

Studies on Changes in Some Indices of Liver Function in HIV/AIDS Patients in Highly Active Anti-Retroviral Therapy (HAART) in Imo State, Nigeria

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Abstract

A study on changes in some indices of liver function amongst HIV/AIDS patients on highly active anti-retroviral therapy (HAART) was conducted in Imo State between February 2022 and August 2023. A total of 722 Venous blood samples, comprising 24 serum 1 from HIV seropositive patients receiving highly active anti-retroviral therapy (HAART), 241 samples from HIV seropositive patients that have not commenced HAART and 240 samples from the control group. The blood samples were collected using sterile needles and syringes and were analyzed using standard biochemical methods for serum bilirubin, aspartate aminotransaminase, alanine aminotransaminase, alkaline phosphatase, serum protein and lipid profile. HIV/AIDS Seropositive patients on HAART showed a significant increase ($P < 0.25$) in the mean aspartate aminotransaminase, alanine aminotransaminase, and alkaline phosphatase (10.32 ± 2.74 , 8.71 ± 0.85 , and 33.91 ± 0.37 in/L) and the control group (11.43 ± 2.73 , 8.58 ± 0.49 and 34.17 ± 0.39 in/L). However, HIV/AIDS seropositive patients receiving HAART with hepatitis B virus co-infection showed a more significant increase ($P < 0.05$) in the mean total and conjugated bilirubin, alanine aminotransaminase, aspartate aminotransaminase (48.2 ± 0.79 , 30.90 ± 0.79 , 37.72 ± 3.58 , 49.70 ± 3.58 and 186.14 ± 13.15 in/L). No significant difference ($P > 0.05$) was observed in the mean total cholesterol, triacylglycerol, HDL-Cholesterol, LDL-Cholesterol, Serum protein and bilirubin among HIV seropositive patients receiving HAART, when compared with the control group. The study has revealed that HIV/AIDS Patients on HAART are more likely to be predisposed to hepatic toxicity due to long-term dependency on HAART. Therefore, early diagnosis of hepatic dysfunction could promote safe and effective use of highly active anti-retroviral therapy among these patients.

Keywords: HIV/AIDS, Liver Function, HAART, Indices, Imo State.

Introduction

In Nigeria, the first case of HIV/AIDS was reported in 1986, in a sexually active 15years old girl (NBE, 1992). Since then, the number of people living with HIV/AIDS steadily increased and the pandemic has moved into a generalized state with decrease in sero-prevalence from 11.8% in 1991 to 5.8% in 2020 (Morgan et al., 2020). More than 2.1 million Nigerians are infected with HIV virus, the fifth highest in the world (Spinetti and Donalds, 2020). Since

the reported case of HIV/AIDS in Nigeria, the Federal Government has continued to respond to the pandemic through the implementation of various programs aimed at preventing, controlling and reducing its impact to the barest minimum. One of those programs initiated in the year 2001 was to provide affordable antiretroviral drugs to many Nigerians living with HIV/AIDS. The implementation of the program has not been without challenges and constraints, some of which include very high demand for drugs, low funding and notable side effects of the drugs (Barnett et al., 2021).

Highly active antiretroviral therapy (HAART) developed for the treatment of HIV/AIDS has resulted in significant decrease in morbidity and mortality among HIV infected patients (Rutschman et al., 2022).

Antiretroviral drugs now licensed for use in combination therapy include nucleoside analog reverse transcriptase (e.g., Zidovudine, Didanosine, Zalcitabine, Stavudine and Lamivudine), non-nucleoside reverse transcriptase inhibitors (e.g., delavirdine, and nelfinavir) and protease inhibitors (e.g., zalcitabine, zalcitabine, zalcitabine and nelfinavir) (Becher, 2020).

Several works have been carried out on the toxicological potentials of antiretroviral drugs, but in Nigeria where the scourge of HIV/AIDS epidemics is still high, little or no work has been carried out on the effects of HAART on the liver and this necessitated this research work in Imo State.

Since the liver is the central organ that modulates and coordinates most biochemical activities of the body, measurement of plasma enzymes of the liver can be of diagnostic importance. Plasma enzymes are those, though produced in the liver are present in the body in an equivalent or even higher concentration than in the tissue. The non-functional enzymes, though present in the plasma of normal individuals perform a known physiological function. Their presence in the plasma at levels elevated above normal values suggests an increased rate of tissue destruction. Therefore, measurement of these classes of plasma enzymes can provide valuable evidence on the toxicity of the liver tissue (Rutschman et al., 2022).

