirubinemia etc, has made it necessary to investigate the effect of ARVs to the patients. The present study was therefore undertaken to evaluate the effect of HIV infection on the liver marker enzymes and the risk associated with the use of antiretroviral drugs.

MATERIALS AND METHOD

Research location

The research was carried out at Specialist Hospital, Sokoto. Samples were collected from antiretroviral therapy centre (ART) at Specialist Hospital Sokoto.

Study population

The study population comprised 75 subjects, which includes 25 HIV positive non-treated with antiretroviral drugs, 25 HIV positive treated, and 25 HIV negative (Healthy adult as control) subjects.

Sample collection

On enrollment, 5mls of blood were aseptically collected from each subject using a sterilized syringe into a clean dry glass test tube and centrifuged to obtain the serum.

Ethical Clearance and Patients Consent

The ethical clearance of ethical and research committee of Specialist Hospital Sokoto was obtained prior to the commencement of the study. Standard informed consent form was used to inform the patients that give their consent to participate in the study.

Patient's Treatments Combination

Patients were treated based on the National recommended guideline for the treatment of HIV positive patients. The combinations used were as follows:

For patients on first line therapy, the combinations are:

- i. 2 NRTIs + NNRTIs

For second line ART regimen, the following combinations were used:

- ii. 2 NRTIs + A boosted protease inhibitor (Indinavir or Ritonavir)

The drugs dosages are:

- iii. NRTIs: Zidovudine – 300mg BD, Lamivudine – 150mg BD or 300mg OD, Didanosine – <60kg: 250mg OD, >60kg: 400mg, Tenofovir – 300mg OD.
- iv. NNRTIs: Nevirapine – 200mg OD for 1st 2-week, 200mg BD thereafter, Efavirenz – Nocte: 600mg, >60kg: 800mg.
- v. PIs: Ritonavir – 100mg BD boosting, Indinavir – 400mg BD.

Inclusion Criterion

- i. HIV-positive patients not on treatment and those on treatment for one year or less.
- ii. Individuals between the ages of 15-60 years old.
- iii. Individuals with no physical sign of metabolic syndrome.
- iv. Hepatitis (A, B, or C) negative individuals

Exclusion Criterion

- i. Individuals with physical sign of liver cirrhosis or any metabolic syndrome.
- ii. Alcoholic consumption, Cigarette smokers or drug abusers.
- iii. Hepatitis A, B or C positive patients

Liver Toxicity Markers

Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT)

AST and ALT was measured according to the method of Reitman and Frankel (1957).

Alkaline Phosphatase (ALT)

Alkaline phosphatase was determined according to the method of King Armstrong (1980).

CD₄ Cells Count

The CD₄⁺ T- cells were enumerated using flow cytometry (FCM) method (Cassens *et al.*, 2004), using Cyflow Counter (Partec, Munster, Germany).

Statistical analysis

Statistical analysis was performed using Graph pad Instat version 3.02 (Graph pad Corp., San Diego, USA). The data was analysed using descriptive statistics and analysis of variance (Benferroni compare all columns) was used to test for the level of significant between mean. A P value < 0.05 was taken as statistically significant.

RESULTS

The effects of antiretroviral drugs on serum AST, ALT, and ALP were investigated. The age groups were observed to be within the middle age group of 20 to 39 years, with a significant difference between the age group of HIV positive non-treated group and control subject.

The result also shows that majority of HIV infected patients are females and constituted 72% and 64%, while male constituted 28% and 36% in HIV positive non-treated group and treated subjects respectively.

All the 25 HIV positive patients non-treated with antiretroviral drugs have the serum AST and ALT activities significantly higher ($p < 0.05$) than the control group, which is three (3) folds the value of control group. A significant difference ($p < 0.05$) in the activities of these two enzymes (AST and ALT) of HIV positive treated group was observed compared to the non-treated group. ALT activity of HIV positive treated group is significantly lower ($p < 0.05$) compared to non-treated group. The CD4 T-cells of HIV positive non-treated group were significantly lower ($p < 0.05$) than the control

group, likewise the CD4 T-cells number in HIV positive treated with antiretroviral drugs were significantly higher ($p < 0.05$) compared to the non-treated group (Table 1).

DISCUSSION

Highly active antiretroviral therapy (HAART) has dramatically improved the prognosis of HIV infection, therefore AIDS deaths has been reduced dramatically with the initiation of the therapy (Holtzer and Roland, 1999).

Table 1: Serum Transaminases, and Alkaline Phosphatase of HIV/AIDS Positive non-treated and treated with Antiretroviral Drugs.

