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Evaluation of the Liver Enzyme (AST, ALT & ALP) Levels of Adult HIV Patients on HAART in UPTH

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Authors' contributions

This work was carried out in collaboration among all authors. Authors CFA and EOAJ conceptualized the research and formulated the topic, co-designed the study, co-drafted the ethical clearance proposal. Author EOAJ analyzed the data and author TOJ managed the literature searches, harmonized sectional write-ups and provided the final manuscript. All authors participated in data collection, read, reviewed and approved the final manuscript.

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ABSTRACT

This study evaluated the liver enzyme levels of HIV-infected adult patients on highly active antiretroviral treatment (HAART) for not less than 1 year. The study was designed as a randomised cross-sectional study that evaluated the differences in the aspartate aminotransferase [AST], alanine transaminase [ALT] and alkaline phosphatase [ALP] of 129 (46 males and 83 females) HIV-infected adult patients. Before the study, ethical clearance (Ref: UPH/CEREMAD/REC/19) was obtained from the University of Port Harcourt Ethics Committee. Venous blood samples were obtained and the liver enzyme levels were analysed using Clinical Chemistry Analyser (VS10) manufactured by Vitro Scient. Values were further categorised into; normal or abnormal. SPSS version 21 (IBM® Armonk, USA) and Graph Pad Prism (Version 8.0.2) was used to analyse the data. T-test compared the sex differences in mean, while Chi-square analysis tested the sex differences in the categorised data. From the result, the mean AST (27.65±17.93 IU/L) and ALP (135.13±10.87 IU/L) values for males were higher than females AST (27.25±18.93 IU/L) and ALP (132.65±9.96 IU/L) values, while ALT was higher in female (34.66±22.29 IU/L) than males (33.75±18.14 IU/L); however, the differences were not significant (p>0.05). Generally, abnormal

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AST, ALT and ALP levels were 31%, 34% and 82% respectively; with no sex-associated differences (p>0.05). 45.7% of the patients (males: 25 [54.3%] and females: 34 [41.0%]) had cholestatic abnormality, while 8.5% (5; 10.9% males and 6; 7.2% females) had hepatocellular abnormality, 18.6% (6; 13.0% of males and 18; 21.7% of females) and mixed abnormality (AST/ALT/ALP). In conclusion, cholestatic abnormalities were observed in more than 80% of the patients as opposed to hepatocellular abnormalities, which were less than 35%. More females were associated with mixed abnormality when compared to males with independent (ALT/AST) abnormality. This study, therefore, suggests the need for a randomised case-control study to highlight the extent of deviation from normal values.

Keywords: Enzyme levels; liver; HAART; HIV-infected adults; sex.

1. INTRODUCTION

The term Highly Active Antiretroviral Therapy (HAART) refers to the customized combination of three or more antiretroviral agents [1] which generally consists of a combination of Nucleoside Analog Reverse Transcriptase Inhibitors (NRTI) plus a Protease Inhibitor (PI) and Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTI) [2]. The increased availability of the different combinations has been identified to have the potential to increase the hepatotoxicity susceptibility. Studies by Ngala et al. [3] Wambani et al. [4] and Neukam et al. [5] suggest that prolonged use of any class of ART is implicated in hepatotoxicity that is often seen in patients undergoing drug treatment; however, the extent to which each of these drugs induces or contributes to hepatotoxicity is varied [3].

Alanine transaminase (ALT) is an enzyme found in the liver that helps convert proteins into energy for the liver cells. When the liver is damaged, ALT is released into the bloodstream and levels increase. Aspartate transaminase (AST) is an enzyme that helps metabolize amino acids, it is also normally present in blood at low levels, and an increase may indicate liver damage, disease or muscle damage. Alkaline phosphatase (ALP) is an enzyme found in the liver and bone and is important for breaking down proteins. Higher-than-normal levels of ALP may indicate liver damage or diseases, such as a blocked bile duct, or certain bone diseases [6].

