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Hepatotoxicity of Antiretroviral Drugs in HIV Seropositive Nigerian Patients

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Abstract: Hepatotoxicity of antiretroviral (ARV) drugs using liver function parameters were assessed in 40 HIV seropositive subjects on ARV, 40 HIV seropositive subjects not on ARV and another 40 HIV seronegative subjects. They were all of the same age range of 19 – 56 years old. Results show that the serum activities of the liver enzymes and the concentrations of total and conjugated bilirubin were significantly ($p<0.05$) higher in the seropositive patients compared to the seronegative subjects. Similarly, total protein concentration of the HIV positive patients were significantly ($P<0.05$) reduced. A study of these parameters among the HIV seropositive groups, showed that administration of ARV drugs caused significantly ($p<0.05$) more effects on the parameters among the HIV seropositives on ARV drugs than on those not on any ARV drug. Sex of the patients was found not to significantly ($P>0.05$) influence the effects of HIV seropositivity and ARV drugs intake on the liver function parameters. The study affirms the potential risk of hepatotoxicity for HIV seropositive patients on ARV drugs and calls for continuous monitoring of ARV administration so as to prevent fatal effects of hepatotoxicity.

Key words: Antiretroviral drugs, Hepatotoxicity, liver function test, Nigeria.

INTRODUCTION

Human Immunodeficiency Virus (HIV) is a retrovirus known to be the primary aetiologic agent of Acquired Immunodeficiency Syndrome (AIDS). The HIV/AIDS epidemic has claimed more than 25 million lives and an estimated 39 million people globally are living with HIV, out of which approximately 4 million were newly infected with HIV in the year 2005. Of the estimated number of adults and children living with HIV or AIDS, 24.5 million (5.1%) reside in Sub-Saharan Africa (UNAIDS, 2006). The prevalence rate of HIV infection in Nigeria rose from 0.95% in 1988 to hit the red mark of 5.8% in 2002 (UNAIDS/WHO, 2002), with marked prevalence variations in different Nigerian cities (Ezioba, 2006).

People living with HIV and AIDS are prone to developing other illnesses and infections because of their suppressed immune system. Thus, without life saving drugs, one-third of children who are born infected with HIV die before their first birthday, and about 60 percent die by age 5 (Lamptey *et al,* 2006). Furthermore, because AIDS-related deaths are concentrated on the 25 to 45 age group, communities with high rates of HIV infections lose disproportionate numbers of parents and experienced workers, which has not only affected families, but also farms and other workplaces, schools, health systems and governments (Ashford, 2006).

During the acute or primary stage of HIV infection, the body's immune system tries to combat and control the spread of the viral particles. This ability is gradually lost due to the unique characteristic of the virus to seed and multiply in CD^{+4} T cells, thereby compromising the body's immunity (NIAID, 1999). The high HIV/AIDS related morbidity and mortality in developed countries has been dramatically reduced by the advent of effective combination of antiretroviral therapy (Harries, 2001). The antiretroviral therapies hamper the growth of the virus, thereby causing the suppression of viral particle multiplication and eventually leads to a decreased viral load, thereby prolonging the patient's life span. The impediments to effective use of triple antiretroviral therapy or highly active antiretroviral therapy (HAART) in public health systems in Sub-Saharan Africa include the cost of the drugs, the difficulties in administering and monitoring the drugs, fragile health infrastructures, weak drug procurement and distribution systems, as well as the side effects associated with the drugs and the development of drug resistance by the virus (Harris, 2002).

Liver toxicity is an important complication of HIV infection. As HIV-infected patients live longer, they develop long term manifestation of chronic HIV infection and treatment complication. Drug-induced hepatotoxicity is a frequent cause of liver injury. The pathogenesis of drug-induced liver disease normally

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involves the participation of the parent drug or its metabolite that either affects the cell biochemistry directly or indirectly by eliciting an immune response (Neil, 2004). All classes of antiretroviral drugs have been associated with potential risk of hepatotoxicity. However, the complexity of medication used in antiretroviral therapy complicates the understanding of the independent effects of each drug in the development of drug-induced liver injury (Sulkowski, 2004). Meanwhile, the hepatotoxicity of antiretroviral therapy in general and in relation to sex among the highly vulnerable black race has not been well documented particularly within this locality of Eastern Nigeria. Thus, this study was aimed at determining changes in the liver function profile of HIV/AIDS seropositive Nigerian patients on antiretroviral therapy.