Selection of the combination regimen must take into account the patient's prior history of antiretroviral use, the side effects of the drugs and drug interaction that occur among these agents and with other drugs as well. Therefore, routine investigation of the liver function will facilitate safe and effective use of highly active antiretroviral therapy (HAART) and can help to prevent liver damage that usually occurs in the course of managing HIV/AIDS patients.

Research Questions

1. Do HIV/AIDS patients on highly ant-retroviral therapy experience change in liver enzymes?
2. What is the effect of anti-retroviral therapy in liver function?
3. Can monitoring liver enzymes of HIV/AIDS patients on highly active anti-retroviral therapy (HAART) be used to predict liver failure?

Objectives

1. To determine the effect of highly active anti-retroviral therapy (HAART) or liver enzyme of HIV/AIDS patients.
2. To identify the potential risk factors associated with toxicity in HIV/AIDS patients on anti-retroviral drugs.
3. To relate the effects of HAART on liver enzymes to age and sex of the patients.

Materials and Methods

Study Area

This study was carried out in Imo State. Imo State is in south Eastern Nigerian and lies on the attitude $5^{\circ}21^1$ N, $7^{\circ}2^1$ E, with a temperature range of between 20°C – 30°C and a high relative humidity of about 33% - 57% during the rainy season.

The State comprises of three senatorial zones, namely Owerri, Orlu and Okigwe, with a total of 27 local government areas. There are six university, two polytechnics and one college of education. Also, within the state, there are 17 general hospitals, and two teaching hospitals.

The inhabitants are mainly Igbos, while Christianity is the predominant religion in the state.

Study Group (Patients)

This group comprised of two hundred and forty- one (121 males and 120 females) HIV/AIDS seropositive patients aged $<1-70$ years from Imo State receiving highly active antiretroviral therapy (HAART) at specialist hospital Owerri, Imo State. Another group comprised of two hundred and forty-one (121 males and 120 females) who were newly diagnosed to be HIV positive and have not commenced HAART.

The diagnosis of these patients was based on two separate tests (routine HIV screening and a confirmatory test).

Patients' histories of alcoholism and drugs that can cause liver damage were taken. Also post exposure to viral hepatitis (HBsAg and HCV), were noted.

Control Group

This group comprised of two hundred and forty (120 males and 120 females) volunteers who were resident in Imo State. They were screened of HIV and confirmed to be negative. History of drugs and other substances that can affect the liver were taken.

Ethical Clearance

Ethical clearance was obtained from the Department of Medical Laboratory Sciences, Post Graduate Board of Imo State University Owerri. The Patients were duly informed of the purpose and details of the research work. Written consent was obtained from them as recommended by World Health Organization (WHO 2020).

Specimen Collection

Eight (8ml) of blood were collected from each subject and allowed to clot. The serum sample were obtained by centrifuging the clotted blood sample in a wisperfuge (model 684) centrifuge at 2500rpm for 5 minutes and serum obtained were used to assay alanine amino transaminase, aspartate amino transaminase, alkaline phosphatase, bilirubin, total protein, and lipid profile.

Statistical Analysis

Data collected were summarized as mean $\pm$ SD. Differences between individual groups were assessed by analysis of variance (ANOVA) and by students T-test. A P-value of less than 0.05 was considered significant.

Methodology for Total Cholesterol Estimation (*Friedwald et al., 1972*)

Principle:

Cholesterol in acetic acid gives a colour complex with ferric chloride and sulphuric acids. The colour intensity is proportional to the cholesterol contents in the plasma when measured calorimetrically.

Method:

Three centrifuge tubes were set up and labeled as follows: Test, Standard and Blank Tubes. 0.1 ml of plasma was added into the 'Test' tube while 0.1 ml of standard solution into the 'Standard' tube and 0.1 ml of distilled water to the 'Blank' tube. After that, 4ml of FeCl_2 was added to the three tubes respectively. The tubes were mixed and allowed to stand at room temperature for 10mins, then spun for 10mins.

Thereafter, three long test tubes were set up and labeled as Test, Standard and Blank; 2ml of the supernatant and 1.0ml of conc. H_2SO_4 were added and incubated at room temperature in the dark for 25mins then read at 540nm using spectrophotometer.

Calculation:

$$\frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \text{Conc. Of standard} = \text{mg cholesterol/100ml}$$

Methodology for High Density Lipoprotein Cholesterol Estimation (*Loffter, 2017*)

Principle:

Phosphotungstic acids extract HDL from total cholesterol, which gives a reddish colour with the ferric chloride reagent and sulphuric acid.

