Abnormalities in Liver Function Tests in HIV Positive Children on Highly Anti-retroviral Therapy in a Tertiary Hospital in Abuja, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Authors AAO, MSD and JOL designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors AAO and UDI managed the analyses of the study. Authors AAO and JOL managed the literature searches. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

Background: Liver related deaths are becoming common amongst HIV-infected individual with longer survival on antiretroviral therapy. This study was undertaken to document derangement in liver function tests with associated risk factors in HIV infected children and adolescents on highly active antiretroviral therapy overtime in our health facility, for guide for intervention, and baseline information.
Methods: A cross sectional hospital based study was conducted among HIV-infected children and adolescents aged 6 months-18 years on antiretroviral therapy in our health institution from February to May 2016 for the above objectives.
Results: Of the total of 161 patients studied with a mean age of 10.13±4.5 years, 103(64.0%) were males, and 137 (85.1%) on 1st line HAART. Hepatotoxicity was seen in 37(22.9%) of

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patients, with grade 3 and 4 toxicity (alanine aminotransaminase of 5.0 - >10.0 x ULN) being recorded in 3.4% of those on antiretroviral therapy for <10 years and 13.3% of those >10 years. Grade 3 and 4 hepatotoxicity was also found to be commoner in patients on zidovudine + lamivudine + nevirapine (5.7%), zidovudine + lamivudine + lopinavir-ritonavir (18.2%), and tenofovir + emtricitabine + lopinavir-ritonavir (100%). Risk factors for hepatotoxicity were 2nd line medication [OR of 0.26 (CI 0.81-0.97), p value = 0.013, and co-administration with trimethoprim/sulfamethoxazole [OR 0.18, (CI 0.55-9.59), p value =0.026)].

Conclusion: HAART was well tolerated by most children and adolescents in this study, however, those on 2nd line medication and co-administration with trimethoprim/sulfamethoxazole need more regular monitoring for hepatotoxicity.

1. INTRODUCTION

Over thirty-five million of the world population is estimated to be living with the Human Immunodeficiency Virus (HIV), and sub-Sahara Africa alone is accounting for more than 71% of this infection [1,2]. Nigeria which ranks second in the global burden has 3.3 million of his population living with the virus out of which 360,000 are children below 15 years [3]. Following increasing survival of infected patients with highly antiretroviral therapy (HAART), liver disease is an emerging clinical problem among these groups of individuals [4]. Liver diseases which ranges from abnormal liver function tests, to liver decompensation, non-alcoholic liver disease (NALD), non-alcoholic steatohepatitis (NASH) and hepatocellular cancer (HCC) are becoming common events among infected on HAART [5-7]. Abnormal liver function test defined as either aspartate aminotransferase (AST) or alanine aminotransferase (ALT) ≥1.25 times the upper limit of normal, and low serum albumin level of <3.5 gm/dl are measures of chronic liver disease [8]. This was documented in 16% of cases in a Swiss cohort study of 2,365 HIV-infected individuals who were not co-infected with either hepatitis B or C virus by [8]. Risk factors associated with elevated ALT were high HIV RNA, prolonged use of antiretroviral therapy (ART), high body mass index (BMI), alcohol abuse and increasing age [8-10]. Ocama et al. [11] in their 2010 cohort study of 546 HIV infected individuals in Kampala also documented 1.5% grade 3 AST elevations after 36 months of ART.

Affectation of the liver can result from HIV infection itself, from use of ART, from co-morbidities, and alcohol use. HIV is known to infect predominantly CD4+ T-cells, monocyte/macrophages and dendritic cells; however a wide range of non-haemapoietic cells, including liver cells can also be infected by the virus. HIV can directly infect hepatocytes, hepatocyte cell lines, sinusoidal cells, hepatic stellate cells (HSCs) and Kupffer cells (KCs) [12]. An indirect effect of HIV on the liver is via the gastrointestinal tract (GIT). HIV infection of GIT associated CD4+ T-cells leads to increased permeability to bacterial endotoxins such as lipopolysaccharide (LPS) which can stimulate hepatocytes, KCs and HSCs to produce pro-inflammatory cytokines and chemokines that attracts activated lymphocytes and monocytes to the liver thus further inducing fibrosis [13,14]. Increased systemic levels of LPS are hypothesized to contribute to chronic immune activation in HIV-infected patients *via* activation of monocytes [13,14]. Kupffer cells are the main cell type in the liver that responds to LPS. When stimulated through ligation of the LPS receptor, toll like receptor (TLR)-4, KCs produce pro-inflammatory cytokines [15].

Hepatotoxicity from use of HAART may be related to different classes of ART combinations. These include nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs) and protease inhibitors (PI) [16-18]. The severity of hepatotoxicity may range from transient elevations in transaminase levels to hepatic failure and death, *via* a variety of mechanisms. NNRTI such as nevirapine and efavirenz may cause hypersensitivity [19]. NRTI, primarily diadanosine may cause direct mitochondrial toxicity leading to abnormal liver function [20]. Other mechanisms by which ART causes liver-related toxicity include direct cell stress and disturbances in lipid/sugar metabolism and steatosis, as seen with PI [18]. The PI such as

ritonavir, tipranavir and darunavir has all been associated with elevations in ALT [18]. Mild liver toxicity was observed in 19.7% of the HIV-infected children and adolescents on ART by [19]. Drugs commonly used in combination with HAART in infected patients are trimethoprim/sulfamethoxazole (TMP-SMX) for prevention of opportunistic infections, and anti-tuberculous drugs (isoniazid and rifampicin) in TB/HIV co-infection can induce cholestatic or hepatocellular hepatotoxicity [20-22]. A meta-analysis by Steele et al. [23] also showed that when rifampicin and isoniazid were co-administered with ART might lead to synergistic hepatotoxicity. The mean incidences of drug-related toxic hepatitis were found to be 1.6% (isoniazid), 1.1% (rifampicin) and 2.6% (isoniazid + rifampicin) [23].

