



Derangement in Enzymatic and Non-Enzymatic Markers of Liver Function in Human Immunodeficiency Virus (HIV) Infected Patients in Makurdi, North Central Nigeria

Ochalefu, D.O.¹, Adoga, G.I.², Alonyenu, E.I. ¹ and Agada, S.A.¹

Department of Medical Biochemistry, College of Health Sciences, Benue State University, Makurdi, Nigeria.¹

Department of Biochemistry, College of Medicine, University of Jos, Jos, Plateau State, Nigeria.²

Correspondence Author: dickochalefu@gmail.com.

Abstract

Liver disease has emerged as a critical issue in the management of human immunodeficiency virus (HIV) victims. This study was carried out to investigate the derangement in indices of liver function in HIV-infected patients in Makurdi, North Central Nigeria. One hundred and fifty males and females adults within the age range of 21- 50 years were enrolled for this study. One hundred of these participants were confirmed HIV positive, out of which fifty were on various antiretroviral drugs including the nucleoside / nucleotide reverse transcriptase inhibitors and the non-nucleoside transcriptase inhibitors classes of antiretroviral drugs. The remaining fifty persons who were HIV- negative served as the control group. Blood samples from the studied persons were analysed for alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, total protein, albumin, total bilirubin and conjugated bilirubin using automated clinical chemistry analyser, Hitachi 902 (Roche Diagnostic GMBH, Germany). The HIV- patients with or without antiretroviral drugs had their serum levels of alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase significantly raised ($p < 0.05$) compared with the control subjects. A significant rise ($p < 0.05$) was observed in serum level of total protein in the HIV patients who were yet to commence antiretroviral drugs when compared to the control subjects. However, the albumin levels of the HIV patients on the antiretroviral drugs and those who were yet to commence the antiretroviral drugs decreased significantly ($p < 0.05$) when compared with that of the control. The total bilirubin level of the HIV patients on antiretroviral drugs showed significant increase ($p < 0.05$) compared with the control subjects and the HIV patients who were not on antiretroviral drugs. This study reveals that both HIV infection itself and the antiretroviral drugs cause hepatic malfunction.

Keyword: Human immunodeficiency virus, Enzymatic markers, Non-enzymatic markers, Antiretroviral drugs.

Introduction

The advent and use of highly active antiretroviral therapy which consists of the nucleoside / nucleotide reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, integrase inhibitors, entry inhibitors, protease inhibitors and post attachment inhibitors in the management of human immunodeficiency virus (HIV) infected patients has put smile on the faces of these patients since the medication leads to non-progressive viral infection and increases their life span (Mastroianni, Lichtner and Mascia, 2014). Since HIV patients now live for longer period, it is imperative to investigate the functional status of the vital organs like the liver, kidney and the heart in this category of patients with a view to ascertaining the effect of this disease and or the highly active antiretroviral therapy on these organs.

Previous studies show that the complex nature of the medication used in antiretroviral therapy makes it difficult to understand the independent contribution of the individual drug in the development of liver injury by the drugs (Mankhatithan *et al.*, 2011; Sulkowski, 2004). As a result of the foregoing, the prevention and management of liver injury due to antiretroviral therapy has emerged as a major concern in the treatment of HIV/AIDS patients (Law, Dore and Duncombe, 2003; Mankhatithan *et al.*, 2011; Wondemagegn *et al.*, 2013). The longer HIV- infected patients live, the more they are predisposed to developing long term manifestations of long standing HIV infection and treatment complications (Emejulu, Ujowundu and Igwe, 2010). The destruction of the liver cells (hepatocytes) results in the release to the plasma the marker enzymes: aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase (Mankhatithan *et al.*, 2011). The analyses of the activities of these enzymes in the plasma are used to indirectly assess the activity of the liver. A rise in the plasma concentrations of these enzymes above the homeostatic limits implies a disorder to the liver tissue (Friday, Iniobong and Moses, 2010). In addition, abnormalities in the serum levels of total protein, albumin, total and conjugated bilirubin

are used in assessing the functional status of the liver (Okpa *et al.*, 2015; Jemikalajah and Adu, 2015).

In this study the enzymatic markers (alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase) and non- enzymatic markers (total protein, albumin and bilirubin) of the functional status of the liver in HIV patients with or without highly active antiretroviral drugs are investigated. Although some studies have been carried out on liver function in HIV patients elsewhere, there is lack of wealth of information in Makurdi.

