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ORIGINAL RESEARCH ARTICLE

Liver function test abnormalities in Nigerian patients with human immunodeficiency virus and hepatitis B virus co-infection

M O Iroezindu FWACP FMCP*, **O O Agbaji** FMCP†‡, **C A Daniyam** FWACP†‡, **G C Isiguzo** FWACP†, **C Isichei** FMCPath MSc§ **and M O Akanbi** FMCP MSCI†‡

*Department of Medicine, University of Nigeria Teaching Hospital, Enugu; †Department of Medicine; ‡AIDS Prevention Initiative Nigeria (APIN) Centre, Jos University Teaching Hospital; §Department of Chemical Pathology, University of Jos, Jos, Nigeria

Summary: Data on baseline hepatic function of HIV and hepatitis B virus (HBV) co-infected patients are limited in sub-Saharan Africa. We assessed liver function test (LFT) abnormalities in Nigerian patients with HIV/HBV co-infection to highlight the impact of HIV on HBV-related liver disease in sub-Saharan Africa. A cross-sectional study involving 100 HIV/HBV co-infected patients and 100 age- and sex-matched HBV mono-infected controls. Blood testing for HIV antibodies, CD4+ cell count, hepatitis B surface antigen (HBsAg), hepatitis B e antigen (HBeAg), LFTs, platelet count, fasting blood glucose and lipid profile were carried out. Non-invasive hepatic fibrosis scores (aspartate aminotransferase-platelet ratio index [APRI] and FIB-4) were also calculated. Co-infected patients had deranged liver enzymes more than the controls (77% versus 64%, $P = 0.04$). The predominant patterns of enzyme derangement in co-infected patients were either predominantly ↑ALP (30% versus 4%, $P < 0.0001$) or mixed (30% versus 15%, $P = 0.01$) but predominantly ↑AST/ALT in the controls (25% versus 9%, $P = 0.003$). Co-infected patients had higher fibrosis scores for both APRI ($P = 0.002$) and FIB-4 ($P = 0.0001$). On further analysis, LFT abnormalities and fibrosis scores were only significantly higher in co-infected patients in the immune clearance and HBeAg-negative chronic hepatitis phases. LFT abnormalities are common in Nigerians with HBV infection and co-infection with HIV negatively impacts on hepatic function.

Keywords: human immunodeficiency virus, HIV, AIDS, hepatitis B virus, co-infection, liver function test, hepatic fibrosis

INTRODUCTION

In sub-Saharan Africa, hepatitis B virus (HBV) has a high endemicity and is the major aetiological factor for chronic liver disease.[1,2] Over the past two decades, the burden of human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS) has reached alarming proportions in several countries of sub-Saharan Africa.[3] Due to shared transmission mechanisms, HIV and HBV co-infection has become an important public health problem in the region.[4] While HIV infection and its treatment have been associated with increased risk of liver disease,[5] HIV itself negatively impacts on the natural history of HBV infection making HBV-related liver disease a major cause of death in people living with HIV/AIDS.[6]

Liver enzymes are useful surrogates of the degree of liver damage and are readily available for the evaluation of patients with liver disease in developing countries where the burden of disease is high but access to invasive diagnostic facilities such as liver biopsy is limited. In patients with HIV/HBV co-infection, several processes involved in HBV-related liver damage including HBV replication as well as host immune-mediated injury are affected by HIV infection.[7] The tendency of HIV-related immunosuppression to obscure hepatic necro-inflammatory activity and its liver enzyme surrogates may limit the utility of liver enzymes in assessing severity of liver damage. Non-invasive hepatic fibrosis scores such as aspartate aminotransferase-platelet ratio index (APRI) and FIB-4 score, which can readily be obtained from investigations routinely available in clinical practice, have been found to have high utility in the assessment of severity of liver damage.[8]

Using APRI score in the Multicenter AIDS Cohort Study, it was shown that antiretroviral treatment-naïve HIV/HBV and HIV/HCV co-infected patients had accelerated liver fibrosis compared with those with either HBV or HCV mono-infection, respectively.[8] Although there are limited data in sub-Saharan Africa, the prevalence of significant liver fibrosis assessed by transient elastography has been shown to be higher in HIV/HBV co-infected patients than in those with HBV mono-infection in Uganda.[9] Assessment of liver damage using non-invasive markers in HIV/HBV co-infected individuals is even of immense importance in sub-Saharan Africa considering that majority of people with hepatitis B infection would have had it for longer duration, bearing in mind that they are likely to have acquired it either vertically or in early childhood as compared with the cohorts in the USA and

Correspondence to: Michael O Iroezindu
Email: mikezindu@yahoo.com

Europe, many of whom would have contracted it as teenagers or adults.