Most information relating to therapeutic toxicity of HAART among HIV infected children and adolescents are coming from developed nations, with little or no information from the developing countries where HIV is very common. Management of HIV associated hepatoxicity is usually very difficult because of the intricacies involved in pathogenic mechanisms of liver functioning. We therefore conducted this study to document the markers of hepatocellular damage [AST, ALT and alkaline phosphatase (ALP)], liver synthetic functions [total protein albumin, bilirubin] and liver/splenic sizes and their risk factors among HIV infected children and adolescents on HARRT overtime in our health facility for timely intervention and provision of baseline information in our environment.

2. METHODS

A cross sectional survey of HIV infected children and adolescents on HAART and being monitored and followed up at the paediatric out-patient special treatment clinic (POSTC) of the university of Abuja teaching hospital (UATH) was carried over a 4 months period of February to May 2016. POSTC is an out-patient clinic service area of the health institution where HIV infected children and exposed babies were followed up for treatment and monitoring. It has consulting rooms for the doctors, nurses, and adherence counselors. Record clerks, pharmacists, and nutritionists and home base-care providers are also at their disposal on week days (Monday-Friday, from 7.30 am to 4 pm.). UATH is a 350 bed capacity referral hospital, sub-serving the people of Federal Capital Territory (FCT) Abuja and five

neighbouring states. Is one of the first centers in the country to start offering free HIV/AIDS services through the President Emergency Plan for AIDs Relief (PEPFAR) since 2005.

The subjects were paediatric HIV infected patients aged 6 months to 18 years and on HAART. Consecutive eligible children attending the POSTC were recruited and enrolled into the study after caregivers has provided written informed consent and children 7 years and above provide written inform assent. All the enrolled children were evaluated clinically in POSTC. Weight, length/height were measured using electronic Seca beam weighing scale accurate to the nearest 0.01 kg, and Roche standiometer /infantometer accurate to 0.1 cm, and body mass index (BMI) calculated. Blood pressure (B/P) was measured using (*Accosson Sphygomanometer*, *Accoson Works, Parkway, CM19 5QP England*) with appropriate cuff for age, together with CD4 cell count, using automated Partec Cyflow easy count kit (*Partec code no. 05-8401 Western Germany*) for those who has not done their CD4 cell count for more than 4 weeks prior to the study, and viral load (VL) measurement with (*Roche Smp /prep /cobs Taqman 96, USA*) for those who have not done their VL measurement in the last 6 months prior to the study.

Plasma total protein and albumin were estimated using Biuret and Bromocresol Green methods, while serum ALT, AST and ALP were determined by using Reflotron® Plus at 37°C (*Roche Diagnostics, Mannheim, Germany*). Titers were expressed in IU/l. Bilirubin estimation was done using spectrophotometric Malloy and Evelyne method. Hepatic toxic effects were graded according to the toxicity tables of the Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse events as follows: Grade 1 [mild] (1.2 - 2.5 x upper limits of normality [ULN]), grade 2 [moderate] (2.5 - < 5.0 x ULN), grade 3 [severe] (5.0 - <10.0 x ULN), and grade 4 [potential life threatening] (>10.0 x ULN) for ALT,AST, and ALK [24]. Hepatotoxicity was considered to be present when ALT and AST levels rose above the ULN. Normality was considered to be the values defined by Fischbach & Zawta [25]. ALT levels up to 60 IU/L, AST levels up to 40 IU/L, protein up to >5.7 g/dl, albumin >3.2 g/dl, and total bilirubin <1.0 mg/dl are defined as normal. Sonographic assessment of liver and spleen sizes was done using high-resolution real-time sonographic scanners with 3.5-MHz convex transducers.

Ethics clearance was obtained from the ethics committee of the health institution before the commencement of the study. Data analysis was computed using SPSS version 16.0 computer software packages. Tests of significance were the Chi-square or Fisher exact test (whenever the expected frequency in one of the cells was less than 5), or analysis of variance or Student's *t*-test for continuous variables. Associations between dependent and independent variables were initially assessed using univariate analysis, and Odds ratios (OR) calculated. Variables that showed significant associations with hepatotoxicity risk were then assessed by multivariate logistic regression. Differences between groups were considered significant at p<0.05.

