MONITORING OF HIGHLY ACTIVE ANTIRETROVIRAL THERAPY AMONG ASYMPTOMATIC HIV-INFECTED PATIENTS IN AKWA-IBOM: EFFECT ON LIVER AND KIDNEY FUNCTIONS

OLUGBENGA M. AJULO[1*] and CHINYERE NWAMURAMU[2]

1. Department of Clinical Pharmacy & Biopharmacy
   Faculty of Pharmacy
   University of Uyo
   Uyo. Akwa-Ibom State
   Nigeria
2. Faculty of Pharmacy
   University of Uyo
   Uyo. Akwa-Ibom State
   Nigeria

***Correspondence:** Dr. Olugbenga M. Ajulo
Email: ajugbeng@gmail.com
Phone: +2347030262468

Abstract

Introduction: New target is driven at achieving 90-90-90 treatment goal by 2020, whereby 90% of HIV-infected persons will know their HIV status, access HAART and have viral suppression. Improved access to HAART in sub-Saharan Africa led to the emergence of HAART-related toxicity and its management as component of HIV care in developing countries. The aim of this study was to evaluate the effects of HAART on liver and kidney of HIV-infected adult participants in a secondary healthcare facility.

Method: The participants came to the hospital after 3 months of adherence to the Highly Active Anti-retroviral Therapy (HAART) where phlebotomist collected 3mL blood from venous circulation for analysis of biochemical parameters. The blood samples were spun to obtain sera which were used for determining the amount of ALT, AST and creatinine by using ALT, AST and creatinine kits made by Randox® laboratory limited, United Kingdom. Creatinine clearance was calculated by using Cockcroft-Gault equation.

Result: ALT of 198 HIV-infected participants on HAART (11.21±9.78IU/L) was significantly (p=0.01) elevated than the 74 control participants (6.28±5.17IU/L). AST of HIV-infected participants on HAART (18.51±10.38IU/L) was significantly (p=0.00) elevated than the control participants (13.43±6.07IU/L). Creatinine clearance of HIV-infected participants on HAART (79.19±72.27mL/min/1.732m^2) was significantly (p=0.00) reduced than the control participants (132.34±53.06mL/min/1.732m^2).

Conclusion: Both biochemical parameters of liver were significantly elevated in HIV-infected participants on HAART which indicated hepatotoxicity. The biochemical parameter of kidney, creatinine clearance was significantly reduced in HIV-infected participants on HAART indicating renotoxicity.

Keywords: Toxicity, Liver, Kidney, HIV, HAART

INTRODUCTION

A recent report on global HIV infection indicated that 36.7 million people were living with HIV-infection with a 2.1 million new HIV-infection in 2015[1]. People living with HIV-infection who are enrolled on HAART reached 17 million globally in 2015[1]. Factors such as international donors, advanced clinical experience and research, improved treatment regimens and diagnostics and reduction in price of medicine have resulted to gains in efficiency and effectiveness of controlling HIV-infection[1]. The new inspiration is driven at achieving 90-90-90 treatment goal by 2020, whereby 90% of HIV-infected persons will know their HIV status, 90% of HIV-infected persons who know their HIV-positive status are accessing HAART and 90% of persons on treatment have viral suppression[1].

In developing countries, the annual expenditures of HIV/AIDS interventions have been tremendously increased over the last ten years 10 years with an estimated US$20 billion to be spent in 2017[2]. Highly active antiretroviral therapy (HAART) has been shown to increase survival of people living with HIV infection from 30% to 90% in developing countries[2]. Africa is leading the world at expanding access to HAART with 7.6 million people on HAART in 2013[3].

HAART was shown to avert 5.5 million deaths in developing countries from the peak in 1995 until 2012. Most of the averted deaths occurred in Sub-Saharan Africa[3]. HAART was shown to reduce the risk of HIV transmission by up to 96% and the risk of tuberculosis infection among people living with HIV by 65%[4,5]. HAART is highly effective at viral loads reduction in HIV-infected persons thereby slowing the spread of HIV infection across communities. HAART was reported to reduce the social costs of the HIV epidemic[6].

In 2013, WHO recommended that HAART should be commenced in all adult patients with a CD4 count of 500 cells per mm^3 and lower[7]. However, a new recommendation in 2015 stated that HAART should be initiated in all adults living with HIV regardless of WHO clinical stage and at any CD4 cell count[8]. This modification is due to the available evidences from systematic reviews and cohort analyses which suggested that untreated HIV infection might be associated with many non-AIDs associated conditions such as cardiovascular, liver and kidney diseases, cancer and neurocognitive disorders[9-12]. Earlier initiation of HAART has been shown to reduce non-AIDS associated conditions and improve survival. A recent study showed that earlier initiation of HAART among heterosexual serodiscordant couples substantially reduced sexual transmission of HIV to sexual seronegative partners[13].The new recommendation also stated that HAART should be initiated in all pregnant women and breast-feeding women living with HIV regardless of their WHO clinical stages and at any

CD4 cell count and continued unstopped[8]. Scientific evidences have shown numerous benefits for lifelong HAART in pregnant and breast-feeding women suggesting that all HIV-infected adults benefit from HAART at any CD4 cell count regardless of their WHO clinical stage of the disease[14,15].