So far, the impact of HIV on hepatic function of patients with HIV/HBV co-infection has not been sufficiently characterized in sub-Saharan Africa. Considering that Nigeria is one of the countries with the highest burden of HIV and HBV infections in sub-Saharan Africa, we determined liver function test (LFT) abnormalities and non-invasive hepatic fibrosis scores in Nigerian patients with HIV/HBV co-infection compared with those with HBV mono-infection in order to highlight the impact of HIV on HBV-related liver disease in sub-Saharan Africa.

PATIENTS AND METHODS

We carried out a cross-sectional study in which antiretroviral treatment (ART)-naïve HIV-infected patients with positive hepatitis B surface antigen (HBsAg) serology were recruited from the HIV clinic of Jos University Teaching Hospital (JUTH), Nigeria. Age- and sex-matched HIV-negative HBsAg-positive subjects attending the gastroenterology clinic of JUTH were enrolled as controls. The co-infected patients were at entry point into the adult ART programme of the hospital and had not been commenced on any medications for HIV or HBV. The co-infected patients comprised mainly subjects whose HBsAg-positive status was diagnosed as part of routine HIV care as well as few patients who were symptomatic for HBV-related liver disease but this was not the primary reason for their hospital visit. A majority of the patients in the control group (87%) were referred to the gastroenterology clinic for expert evaluation following a positive HBsAg test during pre-blood donation screening, premarital screening and pre-employment medicals or during evaluation for other medical/surgical disorders. The remaining patients (13%) were those who either presented or were referred as cases of symptomatic HBV-related chronic liver disease but had not commenced any medications for HBV. The study lasted from May to November, 2010. Only individuals who were 18 years and above were considered eligible. Patients were excluded if they had evidence of hepatitis C virus (HCV) infection, previous anti-HBV or antiretroviral therapy (ART) experience, pregnancy, diabetes mellitus, dyslipidaemia, obesity or clinical evidence of on-going systemic infection other than HIV and HBV. Those receiving potentially hepatotoxic drugs such as antituberculous medications, statins and systemic antifungals were also excluded. Patients who were taking medications that could not be readily identified were also excluded. Due to the rampant use of over-the-counter medications such as paracetamol, non-steroidal anti-inflammatory drugs (NSAIDS) and haematinics in our environment, they were not considered exclusion criteria. Patients currently using herbal medications were not included in the study.

We obtained information regarding each participant's demographic characteristics such as age and gender, medical history, alcohol use and history of medication use. Height and weight measurements were carried out for each subject. Physical examination was performed to identify individuals with signs of liver disease and to exclude those with features of diseases that were exclusion criteria. HBsAg seropositivity was confirmed using third generation enzyme-linked immunosorbent assay, ELISA (ETI-MAK-2 Plus, Diasorin S.P.A kit, Saluggia, Italy). Each subject also had blood testing for hepatitis B e antigen (HBeAg) using semiquantitative ELISA technique (ETI-EBK Plus Diasorin S.P.A kit, Saluggia, Italy) to identify those with HBV replication markers. HIV antibody was initially detected by ELISA test and subsequently confirmed by Western blot. The HIV-positive patients also had CD4+ cell quantification by flow cytometry (Partec, Münster, Germany).

Liver function tests including serum total protein, albumin, bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were carried out for each subject using a blood chemistry autoanalyser (Hitachi 902, Mannheim, Germany). Gamma glutamyl transpeptidase (GGT) test was carried out using a dry reagent chemistry technique (Reflotron® System, Roche Diagnostics, Mannheim, Germany). Platelet count was also carried out. Serum fasting blood glucose (FBG) and lipid profile were also carried out in order to exclude those with diabetes mellitus and dyslipidaemia. Elevated liver enzymes were defined as greater than the upper limits of normal by the local laboratory standards: ALT $>$ 40 IU/L, AST $>$ 40 IU/L, $>$ 117 IU/L and GGT $>$ 58 IU/L for men and $>$ 32 IU/L for women while hypoalbuminaemia was defined as serum albumin $<$35 g/L. Significant alcohol consumption was defined as alcohol use in excess of 21 units per week for men or 14 units per week for women.[10]

Two simple non-invasive scores for hepatic fibrosis, which make use of parameters easily assessed in routine clinical practice, were calculated for each participant. The index by Wai *et al.*,[11] known as APRI, was calculated as AST level (IU/L)/AST upper level of normal (i.e. 40 IU/L)/platelet counts (10^9/L) $\times$ 100.

