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Full Length Research Article

Influence of ARVs on Some Biochemical Changes in Liver Non Enzymatic Markers of HIV Positive Patients Attending Specialist Hospital Sokoto, Nigeria.

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ABSTRACT: Both HIV infection and antiretroviral drugs (ARVs) are associated with abnormalities of liver function, revealed by both enzymatic and non-enzymatic markers. This study evaluated the effect of HIV infection and antiretroviral drugs on the liver non enzymatic marker (total and direct bilirubin, total protein and albumin level) of HIV positive patients (pre-highly active antiretroviral therapy and those on therapies) attending the voluntary counselling and testing units in the state. Seventy five subjects were enrolled into the study, which constituted 25 HIV negative individuals (control group), 25 HIV positive patient not on antiretroviral therapy and 25 on the therapy. Bilirubin, albumin, total protein and CD4 cell count were determined using standard methods. Significantly ($p<0.05$) higher level of total bilirubin, direct bilirubin and total protein in the HIV positive non-treated with ARVs was observed compared to the control group. At the initiation of antiretroviral therapy the levels of the three parameters were lower, though statistically not significant except for total protein. Albumin level and CD4 cell count were significantly ($p<0.05$) lower in the non-treated group compared to the control subjects. But at the initiation of therapy the serum levels of Albumin and CD_4 count was significantly ($p<0.05$) higher except for albumin level. HIV infection lowers the level of liver non enzymatic markers, which increase at the initiation of antiretroviral therapy.
Keywords: Bilirubin, Albumin, Total protein and CD_4 cell.

INRODUCTION

Hepatotoxicity is the most frequent (30%) toxicity of antiretroviral drugs, which has appeared as one of the leading causes of HIV related illness, death and treatment withdrawal (Nunez *et al.,* 2006). The extensive use of antiretroviral drugs by HIV positive patients as well as the new antiretroviral medications resulted in negative impacts on the clinical outcome of the patients (Palella*et al.,* 2006).

Abnormalities of liver function are frequent in HIV positive subjects and may be caused by other agents beside HIV itself; others may include: hepatitis viruses (major cause of death to the patients), systemic opportunistic infections, malignancies and ARVs induced hepatotoxicity (Kreisberg, 1995). The hepatotoxicity caused by antiretroviral drugs (ARVs) may be related to all the agents of the ARVs classes (Nickolas and Douglas, 2003). Severe and life threatening hepatotoxicity and fatal fulminant hepatitis have been reported in HIV positive patient taking mono therapy of antiretroviral drugs (Deniz and Tansu, 2002). The NRTIs (the back bone of ARVs) has severe hepatotoxic effect particularly zidovudine mono therapy, which subsequently increases the risk during combination therapy (Nickolas and Douglas, 2003). For the NNRTIs, the hepatotoxicity is commonly seen with nevirapine therapy which typically occurs after 4-5 months (Martínez *et al.,* 2001). Hepatotoxicity is also a well known adverse effect of protease inhibitors with ritonavir being the most frequent cause of hepatotoxicity (Nickolas and Douglas, 2003).

The severity of the liver disease may range from the absence of symptoms to liver decomposition, and the outcome can range from spontaneous resolution to liver failure and death (Clark *et al.,* 2002 and Kramer *et al.,* 2005). The severity of hepatotoxicity in HIV positive patients that 2% of patients are having acute hepatic necrosis and are dying from liver disease (Reisler *et al.,* 2003).

After the commencement of HAART, the reported incidence of severe liver toxicity ranges from 2 to 18% (Rodríguez-Rosado *et al.,* 1998 and Servoss *et al.,* 2006). Liver failure is the major leading cause of death among individuals being treated with antiretroviral drugs (Alive and Well, 2002). Other risk of liver disease includes alcohol consumption, because alcohol is a known hepatotoxin and its use has been associated with an increased risk of liver dysfunction (Nuñez *et al.,* 2006). Furthermore, cocaine is one of the causative agents of hepatotoxicity via a toxic oxidative metabolite, which induces mitochondrial damage

(Campos *et al.,* 2002). Other risk factors associated with liver disease include old age (Campos *et al.,* 2002), female gender (Martín-Carbonero *et al.,* 2003), first exposure to antiretroviral treatment (Wit *et al.,* 2002) and significant CD_4 cell gains following HAART initiation (Sulkowski *et al.,* 2002).