3. RESULTS

Table 1 showed the characteristics of the study population. Of the total of 161 patients studied, 103(64.0%) were males, 137 (85.1%) were on 1st line HAART, and 110 (68.3%) were Christians. Their mean weight, length/height, BMI, CD4 cell count, VL, and duration on HAART were 30.62 ±12.3 kg, 136.06 ±17.8 cm, 16.01±3.0 kg/m2, 958.57 ±37.8 cells/µl, 9,717.38±47.2 copies/ml and 7.90 ±3.2 years respectively. While their mean systolic and diastolic blood pressures remain below the 95th centile for their age and sex, their mean serum bilirubin, protein, albumin, liver, and splenic sizes were all also within normal limit. However, there was slight elevation in their mean AST (49.50±5.1 IU/ml),

Table 1. Characteristics of the study population on first and second line antiretroviral therapy

Characteristics	1st line ARV (%)	2nd line ARV (%)	Total (%)	P value
Study population	137(85.1)	24(14.9)	161 (100.0)	0.004
Age (years)	9.6±4.4	11±3.0	10.13±4.5	0.386
Sex				
Male	90 (64.7)	13(59.1)	103(64.0)	0.003
Female	49 (35.3)	9(40.9)	58(36.0)	0.042
Religion				
Christianity	96 (69.1)	14(63.6)	110(68.3)	0.038
Islam	43 (30.9)	8(36.4)	51(36.7)	0.027
Anthropometry				
Weight (kg)	29.30±1.0	34.64±3.0	30.62±12.3	0.058
Length/Height (cm)	133.13±1.66	143.48±3.7	136.06±17.8	0.021
BMI (kg/m^2)	16.37±0.69	15.92±0.6	16.01±3.0	0.821
ARV duration (years)	9.40 ± 0.56	6.41±0.28	7.91±3.2	0.035
Blood pressure (mmHg)				
Systolic BP	93.04±1.82	94.91±±2.2	93.50±9.9	0.405
Diastolic BP	57.04±0.78	55.46±2.3	56.43±10.3	0.463
Liver function tests				
Total bilirubin (mg/dl)	2.85±1.3	4.0± 0.6	3.0±1.8	0.008
Serum protein (gm/dl)	17.39±2.6	10.49±5.6	11.4±3.4	0.310
Serum albumin (gm/dl)	4.6±0.6	3.1 ±1.4	3.3±2.5	0.007
AST (IU/mL))	45.99±3.9	71.68 ±15.0	49.50±5.1	0.028
ALT (IU/mL)	80.39±18.30	94.27±16.6	92.64±22.37	0.759
ALP (IU/mL))	347.14±22.65	450.09 ±36.1	436.06±57.73	0.022
Liver & splenic sizes				
Liver size (cm)	11.28±0.13	11.99 ±0.35	8.08±1.2	0.954
Splenic size (cm)	7.03±0.10	7.90±0.18	7.79±1.2	0.874
CD4 & viral load				
CD4 (cells/µl)	963.80±40.40	925.10.9 ±10.9	944.49±25.7	0.027
Viral load (copies/ml)	15,702.73±79.4	3,732.03±15.82	9,717.38±47.2	0.003

ALT: Alanine transaminase, AST: Aspartate transaminase, ALP: Alkaline phosphatase, BMI: Body mass index

Table 2. Age in years of study population and liver function test

Parameter	0.25 – 5 (n=24)	6 – 10 (n=73)	11 – 15 (n=53)	>16 yrs (n=12)	P value
Wt (kg)	17.03±0.58	25.52±0.85	37.28±1.40	51.00±3.03	0.000
Lt/Ht (cm)	110.42±1.85	130.97±1.39	145.03±2.65	157.92±4.50	0.000
BMI (kg/m^2)	14.97±0.29	16.89±0.40	17.92±3.77	18.70±1.18	0.821
SBP (mmHg)	85.04±1.69	90.33±0.96	98.79±1.05	103.33±2.24	0.013
DBP (mmHg)	53.62±1.77	54.92±1.04	60.43±1.35	58.82±0.73	0.175
Serum protein (gm/dl)	7.27±0.36	15.08±5.2	7.63±0.36	13.86±5.39	0.621
Serum albumin (gm/dl)	2.96±0.17	3.53±0.43	3.11±0.11	3.11±0.11	0.773
Serum bilirubin (mg/dl)	2.44±0.24	3.25±0.24	2.83±0.18	3.39±0.80	0.867
AST (IU/mL)	36.21±3.9	62.34±7.44	38.25±4.9	48.77±16.20	0.130
ALT (IU/mL)	54.04±8.84	129.92±34.3	63.60±9.5	74.33±23.4	0.225
ALK (IU/mL)	466.58±63.3	415.02±18.2	477.28±45.2	319.17±76.58	0.020
Liver size (cm)	11.48±0.21	12.41±0.22	12.17±0.18	12.69±0.03	0.702
Splenic size (cm)	7.55±0.14	8.29±9.28	7.83±0.14	7.92±9.29	0.024
CD4 cell count (cells/μl)	1202.9±10.9	933.62±57.0	937.81±59.2	711.33±10.7	0.063
Viral Load (copies/ml)	2869.1±596.6	9561.9±883.5	4980.5±582.9	57.5±12.4	0.002

ALT: Alanine transaminase, AST: Aspartate transaminase, ALP: Alkaline phosphatase, BMI: Body mass index

ALT (92.64±22.37 IU/ml), and ALP (436.06 ±57.73 IU/ml) levels respectively. Statistical significant difference was seen in the mean total values for serum bilirubin, albumin, AST, ALP, CD4 cell count, and VL among those on 1st and 2nd line HAART, (p values were 0.008, 0.007, 0.028, 0.022, 0.027, and 0.003 respectively).