The FIB-4 score was calculated as Age (in years) $\times$ AST level (IU/L)/platelet count (10^9/L) $\times$ $\sqrt{}$ALT (IU/L).[12]

In various studies,[11–13] an APRI value $>$1.5 or FIB-4 score $>$3.25 have been found to have high positive predictive values for significant hepatic fibrosis and these cut-off values were adopted for this study.

In the absence of HBV viral load, the patients were preliminarily classified according to the natural history of HBV,[14] using HBeAg status and serum ALT levels thus:

Immune tolerant phase: HBeAg positive with normal ALT
Immune clearance phase: HBeAg positive with elevated ALT
Inactive HBsAg carrier: HBeAg negative with normal ALT
HBeAg-negative chronic hepatitis: HBeAg negative with elevated ALT

Ethical approval to conduct the study was obtained from the Research and Ethics committee of JUTH. Participation was voluntary and informed consent was obtained from each participant. Patients diagnosed to have liver function abnormalities were counselled and their managing physicians were notified for appropriate management.

Data analysis was carried out using the Epi Info version 3.5.3 statistical software (CDC, Atlanta, GA, USA). Means ($\pm$SD) and proportions were used to describe continuous and categorical variables respectively. The Student's '*t*'-test was used to compare the mean values of uniformly distributed variables while non-uniformly distributed continuous data were compared using the Kruskal–Wallis test. The Chi-square test or the Fisher's exact test were used to compare observed differences in dichotomous variables. A *P* value less than 0.05 was considered significant.

Table 1 **Characteristics of the study participants**

Characteristic	HIV/HBV co-infected patients (*N* = 100)	HBV mono-infected control (*N* = 100)	*P* value
Age (years), mean ± SD	33.81 ± 8.46	33.53 ± 8.44	0.82
Gender (F/M)	61/39	61/39	1.00
BMI (kg/m^2), mean ± SD	22.50 ± 3.14	24.46 ± 2.64	<0.0001
Significant alcohol consumption (%)	18.0	17.0	0.85
Jaundice (%)	7.0	4.0	0.19
Ascites (%)	11.0	8.0	0.47
Leg oedema (%)	9.0	6.0	0.42
Liver span (cm) (%)			
<8	7.0	4.0	0.40
8–12	90.0	90.0	
>12	3.0	6.0	
Splenomegaly (%)	5.0	3.0	0.72
FBG (mmol/l), mean ± SD	5.09 ± 1.19	4.98 ± 0.90	0.46
Total cholesterol (mmol/L), mean ± SD	3.97 ± 0.96	4.55 ± 1.02	<0.0001
Triglyceride (mmol/L), mean ± SD	1.66 ± 0.58	1.50 ± 0.47	0.01
Platelet count (10^9/L)	152.31 ± 69.82	258.69 ± 80.92	<0.0001
HBeAg seropositivity (%)	28.0	15.0	0.03
CD4 cell count (cells/μL), median (IQR)	222 (107–320)	–	–

BMI = body mass index; FBG = fasting blood glucose; HBV = hepatitis B virus; HBeAg = hepatitis B e antigen

RESULTS

Characteristics of the study participants

The study population included 100 HIV/HBV co-infected patients and 100 age- and sex-matched HBV mono-infected control. The characteristics of the study participants are shown in Table 1. The mean ages of the co-infected patients (33.81 ± 8.46) and controls (33.53 ± 8.44) were similar, $P = 0.82$. A majority of the subjects were women (61%). There was no difference between co-infected patients and control in the proportion of subjects with significant alcohol consumption, jaundice, ascites or leg oedema. Both groups were also similar in terms of liver span and the presence of splenomegaly as sonographicaly determined (Table 1). The mean body mass index (BMI), FBG, total cholesterol and triglyceride in each group were within normal limits although co-infected patients had a significantly lower BMI (22.50 ± 3.14 versus 24.46 ± 2.64 kg/m^2, $P < 0.0001$) and total cholesterol (3.97 ± 0.96 versus 4.55 ± 1.02, $P < 0.0001$) but higher triglycerides (1.66 ± 0.58 versus 1.50 ± 0.47, $P = 0.01$). Platelet counts for the co-infected patients were significantly lower than in the controls (152.31 ± 69.82 versus 258.69 ± 80.92, $P < 0.0001$). The co-infected patients were significantly more likely to be HBeAg seropositive (28.0% versus 15%, $P = 0.03$). The median CD4+ cell count of the co-infected patients was 222 cells/μL (IQR 107–320) and 46% of them had severe immunosuppression as reflected by CD4+ cell counts below 200 cells/μL.