Method:

Into a long tube, 0.1ml of fresh serum, 0.05ml of MgCl_2 solution and 0.05ml of phosphotungstic acid were added. The tube was mixed and allowed to stand on the bench

for 60mins, then centrifuged at 3000rpm for 20mins. After that three long test tubes were set as follows: test, standard and blank and the following were added.

0.1ml of the supernatant was added into the tube for test, 0.1ml of standard solutions into the standard tube and finally 0.1ml of distilled water was added into the tube for blank. Also 3.0ml of colour reagent, was added to all the tubes.

The tubes were properly mixed and 1.0ml of conc. H_2SO_4 was added into each of the tubes respectively. They were mixed and incubated at room temperature in the dark for 25mins. The absorbance was measured at 540nm using spectrophotometer.

Calculation:

$$\frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \text{concentration of standard} = \text{Mg/dl}$$

Methodology for Triacylglycerol Estimation (Mendez et al., 1975)

Principle:

Triacylglycerol was extracted with heptane and hydrolyzed with conc. Potassium hydroxide solution to glycerol, which is oxidized by sodium metaperiodate to formaldehyde. Formaldehyde is condensed with acetylacetate and ammonia. The intensity of the final yellow colour produced is proportional to the concentration of triglycerides present in the sample.

Method:

Three long test tubes were set up and labeled as follows Test, Standard and Blank. Then 0.5ml of serum standard and distilled water was added to the respective tubes.

After that, 2ml of heptane's and isopropanol were added respectively, followed by addition of 1.8ml of sulphuric acid to the respective tubes.

The tubes were mixed for 30secs and allowed to separate without centrifugation.

After 3 test tubes were set as Test, Standard and Blank, followed by addition 2.0ml of heptane (Upper Layer) was added to all the tubes, followed by the additional 2.0ml of isopropanol and 0.6ml of saponification reagent to all tubes.

These tubes were mixed and allowed to stand for at room temperature for 5mins. After that, 1.5ml of metaperiodate and 2ml of acetyl acetate were added to all the tubes.

The tubes were mixed after each addition, covered and incubated at 60-70°C (Water bath) for 15mins. The tubes were removed and allowed to cool at room temperature.

The absorbance was read at 415nm against blank using a spectrophotometer

Calculation:

$$\frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \text{concentration of standard} = \text{mg/dl}$$

Methodology for Low Density Lipoprotein Cholesterol (LDL-C) (Sandkamp, 1990)

Calculation:

low density lipoprotein –cholesterol was calculated by modification of the fried Wald formula (Sand Kamp, 2001)

LDL – C =

$$\text{Total: } \frac{\text{cholesterol-HDL cholesterol} - \text{triacylglycerol}}{5}$$

Methodology for Serum Aspartate Aminotransaminase (Reitman and Frankel, 1957)

Principle is incubated at 37°C for exactly 60 minutes in a PH. 7.5 buffered substrate containing aspartate and ∞ -ketoglutarate.

Aspartate catalysis the transfer of the amino group from aspartate to ketoglutarate forming oxaloacetate and glutamate.

The oxaloacetate reacts with 2,4, dinitrophenyl hydrazine (DHpH) to form 2,4, dinitrophenyl hydrazine which in an alkaline medium gives a red brown colour. The absorbance of the colour produced was measured with a spectrophotometer at 540nm wavelength.

Method:

Three long tubes were set up and labeled as follows: test, standard and blank tubes.

In all the tubes 0.5ml of aspartate substance was added to the three test tubes and was incubated in water bath at 37°C for 5minutes.

Also, 0.1ml of serum was added to the 'test' tube, standard and blank tubes.

The tubes were thoroughly mixed and allowed to stand for 30 minutes in the water bath. Thereafter, 0.5ml of 2.4DNpH was added to all the tubes and was allowed to stand for 20 minutes at room temperature. Five milliliters of 0.5 normal sodium hydroxide was added to all the tubes.

Finally, 0.1ml of serum was added to the "blank" tube. The content of the tubes were read at 540nm wavelength using spectrophotometer.

Calculation:

$$\frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \text{concentration of standard} = \mu\text{g/l}$$

Methodology for Serum Alanine Aminotransminase (Reitman and Frankel, 1957)

Principle:

L-Alanine + ∞ $\rightarrow$ pyruvate + Glutamate

Pyruvate + 2, 4, DHPH-ine $\rightarrow$ pyruvate + 2,4, DHPH.

In this procedure alanine aminotransferase catalyzes L-alanine and, ∞ — ketoglutarate to form pyruvate and glutamate. The pyruvate then reacted with 2,4 dinitrophenylhydrazone, and the product was to be measured at 540nm wavelength.