	CONTROL	HIV+ NON-TREATED	HIV+ TREATED
AGE (YEARS)	38.12±1.65 ^a	29.68±1.41 ^a	30.60±1.84
GENDER	15M and 10F	7M and 18F	9M and 16F
AST (U/L)	7.57±0.32 ^a	44.89±1.13 ^{ab}	9.86±0.21 ^b
ALT (U/L)	9.23±0.28 ^a	36.08±0.83 ^{ab}	29.51±0.91 ^b
ALP (U/L)	47.58± 2.37	49.39± 1.45 ^a	34.33± 0.91 ^a
CD4 T-CELLS (cell/ μ L)	791.12±33.49 ^a	337.74±59.29 ^{ab}	516.28±37.51 ^b

Key: n= Sample size (25/group) Values are expressed as Mean \pm SEM Values with the same superscript in column are statistically significant ($P < 0.05$)
 AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, ALP: Alkaline phosphatase, U/L: Unit per litres, M: Male, F: Female

The result of this study revealed that, HIV infected patients are at middle age of 20 to 39 years and females are the majority of HIV infected patients. This finding is inconsistent to report of UNAID, 2010 in which they reported that the rate of women living with HIV/AIDS rose from 43% in 1999 to 50% in 2010 and in Sub-saharan Africa women comprised 59% of the adult infected with HIV. A report of study by Babadoko, (2005) indicated that in northern Nigeria women comprised of 59% of the people living with HIV/AIDS in 2010.

Many factors were indicated to be attributed to the women becoming infected with HIV which may include to certain traditional practices such as female genital mutilation (FGM) and unfaithful multiple sex partners as may occur in polygamous relationships (UNAIDS, 2003). Moreover poverty and ignorance in addition to other socioeconomic conditions may be the driving forces increasing the risk of women getting infected through increased commercialization of sex (FMOH, 2010).

The number of people infected with HIV was estimated to be 33 million globally, and by increase in the accessibility of antiretroviral therapy, the number of patients persisting with the HIV increases and also

increases in the presentation of liver disease (Smith *et al.*, 2010). Hepatotoxicity is a major side effect described for all antiretroviral drugs (Reisler *et al.*, 2001, Sulkowski *et al.*, 2000, and Gisolf *et al.*, 2000). Hepatotoxicity caused by antiretroviral therapy may be associated to number of agents in antiretroviral drug classes which include nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs) and protease inhibitors (PIs) though the severity of hepatotoxicity may range from transient elevation of transaminase to hepatic failure and death (Nunez, 2010).

Mechanism by which the ART causes liver related toxicity particularly NRTIs which causes direct mitochondrial toxicity leading to abnormal liver function (Murphy *et al.*, 2007), while NNRTIs may cause hypersensitivity (Mbougua *et al.*, 2010). Other mechanisms include direct cell stress and disturbances in lipid or sugar metabolism associated to protease inhibitors (Nunez, 2010).

Many studies around the globe showed that HIV infection alters the liver function enzymes by either direct or indirect mechanism. Megan *et al.* (2012) reported that multiple studies showed that HIV infects a wide range of non-hematopoietic cells, including cells in

the liver. This may serve as a reason for the elevation the liver enzymes observed in the non-treated subjects when compared to control group. Our finding was also in conformity with the study conducted by Lebovics et al. (1988); Cappell (1991) and Lefkowitz (1994) who reported that liver enzymes elevation are frequent in HIV infected patients.

The finding of our study was contrary to many studies that reported antiretroviral drugs are associated with liver enzyme elevation in 6-30% of the patients (Sulkoski, 2012). Our finding observed that at the commencement of ART the activities of the liver enzymes decreases in comparison to the non-treated group. It has believed that factors such as alcohol consumption, illicit drug or medication abuse, abnormal metabolic syndrome and HBV or HCV co-infection are among many risk factors for liver enzymes elevation in HIV infected patients. All these risk factors for the elevation of liver enzymes were among the exclusion criterion for this study particularly to the treated subjects, this and the duration of treatment used for the study (one year) may serve as a reason for the decrease in the activities of the liver enzyme noticed in the study.

The CD4 T- cells and other component of the immune system were among the principal target cells by the HIV (Alimonti et al., 2003). Alimonti et al. (2003) also reported that antiretroviral drugs were known to inhibit the growth and replication of HIV, thereby hindering the negative effect of the virus to the CD4 T-cells and other cells of the immune system. This may serve as a reason for substantial decrease in the CD4 cell count of HIV non-treated group compared to the control subjects, but at the initiation of ART in the treated group the level of CD4 cell increases significantly compared to the non-treated group.

CONCLUSION

In this study, liver enzymes were found to be elevated in HIV positive non-treated with antiretroviral drugs compared to control subjects, which may be due to the fact that studies. For clinical significance, it is important to investigate the activities of liver enzymes markers in order to monitor the diagnosis and advanced infection of the liver cells by HIV. The study was restricted to one year duration of ART which may be short to examine the effects of ARVs on the liver function enzymes.