Natural history of HBV infection

We preliminarily classified the study population in terms of the phases of the natural history of HBV infection using the HBeAg status and serum ALT levels (Table 2). The majority of HIV/HBV co-infected patients and the HBV mono-infected control were either inactive HBsAg carriers (51% for co-infected group and 58% for mono-infected control) or had HBeAg-negative chronic hepatitis (21% for co-infected group and 27% for mono-infected control).

Liver function test abnormalities

The liver function test abnormalities in the study population are shown in Table 2. Co-infected patients had a significantly lower mean serum albumin (30.59 ± 8.45 versus 37.17 ± 9.00, $P < 0.0001$), higher mean bilirubin, both total ($P = 0.0004$) and conjugated ($P = 0.0001$). They also had a higher proportion of participants with at least one elevated liver enzyme than the controls (77% versus 64%, $P = 0.04$). In terms of specific liver enzymes, co-infected patients had a significantly higher mean ALP (154.39 ± 67.79 versus 98.96 ± 39.16, $P < 0.0001$) while the controls had a significantly higher mean AST (56.45 ± 81.49 versus 37.07 ± 26.52, $P = 0.04$). There was no difference between the two groups in the mean ALT ($P = 0.34$) and GGT ($P = 0.59$). Overall, the pattern of liver enzyme derangement (Table 2) shows that co-infected patients had either predominantly raised ALP (30% versus 4%, $P < 0.0001$) or mixed pattern (30% versus 15%, $P = 0.01$) while HBV mono-infected controls had predominantly raised transaminases (25% versus 9%, $P = 0.003$).

We further analysed liver function parameters for the study participants according to the phases of HBV natural history to allow for better comparability of the co-infected patients and the control (Table 3). For those in the *immune tolerant phase*, LFT parameters were mostly within normal limits with the HIV/HBV co-infected group and the HBV mono-infected controls having similar LFT parameters except for significantly lower serum albumin (30.03 ± 3.39 versus 34.57 ± 9.90, $P = 0.046$) and higher ALP ($P = 0.047$) in the co-infected patients. Derangements in LFT parameters were most marked among patients in the *immune clearance phase*. In this phase, co-infected patients had significantly higher total bilirubin (20.28 ± 14.89 versus 10.64 ± 11.15, $P = 0.01$), conjugated bilirubin (14.49 ± 17.10 versus 4.44 ± 7.70, $P = 0.01$) and ALP ($P = 0.04$) but significantly lower albumin (21.85 ± 4.98 versus 34.64 ± 7.30, $P = 0.0002$). The mono-infected group had significantly higher AST ($P = 0.02$) and ALT ($P = 0.03$). Among the *inactive HBsAg carriers*, LFT parameters were all essentially within normal limits although the levels of albumin were

Table 2 **HBV natural history phase, liver function tests and hepatic fibrosis scores of the entire study population**

Parameter	HIV/HBV co-infected patients (*N* = 100)	HBV mono-infected control (*N* = 100)	*P* value
Phase of HBV natural history, %			0.10
Immune tolerant phase	13	4	
Immune clearance phase	15	11	
Inactive HBsAg carriers	51	58	
HBeAg-negative chronic hepatitis	21	27	
LFT, mean ± SD			
Total protein (g/L),	68.74 ± 7.96	70.47 ± 12.35	0.31
Albumin (g/L),	30.59 ± 8.45	37.17 ± 9.00	<0.0001
Total bilirubin (mmol/L)	10.91 ± 10.37	6.93 ± 5.24	0.0004
Conjugated bilirubin (mmol/L)	5.40 ± 8.53	2.47 ± 3.10	0.0001
ALT (IU/L)	45.03 ± 36.26	57.85 ± 98.21	0.34
AST (IU/L)	37.07 ± 26.52	56.45 ± 81.49	0.04
ALP (IU/L)	154.39 ± 67.79	98.96 ± 39.16	<0.0001
GGT (IU/L)	72.53 ± 82.70	60.91 ± 53.27	0.59
Enzyme pattern, %			
Normal	23.0	36.0	0.04
Predominantly ↑ALT/AST	9.0	25.0	0.003
Predominantly ↑ALP	30.0	4.0	<0.0001
Mixed	30.0	15.0	0.01
Non-invasive hepatic fibrosis scores			
APRI	1.18 ± 1.98	0.53 ± 0.60	0.002
FIB-4	2.03 ± 2.54	0.98 ± 0.76	0.0001

ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; GGT = gamma glutamyl transpeptidase; LFT = liver function test; APRI = aspartate aminotransferase-platelet ratio index; HBV = hepatitis B virus

significantly lower in the co-infected patients (34.64 ± 7.30 versus 38.97 ± 8.36, $P = 0.01$) and they also had higher conjugated bilirubin (2.81 ± 1.73 versus 1.97 ± 1.60, $P = 0.01$) and ALP ($P < 0.0001$). Derangements in LFT parameters were also observed among patients with *HBeAg-negative chronic hepatitis*. In this phase, co-infected patients had significantly higher total bilirubin (16.91 ± 16.82 versus 5.75 ± 3.72, $P = 0.02$), conjugated bilirubin (10.56 ± 9.24 versus 1.65 ± 0.48, $P = 0.001$) and ALP ($P = 0.0003$) but significantly lower albumin (23.28 ± 4.65 versus 35.73 ± 13.08, $P = 0.01$). Although both groups had elevated transaminases in this phase, there was no difference in the ALT ($P = 0.70$) and AST ($P = 0.31$) levels.

Non-invasive hepatic fibrosis scores

Overall, HIV/HBV co-infected patients had significantly higher values of non-invasive hepatic fibrosis scores for both APRI ($P = 0.02$) and FIB-4 ($P = 0.0001$) as shown in Table 2. Significant fibrosis defined by APRI was found in 17 (17%) co-infected patients compared with 4 (4%) in the control, $P = 0.006$. Similarly, based on FIB-4 score, co-infected patients were also more likely to have values suggestive of significant fibrosis, 13% versus 2%, $P = 0.007$.

Hepatic fibrosis scores between the two groups were further compared according to the natural history of HBV infection (Table 3). In the *immune tolerant phase*, there was no difference in the fibrosis scores of co-infected and mono-infected patients both for APRI ($P = 0.19$) and FIB-4 ($P = 0.28$). For those in the *immune clearance phase*, co-infected patients had significantly higher fibrosis scores for both APRI (0.03) and FIB-4 (0.01). Among the *inactive HBsAg carriers*, APRI and FIB-4 scores were higher in the co-infected patients but this did not attain statistical significance ($P = 0.06$ and 0.08, respectively). In those with *HBeAg-negative chronic hepatitis*, liver fibrosis indices were significantly higher in the co-infected patients, APRI ($P = 0.01$) and FIB-4 ($P = 0.009$).

DISCUSSION

We assessed the LFT and non-invasive hepatic fibrosis scores of HIV/HBV co-infected and HBV mono-infected Nigerians in order to determine the impact of HIV on HBV-related liver disease in a sub-Saharan African population. We found that co-infected patients were generally more likely to have deranged LFT than those with HBV mono-infection. Co-infected patients had significant hypoalbuminaemia despite normal total protein and relatively higher bilirubin compared with those with HBV mono-infection. Seventy-seven percent (77%) of co-infected patients had at least one deranged liver enzyme with elevated ALP being the commonest enzyme abnormality. In terms of pattern of liver injury, co-infected patients had either predominantly raised ALP or mixed patterns while the mono-infected group had predominantly raised transaminases. In addition, co-infected patients had significantly higher hepatic fibrosis scores for both APRI and FIB-4. When the patients were preliminarily classified according to the phases of the natural history of HBV infection, LFT derangements and elevated fibrosis indices were essentially limited to patients in the immune clearance and HBeAg-negative chronic hepatitis phases.

Although HIV/HBV co-infected patients have also been reported to have hypoalbuminaemia compared with individuals with HBV mono-infection in other studies,[15–17] the clinical significance of hypoalbuminaemia with normal total protein cannot readily be stretched too far as liver synthetic dysfunction, poor nutritional status, and immunoglobulin response to infections may all be contributory.[17–19]

The finding of significantly higher serum ALP in co-infected patients than in the mono-infected controls was seen in all four phases of the natural history of HBV infection but it was only

Table 3 **Liver enzymes and hepatic fibrosis scores of patients according to phases of HBV natural history**