Method:

Three long tubes were set up and labeled as follows test, standard and blank tubes. About 0.5ml of alanine substance was added to all the tubes and incubates for 5 minutes in water bath at 37°C.

After that 0.1ml of serum was added to the 'test' tube and allowed to stand for another 30 minutes in a water bath at 37°C. Also 0.5ml of 2,4 DNPH was added to all the tubes and was allowed to stay for 20 minutes at room temperature.

After that, 5ml of 0.5N NaOH, as well as 0.1ml of serum was added to all the tubes. They were incubated at 37°C for 30 minutes and were all read at 540nm wavelength using spectrophotometer.

Calculation:

$$\frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \text{concentration of standard} = i\mu/l.$$

Methodology for Serum Alkaline Phosphates (King – Armstrong, 1964)**Principle:**

Disodium phenyl phosphate was used as substrate; this is hydrolyzed liberating phenol and phosphoric acid. The phenol is estimated by its reaction with 4-amino phenazone to produce an orange colour in the presence of alkaline oxidizing agent.

Method:

Three long test tubes were set up and labelled test, blank and standard. In all the tubes, 1ml of phosphatase substrate was added, to all the test tubes, followed by 1ml of alkaline buffer.

Thereafter, 0.8ml of 0.5N NaOH was added to the test tube labeled blank.

The content of tubes were thoroughly mixed and allowed to stand for 5 minutes in a water bath at 37°C.

After the incubation 1.0ml serum was added in the 'test' tube while 0.1ml of standard was added to the 'standard' tube.

The tubes were allowed to stand for 15 minutes in the water bath followed by the addition of 0.8ml of 0.5N NaOH to the 'test' tube and 'standard' test tubes. The content of the tubes were thoroughly mixed.

Also 1.2ml of 0.5N NaOH was added to all the tubes followed by the addition of 1ml of 4-amino phenazone. Finally, 1ml of potassium ferricyanide was added to all the tubes and was read at 520nm wavelength using spectrophotometer.

Calculation:

$$\frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \text{concentration of standard} = i\mu/l.$$

Methodology for Estimation of Serum Bilirubin (Malloy and Evelyn, 1937)

Principle:

Serum bilirubin is coupled with diazotized sulphonilic acid to form diazotized bilirubin. The intensity of the colour developed is proportional to the bilirubin concentration in the sample.

Alcohol accelerates the reaction of the unconjugated bilirubin in the serum.

Method:

Three long test tubes were labeled as follows: standard, test and blank.

Also 1.8ml of distilled water was added to all the tubes, followed by addition 0.2ml of serum. About 0.5ml of diazo reagent was added to the 'test' test tubes, while 0.5 of diazo reagent was added to blank tube. Methanol was added to conjugated bilirubin, while distilled water was used for the unconjugated bilirubin. The contents of the tubes were read at 540nm using spectrophotometer.

Methodology for Hepatitis B Surface Antigen Screening

One drop of serum was added to HBs Ag strip and allowed to stay for 2 minutes. The presence of a pink line at the control portion of the strip indicates that strip is working. Also, the presence of a pink line at the portion specified patient indicates a positive result.

Diagnosis of HIV

Spot test: Two drops of buffer solution were added to the cassette, followed by the addition of two drops of serum. The serum samples were allowed for 2 minutes and washed off with 2 drops of buffer solution. Two drops of gold conjugation solution were added to the cassette. The presence of two pink dots at the control and test portions of cassette were interpreted as positive, while the presence of one pink dot at the control side only were also interpreted as negative.

HIV Confirmatory Test using Blot Method

The antigen samples were prepared by ultracentrifugation of cell free supernatant from HIV producing cell line while the proteins were separated by electrophoresis on a polyacrylamide gel. These resolved proteins were transferred onto nitrocellulose sheet between several sheets of absorbent paper, soaked in buffer for 2-3 days. The non-specific binding sites on the nitrocellulose paper were blocked by placing it in albumin at 37°C for 1 hour. Patients' serum were added and antibodies in patients' serum bound to their corresponding antigens. Antigen-antibody binding were detected by the addition of anti-human globulin labeled with horse-radish peroxidase which produced colored bands at the electrophoretic positions of the corresponding HIV antigen i.e. envelope glycoproteins gp 160, gp 120, gp 41. Reactivity to at least one antigen was considered positive.

Results

Table 1 shows the mean and standard deviation values for liver function and lipid profile in HIV seropositive patients before and on HAART, those co-infected with HBs Ag that are on and not on HAART and the control group.