Despite the increasing number of people using HAART in Africa, it was reported that seventy-five percent (75%) of HIV-infected adults in sub-Saharan Africa did not achieve viral suppression due to inadequate healthcare facilities and healthcare practitioners[3]. The consequence of improved access to HAART in sub-Saharan Africa is the emergence of HAART related toxicity and its management which has become an important component of HIV care in developing countries[16].

The incidence of drug induced hepatotoxicity in the US and Europe was reported to range from 1% to 10%[17-20]. Cohort studies from Haiti, Thailand, India, Zambia, and Malawi reported similar rates of nevirapine-induced hepatotoxicity, ranging from 1% to 7%[21,22-26]. A South African study showed a 17% incidence of life-threatening hepatotoxicity[27]. The aim of the study is to evaluate the effect of HAART on liver and kidney of adult HIV-infected patients attending a secondary healthcare facility in Akwa-Ibom state, Nigeria.

METHOD

The General Hospital, Anua was selected by convenience sampling. Ethical approval was granted by the management of the hospital for the study. The participants were acquainted with the study and were given consent forms to fill. Those who accepted to participate in the study and returned the filled consent forms were recruited into the study.

Inclusion criteria

The eligible participants for the study were confirmed asymptomatic HIV-infected ambulatory patients who received HAART regimens from St Luke General Hospital, Anua, Uyo for a year. They were 18 years of age and above. Both male and female HIV-infected patients were eligible.

Exclusion criteria

Symptomatic HIV-infected patients were excluded from the study. HIV-infected patients below the age of 18 years were excluded from the study.

Study design

The study was a prospective observational study.

Study site

The study was conducted in HIV clinic at the St Luke General Hospital Anua, Uyo, Akwa-Ibom State. The hospital was built by Christian missionaries in 1939. The hospital registered over 1000 HIV-infected patients since inception of the clinic but not all of them were attending the healthcare facilities regularly. The hospital is a 150 bed space facility with treatment focus on most areas of medicine. The hospital is surrounded by University of Uyo Teaching Hospital, University Health Centre and a lot of private hospitals in Uyo.

Study population

Having received ethical permission from the Hospital ethical committee to commence the study, the principal investigator talked with the ambulatory HIV-infected patients after the counselling session on HIV clinic visit to acquaint them with the study. The volunteered

patients received questionnaires and submitted filled questionnaires to the principal investigator who screened the volunteered patients for eligibility and recruitment to the study.

Sample size

There was no sample size calculation for the study because the focus of the study was to observe biochemical parameters of both renal and hepatic functions of all the eligible participants. The study involved 198 HIV-infected participants including male and female who had started Highly Active Anti-retroviral Therapy and 74 HIV-uninfected control participants. All eligible volunteers were recruited for the study.

Data collection

Participants' parameters such as age, sex, height, weight were determined and documented. Biochemical parameters of participants such as ALT, AST, creatinine were measured and documented.

Laboratory investigations

The participants underwent laboratory tests to evaluate the biochemical parameters of liver such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT) and kidney such as serum creatinine and creatinine clearance.

The participants came to the hospital after 3 months of use of Highly Active Anti-retroviral Therapy (HAART) where a phlebotomist collected 3 ml blood from venous circulation for analysis of biochemical parameters. The blood samples were spun to obtain sera which were used for determining the amount of ALT, AST and creatinine by using ALT, AST and creatinine kits made by Randox® laboratory limited, United Kingdom.

Determination of alanine aminotransferase (ALT) was done by using pipette to add 0.5 mL of buffer into a test tube without serum which was known as the sample blank tube. Pipette was used to add 0.5 mL of buffer and 0.1 mL of serum into another test tube which was known as the sample. Each of the test tubes was mixed and incubated for exactly 30 minutes at 37°C. Pipette was used to add 0.5 mL of 2, 4-dinitrophenylhydrazine and 0.1 mL of sample into the sample blank tube. A 0.5 mL of 2, 4-dinitrophenylhydrazine was added into the sample tube with the use of pipette. Each of the tubes was mixed and allowed to stand for exactly 20 minutes at 25°C. Then, 5.0 mL of Sodium Hydroxide (0.4 mol/L) was added both into the sample blank tube and the sample tube. Each tube was mixed and put in the Unispec 23D Spectrophotometer (UNISCOPE) made by Surgifriend Medicals, England. The absorbance of the sample was read against the sample blank after 5 minutes. The absorbance was compared with established values on the manufacturer's leaflets[28].