Phase of HBV natural history	HIV/HBV co-infected patients	HBV mono-infected control	*P*-value
Immune tolerant phase *(i.e. HBeAg +ve with normal ALT)*	*N = 13*	*N = 4*	
ALP (IU/L)	168.15 ± 71.42	100.25 ± 38.63	0.047
AST (IU/L)	31.25 ± 9.11	43.38 ± 42.03	0.58
ALT (IU/L)	31.77 ± 11.34	39.00 ± 13.37	0.30
APRI	1.03 ± 0.67	0.55 ± 0.43	0.19
FIB-4	1.58 ± 0.83	1.21 ± 1.10	0.28
Immune clearance phase *(i.e HBeAg +ve with ↑ALT)*	*N = 15*	*N = 11*	
ALP (IU/L)	198.76 ± 97.89	125.18 ± 55.74	0.04
AST (IU/L)	74.40 ± 23.46	124.82 ± 84.92	0.02
ALT (IU/L)	86.60 ± 34.04	145.00 ± 51.61	0.03
APRI	2.85 ± 2.46	0.87 ± 0.98	0.03
FIB-4	5.15 ± 4.73	1.35 ± 1.17	0.01
Inactive HBsAg carriers *(i.e. HBeAg −ve with normal ALT)*	*N = 51*	*N = 58*	
ALP (IU/L)	128.96 ± 46.83	86.47 ± 25.57	<0.0001
AST (IU/L)	22.49 ± 8.91	28.26 ± 11.31	0.40
ALT (IU/L)	22.05 ± 8.08	25.86 ± 9.50	0.30
APRI	0.52 ± 0.72	0.32 ± 0.16	0.06
FIB-4	1.26 ± 1.25	0.83 ± 0.47	0.08
HBeAg −ve chronic hepatitis *(i.e HBeAg −ve with ↑ALT)*	*N = 21*	*N = 27*	
ALP (IU/L)	175.93 ± 61.33	114.90 ± 46.21	0.0003
AST (IU/L)	63.33 ± 22.46	92.89 ± 130.36	0.31
ALT (IU/L)	79.34 ± 38.26	93.85 ± 169.09	0.70
APRI	2.31 ± 1.65	0.77 ± 0.45	0.01
FIB-4	2.99 ± 3.39	0.88 ± 0.46	0.009

ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; APRI = aspartate aminotransferase-platelet ratio index; HBV = hepatitis B virus; HBeAg = hepatitis B e antigen

in the immune clearance and the HBeAg-negative chronic hepatitis phases that it was accompanied by predominantly conjugated hyperbilirubinaemia. It is worthy of note that while the predominance of raised ALP, especially when accompanied by predominantly conjugated hyperbilirubinaemia, in co-infected patients is suggestive of a cholestatic pattern, there was no significant difference in the GGT levels between them and the controls. This appears rather unusual as cholestasis is typically associated with raised GGT. The contribution of alcohol intake to GGT rise in the study population cannot be convincingly ascertained, especially considering the fact that alcohol consumption is often underestimated by patients. Nevertheless, the lack of difference in GGT levels cannot readily be attributed to alcohol consumption as both groups were similar in the proportion of subjects with history of significant alcohol consumption. In consideration of the fact that we did not specifically assay for liver-isoenzyme of ALP in our patients, it is possible that extra-hepatic sources of ALP may be contributing to ALP elevation in the co-infected patients, thereby making ALP and GGT patterns appear different. In addition, the observation that significantly higher ALP was seen in co-infected patients in all phases of the natural history of HBV infection, including the phases usually associated with minimal hepatic injury, further calls for cautious interpretation in attributing this phenomenon to HBV-related hepatic disorder.

A number of studies have documented a predominantly cholestatic liver enzyme abnormality in HIV/HBV co-infected patients.[15,16,20] In Uganda,[20] it was found that HIV/HBV co-infected patients predominantly had cholestatic liver injury (64%), followed by hepatocellular injury (27%) and mixed pattern (9%). The proportion of co-infected patients with at least one liver enzyme elevation in our study (77%) is comparable with 88% reported by Lodenyo *et al.*[17] in South Africa. In agreement with our findings, 56% of the co-infected patients were found to have elevated ALP in South Africa.[17] Unlike our study where 23% of the co-infected patients had normal liver enzymes, none of the Ugandan co-infected patients had normal liver enzymes. There are a number of reasons to account for this difference. While our study involved ART-naïve co-infected patients, 29% of the Ugandan co-infected population was on ART. In addition, the proportion of HIV-positive patients who had severe immunosuppression in Uganda was twice that of our study. Our exclusion of individuals with obvious infective and metabolic co-morbidities that readily affect the liver could also have contributed.