There is evidence that when patients commence ART, 14–20% will present with elevated hepatic enzymes [7] with a recent report suggesting up to 60% [8]. However, co-infection with hepatitis B or C virus [9,10,11] and tuberculosis treatment [12] have also been implicated in mild hepatotoxicity. In Tanzania, efavirenz and rifampicin-based hepatotoxicity occurred in HIV patients with or without tuberculosis (TB) co-infection; however, the effects were mild, thus therapeutic modifications were not needed [13]. Whereas, in a recent case-study by Segamwenge and Bernard [14] four patients were reported to been diagnosed with acute liver failure within one month of switching to an efavirenz-based antiretroviral therapy.

It has been suggested that sex-specific thresholds be applied when investigating liver enzyme levels because women have slightly lower normal ALT levels than men [15]. This was evident in a study conducted in the U.S. that identified an ALT upper limit of 29 IU/L for men and 22 IU/L for women [16]. Additionally, in the study by Wu et al. [17] suggesting that liver enzymes in the aetiology of diseases, abnormal elevations may differ by sex, it is therefore imperative to investigate the role of sex in liver enzyme elevation among HIV-infected patients on HAART.

2. MATERIALS AND METHODS

2.1 Research Design

This study was designed as a “cross-sectional” part of the randomized comparative study (CEREMAD, 2016) by Anyanwu et al. [18]. The study population involved HIV-positive patients, who were on HAART for not less than 1 year and whose CD4 count was not below the critical value of 200 cells/mm^3. Personal information such as age, sex, lifestyle (alcohol and cigarette consumption), pregnancy state for the females, duration of HAART and HIV co-infections (such as pulmonary tuberculosis, hepatitis B & C) history were obtained from their hospital folders and pre-tested questionnaires.

2.2 Study Population

The clinic had over twelve thousand (12,000) registered HIV patients. Data for 900 HIV-

infected patients undergoing HAART at University of Port Harcourt Teaching Hospital as at 2015 were collected. However, 392 patients were physical present for recruitment.

2.3 Sampling Technique

This study utilized randomised purposive sampling method.

2.4 Eligibility and Enrolment

1. Inclusion criteria - patients: (i) diagnosed with HIV infection and undergoing HAART; (ii) aged >20 years; (iii) continuing HAART (≥12 months).
2. Exclusion criteria - patients (i) with acute HIV infection; with (ii) co-infections or severe, life-threatening complications; (iii) who were pregnant; (iv) with autoimmune diseases; (v) with incomplete data. (vi) older than 55 years of age [19,20].

2.5 Methods of Data (sample) Collection/Instrumentation

Venous blood samples were collected and serum levels of Aspartate aminotransferase [AST], Alanine transaminase [ALT] and Alkaline phosphatase [ALP] were determined using Clinical Chemistry Analyser (VS10) manufactured by Vitro Scient. The machine utilises the operational principle guided by Beer-lambert's law (that is; the linear relationship between absorbance and concentration of an absorbing species). Normal range values were established in units per litre as follows; ALT (7 to 55 U/L), AST (8 to 48 U/L) and ALP (40 to 129 U/L) [6].