The mean alanine amino transaminase, aspartate aminotransaminase and alkaline phosphate (29.07 ± 5.13 , 36.89 ± 3.81 and $123.56 \pm 9.13 \mu\text{L}$) in HIV seropositive patients receiving HARRT revealed a significant increase ($p < 0.05$) when compared with those of HIV-seropositive patients that have not commenced HARRT (8.71 ± 0.85 , 10.32 ± 2.74 and $33.91 \pm 0.037 \mu\text{L}$) and the control group (8.58 ± 0.49 , 11.43 ± 2.73 and $17 \pm 0.39 \mu\text{L}$) respectively.

Also, a significant increase ($p < 0.05$) was shown in the mean total and conjugated bilirubin, alanine aminotransaminase, aspartate aminotransaminase, and alkaline phosphatase.

(48.21 ± 0.79 and $30.90 \pm 0.79 \text{ mmol/L}$) (37.72 ± 3.58 and $186.14 \pm 13.15 \mu\text{L}$) respectively in HIV seropositive patients with hepatitis B virus co-infection when compared with those of HIV patients on HARRT (19.80 ± 0.14 , $4.93 \pm 0.20 \text{ mmol/L}$). (8.71 ± 0.85 , 10.32 ± 2.74 , $33.91 \pm 0.37 \mu\text{L}$), in HIV patients not on HARRT (9.21 ± 0.16 , $7.02 \pm 0.14 \text{ mmol/L}$), (29.07 ± 5.31 , 36.89 ± 3.81 , $123.56 \pm 9.13 \mu\text{L}$) and the control group (11.80 ± 0.14 , $5.33 \pm 0.20 \text{ mmol/L}$) (8.58 ± 0.49 , 11.43 ± 2.73 , $34.17 \pm 0.39 \mu\text{L}$) respectively.

The mean total and conjugated bilirubin, total cholesterol, triacylglycerol, HDL-cholesterol. LDL-cholesterol and total protein. (9.21 ± 0.16 , $7.02 \pm 4.78 \pm 0.63$, 1.50 ± 0.30 , 2.14 ± 0.92 and $1.32 \pm 0.82 \text{ mmol/L}$) ($7.20 \pm 1.16 \text{ g/l}$) in HIV-infected patients on HAART showed no significant difference ($P < 0.05$) when compared with those of HIV-seropositive patients that are not on HAART (10.80 ± 0.14 , 4.93 ± 0.20 3.82 ± 1.67 1.28 ± 0.62 1.60 ± 0.14 , and $1.13 \pm 0.21 \text{ mmol/l}$) ($7.43 \pm 1.20 \text{ g/l}$) and the control group (11.80 ± 0.14 , 5.33 ± 0.20 , 3.92 ± 0.75 , 1.35 ± 0.93 , 1.71 ± 0.14 and $1.60 \pm 0.52 \text{ mmol/l}$) and ($6.92 \pm 1.30 \text{ g/l}$) respectively. The sex related indices of liver function and lipid profile in HIV seropositive patients on HAART, before HAART, those co-infected with HBsAg on and not on HAART and the control group showed no significant difference ($p < 0.05$) in males when compared with female (Table 2).

As shown in table 4 the age group 51-70 years showed a more significant increase ($p < 0.05$) in the mean alanine aminotransaminase, aspartate aminotransaminase alkaline phosphate, total cholesterol (32.24 ± 2.14 , 37.9 ± 7.93 , $133.4 \pm 8.40 \mu\text{L}$) respectively when compared with the age group <1-10 years (18.16 ± 0.94 , 17.10 ± 4.33 , $31.40 \pm 2.42 \mu\text{L}$ and 2.02 ± 0.07 , 0.54 ± 0.47 , 0.71 ± 1.18 , $0.38 \pm 0.04 \text{ mmol/L}$) respectively.

TABLE 1: LIVER FUNCTION AND LIPID PROFILE LEVELS IN HIV/AIDS SEROPOSITIVE PATIENTS ON AND BEFORE HAART, THOSE CO-INFECTED WITH HBs Ag AND THE CONTROL GROUP.