Although the clinical significance and mechanisms of cholestatic liver damage in HIV-infected patients is still undergoing further investigation, a lot of explanations have been put forward. Some of these include subclinical cholangitis from viral hepatitis and opportunistic conditions such as cytomegalovirus (CMV) and mycobacteria, side-effects of drugs such as co-trimoxazole and the well-recognized entity known as AIDS-related sclerosing cholangitis.[15,21,22] The fact that 46% of our HIV/HBV co-infected patients had severe immunosuppression means that a good number of them were at risk of AIDS-related sclerosing cholangitis. Our patients were unlikely to be on co-trimoxazole as they were recruited at entry point when they were still being accessed for treatment. None of our subjects had clinical evidence of tuberculosis.

On the other hand, our HBV mono-infected subjects were more likely to have liver enzyme derangement suggestive of hepatocellular injury, with higher AST and ALT levels. However, when the patients were analysed according to the phases of the natural history of HBV infection, significant difference in necro-inflammatory response between co-infected and mono-infected patients was only seen in patients in the immune clearance phase. Although available reports on LFT abnormalities of HIV/HBV co-infected patients did not readily compare them with HBV mono-infected patients according to the natural history of HBV infection, the tendency of HIV/HBV co-infected patients to have relatively lower levels

of markers of hepatocellular injury compared with HBV mono-infected populations has been reported by other workers, with ALT rather being the most consistently affected.[15,16,23,24] Colin *et al.*[16] in London and Otedo *et al.*[24] in Kenya found that the serum ALT of their HBV mono-infected population was higher than that of the co-infected patients while the differences in the AST levels of the two groups were not significantly different. In a French population that was further classified according to their HBeAg status, Gilson *et al.*[12] found that ALT levels were lower in HBeAg-positive HIV/HBV co-infected patients than in a HBeAg-positive HBV mono-infected group. The phenomenon of relatively lower levels of necro-inflammatory markers in HIV/HBV co-infected populations can be explained by the fact that HIV-induced immunosuppression affects adaptive and innate host mechanisms that play a central role in the pathogenesis of HBV-related liver disease.[5,7]

Perinatally-acquired HBV infection is characterized by a prolonged immune tolerant phase with HBeAg positivity, high levels of serum HBV DNA and minimal liver damage if at all (hence normal ALT). There is virtually no immune response by the host to HBV in this phase.[14] The normal LFT with no difference between our co-infected and mono-infected patients in this phase is therefore understandable.

The immune clearance phase usually occurs in persons who acquired HBV infection in adulthood, or later in life in persons who acquired HBV infection perinatally and have initially been through the immune tolerant phase. This phase involves several HBV-specific immune responses with various degrees of hepatic damage (hence elevated ALT) and half of the affected patients may clear the HBeAg (seroconversion) within five years.[14] It therefore stands to reason that the most marked derangements in both our co-infected and mono-infected patients occurred in this phase. In addition, the fact that this phase depends on several HBV-specific immune-mediated processes which are impaired by HIV-induced immunosuppression readily explains why the HBV mono-infected patients had a stronger necro-inflammatory response. When established, seroconversion from HBeAg seropositive to HBeAg seronegative status (with appearance of antibody to HBeAg) marks the transition to inactive HBsAg carrier state with low or undetectable serum HBV-DNA, and normal aminotransferases.[14] There is little or no HBV-specific immune-mediated response in this phase. It is therefore not surprising that co-infected and mono-infected patients in this phase had similar transaminases which were within normal limits. HBeAg-negative chronic hepatitis is a late phase in the natural history of chronic HBV infection and represents those who continue to have or re-develop high levels of HBV-DNA and active hepatitis despite HBeAg seroconversion. These individuals still have variable degrees of ongoing liver damage. Although the serum transaminases were higher among our HBV mono-infected patients in this phase suggestive of a relatively more robust necro-inflammatory response, the difference between them and co-infected patients was not statistically significant. Our findings therefore suggest that the impact of HIV-induced immunosuppression on hepatic necro-inflammation in HIV/HBV co-infected individuals is probably most significant in individuals in the immune clearance phase of HBV infection.