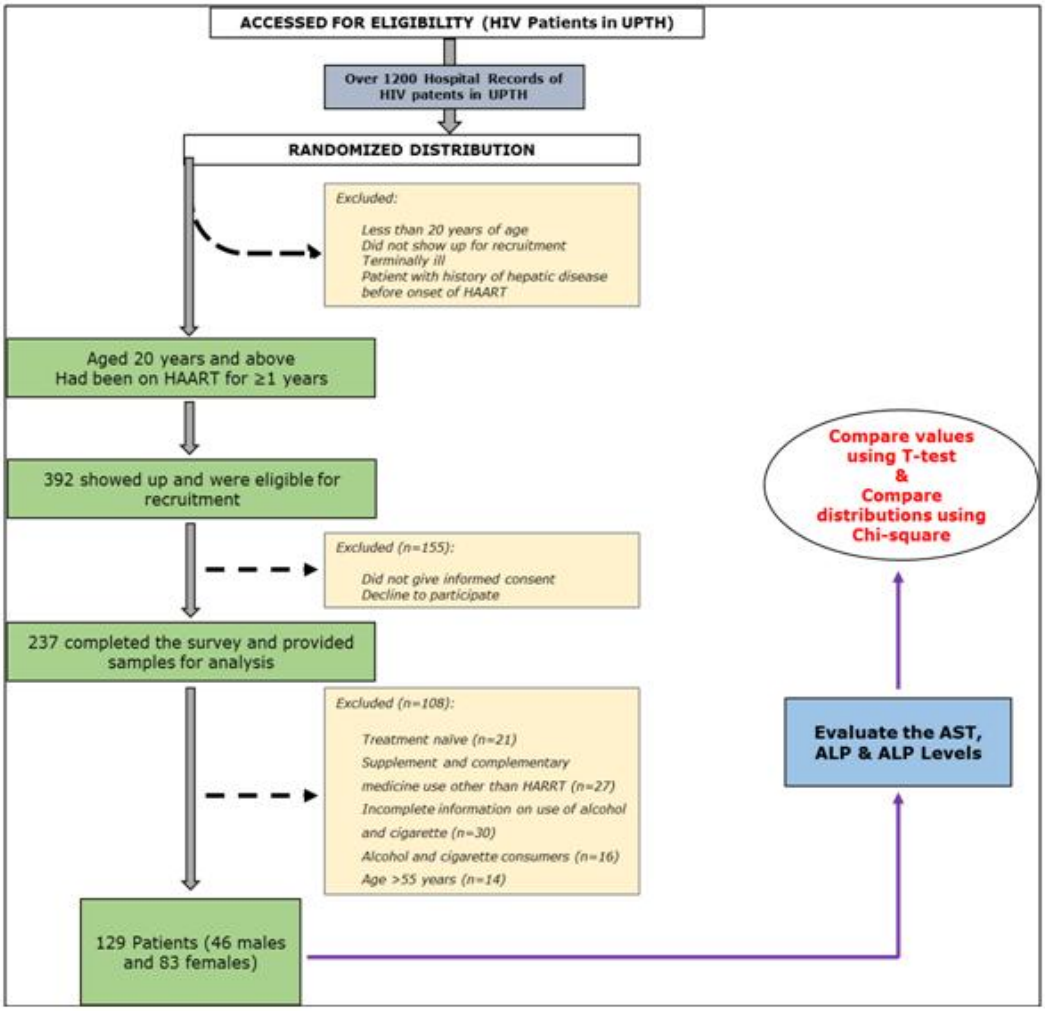

Fig. 1. Flow diagram for the research lifecycle

2.6 Aspartate Aminotransferase (AST)

In a water bath for five minutes at 37ºC, 500 μL of reagent 1 (L-Aspartate 200 mmol/L and 2-oxoglutarate 2 mmol/L) was pipetted into a clean test tube, mixed and incubated. 100 μL of the serum were added, mixed, and incubated at 37ºC for 30 minutes. 500 μL of developer (2, 4-dinitrophenylhydrazine) were added to the reacting tube, mixed and left to stand for 20 minutes at room temperature. 5 mL of 0.4N NaOH were added to the tube and left for 5mins at room temperature. Absorbance of the test samples were read against water blank at 500ηm in a light path cuvette spectrophotometerically. Results were obtained by comparing the absorbance of the test samples to a calibration values provided in the manual of the kit.

2.7 Alanine Transaminase (ALT)

Alanine aminotransferase (ALT) activity is measured at 546 ηm by observing the conc. of pyruvate hydrazine formed when pyruvate reacts with 2,4-dinitrophenyl hydrazine. The rate at which the NADH is expended is proportionate to ALT catalytic activity measured at 340 ηm. Procedure is the same as AST above but only varies in the substrate. The substrate here is L-Alanine and α- ketoglutarate.

2.8 Alkaline Phosphatase (ALP)

This enzyme requires the use of colorimetric method. This procedure is an enhanced standard technique recommended by the Deutsche Gesellschaft für Klinische Chemie und Laboratoriumsmedizin e.V. (DGKL). The assay was read at a wavelength of Hg 405 nm. 0.05 ml of the sample was pipetted against 3 ml of the reagent in the micro plate wells. It was mixed and read, the initial absorbance was recorded and stop watch started. It was read again after 1, 2 and 3 min, all samples collected were stored at 4ºC and tested within 72 hrs of collection.

2.9 Data Analysis

Statistical Package for the Social Sciences (SPSS IBM® version 23) and GraphPad Prism (Version 8.0.2) were used in analysing the data. Descriptive statistics were performed for continuous data and represented as mean (S.D) while frequencies (%) were used to express categorical data. Z-test was used to determine mean difference while Fisher's Chi-square analysis was used to determine trends and association. Confidence level was set at 95% and P-value less than 0.05 was considered significant.