Table 2 showed LFTs for different age groupings. While liver synthetic function such as serum albumin level was non significantly lower in 0.5-5 years (2.96 ±0.17 gm/dl) than in the others, marker for hepatocellular damage, the AST, ALT, ALK were also non significantly higher in 6-10 years than in the others, 62.34±7.44 IU/ml, 129.92±34.3 IU/ml, 415.02±18.2 IU/ml, for AST, ALT, and ALP respectively. There was no significant difference in the liver sizes for different age ranges despite their age differences, (p=0.702), however significant difference was noted in splenic sizes for age range 6-10 years and the other age ranges, (p=0.024).

LFT and types of 1st and 2nd Line HAART was shown in Table 3. For the different types of 1st and 2nd HAART, VL was the only study variable that showed statistical significant difference for the different line of HAART (p= 0.008 for 1st line, and 0.032 for 2nd line). AST, ALT, and ALP was higher for those zidovudine (AZT) + lamivudine (3TC) + nevirapine (NVP) 1st line: 69.27±4.3 IU/ml, 192.69±16.5 IU/ml, 410.18±13.2 IU/ml for AST, ALT and ALP, and those on tenofovir (TDF)+ emtricitabine (FTC)+ lopinavir-ritonavir (LP/r): 161.3±21.3l U/ml, 295.5±54.5 IU/ml,

593.14±56.0 IU/ml for AST, ALT and ALP, and abacavir (ABC)+3TC+LP/r 2nd line medication: 68.09±24.1 IU/ml, 197.2±74.1 IU/ml, 458.14±54.0 IU/ml for AST, ALT and ALP. While 146 (90.7%) were on HAART for less than 10 years, only 15 (9.3%) were on the drugs for greater than 10 years (table not shown). All LFT parameters studied except for ALP were all statistically non-significant for < and > 10 years (table also not shown).

Fig. 1 and Table 4 depicts grades of hepatotoxicity Vs duration and types of HAART. Hepatotoxicity was documented in 37(22.9%) of the patients reviewed using ALT level of 1.2 to >10 ULN, while no toxicity was seen in 124(77.1%). Greater than 70.0% of the patients also had normal liver and splenic sizes, however, while 3.4% of those on ARV for <10 years had 3 and 4 grade of hepatotoxicity (ALT of 5.0 - >10.0 x ULN), 13.3% of those on ART >10 years had same grade of hepatotoxicity. Grade 3 and 4 hepatotoxicity was also found in patients on AZT+3TC+NVP combination (5.7%) for 1st line HAARTs, and TDF+ FTC+ LP/r (100%), and AZT+3TC+LP/r (18.2%) for 2nd line combinations.

Table 5 showed multiple logistic regression and risk factor for hepatotoxicity using ALT levels. Use of 2nd line HAART, and co-administration with TMP-SMX were the only study variable found have risk factors for hepatotoxicity in this study, [OR of 0.26 (CI 0.81-2.97), p value =0.013] for 2nd line, and [OR 0.18,(CI 0.55-4.89), p value =0.026)] for TMP-SMX.

Table 3. Liver function tests and type of 1st and 2nd Line Haart

LFT	1ST Line HAART				2ND Line HAART			
	AZT+3TC+ NVP (n= 88)	ABC+3TC+ NVP(n= 45)	D4T+3TC+ EFV (n= 4)	P value	AZT+3TC+ LP/r(n= 11)	ABC+3TC+LP/r(n= 6)	TDF+3TC+ LP/r(n= 4)	TDF+FTC+ LP/r(n=3)
Protein (gm/dl)	7.54±1.7	8.03±0.7	7.26±2.30	0.99	7.55±2.37	7.54±1.55	8.74±2.63	8.38±1.47
Albumin (gm/dl)	3.14±0.8	3.01±0.9	2.84±0.5	0.97	2.97±0.7	3.12±0.7	2.91±0.45	3.08±0.53
Bilirubin (gm/dl)	2.97±0.2	4.60±0.7	2.80±0.3	0.39	3.75±0.6	3.68±1.0	1.98±0.44	3.35±1.24
AST (IU/mL)	69.27±4.3	43.01±6.3	54.6±13.3	0.71	99.28±15.1	68.09±24.1	25.86±7.3	161.3±21.3
ALT (IU/mL)	192.69±16.5	90.75±33.1	82.2±44.643	0.84	159.0±23.5	197.2±74.1	54.0±17.7	295.5±54.5
ALP (IU/mL)	410.18±13.2	398.14±34.0	435.14±56.5	0.06	458.14±54.0	418.37±45.0	388.14±64.3	593.14±56.0
Liver size (cm)	11.93±1.6	11.90±1.83	11.26±0.64	0.75	11.70±2.1	12.11±1.5	11.28±1.42	12.12±1.92
Splenic size (cm)	7.78±1.16	8.83±2.17	7.76±0.96	0.30	8.21±1.44	7.35±0.83	8.70±1.63	8.67±1.95
CD4 +(ul/ml)	889.47±36.3	869.5±211.6	1185.51±6.1	0.79	892.7±35.9	901.7±13.3	1183.7±12.8	938.5±14.5
VL (copies/ml)	6699.4±34.2	281.67±22.8	59.0±2.1	0.008	2994±35.6	481.1±15.8	54.7±2.8	20.0±0.0