The limited utility of liver enzymes for assessment of liver damage has been substantiated by the finding that our co-infected patients were more likely to have elevated values of non-invasive hepatic fibrosis scores for both APRI and FIB-4 irrespective of relatively lower levels of transaminases. When the patients were classified according to the phases of the natural history of HBV, this observation was limited to patients in the immune clearance and HBeAg-negative chronic hepatitis phases. Co-infection of HIV and viral hepatitis has been associated with higher non-invasive fibrosis scores than either viral hepatitis or HIV mono-infection in American populations.[8,25] In the Multicentre AIDS Cohort Study, baseline hepatic fibrosis score assessed by APRI was significantly higher in HIV-positive patients with either HBV or HCV co-infection (APRI = 0.81) compared with those with either HBV or HCV mono-infection (APRI score = 0.41) or HIV mono-infection (APRI = 0.41), $P = 0.0001$.[8] In the work of Blackard *et al.*,[25] the median FIB-4 scores were 0.60 in subjects without HIV or HCV, 0.83 in HCV mono-infected subjects, 0.86 in those with HIV mono-infection but 1.30 in HIV/HCV co-infected subjects. Other studies have also suggested that immunosuppressed patients with viral hepatitis may progress to extensive fibrosis and cirrhosis with only minimal inflammatory response as reflected by serum ALT.[26,27] From a clinical point of view, it deserves to be said that relatively lower transaminases in HIV/HBV co-infected patients should be cautiously interpreted since it may not be reflective of the extent of hepatic damage. While our study has provided useful baseline data on non-invasive fibrosis scores for treatment-naïve HIV/HBV co-infected African patients, it should be highlighted that the clinical utility of fibrosis scores in the management of patients with HIV and viral hepatitis co-infection goes beyond baseline assessment. Increased hepatic fibrosis has been associated with elevated plasma levels of various classes of antiretroviral drugs with increased risk of toxicity and this could have a negative impact on ART adherence.[28,29] More importantly, baseline fibrosis scores and subsequent rise in fibrosis scores have been shown to be predictive of mortality in HIV-infected patients with HBV or HCV co-infection.[30] This has led to suggestions for possible increased utility of non-invasive hepatic fibrosis scores for improved management of HIV-infected patients with viral hepatitis co-infection. Furthermore, non-invasive fibrosis scores have a role in monitoring the impact of ART on liver disease in patients with HIV/viral hepatitis co-infection. In the work of Price *et al.*,[8] lower APRI scores following commencement of ART showed that ART mitigated the progression of liver disease in HIV/viral hepatitis co-infected patients.

Our study was limited in some ways. We were unable to do HBV viral load due to cost implications which probably limited some of our conclusions especially regarding the phases of the natural history of HBV infection. Lack of HBV viral load measurement might have led to misclassification of some persons with negative HBeAg and normal ALT, a portion which particularly among the co-infected patients might have had HBeAg-negative chronic hepatitis. In addition, the small sample size for some of the HBV natural history groups such as the immune tolerant phase could have also limited the conclusions drawn from the comparison. Further studies on key natural history groups such as immune clearance phase are needed for better understanding of the pathophysiology of liver function abnormalities between co-infected and mono-infected patients. The recruitment of the control group from the gastroenterology clinic where they have been referred for evaluation and management of HBV-related liver disease may suggest some sampling bias in terms of severity of liver

disease. However, the observation that the clinical characteristics of the co-infected subjects and the control were found to be similar suggests that both groups may not be remarkably different. Other than relatively common disorders such as tuberculosis, pneumonia, candidiasis, meningitis, urinary tract infection, sepsis, helminthiasis and malignancies that were excluded in the patients on either strong clinical grounds or after laboratory screening, other less common opportunistic infections which have been found to play some role in liver disease in HIV-positive patients such as non-tuberculous mycobacteria and CMV were not screened for as the screening serological tests are not readily available in our centre. Our inability to assay for liver isoenzyme of ALP also made it difficult to ascertain the contribution of extra-hepatic sources of ALP in patients with elevated ALP.

In conclusion, LFT abnormalities are common in Nigerians with HBV infection and co-infection with HIV negatively impacts on hepatic function. While liver function tests may be beneficial as baseline pre-ART evaluation of HIV-infected patients, use of non-invasive hepatic fibrosis scores should be considered for better assessment of severity of liver damage in HIV/HBV co-infected patients especially in resource-limited settings. Beyond providing baseline information on the severity of liver damage, use of non-invasive fibrosis scores would further help to identify patients at higher risk of ART toxicity due to hepatic dysfunction. Considering the observation that ART mitigates the progression of liver disease in HIV/viral hepatitis co-infected patients,[8] serial assessment of non-invasive fibrosis scores in such patients will further help to evaluate for differences in progression of liver disease especially in individuals whose antiretroviral drug combination does not contain efficacious anti-HBV agents such as tenofovir. More importantly, since rise in fibrosis scores in HIV/HBV co-infected patients has been identified as a predictor of mortality, serial assessment of non-invasive fibrosis scores should be implemented in HIV/HBV co-infected patients in order to identify those at higher risk of mortality from advanced liver disease despite ongoing antiretroviral treatment. Ultimately, efforts to minimize the co-infection of HBV and HIV should be strengthened in sub-Saharan Africa.

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