PARA – METERS	HIV PATIENTS BEFORE HAART	HIV PATIENTS ON HAART	HIV NEGATIVE (CONTROL)	HIV POSITIVE WITH HBs Ag, WITHOUT HAART	HIV POSITIVE WITH HBs Ag AND ON HAART
TB $\mu\text{mol/L}$	10.80 $\pm$ 0.14	9.21 $\pm$ 0.16	11.80 $\pm$ 0.14	26.50 $\pm$ 0.10.15*	48.21 $\pm$ 0.79*
CB $\mu\text{mol/L}$	4.93 $\pm$ 0.20	7.02 $\pm$ 0.14	5.33 $\pm$ 0.20	11.1 $\pm$ 0.25	30.90 $\pm$ 0.79*
ALT $\mu\text{mol/L}$	8.71 $\pm$ 0.85	29.07 $\pm$ 5.31**	8.58 $\pm$ 0.49	22.30 $\pm$ 3.25*	37.72 $\pm$ 3.58*
AST $\mu\text{mol/L}$	10.32 $\pm$ 2.74	36.89 $\pm$ 3.81**	11.43 $\pm$ 2.73	29.98 $\pm$ 4.52*	49.70 $\pm$ 3.58*
ALP $\mu\text{mol/L}$	33.91 $\pm$ 0.37	123.56 $\pm$ 9.13**	34.17 $\pm$ 0.39	119.22 $\pm$ 9.30*	186.14 $\pm$ 13.15*
PROTEIN mg/dl	7.43 $\pm$ 1.20	7.20 $\pm$ 1.16	6.92 $\pm$ 1.30	6.49 $\pm$ 1.16	6.81 $\pm$ 1.34
CHOL mg/dl	3.82 $\pm$ 1.67	4.78 $\pm$ 0.63	3.92 $\pm$ 0.75	4.04 $\pm$ 0.99	3.55 $\pm$ 0.73
TRIGLCERIDE mg/dl	1.28 $\pm$ 0.62	1.50 $\pm$ 0.30	1.35 $\pm$ 0.93	1.22 $\pm$ 1.73	1.29 $\pm$ 0.24
HDL-CHOL	1.60 $\pm$ 0.04	2.14 $\pm$ 0.92	1.71 $\pm$ 0.14	1.53 $\pm$ 0.42	1.99 $\pm$ 0.93
LDL-CHOL	1.13 $\pm$ 0.21	1.32 $\pm$ 0.82	1.60 $\pm$ 0.52	1.45 $\pm$ 0.99	1.50 $\pm$ 0.33

*Significantly different from HIV patients before HAART and the control group

**Significantly different from HIV Patients before HAART and the control group

TABLE 2: LIVER FUNCTION AND LIPID PROFILE LEVELS IN RELATION TO SEX OF HIV/AIDS SEROPOSITIVE PATIENTS ON AND BEFORE HAART, AND CONTROL GROUP

PARA-METERS	HIV POSITIVE BEFORE HAART		HIV POSITIVE ON HAART		HIV NEGATIVE (CONTROL)	
	MALE	FEMALE	MALE	FEMALE	MALE	FEMALE
TB $\mu\text{mol/L}$	8.20 $\pm$ 0.20	9.06 $\pm$ 0.24	4.61 $\pm$ 0.59	3.93 $\pm$ 0.62	10.61 $\pm$ 0.12	9.42 $\pm$ 0.29
CB $\mu\text{mol/L}$	3.42 $\pm$ 0.13	3.87 $\pm$ 0.98	4.93 $\pm$ 0.08	4.10 $\pm$ 0.09	5.21 $\pm$ 0.04	5.04 $\pm$ 0.03
ALT $\mu\text{mol/L}$	9.13 $\pm$ 1.13	8.94 $\pm$ 0.98	24.84 $\pm$ 5.03	22.62 $\pm$ 0.41	8.43 $\pm$ 0.21	7.84 $\pm$ 0.49
AST $\mu\text{mol/L}$	27.14 $\pm$ 0.77	31.09 $\pm$ 0.43	119.32 $\pm$ 0.63	117.09 $\pm$ 8.07	31.82 $\pm$ 1.24	11.12 $\pm$ 1.17
ALP $\mu\text{mol/L}$	5.81 $\pm$ 0.84	6.13 $\pm$ 1.04	6.80 $\pm$ 0.16	7.10 $\pm$ 1.10	6.53 $\pm$ 1.13	7.21 $\pm$ 1.51
PROTEIN mg/dl	3.21 $\pm$ 0.47	3.40 $\pm$ 0.24	4.63 $\pm$ 0.31	4.71 $\pm$ 0.15	3.61 $\pm$ 1.19	4.11 $\pm$ 0.97
CHOL mg/dl	1.03 $\pm$ 0.18	1.12 $\pm$ 0.66	1.3 $\pm$ 0.33	1.21 $\pm$ 0.52	1.50 $\pm$ 0.69	1.21 $\pm$ 0.82
TRIG mg/dl	1.41 $\pm$ 0.59	1.34 $\pm$ 1.12	2.01 $\pm$ 1.14	2.10 $\pm$ 0.55	1.142 $\pm$ 0.21	1.95 $\pm$ 0.72
HDL-CHOL	1.04 $\pm$ 0.04	1.09 $\pm$ 0.30	1.22 $\pm$ 0.14	1.31 $\pm$ 0.42	1.81 $\pm$ 0.39	1.39 $\pm$ 0.63