Table 4. Grades of hepatotoxicity vs duration and types of Haart

| LFT	HAART Duration		Types of ART (1st Line)			Type of ART (2ND Line)			
	< 10 yrs [n=146] (%)	> 10 yrs [n= 15] (%)	AZT+ 3TC+ NVP [n= 88] (%)	ABC+ 3TC+ NVP [n= 45] (%)	D4T+ 3TC + EFV [n= 4] (%)	AZT + 3TC+ LP/r [n= 11] (%)	ABC+ 3TC+ LP/r [n= 6] (%)	TDF+ 3TC+ LP/r [n= 4] (%)	TDF+ FTC+ LP/r [n=3] (%)
Normal Value	93(63.7)	14(93.3)	60(68.2)	35(77.8)	2(50.0)	10(90.9)	3(50.0)	2(50.0)	0(0.0)
Grade 1 AST: 1.2 < 2.5 x ULN	35(24.0)	1(6.7)	18(20.5)	9(20.0)	1(25.9)	0(0.0)	1(16.7)	2(50.0)	0(0.0)
Grade 2 AST: 2 < 5.0 x ULN	15(10.3)	0(0.0)	7(8.0)	0(0.0)	1(25.0)	1(9.1)	2(33.3)	0(0.0)	0(0.0)
Grade 3 AST: 5.0 <10.0 x ULN	3(2.1)	0(0.0)	3(3.3)	1(2.2)	0(0.0)	0(0,0)	0(0.0)	0(0.0)	2(66.7)
Grade 4 AST: >10.0 x ULN	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(33.3)
Normal Value	111(76.0)	13(92.9)	64(72.7)	42(93.3)	3(75.0	10(90.9)	3(50.0)	2(50.0)	0(0.0)
Grade 1 ALT: 1.2 < 2.5 x ULN	11(7.5)	0(0.0)	7(8.0)	2(4.4)	1(25.0)	0(0.0)	2(33.3)	0(0.0)	0(0.0)
Grade 2 ALT: 2 < 5.0 x ULN	19 (13.0)	0(0.0)	12(13.6)	1(2.2)	0(0.0)	0(0.0)	1(16.7)	2(50.0)	0(0.0)
Grade 3 ALT: 5.0 <10.0 x ULN	2 (1.4)	1(7.1)	2(2.3)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(33.3)
Grade 4 ALT: >10.0 x ULN	3(2.1)	1(7.1)	3(3.4)	0(0.0)	0(0.0)	1(9.1)	0(0.0)	0(0.0)	2(66.7)

Key: *for extreme
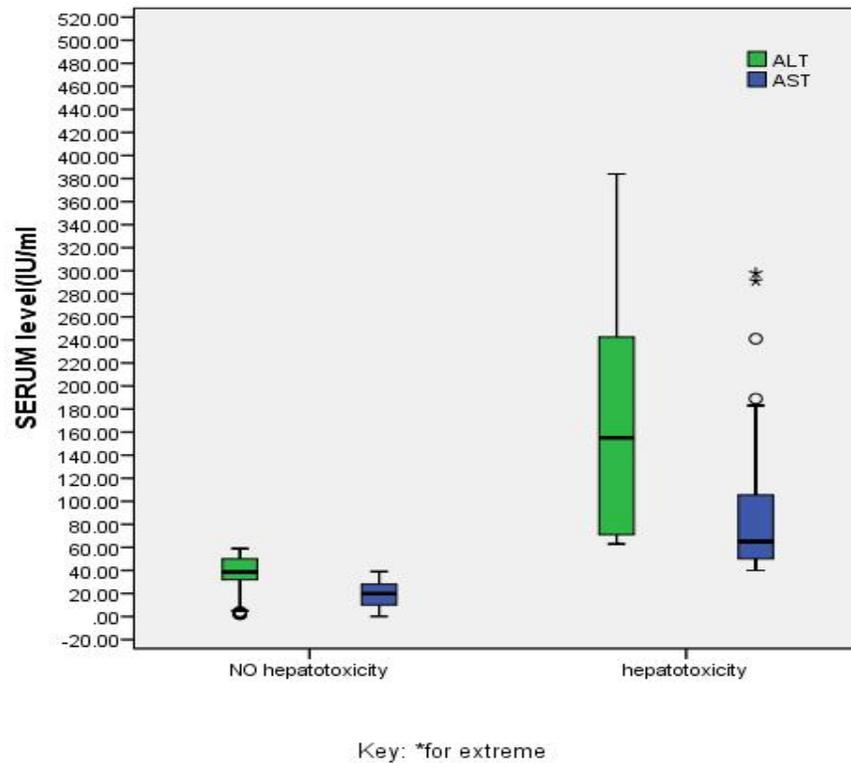
Fig. 1. Hepatotoxicity and liver function test

Table 5. Multiple logistic regression and risk factor for hepatotoxicity using alanine transaminase