3. RESULTS

The mean values of the hepatic enzyme markers presented in Table 1 showed that the mean(S.D) AST (27.65±17.93 U/L) and ALP (135.13±10.87 U/L) values for males were higher than the female values (AST = 27.25±18.93 U/L and ALP (132.65±9.96 U/L), whereas females (34.66±22.29 U/L) had higher ALT values than males (33.75±18.14 U/L). However, the t-test of mean differences presented in Fig. 2 did not reveal any significantly differences (P>0.05) (Fig. 2).

The values obtained were categorized; within [normal] and outside normal limit [abnormal] and the Chi-square analysis of sex association were represented in Table 2. The result for AST and ALT showed that males patients (AST = 34; 73.9% and ALT = 31; 67.4%) were observed to have more normal values than females (AST = 55; 66.3% and ALT = 53; 63.9%). But slightly lower normal ALP values were observed for males (8; 17.4) when compared to females (15; 18.10). The differences in AST (χ^2=0.809; *p*=0.368), ALT (χ^2=0.163; *p*=0.686), and ALP (χ^2=0.009; *p*=0.923) were not statistically significant.

Among the patients, 17.8% (6; 13.0% males and 17; 20.5% female) were normal, while no patient (male or female) had abnormal AST levels. 3; 2.3% of the patents had abnormal ALT levels. 59; 45.7% of the patients (males: 25 [54.3%] and females: 34 [41.0%]) had cholestatic abnormality (higher than normal ALP levels). For hepatocellular abnormality, 8.5% (5; 10.9% males and 6; 7.2% females) were observed for this group, while for mixed abnormality (AST/ALT/ALP), 18.6% (6; 13.0% of males and 18; 21.7% of females) fell into this class (Table 3).

Table 1. The mean, standard deviation of the liver enzymes of HIV patients on HAART

	Mean±S.D	
	Male (n=46)	**Female (n=83)**
AST (U/L)	27.65±17.93	27.25±18.93
ALT (U/L)	33.75±18.14	34.66±22.29
ALP (U/L)	135.13±10.87	132.65±9.96

Note: Aspartate Aminotransferase [AST], Alanine Transaminase [ALT], Alkaline Phosphatase [ALP]

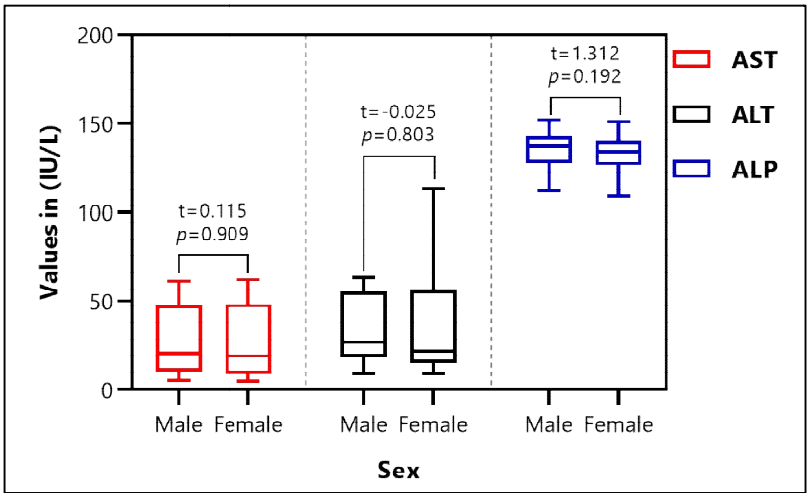

Fig. 2. Comparison of the mean AST, ALT and ALP levels in males and female

Table 2. Liver enzyme characteristics and test of sex-associated distributional test differences

		Normal (%)	Abnormal (%)	Chi-sq. (χ^2)	P-value
AST	Male	34 (73.9)	12 (26.1)	0.809	0.368
	Female	55 (66.3)	28 (33.7)		
	Total	89 (69.0)	40 (31.0)		
ALT	Male	31 (67.4)	15 (32.6)	0.163	0.686
	Female	53 (63.9)	30 (36.1)		
	Total	84 (65.1)	45 (34.9)		
ALP	Male	8 (17.4)	38 (82.60)	0.009	0.923
	Female	15 (18.10)	68 (81.90		
	Total	23 (17.8)	106 (82.2)		