TABLE 3: LIVER FUNCTION AND LIPID PROFILE LEVELS IN RELATION TO AGE OF HIV/AIDS SEROPOSITIVE PATIENTS BEFORE HAART

AGE	TB μmol/L	CB μmol/L	ALT iμ/L	AST iμ/L	ALP iμ/L	CHOL Mg/dl	TRIG Mg/dl	HDL- CHOL Mg/dl	LDL- CHOL Mg/dl	PROTEIN Mg/dl
<1-10	6.60± 0.04	3.41± 0.14	5.62± 0.22	7.01± 2.02	19.41± 0.33	2.10± 0.27	0.6± 0.04	0.52± 0.12	0.43± 0.07	6.21± 0.52
11-30	8.51± 0.22	4.21± 0.42	7.91± 2.01	9.21± 1.02	23.41± 0.08	2.72± 1.05	0.9± 0.55	0.85± 1.18	0.65± 0.94	6.84± 1.33
31-50	10.9±	5.11± 0.49	8.52± 0.44	10.71± 2.10	35.34± 0.52	3.03± 1.63	1.14± 0.94	1.4± 1.04	0.94± 0.04	7.94± 1.33
51-70	11.94± 0.09	6.96± 0.57	9.66± 0.76	12.13± 0.39	34.21± 0.11	3.99± 2.14	1.37± 0.50	1.73± 1.06	1.24± 0.26	8.34± 1.13

TABLE 4: LIVER FUNCTION AND LIPID PROFILE LEVELS IN RELATION TO AGE OF HIV/AIDS SEROPOSITIVE PATIENTS ON HAART

AGE	TB μmol/L	CB μmol/L	ALT iμ/L	AST iμ/L	ALP iμ/L	PROTEIN Mg/dl	CHOL Mg/dl	HDL- CHOL Mg/dl	LDL- CHOL Mg/dl	TRIG Mg/dl
<1-10	5.84± 0.52	3.04± 0.21	18.16± 0.94	17.10± 4.33	31.4± 2.42	6.33± 0.34	2.02± 0.07	0.71± 1.18	0.38± 0.04	0.54± 0.47
11-30	8.82± 0.09	5.22± 0.14	26.18± 1.34	29.41± 2.41	120.11± 2.37	7.94± 1.54	2.98± 0.18	1.16± 0.93	0.71± 0.36	1.01± 0.73
31-50	9.88± 0.01	6.82± 1.14	28.44±* 1.50	35.93±* 5.32	130.24±* 4.41	8.21±* 0.94	4.11±* 1.59	2.21±* 1.59	1.11±* 1.43	1.27±* 1.08
51-70	10.19± 1.23	7.94±	32.24±* 2.14	37.9±* 7.93	133.4±* 8.40	7.99±* 1.09	5.21±* 1.39	1.94±* 1.36	1.40±* 1.35	1.66±* 1.34

Discussion

Liver toxicity is a growing problem among HIV patients, particularly in those who have been on highly active antiretroviral therapy (HAART) for many years, and among those who are co-infected with hepatitis B and C virus (Marks and Bell, 2021).

This study shows a significant increase ($P < 0.05$) in the mean alanine aminotransaminase, aspartate aminotransaminase and alkaline phosphate in HIV- seropositive patients on highly active antiretroviral therapy (HAART) when compared with those of HIV seropositive patients that have not commenced HAART.

This finding is in conformity with the report of earlier work by Maat et al (2019) in Maryland, London, who reported that liver enzymes are elevated in HIV infected patients especially those on highly active antiretroviral therapy.

Also, Pout et al (2023) in their work in Netherland reported that HIV related liver toxicity has become an important cause of 8.6% morbidity and 4.8% mortality of HIV hospitalized patients.

Pezzotti et al (2020) in a prospective study of patient who initiated antiretroviral therapy in Italy reported a significant increase in hepatic enzymes, among 1500 patients initiating HAART, 3% were found to have acute hepatic diseases for over 2 years.

In a related study by Spinetti and Donalds (2020) in Brescia, Italy, a retrospective comparison of in-hospital liver-related mortality before, and during the advent of HAART was studied. They reported that liver failure contributed to death in 12% of 308 patients who died between 2017 and 2018 compared with 33% of 46 patients who died between 2019 and 2020.