MATERIALS AND METHODS

Study Area:

The study was carried out in Owerri, the capital city of Imo State, Nigeria. The State has a temperature range of between 20ºC and 30ºC, with a very high relative humidity of about 75% - 55% during the rainy season months of May – October. The inhabitants are mainly Ibos, with farming as their main occupation.

Study Groups:

The study group comprised of 80 (40 males and 40 females) HIV seropositive patients, aged 19 – 56 years old, attending HIV Clinic at Federal Medical Centre, Owerri. Out of the 80 seropositive subjects, 40 were newly diagnosed of the disease and were not yet on any antiretroviral drugs, while the other 40 were previously diagnosed HIV patients on antiretroviral drugs: Lavudine-SNP (CIPLA, INDIA) involving a combination of *Lamivudine, Stavudine* and *Nevirapine.* The HIV seropositive patients were age-matched with 40 (20 males and 20 females) HIV seronegative volunteers. All the subjects underwent HIV antibody assay with an ACON HIV1/2 Rapid Human Immunodeficiency Virus test strip (ACON Laboratories, USA). This confirmed the seronegative control subjects, while the seropositive subjects were further confirmed using Immunocomb HIV1/2 Biospot (Orgenics, Isreal). Two kits were used based on WHO (1992) recommendation of 2 different testing strategies (algorithms) involving ELISA and simple or rapid assays.

The study was approved by the Ethical Committee of Federal Medical Centre, Owerri. Informed consent was obtained from all participating subjects as recommended by WHO (TDR, 2003). This was done via an informed consent form duly completed by all the subjects.

Collection of Sample:

Five milliliters (5ml) of venous blood was obtained from each subject, dispensed into plain sample container without anticoagulant, allowed to clot and retract. The serum was separated from the clotted blood after centrifugation and used for the analyses.

Liver Function Tests:

The activities of alanine aminotransferase (ALT). aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were determined by Reitman and Frankel method and King Armstrong method as described by Balistreri and Shaw (1987). Total protein, total bilirubin and conjugated bilirubin concentrations were determined by the use of an automated chemistry analyzer (ciba-corning 550 Express plus, USA).

Statistical Analysis:

Data obtained were analyzed with student's "t" test and one-way ANOVA. Values for $p<0.05$ were considered statistically significant.

Results:

The liver function profiles of both the HIV seropositive and seronegative subjects are given in Table 1. It shows that the ALT activities of the HIV seropositive patients, both those on ARV (13.00 ± 4.70 IU/L) and those not yet on ARV (8.00 ± 2.45 IU/L) were significantly ($p<0.05$) higher than that of the seronegative control group (6.00 ± 1.78 IU/L). Similarly, the seropositive subjects' serum activity of ALP and concentrations of total and conjugated bilirubin were significantly ($p<0.05$) raised when compared to those of the control. Meanwhile, significant ($p<0.05$) changes in serum activities of AST and total protein concentration were seen only in the HIV seropositives on ARV, in comparison with the control. A comparison of the levels of the liver function parameters between the HIV seropositive patients on ARV and those not on ARV indicate significantly ($p<0.05$) higher values for patients on ARV, except for total protein concentration, where the

lower value obtained for patients on ARV (70.00±10.00 g/L) was also significant (P<0.05) as against that of their counterparts that were not on ARV (77.00 ± 9.20 g/L).

Table 2 shows the liver function profile of HIV seropositive subjects on antiretroviral drug with respect to sex. It shows slight but non significant (p> 0.05) increases in the values of almost all the liver function parameters of the male patients compared with those of their female counterparts.

Table 1: Liver Function Profile of the Subject

Parameter	Subjects		
	HIV seronegative (control; n = 40)	HIV Seropositive (on ARV; n = 40)	HIV Seropositive (not on ARV; n = 40)
ALT (IU/L)	6.00 ± 1.78[a]	13.00 ± 4.70[b]	8.00 ± 2.45[c]
AST (IU/L)	8.00 ± 2.42[b]	19.00 ± 6.70[b]	9.00 ± 2.50[a]
ALP (IU/L)	52.00 ± 12.23[a]	79.00 ± 28.70[b]	67.00 ± 13.20[c]
Total Protein (g/L)	80.00 ± 8.50[a]	70.00 ± 10.00[b]	77.00 ± 9.20[a]
Total Bilirubin (µmol/L)	8.62 ± 2.24[a]	13.79 ± 3.97[b]	10.34 ± 3.79[c]
Conjugated Bilirubin (µmol/L)	3.45 ± 1.72[a]	6.90 ± 2.59[b]	5.17 ± 2.07[c]

Values are in mean ± standard deviation. Values with different superscripts per row are significantly different (p<0.05). (ARV = Antiretroviral drug; n = number of sample).