Materials and Methods

A total of one hundred and fifty persons selected over three months duration from the HIV/AIDS clinic and the blood bank of the Federal Medical Centre, Makurdi were used for the study. They were stratified into 3 groups of fifty persons each. Group 1 comprised of fifty persons confirmed to be negative for HIV antibodies. These were blood donors at the blood bank who were used as the control subjects, group 2 consisted of fifty persons confirmed HIV positive but were yet to start antiretroviral therapy whereas group 3 was made up of fifty confirmed HIV patients who were already on antiretroviral drugs.

The HIV status of the participants was confirmed using Western Blot. The subjects comprised of adults of both sexes of age range 21- 50 years. The nature and purpose of the research were explained to the participants and they consented to it. Structural questionnaires were administered to the participants in order to obtain their demographic data and clinical history. It was ensured that the participants had no hepatitis B, C and E and that they had no past history suggestive of liver or renal disease.

Ethical Consent

The study was approved by the Ethical Committee on research of the Federal Medical Centre Makurdi, Benue State, Nigeria.

Sample Collection

Five milliliters of blood sample each were collected by venepuncture from the

participants. The site of collection was sterilized with cotton wool soaked in 70% alcohol. The blood samples were collected with minimal stasis (to prevent haemolysis) using disposable syringes and needles; and were put into plain sample bottles. These were allowed to stand at room temperature for the blood to clot and retract. This was followed by centrifugation at 4000rpm for 4 minutes. Analyses of the samples were done within twenty-four hours. Universal Safety measures were considered while handling the blood samples.

Determination of Biochemical Parameters

An Automated Clinical Chemistry Analyser, Hitachi 902 (Roche Diagnostic GMBH, Germany) was used to analyze alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, total protein, albumin, total bilirubin and conjugated bilirubin.

Statistical Analysis

The data obtained were analyzed for Mean \pm Standard Deviation. The level of significance was set at $p < 0.05$ confidence limit using paired student's t- test.

Results

There was significant rise ($p < 0.05$) in the serum levels of alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase of the HIV patients without antiretroviral drugs and those of the patients on antiretroviral drugs when compared with the control subjects. While there was no significant difference ($p < 0.05$) in the serum levels of alanine aminotransferase in both HIV patients on antiretroviral drugs and those who were yet to commence the treatment, the serum level of aspartate aminotransferase in the HIV patients

on antiretroviral drugs decrease significantly ($p < 0.05$) from those of the HIV patients yet to be treated with antiretroviral drugs. There was significant rise ($p < 0.05$) in the serum level of alkaline phosphatase of the HIV patients on antiretroviral drugs when compared with those living with the virus but had not commenced antiretroviral therapy (Table 1).

The serum total protein of the HIV patients who were yet to commence antiretroviral drugs increased significantly ($p < 0.05$) when compared with the control subjects. Treatment with antiretroviral drugs was seen to reduce the serum total protein significantly ($p < 0.05$) when compared with those of the HIV patients without antiretroviral drugs. The HIV patients on antiretroviral drugs and those not on antiretroviral drugs had their serum albumin levels reduced significantly ($p < 0.05$) when compared with those of the control subjects. The albumin level of the patients without antiretroviral drugs decreased significantly ($p < 0.05$) when compared with HIV patients that are on antiretroviral drugs. There was significant rise ($p < 0.05$) in the serum level of total bilirubin of the HIV patients on antiretroviral drugs when compared with those of the HIV- positive patients that were yet to commence antiretroviral drugs and those of the control subjects. The total bilirubin level of the HIV patients without antiretroviral drugs did not show any significant difference ($p > 0.05$) when compared with those of the control subjects. The serum levels of conjugated bilirubin of both the HIV patients on antiretroviral drugs and those not on antiretroviral drugs did not show any significant difference ($p > 0.05$) when compared with that of the control subjects (Table 1).

Table 1: Serum levels of liver marker enzymes, total protein, albumin and bilirubin in HIV patients and normal subjects.

Parameter	Control	HIV positive without antiretroviral drugs	HIV positive on antiretroviral drugs
Alanine aminotransferase (IU/L)	24.84 ± 7.30	28.20 ± 17.16 ^a	28.97 ± 15.15 ^a
Aspartate aminotransferase (IU/L)	31.84 ± 7.93	35.34 ± 14.14 ^a	33.54 ± 20.22 ^{ab}
Alkaline phosphatase (U/I)	73.26 ± 24.63	103.60 ± 36.46 ^a	120.28 ± 48.42 ^{ab}
Total protein (G/L)	83.35 ± 6.46	87.52 ± 9.26 ^a	83.00 ± 6.52 ^b
Albumin (G/L)	44.91 ± 3.38	38.74 ± 3.80 ^a	41.44 ± 3.22 ^{ab}
Total bilirubin (µmol/L)	10.03 ± 3.45	10.76 ± 5.54	14.57 ± 3.71 ^{ab}
Conjugated bilirubin (µmol/ L)	2.05 ± 0.89	2.68 ± 1.62	2.50 ± 1.34

Results with superscripts show significant difference ($p < 0.05$).

a = statistically significant from normal control ($p < 0.05$).

b = statistically significant from HIV positive without antiretroviral drugs ($p < 0.05$).