REFERENCES

- Alimonti, J.B., Ball, T.B. and Fowke, K.R. (2003). Mechanism of CD₄ T-lymphocyte cell death in HIV infection and AIDS, *Journal of Genetic Virology*, **84(7)**: 1649-1661
- Cappell, M.S. (1991). Hepatobiliary manifestations of the acquired immune deficiency syndrome, *American Journal of Gastroenterology*, **86**:1-15.
- Cassens, U., Göhde, W., Kuling, G., Gröning, A., Schlenke, P., Lehman, L.G., Traore, Y., Servais, J. and Henin, Y. (2004). Simplified Volumetric Flow Cytometry allows Feasible and Accurate Determination of CD4 T- Lymphocytes in Immunodeficient Patients Worldwide. *Antiviral Therapy*. **9**: 395-405.
- Coffin J., Haase, A., Levy, J.A., Montagnier, L., Oroszian, S., Teich, N., Temin, H., Toyoshima, K., Varmus, H., Vogt, P. and Weiss R.A. (1986). What call to AIDS virus? (Letter), *Nature*, **321**:10-15.
- Department of Health and Human Services (DHHS) (2005). A Pocket Guide to adult HIV/AIDS treated January edition cited on 5th – 07 0 2008.
- Federal Ministry of Health (FMOH) (2010). National Guidelines for HIV and AIDS Treatment and Care in Adolescents and Adults. Federal Ministry of Health Abuja, Nigeria.
- Greener, R. (2002). *AIDS macroeconomic impact*, in S., Forsynth (ed.) state of the Art: AIDS and Economics, *IAEN*, 47-55.
- Holtzer, C.D. and Roland, M. (1990). The use of combination antiretroviral therapy in HIV infected patient, *Annal of Pharmacotherapy*, **33(2)**: 198-209.
- King, E.J. and Armstrong, A.R. (1980). Calcium, Magnesium, Phosphatase, In *practical clinical Biochemistry*, vol I, varley, B., Gowenlock A.H. and Bell M. (eds), London, Pp 850
- Lebovics, E., Dworkin, B.M., Heier, S.K. and Rosenthal W.S. (1988). The hepatobiliary manifestations of human immunodeficiency virus infection, *American Journal of Gastroenterology*, **83**:1-7.
- Lefkowitz, J.H. (1994). Pathology of AIDS-related liver disease, *Digestive Disease*, **12**:321-30.
- Mbougua, J.B., Laurent, C., Kouanfack, C., Bourgeois, A., Ciaffi, L., Calmy, A., Gwet, H., Koulla-Shiro, S., Ducos, J. and Mpoudi-Ngolé, E. (2010). Hepatotoxicity and effectiveness of a Nevirapine-based antiretroviral therapy in HIV-infected patients with or without viral hepatitis B or C infection in Cameroon. *BMC Public Health*, **10**:105.

- Megan, C., David, I. and Sharon, R.L. (2012). Human Immunodeficiency Virus and the liver, *World Journal of Hepatology*, **4(3)**: 91-98.
- Murphy, R.A., Sunpath, H., Kuritzkes, D.R., Venter, F. and Gandhi, R.T. (2007). Antiretroviral therapy-associated toxicities in the resource-poor world: the challenge of a limited formulary, *Journal of Infectious Disease*, **196(Suppl 3)**: S449-S456.
- Núñez, M. (2010). Clinical syndromes and consequences of antiretroviral-related hepatotoxicity, *Hepatology*, **52**:1143-1155.
- Oladepo, D.K., Idigbe E.O. and Audu R.A. (2008). Establishment of CD₄ lymphocyte baseline in Nigeria, National Institute for pharmaceutical Research and Development, Abuja, Pp 5.
- Reisler, R., Liou, S., Servoss, J., Robbins, G., Theodore, D., Murphy, R. and chung, R. (2001). Incidence of hepatotoxicity and mortality in 21 adult antiretroviral treatment trials, Conference on HIV pathogenesis and treatment, Argentina, Pp 43.
- Reitman, S. and Frankel S. (1957). Determination of serum glutamate oxaloacetate and glutamic pyruvic acid transaminase, *American Journal of Clinical Pathology*, **28**: 56-66.
- Smith, C., Sabin, C.A., Lundgren, J.D., Thiebaut, R., Weber, R., Law, M., Monforte, A., Kirk, O., Friis-Moller, N. and Phillips, A. (2010). Factors associated with specific causes of death amongst HIV-positive individuals, *AIDS*, **24**: 1537-1548.
- Sulkowski, M.S., Thomas, D.L., Chaisson, R.E. and Moore, R.D. (2000). Hepatotoxicity associated with antiretroviral therapy in adults infected with HIV and role of Hepatitis C or B virus infection, *JAMA*, **283**: 74-80
- Sulkowski, M.S., Thomas, D.L., Mehta, S.H., Chaisson R.E. and Moore R.D. (2002). Hepatotoxicity associated with nevirapine or efavirenz-containing antiretroviral therapy: role of hepatitis C and B infections, *Hepatology*, **35(1)**: 182–9.
- Thorne, C., Newell, M.L. and Peck, H.C. (1998). Social care of children born to HIV infected mothers in Europe, *AIDS Care*, **10**: 7-16.
- UNAIDS (2003). Global Report: Annexes, adapted from <http://www.who.int/hiv>
- UNAIDS (2010). [UNAIDS report on the global AIDS epidemic](http://www.who.int/hiv), adapted from <http://www.who.int/hiv>