Note: Aspartate Aminotransferase [AST], Alanine Transaminase [ALT], Alkaline Phosphatase [ALP]
Normal values: ALT (7 to 55 U/L), AST (8 to 48 U/L), and ALP (40 to 129 U/L)

Table 3. Liver enzyme characteristics and associated coexistence of abnormalities

Hepatological condition	Sex		Total (%)
	Male (%)	Female (%)	
Normal	6 (13.0)	17 (20.5)	23 (17.8)
AST	-	-	-
ALT	1 (2.2)	2 (2.4)	3 (2.3)
ALP	25 (54.3)	34 (41.0)	59 (45.7)
AST/ALT	5 (10.9)	6 (7.2)	11 (8.5)
AST/ALP	-	2 (2.4)	2 (1.6)
ALT/ALP	3 (6.5)	4 (4.8)	7 (5.4)
AST/ALT/ALP	6 (13.0)	18 (21.7)	24 (18.6)

4. DISCUSSION

Since the alarming rise in HAART-induced hepatotoxicity, prevention and management of ART-related toxicity has emerged as a major issue for HIV/AIDS treatment and care. In this study, the mean AST and ALT of male and female HIV patients were within the normal range, while the ALP values were way above upper limit of 129 U/L for normal values [6]; however, more females had ALT and AST values that were above the normal upper limit reported by Ruhl and Everhart, 2012 [16]. The finding in this study agrees with previous observations; that

up to 14-20% and recently 60% of HIV patients will have elevated enzymes levels upon commencement of ART [7,8,21,22].

The findings in this study do not agrees with the reports of more independent association with mild increase in ALT and AST values; [23] as independent ALT and AST abnormality were less common when compared to both abnormalities (ALT/AST). On the other hand, ALP as an independent abnormality was the most observed; accounting for more than 40%. Predominant abnormal ALP levels in HIV patients without hepatitis co-infection has been reported [23,24]. In the study by Maida, [24] among 17 patients with increased liver enzymes, ALP was elevated in 16. Sterling et al. [23] also found that evaluated ALP was more common than AST or ALT. It is also important to note that mixed (hepatocellular + cholestatic abnormality) was commoner among females while independently, hepatocellular and cholestatic abnormality was more observed in males.

There are several possible mechanisms that are specific to HIV medications capable of causing liver enzyme elevations; however, most HAART contain PIs which has been associated with the development of insulin resistance (IR) and dyslipidaemia, both risk factors for steatosis [25-29]. Additionally, BMI > 30 and Diabetes Mellitus (DM) have been associated with increased liver enzymes in HIV patients on HARRT [23].

The findings in this study do not categorically exclude sex influence in hepatic enzyme abnormalities in HIV-infected patients (without hepatitis co-infection), because the mechanism of action of HIV drugs in inducing hepatotoxicity may be significantly influenced by hormones (which vary with sex), and other clinical conditions such as BMI, diet type and DM, which are not directly associated the HIV infection.

5. CONCLUSION

Generally, among HIV-infected patients on HAART in UPTH, cholestatic abnormalities were observed in more than 80% of the patients as opposed to hepatocellular abnormalities, which were less than 35%. More females were associated with mixed abnormality when compared to males with independent (ALT/AST) abnormality. Any observed sex-associated difference could be associated with a series of unidentified mechanisms of action of HAART. This study, therefore, suggests the need for a randomised case-control study to highlight the extent of deviation from normal values.

Ultimately, there is a need for hospitals to design and implement timely intervention and management strategies; to reduce the rate of drug-induced liver damage in HIV-infected patients. Therefore, it is mandatory for policymakers to develop better and newer management strategies; to reduce the burden associated with drug toxicity in HIV-infected patients.

CONSENT AND ETHICAL APPROVAL

Ethical approval with reference number UPH/CEREMAD/REC/18 was obtained from the Research Ethics Committee of the University of Port before commencement of the research. The study was conducted in line with the Declaration of Helsinki, [30] and all other relevant statutory regulations were observed. Informed consents were obtained from all participating patients.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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