Discussion

The abnormalities observed in the serum levels of the aminotransferases, alkaline phosphatase, total protein, albumin, and total bilirubin indicate poor hepatic function (Subir *et al.*, 2013). The significant rise ($p < 0.05$) in the serum levels of alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase agrees with the findings of previous independent studies of Derick *et al* (2015) and Dusingize *et al* (2015) on HIV patients. These enzymes are found in the cytosol therefore their blood concentrations are normally low but whenever there is increased cell membrane permeability, hepatocellular injuries and necrosis of the liver cells, they are released into the blood circulation leading to increase in their concentrations in the blood (El- Hosseiny *et al.*, 2016).

The HIV is known to cause a direct damage to the hepatic cells through the gp 120 receptor which triggers metabolic pathway that results in the formation of reactive oxygen species (ROS). The ROS damages the liver cells leading to the release into the plasma of

these liver marker enzymes (Mastroiami *et al.*, 2014). The antiretroviral drugs are known to adversely affect liver cells in many ways which include causing metabolic abnormalities, fat accumulation in the hepatocytes, mitochondrial toxicity and insulin resistance (Hurlimann, Weber, Enseleit and Luscher, 2005). When these drugs cause injury to the mitochondria leading to mitochondrial dysfunction there is an initiation of a sequence of events beginning with lipid accumulation, membrane deterioration and the disruption of other energy dependent processes that subsequently result in necrosis (Chwiki *et al.*, 2017). Mitochondrial dysfunction leads to the impairment of mitochondrial fatty acid β - oxidation, tricarboxylic acid cycle and oxidative phosphorylation resulting in intracellular lipid accumulation. This contributes to the resulting hepatotoxicity which culminates in elevated serum liver enzymes (Chwiki *et al.*, 2017).

The significant rise in serum total protein level of the HIV positive patients without antiretroviral drugs in our study concurs with the findings of Arinola *et al*

(2005); Sarro *et al* (2010) and Kapenberg *et al* (2004). Our result, however, disagrees with earlier report of Okpa *et al* (2015) that serum total protein in HIV patients decreased significantly when compared with the control subjects.

The significant increase in serum total protein level in this work may be due to increased immunoglobulins (Arinola *et al.*, 2005). The elevation in immunoglobulin levels may be attributed to polyclonal β - cell activation as the HIV disease advances. The polyclonal β - cell activation is induced by viral envelope proteins especially glycoprotein 41 to produce excess abnormal immunoglobulins (Arinola *et al.*, 2005; Jemikalajah and Adu, 2015).

The significant decrease ($p < 0.05$) in the serum albumin level of the HIV positive patients in our finding is in consonance with the result obtained by Dusingize *et al* (2015); Ibeh *et al* (2013) and Subir *et al* 2013. Albumin is a protein produced in the liver. It transports substances like hormones, drugs and waste products through the blood (Melashu *et al.*, 2016). The significant fall in the level of albumin in this study may be as a result of malnutrition or chronic inflammation common in HIV/AIDS infection. This is because albumin is a negative acute phase reactive protein whose level can be depressed by elevated levels of tumour necrosis factor (TNF) and interleukin – 1 (IL-1) during chronic inflammation (Mehta *et al.*, 2006; Jemikalajah and Adu, 2015).

The significant rise ($p < 0.05$) in the serum level of total bilirubin seen in the HIV positive patients on antiretroviral drugs agrees with the findings of Subir *et al* (2013) and Vanwagner and Green (2015) who in their independent studies observed significant elevation in serum total bilirubin of HIV patients on antiretroviral drugs. Bilirubin is a waste product from the breakdown of the red blood cells. Prior to its passage through the liver where it is mixed with sugars to become water soluble, it is referred to as indirect or unconjugated bilirubin. As it passes through the liver, it is processed and then called direct or conjugated bilirubin. A damaged liver may be unable to process bilirubin resulting in an increase in total

bilirubin level as seen in our finding (Vanwagner and Green, 2015; Periklis, Efstathios, Angelos and Dimitios, 2017).

Conclusion

This work unveiled abnormal changes in serum levels of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, albumin, total protein, and total bilirubin in victims of the human immunodeficiency virus infection, both those on the antiretroviral drugs and those who were yet to commence the drugs. Liver function is, therefore, compromised in HIV infection.

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