Variables (n)	Hepatotoxicity		OR (95% CI)	P value
Duration of HAART	Yes (%)	No (%)		
<10 years (146)	35(24.0)	111(76.0)	7.53(1.72-10.68)	0.342
>10 years (15)	2(13.3)	13(86.7)		
Type of 1st line HAART				
D4T+3TC+EFV (4)	1(25.0)	3(75.0)	1.00	0.257
ABC+3TC+NVP (45)	3(6.6)	42(93.3)	1.13 (0.01- 3.46)	
AZT+3TC+NVP (88)	24(27.3)	64(72.7)	1.25 (0.02-4.73)	
Type of 2nd line HAART				
TDF+EMT+LP/r (3)	3(100.0)	0(0.0)	1.00	0.013
ABC+3TC+LP/r (6)	3(50.0)	3(50.0)	0.26 (0.81-2.97)	
TDF+3TC+LP/r (4)	2(50.0)	2(50.0)	0.47 (0.14-3.88)	
AZT+3TC+LP/r (11)	1 (9.1)	10 (90.1)	1.59 (0.85-5.31)	
Co administration with co-trimozaxol				
No (64)	12(18.8)	52(81.3)	0.18 (0.55-4.89)	0.026
Yes (97)	65 (67.0)	34 (35.0)		

4. DISCUSSION AND CONCLUSION

Hepatotoxicity was seen in 22.9% of HIV infected children and adolescents on HAART in this study. This finding was comparable to 27.0% from India [26], 26.5% from Myanmar [27], 19.7% [19] 16.0% [8], 16.0% [28], and 18%-20% [29,30] from Brazil, and Switzerland among adult population. It was however much higher than 2.7% [31] earlier reported in children from the United State of America. These observed differences both in adult and children might not be unconnected to the geographical differences of the study areas, study population, study

design, hepatitis B and C virus co-infection, other co-morbidities, and wide variability in the criteria to categorize the severity of hepatotoxicity among different studies.

Liver diseases are primarily classified into three broad categories namely: hepatocellular implying injury to the hepatocytes, cholestatic being injury is to the bile ducts, and infiltrative where hepatocytes are invaded or replaced by non-hepatic substances. ALT and AST are two of the most useful measures of liver cell injury, although the AST is less liver specific than ALT. The three enzymes AST, ALT, and ALP tests are also most useful markers in making distinction between hepatocellular and cholestatic disease. The vast majority of this study population (77.1%) showed no indication of hepatotoxicity, and >70.0% had normal liver and splenic sizes while on HAART, however the mean plasma levels for their AST (49.50±5.1 IU/ml), ALT (92.64±22.37 IU/ml), and ALP (436.06±57.73 IU/ml) were all slightly elevated indicating mild degree of hepatocellular and cholestatic dysfunction which however remained asymptomatic. This result further suggests that the larger number of the study population tolerated HAART well, probably because there was no much risk factors to the development of severe liver injury as in adults. Such risk factors include: older age, high alcohol intake and use of illicit drugs [8-10]. Well tolerability of HAART in children was also reported by Wai et al. [27] from Myammar where 18/68 (73.5%) of their children on HAART had no hepatic injury.

Severity of hepatotoxicity ranges from being asymptomatic to liver insufficiency and outcomes also ranges from spontaneous resolution to liver failure and death. Regarding the severity of hepatotoxicity among the study populace, of the 37 (22.9%) that had hepatotoxicity in this study, greater majority 30 (81.1%) had mild to moderate hepatotoxicity, while 7 (18.9%) showed grade 3 and 4 liver dysfunction. Similar observation was also made by Wai et al. [27] and [19] both indicating that most of their study population had mild hepatotoxicity. Infact in Wai et al. [27] study, two of his patients that had severe hepatotoxicity (grade 3 and grade 4 respectively) remained asymptomatic, they however continued them on HAART with close monitoring. Their liver enzymes normalized after 4 weeks of initiation of ART. The severity according to them needed no dose adjustment or cessation of ARTs. In the present study, most of our patients with severe grade 3 and 4 liver enzyme dysfunction were

also asymptomatic. They were however closely monitored for deterioration in liver functions and co-existing of hepatitis B and C virus. Three (3) requiring substitution to another type of 2nd line regimen because of high level of liver enzymes.

Several risk factors for hepatoxicity in HIV positive patients on HAART have been well documented in both children and adults [8-10, 21,23,27,30-32]. Established risk factor was a 2.7 to 5 fold risk of severe increase in ALT while on HAART with hepatitis C virus co-infection [8]. Chronic hepatitis B virus co-infection has also been documented to have a 9.2 hazard risk of grade 4 liver enzyme elevations [8]. Underlying liver inflammation as reflected by elevated ALT at baseline has also been well established as a risk factor for HAART liver toxicity [27,33]. Older age, female sex, thrombocytopenia, renal insufficiency, high VL, increased BMI, and non-black ethnicity, are all well-established bio and demographic markers of hepatotoxicity in positive children and adults on HAART in various studies [8-10]. Individual or classes of ART have been independently associated with HAART hepatotoxicity. They include ARVs such as NVP, PIs, high doses of ritonavir and prolonged AZT or D4T [7,15,30-32]. HAART is a combination therapy of several ARVs that can cause hepatotoxic drug interactions by itself. Of particular, importance is the NNRTIs and PIs interact with cytochrome P450 which will mutually alter their individual serum half-lives. There are conflicting results of studies that have evaluated the risk for liver toxicity associated with the use of individual ARVs. The use of several ARVs combined makes it difficult to ascribe the elevation of transaminases to single drugs. In the present study combinations of ARVs were used, and were identified to be risk factor for development of severe hepatotoxicity (grade 3 and 4). This was obvious to be more with use of 2nd line ARVs, and co-administration with TMP-SMX. Grade 3 and 4 hepatotoxicity was seen with administration of AZT+3TC+NVP (5.7%), TDF+ FTC+ LP/r (100%), and AZT+3TC+LP/r (18.2%). This finding was not surprising considering that several studies have implicated ARVs notably NVP, PIs, high doses of ritonavir and prolonged AZT or D4T [7,15,30-32], as one of the causes of hepatotoxicity in positive patients on HAART. Use of combination of emtricitabine (FTC) and tenofovir (TDF) in 2nd line regimen with LP/r for three (3) adolescents that showed grade 3 and 4 hepatoxity was a source of concern that warrant substitution to alternative 2nd line regime. Patients on such ARV