Being antiretroviral drugs the only mainstay for treatment of HIV infection as well as it's widely reported hepatotoxic effect, we assessed the effect of the both HIV infection and antiretroviral drugs on liver non enzymatic markers of HIV positive patients attending the ART centre of Specialist Hospital, Sokoto, Nigeria.

MATERIALS AND METHODS

Experimental Design

The study was conducted at antiretroviral therapy centre (ART) of Specialist Hospital, Sokoto, Nigeria. A total of 75 patients were enrolled into the study, which comprised 25 HIV negative subjects (blood donor and control), 25 HIV positive non treated with ARVs and 25 patients treated with ARVs. Inclusion criterion for the study includes: HIV-positive patients not on treatment and those on treatment for one year or less, individuals between the ages of 15-60 year old, individuals with no physical sign of metabolic syndrome and patient who are hepatitis (A, B, or C) negative.

Ethical Clearance

Ethical clearance was obtained from the research and ethical committee of Specialist Hospital, Sokoto and all patients were informed using a standard informed consent form.

Method used in assessment of parameters

Bilirubin determination: The colorimetric method of Jendrassik and Gorf (1938) was adopted. Bilirubin reacts with diazotized sulphanilic acid in alkaine medium to form a blue coloured complex. Bilirubin is determined in the presence of caffeine which releases albumin bound bilirubin by reacting with diazotized sulphanilic acid.

Albumin determination: The dye binding technique utilizing bromocresol green (BCG) as modified by Biggs and Doumas (1971) as employed. Albumin binds the indicator bromocresol (BCG) in an acidic medium resulting in the formation of a blue green albumin BCG complex. The coloured complex produced is proportional to the concentration of albumin in the serum.

Total Protein determination: This was determined using the method of Doumas (1975). Cupric ions, in an alkaline medium react with the peptide bonds of protein molecules, forming a blue violet coloured complex. The intensity of coloured complex produced is proportional to the amount of protein present in the serum.

CD_4 Cell Count: BD FACS count system automated analysis was used in accordance with the method of Cassens*et al.* (2004).

Analysis

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

RESULTS

In all the two groups of HIV positive subjects, the result show that most of the patients are at the age of 20 to 39 year having 64% and 84% in the non-treated group and treated subjects respectively. Furthermore, 28% and 12% of the HIV positive patients were within 40-59 years of age as shown in the non-treated and treated subject respectively (Table 1).

In the HIV positive patient, 56% and 64% in the non-treated and treated groups respectively were females. Males constituted 44% in the non-treated group and 36% in the treated group (Table 1).

The result further shows that 52% and 48% of the females in the non-treated and treated subjects respectively were mostly housewives (married) followed by single (16% and 32%) and widows (20% and 12%) in the non-treated and treated group respectively.

In the HIV positive non-treated with antiretroviral drugs, 56% of the patients were in clinical stage I of the infection followed by 28% in the stage II. For the treated subjects, majority of the patients (44%) enrolled in to the study were in the stage II of the disease, followed by 36% been in the clinical stage I (Table 1).
The total protein, total bilirubin, direct bilirubin of all the 25 HIV positive patients non-treated with antiretroviral drugs were significantly ($P<0.05$) higher than the

control group. However, in the treated subjects the level of total bilirubin, direct bilirubin and total protein were lower compared to the non-treated group, though the increase is statistically insignificant (P<0.05) except for the total protein. Albumin levels and CD_4 cell count of the non-treated subjects, show a significantly lower ($p<0.05$) compared to the control group. But at the initiation of antiretroviral therapy, the level of albumin and CD_4 cell count in the treated subject were higher though not statistically (P<0.05) significant except for the CD_4 cell count (Table 2).