Table 2: Liver Function Profile of HIV Seropositive Subjects on Antiretroviral Drug With Respect To Sex

Parameter	Sex	
	Male (n = 20)	Female (n = 20)
ALT (IU/L)	13.00 ± 3.68	12.00 ± 3.12
AST (IU/L)	19.00 ± 5.84	17.00 ± 3.63
ALP (IU/L)	87.00 ± 19.90	64.00 ± 20.97
Total Protein (g/L)	70.00 ± 7.80	73.00 ± 7.70
Total Bilirubin (µmol/L)	13.79 ± 2.24	13.45 ± 3.45
Conjugated Bilirubin (µmol/L)	6.90 ± 1.72	6.90 ± 2.24

Values are in mean ± standard deviation; n = Number of samples.

Discussion:

Although every tissue in human body has the ability to metabolize drugs, the liver is the principal organ. Drug biotransformation *in vivo* can occur by spontaneous non-catalysed chemical reaction, but majority are catalysed by specific cellular enzymes. At subcellular levels, these enzymes may be localized in the endoplasmic reticulum, mitochondria, cytosol, lysosomes, nuclear envelope and plasma membrane. The activities of these drug metabolizing enzymes in drug biotransformation have been implicated in the formation, instead of less biologically active metabolites, but of some metabolites with enhanced activity or toxicity including mutagenicity, carcinogenicity and hepatotoxicity (Neil *et al,* 2004).

It has been reported that liver enzyme elevations are common in HIV infected patients, and their diagnosis and management may be difficult because of the intricacies of the pathogenic mechanism involved. These include suspected hepatotoxicity related to highly active antiretroviral therapy (HAART) regimen, and immune allergic mechanisms. Furthermore, liver enzyme abnormalities may also reflect hepatitis B (HBV) or C (HCV) infections (Cacoub *et al,* 2004).

Results of the present study show that the activities of the liver enzymes were significantly (p<0.05) raised by the presence of HIV infection. The increase in the activities of these enzymes were further compounded by intake of the routine antiretroviral therapy. This observation may be associated with the involvement of the hepatocytes and Kupffer cells in metabolism of the drugs, attack and phagocytosis of foreign matters and remnants of damaged cells in blood stream. Furthermore, the increased activity in the liver cells and progressive cell damage occasioned by ravaging HIV infection in the system generally, will cause an increased toll on the liver cells. This will lead to weakening and death of liver cells and subsequent emptying of the intracellularly localized liver enzymes into the blood stream (Maurizio, 2004).

The impairment or depletion of hepatocyte defence mechanism in HIV infection, especially in combination with antiretroviral therapy is further portrayed by decrease in serum total protein concentration and increased bilirubin concentration in the blood, as observed in this study. The liver being the site for synthesis of most serum proteins, when unduly exposed to damage by viral infection and hepatotoxic drugs will inadvertently react by a drop in its biological functions which will include a drop in synthesis of serum proteins and metabolism of bilirubin (Balistreri and Shaw, 1987). This view is further supported by the reported finding that antiretrovirals produce mitochondrial toxicity in HIV seropositive subjects (Julio et al, 2004). Furthermore, apart from direct hepatotoxicity associated with ARVs, severe anemia (with increased serum bilirubin as a

consequence especially in the presence of hepatocellular damage) has been reported as the most frequent serious adverse events (Mandelbrot *et al,* 2001). On the other hand, there was no observed variation in the liver function profile of male and female HIV patients on antiretroviral drugs. This is probably because the courses of HIV pathogenesis and drug metabolism in humans generally are not sex dependent.

This study has shown that HIV infection and intake of antiretroviral drugs to combat the disease may expose the patients, whether male or female, to potential risk of hepatotoxicity. This makes liver disease and injury important factors for consideration in making antiretroviral decisions. Furthermore, more research is needed in this area especially with regards to mechanism, risk and management for antiretroviral therapy in the safest possible manner, so as to alleviate the anxiety of patients and their clinicians. A better understanding of these complex interactions, including adjustments of dosages of antiretroviral drugs, will probably help in the management of HIV – infected patients with liver function abnormalities.

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