The same procedure was used for determination of AST. ALT buffer kit contained phosphate buffer (100 mmol/L), pH 7.4, L-alanine (200 mmol/L) and α-oxoglutarate (2 mmol/L). AST buffer kit contained phosphate buffer (100 mmol/L), pH 7.4, L-aspartate (100 mmol/L) and α-oxoglutarate (2 mmol/L)[28].

Determination of creatinine was done by mixing 10 mL of picric acid with 10 mL of sodium hydroxide at 25°C to make the working reagent. Pipette was used to add 2 mL of working reagent and 0.2 mL of standard solution into cuvette known as standard macro. Pipette was used to add 1 mL of working reagent and 0.1 mL of standard solution into cuvette known as standard semi-micro. Pipette was used to add 2.0 mL of working reagent and 0.2 mL of sample into cuvette known as sample macro. Pipette was used to add 1.0 mL of working reagent and 0.1 mL of sample into cuvette known as sample semi-micro. The cuvettes were mixed and put in the Unispec 23D Spectrophotometer (UNISCOPE) made by Surgifriend

Medicals, England. The absorbance of the standard and the sample were read after 30 seconds as A_1. The second absorbance was read 2 minutes after the first absorbance as A_2[28].

$A_2 - A_1 = \Delta A_{sample}$ or $\Delta A_{standard}$

Concentration of creatinine in serum (µmol/L) =

$\frac{\Delta A_{sample}}{\Delta A_{standard}}$ * Standard concentration (µmol/L)

Creatinine clearance was calculated from the value of creatinine by using Schwartz equation as given below.

Cockcroft-Gault formula[29]:

Creatinine clearance (ml/min) (females) = $\frac{140\text{-age(years)*weight(kg)}}{\text{Serum creatinine(µmol/l)}}$

Creatinine clearance (ml/min) (males) = $\frac{140\text{-age(years)*weight(kg)*1.2}}{\text{Serum creatinine(µmol/l)}}$

RESULTS

The result showed that 198 HIV-infected participants were on HAART with a mean age of 35.17±10.68 years consisting of 50 males and 148 females in group A. Group B involved 74 control participants with a mean age of 37.56±13.96 years consisting of 26 males and 48 females who were not HIV-infected and were not on HAART (Table 1).

The result of biochemical parameters of liver of male participants on HAART showed that ALT (11.68±8.49 IU/L) and AST (18.84±9.52 IU/L) were higher than ALT (6.57±5.97 IU/L) and AST (13.11±6.87 IU/L) of the male control participants respectively. The HIV-infected female participants on HAART had ALT (11.05±10.02 IU/L) and AST (18.40±10.70 IU/L)

which was higher than ALT (6.12±4.74 IU/L) and AST (13.61±5.66 IU/L) of the female control participants. ALT (p= 0.04) of the male HIV-infected participants varied significantly with that of the male control participants. AST (p=0.03) of the female HIV-infected participants on HAART varied significantly with that of female control participants (Table 2). ALT (p=0.01) and AST (p=0.00) of all participants on HAART varied significantly with those of the control participants (Table 3).

The result of biochemical parameters of kidney showed that the serum creatinine (108.22±29.42 µmol/L) of HIV-infected male participants on HAART was higher than the serum creatinine (63.03±17.53 µmol/L) of the male control participants. The serum creatinine (87.94±24.81 µmol/L) of the HIV-infected female participants on HAART was higher than the serum creatinine (62.04±16.38 µmol/L) of the female control participants. The creatinine clearance (71.49±20.25 mL/min/1.732m^2) of HIV-infected male participants on HAART was lower than the creatinine clearance (147.39±59.42 mL/min/1.732m^2) of male control participants (Table 4).

The creatinine clearance (94.13±27.98 mL/min/1.732m^2) of HIV-infected female participants on HAART was lower than the creatinine clearance (124.19±47.96 mL/min/1.732m^2) of female control participants (Table 4).