combination requires close monitoring of their liver enzymes. The risk factor for ARV toxicity in this study was use of 2nd line ARV, and co-administration of TMP-SMX[OR of 0.26 (CI 0.81-2.97), p value =0.013] for 2nd line, and [OR 0.18,(CI 0.55-4.89), p value -=0.026)] for TMP-SMX. Drugs commonly used in addition to HAART in HIV-infected patients for control of opportunistic and TB which can induce liver injury are TMP-SMX (cholestatic) and isoniazid (hepatocellular) patterns [20]. Over half of the patients (60.2%) in this study were on TMP-SMX prophylaxis for opportunistic infection, and non on anti TB therapy. Montes Gil et al. [19] in their study analysis noted hepatotoxicity to be associated with the use of sulfonamides, a component of TMP-SMX (aOR: 3.58; 95% confidence interval, CI: 1.44 – 8.85). Similar finding was also recorded in this study and buttress the support for closer monitoring of liver enzymes of patients on co-administration with TMP-SMX for prevention of opportunistic infections. This is particularly important especially those from resource constrain environment where resources may be lacking for close monitoring.

CONSENT

As per international standard or university standard, patient's written consent has been collected and preserved by the authors.

ETHICAL APPROVAL

As per international standard or university standard, written approval of Ethics committee has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. World Health Organisation. Global health observatory report; 2015.
   Available:http://www.who.int/gho/hiv/en/
   (Accessed 28 December 2015)
2. AVERT. HIV and AIDS in Sub-Saharan Africa; 2014.
   Available:http://www.avert.org/hiv-aids-sub-saharan-africa.htm
   (Accessed 31 May 2015)
3. United Nations General Assembly Special Session Country Progress Report; 2010.
   Available:http://data.unaids.org/pub/report/Nigeria_2010_country_progress_report
   (Accessed 10 July 2013)
4. Smith C, Sabin CA, Lundgren JD, Thiebaut R, Weber R, Law M, Monforte A, Kirk O, Friis-Moller N, Phillips A. Factors associated with specific causes of death amongst HIV-positive individuals in the D:A:D study. AIDS. 2010;24:1537-1548.
5. Panos G, Farouk L, Stebbing J, Holmes P, Valero S, Randell P, Bower M, Gazzard B, Anderson M, Nelson M. Cryptogenic pseudocirrhosis: A new clinical syndrome of noncirrhotic portal hypertension (unassociated with advanced fibrosis) that can be detected by transient elastography in patients with HIV. J of Acquired Immune Deficiency Syndrome. 2009;52:525-527.
6. Ingiliz P, Valantin MA, Duvivier C, Medja F, Dominguez S, Charlotte F, Tubiana R, Poynard T, Katlama C, Lombès A. Liver damage underlying unexplained transaminase elevation in human immunodeficiency virus-1 mono-infected patients on antiretroviral therapy. Hepatology. 2009;49:436-442.
7. Crum-Cianflone N, Dilay A, Collins G, Asher D, Campin R, Medina S, Goodman Z, Parker R, Lifson A, Capozza T. Non-alcoholic fatty liver disease among HIV-infected persons. J of Acquired Immune Deficiency Syndrome. 2009;50:464-473.
8. Kovari H, Ledergerber B, Battegay M, Rauch A, Hirschel B, Foguena AK, Vernazza P, Bernasconi E, Mueller NJ, Weber R. Incidence and risk factors for chronic elevation of alanine aminotransferase levels in HIV-infected persons without hepatitis b or c virus co-infection. Clin Infect Dis. 2010;50:502-511.
9. Belloso WH, Orellana LC, Grinsztejn B, Madero JS, La Rosa A, Veloso VG, Sanchez J, Ismerio Moreira R, Crabtree-Ramirez B, Garcia Messina O. Analysis of serious non-AIDS events among HIV-infected adults at Latin American sites. HIV Medicine. 2010;11:554-564.
10. Garcia-Jardon M, Bhat VG, Blanco-Blanco E, Stepian A. Postmortem findings in HIV/AIDS patients in a tertiary care hospital in rural South Africa. Tropical Doctor. 2010;40:81-84.
11. Ocama P, Castelnuovo B, Kamya MR, Kirk GD, Reynolds SJ, Kiragga A, Colebunders R, Thomas DL. Low frequency of liver

enzyme elevation in HIV-infected patients attending a large urban treatment center in Uganda. Int J of Sexually Trans Dis and AIDS. 2010;21:553-557.