Table 1: Demographic Information of the Study Subjects in Sokoto.

Parameters	Control	HIV+ Non-treated	HIV+ Treated
Age (years)			
1-19	1 (4%)	2 (8%)	1 (4%)
20-39	16 (64%)	16 (64%)	21 (84%)
40-59	8 (32%)	7 (28%)	3 (12%)
Gender			
Male	20 (80%)	11 (44%)	9 (36%)
Female	5 (20%)	14 (56%)	16 (64%)
Marital Status			
Single	15 (60%)	4 (16%)	8 (32%)
Married	5 (20%)	13 (52%)	12 (48%)
Divorced	3 (12%)	3 (12%)	2 (8%)
Widowed	2 (8%)	5 (20%)	3 (12%)
Clinical Stage			
Stage I	-	14 (56%)	9 (36%)
Stage II	-	7 (28%)	11 (44%)
Stage III	-	2 (8%)	3 (12%)
Stage IV	-	1 (4%)	2 (8%)

KEY: n= Sample size (25 per group).

Table 2: Biochemical Effect of HIV and ARVs on Liver non Enzymatic Indices and CD_4 Cell Count of Control, HIV Positive non-treated and HIV Positive treated used in the Study.

Parameters	Control	HIV+ Non-treated	HIV+ Treated
T-Bilirubin (mg/dl)	0.56±0.04*	0.79±0.04*	0.69±0.04
D-Bilirubin (mg/dl)	0.27±0.02*	0.43±0.01*	0.39±0.01
T-Protein (g/dl)	6.35±0.16*	8.75±0.15*a	6.95±0.28*a
Albumin (g/L)	9.93±1.91*	27.55±2.47*	30.40±0.89
CD_4 Cells (cell/µL)	965.76±25.93*	308.88±25.07*a	442.08±49.43a

KEY: n= Sample size (25 per group), Values are expressed as Mean ± SEM;
Values with the same superscript in column are statistically significant (P < 0.05);
D-Bilirubin: Direct Bilirubin, T-Bilirubin: Total Biliburin, T-Protein: Total Protein.

DISCUSSION

In the current study, the effects of HIV infection and antiretroviral drugs (ARVs) on liver non enzymatic markers which include total and direct bilirubin, total protein, albumin and CD_4 cell count were investigated.

Majority of the HIV patients were between the age of 20 to 39 years old, constituting 64% and 84% of the HIV positive non-treated group and HIV positive treated subjects respectively. Many factors may contribute to the higher HIV infection in the middle age people among which include their physical strength and high sexual activity which is major route for the HIV transmission. The finding also agrees with survey by Federal Ministry of Health, Nigeria (2003) and UNAIDS (2004).

Females form the majority of HIV patients, constituting 56% and 64% of HIV positive non-treated and treated subjects respectively. This may be due to practiced of some traditional practices like female genital mutilation (FGM) and having multiple sex partners as may occur in polygamous relationships of paramount importance is

the lack of western knowledge, in addition to other socioeconomic factors like increase in the commercialization of sex (FMOH, 2003). Survey by UNAIDS (2010), show that the rate of women infected with HIV rose from 43% in 1999 to 50% in 2010. The report further revealed that in Sub-Saharan Africa, women comprised 59% of the adult living with HIV infection in 2010. Moreover, another study conducted by the Federal Ministry of Health (FMOH) in 2003 and 2005 reported that in northern Nigeria over 40% of the married females are on polygamous system of marriage with also over 24% of males on the same marriage system. In 2010 National HIV sero-prevalence sentinel survey by the FMOH reported that majority of HIV infected people in Nigeria were women. The high practice of polygamous marriage in the state may be the core factor for the high rate of infection among women.