Table 1: Demographic parameters of study participants

Parameters	Participants on HAART	Control participants
Age (years)	35.17±10.68	37.56±13.96
Sex Male	50	26

Female	148	48
Total	198	74
Height (m)	1.57±0.08	1.60±0.06
Weight (kg)	61.05±12.38	66.55±6.42
*BMI (kg/m^2)	24.97±5.09	25.92±2.99

*p=0.04

Table 2: Biochemical parameters of liver based on gender

Participants	ALT (IU/L)		AST (IU/L)		RATIO	
	Male	Female	Male	Female	Male	Female
Participants on HAART	11.68±8.49	11.05±10.02	18.84±9.52	18.40±10.70	1.87±0.87	2.15±1.27
Control Participants	6.57±5.97	6.12±4.74	13.11±6.87	13.61±5.66	3.11±2.45	3.49±3.04
P-value	0.04	0.09	0.06	0.03	NS	NS

Normal value: ALT= 12IU/L, AST= 12IU/L. NS= Non-significant

Table 3: Biochemical parameters of liver for all participants

Participants	ALT (IU/L)	AST (IU/L)	RATIO
Participants on HAART	11.21±9.78	18.51±10.38	2.08±1.18
Control participants	6.28±5.17	13.43±6.07	3.35±2.83
p-value	0.01	0.00	NS

Normal value: LT= 12IU/L, AST= 12IU/L. NS= Non-significant

Table 4: Biochemical parameters of kidney

	Serum creatinine			Creatinine clearance		
	Male (μmol/L)	Female (μmol/L)	Total	Male (mL/min/1.732 m^2)	Female (mL/min/1.732m^2)	Total
Participants on HAART	108.22±29.42	87.94±24.81	93.90±26.83	71.49±20.25	94.13±27.98	79.19±72.27
Control	63.03±17.53	62.04±16.38	62.39±16.68	147.39±59.42	124.19±47.96	132.34±53.06

participants						
p-value	0.00	0.00	0.00	0.00	0.17	0.00

Normal values: Serum creatinine (male)=53-97μmol/L
Creatinine clearance (male)=97-137mL/min
Serum creatinine(female)=44-80μmol/L
Creatinine clearance (female)=88-128mL/min

The result showed that serum creatinine of the HIV-infected male participants on HAART varied significantly with that of the male control participants (p=0.00). The serum creatinine of the HIV-infected female participants on HAART varied significantly with the serum creatinine of female control participants (p=0.00). The creatinine clearance of HIV-infected male participants on HAART varied significantly with that of the male control participants (p=0.00).

Both the serum creatinine and creatinine clearance of HIV-infected participants on HAART varied significantly with the serum creatinine and creatinine clearance of control participants (p=0.00) (Table 4).

DISCUSSION

This study indicated that significant elevated ALT was observed among the male asymptomatic HIV-infected participants on HAART suggesting hepatotoxicity while significant elevated AST was observed among the female asymptomatic HIV-infected participants on HAART suggesting hepatotoxicity. Both ALT and AST of asymptomatic HIV-infected participants on HAART were elevated suggesting hepatotoxicity probably due to HAART. This report was consistent with earlier report which indicated that HAART was

responsible for hepatotoxicity in adults[30,31]. Puoti *et al*. indicated in their study that liver damage in patients treated with HAART was an emerging problem because of its increasing frequency and severe adverse clinical outcome[31]. Their study focused on incidence of severe hepatotoxicity. They indicated that liver damage could be caused by direct drug toxicity, pre-existing or concomitant liver damage by alcohol or concomitant medications. Sabin *et al.* reported that nevirapine and ritonavir were associated with hepatotoxicities[32]. They also reported that zidovudine, stavudine and didanosine caused hepatic steatosis which resulted to elevated ALT.

The observed abnormal serum creatinine in both male and female asymptomatic HIV-infected participants on HAART was probably due to the effect of HAART. The observed abnormal creatinine clearance in male asymptomatic HIV-infected participants on HAART was probably due to the effect of HAART.

Serum creatinine was abnormally higher in participants on HAART while the creatinine clearance was abnormally lower suggesting reduced renal function. This report was consistent with a previous study in Brazil which indicated that prolong use of HAART was associated with renotoxicity[33]. A study in South Africa reported renotoxicity with use of HAART regimen containing tenofovir after forty-eight months of initiation[34]. This study has shown that the first-line HAART regimen, zidovudine-lamivudine-nevirapine combination (AZT+3TC+NVP) used by the study participants were associated with renotoxicity and hepatotoxicity. Therefore, this cost-free HAART regimen should be considered for withdrawal as first-line HAART regimen in Nigeria as WHO considered tenofovir-lamivudine-efavirenz combination (TDF+3TC (or FTC)+EFV) as the new first-line HAART regimen[8]. The limitation of the study was lack of follow-up of study participants for a longer period as a result of financial constraint.

CONCLUSION

In conclusion, this study observed that there was associated elevation of ALT and AST as well as reduced creatinine clearance indicating hepatotoxicity and renotoxicity respectively among HIV-infected adults receiving HAART regimen.

A regular monitoring of both liver and kidney biochemical parameters of HIV-infected patients receiving HAART is hereby recommended in all HIV treatment centers.

CONFLICT OF INTEREST

There is no any known conflict of interest with the authors.

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