12. Housset C, Boucher O, Girard PM, Leibowitch J, Saimot AG, Bréchot C, Marche C. Immuno-histochemical evidence for human immunodeficiency virus-1 infection of liver Kupffer cells. Human Pathology. 1990;21:404-408.

13. Brenchley JM, Price DA, Schacker TW, Asher TE, Silvestri G, Rao S, Kazzaz Z, Bornstein E, Lambotte O, Altmann D. Microbial translocation is a cause of systemic immune activation in chronic HIV infection. Nature Medicine. 2006;12:1365-1371.

14. Berger EA, Murphy PM, Farber JM. Chemokine receptors as HIV-1 coreceptors: Roles in viral entry, tropism, and disease. Annual Rev Immunology. 1990;17:657-700.

15. Schwabe RF, Seki E, Brenner DA. Toll-like receptor signaling in the liver. Gastroenterology. 2006;130:1886-1900.

16. Núñez M. Clinical syndromes and consequences of antiretroviral-related hepatotoxicity. Hepatology. 2010;52:1143-1155.

17. Chu KM, Boulle AM, Ford N, Goemaere E, Asselman V, Van Cutsem G. Nevirapine-associated early hepatotoxicity: Incidence, risk factors, and associated mortality in a primary care ART programme in South Africa. PLoS One. 2010;5:e9183.

18. Murphy RA, Sunpath H, Kuritzkes DR, Venter F, Gandhi RT. Antiretroviral therapy-associated toxicities in the resource-poor world: The challenge of a limited formulary. Journal of Infectious Disease. 2007;196(3):S449-S456.

19. Montes Gil AC, Lorenzetti R, Mendes GB, Morcillo, AM, Contrera Toro AA, Nolasco da Silva MT, Marluce dos Santos Vilela M. Hepatotoxicity in HIV-infected children and adolescents on antiretroviral therapy. Sao Paulo Medical Journal. 2007;125(4). Available:http://dx.doi.org/10.1590/S1516-31802007000400002

20. Nunez M, Soriano V. Hepatotoxicity of antiretrovirals: Incidence, mechanisms and management. Drug Safety. 1995;28(1):53-66.

21. Bonfanti P, Landonio S, Ricci E, Martinelli C, Fortuna P, Faggion I, et al. Risk factors for hepatotoxicity in patients treated with highly active antiretroviral therapy. Journal of Acquired Immune Deficency Syndrome. 2001;27:316–318.

22. Wit F, Weverling G, Weel J, Jurrians S, Lange J. Incidence and risk factors for severe hepatotoxicity associated with antiretroviral combination therapy. Journal of Infectious Disease. 2002;186:23–31.

23. Steele MA, Burk RF, DesPrez RM. Toxic hepatitis with isoniazid and rifampin. A meta-analysis. Chest. 1991;99(2):465-71.

24. National Institute of Allergy and Infectious Diseases, National Institutes of Health, US Department of Health and Human Services. Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse events Version 2.0 Nov. 2014;24.

25. Fischbach F, Zawta B. Age-dependent reference limits of several enzymes in plasma at different measuring temperatures. Clin Laboratory. 1992;38: 555-61.

26. Kumarasamy N, Vallabhaneni S, Cecelia AJ, Yepthomi T, Balakrishnan P, Saghayam S, Flanigan TP, Carpenter CC, Solomon S, Mayer KH. Reasons for modification of generic highly active antiretroviral therapeutic regimens among patients in Southern India. Journal of AIDS. 2006;41:53-58.

27. Wai LT, Khaing KW, Tin M. Antiretroviral-therapy-related hepatotoxicity in HIV-infected children in integrated HIV-care clinic, Mandalay Childrens Hospital, Myanmar. Clinical Pediatrics. 2016;1:105.

28. Shah I. Adverse effects of antiretroviral therapy in HIV-1 infected children. Journal of Tropical Pediatrics. 2005;52(4):244-8.

29. Servoss JC, Kitch DW, Andersen JW, Reisler RB, Chung RT, Robbins GK. Predictors of antiretroviral-related hepatotoxicity in the adults AIDS Clinical Trial Group. Journal of Acquired Immune Deficency Syndrome; 1989-1999.

30. Ena J, Amador C, Benito C, Fenoll V, Pasquau F. Risk and determinants of developing severe liver toxicity during therapy with Nevirapine and Efavirenz-containing regimens in HIV-infected patients. International Journal of Sexually Transmitted Diseases and AIDS. 2003;14: 776-781. 2006;43:320-323.

31. Baylor M, Ayime O, Truffa M, Denson A, Johann LR. Hepatotoxicity associated with nevirapine use in HIV-infected children. 12th Conference on Retroviruses and

Opportunistic Infections, Boston, Massachusetts, the United States of America. 2005;776.

32. Aceti A, Pasquazzi C, Zechini B, De Bac C. The liver HAART group. Hepatotoxicity development during antiretroviral therapy containing protease inhibitors in patients with HIV. The role of hepatitis B and C virus infection. Journal of Acquired Immune Deficency Syndrome. 2002;29: 41–48.

33. Sanne I, Marin HM, Hinkle J, Bartlett JA, Lederman MM, Maartens G, Wakeford C, Shaw A, Quinn J, Gish RG, Rousseau F. Severe hepatotoxicity associated with nevirapine use in HIV-infected subjects. Journal of Infectious Disease. 2005;191: 825-829.

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