Our results further reveal that 52% and 48% of the HIV positive non-treated group and treated subject respectively were married. The result was contrary to a 2010 sentinel survey report by the FMOH which showed that widows have the highest rate (6.9%) followed by single (5.6%) and married with 4.9% (FMOH, 2010). The possible reason for this may be due to the fact that most of the men in the state possess more than one wife and consequently one infected husband is likely to infect two or more of his wives. Before the introduction of antiretroviral therapy most of the infected men die with the infection leaving their spouse (s) behind, hence the high number of widows than married. With the introduction of ARVs, the rate of death has drastically reduced, which makes the rate of married men and women higher than the widows.

Our study show an significant ($P<0.05$) increase in serum level of total, direct bilirubin and total protein in HIV positive patients not on treatment when compared with treated.

High level of bilirubin in the blood may be as a result of either the body system is producing excess bilirubin or the liver is unable to eliminate the excess bilirubin produced by the system (Naik, 2010). An elevated level of bilirubin in the blood of HIV positive patients may indicate liver disease due to HIV infection or antiretroviral drugs (Onwuliri, 2004).
The finding of this study was in line with a study by Onwuliri (2004) who reported that total and unconjugated bilirubin concentration were significantly higher in HIV infected subjects compared to control. Another study by Ayelagbe *et al.* (2014) reported significant increase in the level of total bilirubin in HAART naive subject compared to control group.

Many documented studies revealed that antiretroviral drugs induce liver damage through an elevation in the level of bilirubin. Our finding do not support these claims, as the levels decreased in treated subjects when compared with non treated group, though the increase is not statistically significant. The result of this finding was also contrary to the study by Ayelagbe *et al.* (2014) as reported significant increase in total and conjugated bilirubin of HIV positive on HAART subjects compared to HAART naïve subjects.

HIV infection is associated with remarkable alteration in the levels of proteins in the liver (Treitinger *et al.,* 2001). Protein abnormalities have been reported in all the stages of HIV infection (Grunfeld *et al.,* 1992 and Jaboor *et al.,* 1999). Jaboor *et al.* (1999) reported that acute phase response to infection is associated with an increase in protein turnover and remarkable degradation of protein. HIV infection induces leukocyte proliferation, increase in the synthesis of cytokines and immunoglobulin which was shown to contribute to protein turnover (Fleck, 1989).

Fuhrman *et al.* (2004) and Banh (2006) reported that serum total protein in HIV positive patients decreases during inflammation, which returns to normal level after resolution. Banh (2006) reported that the level of protein can be affected by factors like capillary permeability and drugs (like ARVs).

Furthermore, the result of this finding show that albumin level and CD_4 T-cell count of HIV positive non-treated with ARVs has significantly ($p<0.05$) decreased when compared to the control group. Comparing the level of these parameters of the non-treated group and treated subjects, the result revealed an increase in the level of albumin and CD_4 cell count in the treated group compared to non-treated group, though only for CD_4 cell count was found to be statistically significant ($p<0.05$).

Albumin synthesized by the liver helps maintain the amount of blood in the vein and arteries. Decrease in albumin level (hypoalbuminemia) in HIV positive patients may be due to increase in transcapillary escape rate and elevated catabolic rate against

decrease in hepatic synthesis caused by HIV infection (Fleck *et al.,* 1989).

Immune system activation as a result of HIV infection leads to inflammation (either acute or chronic) in HIV infected patients, which in turn lowers the levels of albumin through imposing liver to channels it to other proteins needed for immune response (Naik, 2010). Both HIV infection and antiretroviral drugs cause kidney disease, cardiovascular disease, and arthritis, which are potential source of chronic inflammation in people living with HIV infection (Fuhrman *et al.,* 2004).

CONCLUSION

Total bilirubin, direct bilirubin and total protein are significantly ($P<0.05$) higher in the HIV positive non-treated with ARVs compared to the control group. At the commencement of antiretroviral therapy the levels of these three parameters were lower, though statistically not significant ($P<0.05$) except for total protein. Albumin level and CD_4 cell count were significantly ($p<0.05$) lower in the non-treated group compared to the control subject. But at the beginning of the therapy they serum levels of Albumin and CD_4 cell count were significantly ($p<0.05$) higher except for albumin level. HIV infection lowers the level of liver non enzymatic markers, which antiretroviral drugs increase.

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