Report from Manfredi and Hopkins (2022) in Uganda revealed that 69 patients (52.3%) out of 132 patients developed transaminase elevation of any grade during the 24-month study period, while severe hepatotoxicity was observed in 8 (6.1%) patients. However, antiretroviral therapy had to be interrupted in only one case because of clinical jaundice. The peak in the increase of mean transaminase concentration appeared after a mean period of 13.1 months from the start of HAART.

In as much as these finding reported by various researchers agreed with the present study, it disagrees with the reported normal hepatic enzymes by Buski, (2019) in HIV-seropositive patients on HAART in Kenya.

Furthermore, the mean conjugated and unconjugated bilirubin in HIV_seropositive patients on HAART reported in this study did not show any statistical differences ($P > 0.05$) when compared with the control.

This finding agrees with that of Olufemi et al (2023) in Western Nigeria who reported among HIV – patients on HAART, an elevated liver enzyme with no detectable increase in the unconjugated and conjugated bilirubin. Manfredi and Hopkins (2022) reported one case of clinical jaundice out of 132 patients on HAART in Uganda. In this study also the mean unconjugated and conjugated bilirubin, alanine-aminotransaminase, aspartate aminotransaminase and alkaline phosphatase in HIV seropositive patients on HAART, with hepatitis B virus co-infected revealed a more significant increase ($p < 0.05$) when compared with the control group. This conforms with a related work by Martinez et al (2023) in South African, who reported an elevated bilirubin and transaminase in HIV-patients on HAART, con-infected with hepatitis B virus.

Mohammed *et al* (2021) in a retrospective study in Northern parts of Nigeria, on the incidence and risk factors of severe (grade 4) liver enzyme alteration in patients on HARRT, reported an incidence of 6.3% with significant increase in patients with hepatitis B and C virus co-infection.

This finding also conforms with the work of Marks *et al* (2021) in Botswana, who associated HIV-liver enzyme elevation to elevated plasma level of the drug and viral hepatitis B and C co-infection. They reported that serum drug concentration $> 6\mu\text{g/ml}$ was associated with a 92% rate of liver toxicity, in a pharmacokinetic study involving 70 HIV-infected patients on HARRT.

Chirac *et al* (2018) in France reported an elevated transaminases and alkaline phosphatase and serum bilirubin, which was due to concurrent drug therapy and co-infection of hepatitis

B and C virus. This study also show that the age group 51-70 years had a more significant increase ($p < 0.05$) in their hepatic enzyme and bilirubin levels.

This finding disagrees with the work of bursek and jugal (2019) who in a retrospective study of HIV positive patients on HARRT, reported that the incidence of transaminase increase was not associates to sex, age and risk factors, rather the development of liver injury was due to hepatitis B and C virus co-infection.

The reported elevation in the transaminase, alkaline phosphatase and bilirum levels in HIV-seropositive patients on highly active antiretroviral therapy (HARRT) could be due to mitochondrial toxicity of the drugs.

In addition to impairing inhibitors, triphosphate also inhibits a human polymerase called "gamma-polymerase" which is responsible for the replication of mitochondrial DNA (MT DNA) (Carr 2020).

In the liver, mitochondrial toxicity is associated with increased lipid deposits, elevated liver transaminases and alkaline phosphatase (Lambert, 2019).

Mitochondrial liver toxicity is now observed in patients undergoing treatment with all nucleoside reverse transcriptase inhibitions that have a relatively strong potential to inhibit gamma-polymerase, especially with antiretroviral drugs like didanosine, stavudine and zalcitabine.

However, it has been demonstrated in the hepatic tissues of HIV positive patients that antiretroviral drugs can lead to a time dependent mitochondrial DNA depletion. (Loffler 2017).

The study also showed no significant difference ($p > 0.05$) in total cholesterol Triglyceride, HDL Cholesterol and LDL Cholesterol level among HIV/AIDS patients on HAART, when Compared with the control group. The result of the present study is also in disagreement with the report of Maat *et al* (2019) who reported an elevated level of total cholesterol and triglyceride among HIV/AIDS patients on HAART in Sub-Saharan Africa.

The difference in the report of Maat *et al* (2019) and the result of the present study could be due to the fact that most of the HIV/AIDs patients were on HAART for less than one year.

Conclusion

This study has demonstrated that certain indices of liver function like aspartate aminotranaminase, alamine aminotransaminase and alkaline phosphatase are usually elevated in patients.

Since liver toxicity has been identified as a major problem among HIV /AIDS patients on HAART routine investigation of liver function parameters among these patients could facilitate safe and effective use of HAART in the management of HIV/AIDs.

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