Original Article

Prevalence of hepatitis B e antigen among human immunodeficiency virus and hepatitis B virus co-infected patients in Jos, Nigeria

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Abstract

Introduction: Human immunodeficiency virus (HIV) negatively impacts the natural history of hepatitis B virus (HBV) infection, including replication. We determined the prevalence of HBeAg in HIV/HBV co-infected patients compared to HBV mono-infected controls and further investigated the relationship between HBeAg seropositivity and the degree of HIV-induced immunosuppression in co-infected patients.

Methodology: The study design was cross-sectional. One hundred HBsAg-positive HIV-infected adults and 100 age and sex matched HBsAg-positive HIV negative controls were consecutively recruited between May and November 2010. Relevant demographic and HBV-related information was obtained. HBeAg was assayed by semi-quantitative third generation ELISA. The HIV/HBV co-infected patients also had CD4+ cell and HIV viral load quantification measured using flow cytometry and polymerase chain reaction techniques respectively.

Results: In each group, the mean age was 34 ± 8 years and the majority (61%) was female. The prevalence of HBeAg was significantly higher among co-infected patients (n = 28; 28%) than in the controls (n = 15; 15%; p = 0.03). HBeAg seropositivity was independently associated with age < 40 years (AOR = 2.83, 95% = CI 1.29-6.17) and HIV seropositivity (AOR = 2.44, 95% C.I = 1.17-5.07). The prevalence of HBeAg was significantly higher in co-infected patients with CD4 cell count < 200 cell/μL (41.3%) compared to those with 200-499 cell/μL (18.6%) and ≥500 cell/μL (9.1%), p = 0.006.

Conclusion: HIV/HBV co-infected patients have a significantly higher prevalence of HBeAg than HBV mono-infected individuals. HBV-infected patients should be routinely assessed for HBeAg, especially if they are co-infected with HIV.

Key words: CD4 cell count; co-infection; hepatitis B e antigen; hepatitis B virus; human immunodeficiency virus

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Introduction

There are over 350 million hepatitis B virus (HBV) carriers worldwide. The majority of them reside in the developing countries of South East Asia and sub-Saharan Africa, where the lifetime risk of infection is estimated to be greater than 60%, and carriage rates are in excess of 8% [1]. Over 600,000 persons die each year worldwide from complications of HBV infection including liver cirrhosis and hepatocellular carcinoma [1]. Similarly, 70% of the over 33 million people estimated to live with the human immunodeficiency virus/acquired immune deficiency syndrome (HIV/AIDS) globally reside in sub-Saharan Africa [2]. HIV and HBV co-infection is common due to shared routes of transmission. This union is undesirable because HIV negatively impacts on all the phases of the natural history of HBV infection, leading to higher rates of HBV chronicity and replication in co-infected patients compared to those mono-infected by HBV [3,4].

In Nigeria, the carrier rate of hepatitis B surface antigen (HBsAg) is 10-17% in apparently healthy adults [5-7]. In Jos, Plateau State in north central Nigeria, the prevalence of HBsAg among apparently healthy blood donors was found to be 14.3% [6]. Although horizontal transmission is widely recognized as the major means of HBV transmission in areas of high endemicity such as Nigeria [8], the vertical transmission rate for HBV in a Nigerian population of HBsAg-positive pregnant women was found to be 51.6% [9]. Despite the inclusion of the hepatitis B vaccine in the National Programme on Immunization in 1995, the vaccine only became widely available in Nigeria in 2004. Hepatitis B vaccine coverage rate is still poor, with vaccination rates at about 41% [10,11]. On the other hand, the national HIV seroprevalence in

Nigeria is about 4.1% with 3.1 million people living with HIV (PLHIV), 56,681 annual HIV-positive births, and 215,000 annual AIDS deaths [12]. In response to the raging epidemic of HIV/AIDS, the government of Nigeria, in partnership with international collaborators, established the national antiretroviral therapy programme in 2002, which led to increased access to HIV care and treatment [12]. In terms of HIV/HBV co-infection in Nigeria, the prevalence of hepatitis B surface antigen (HBsAg) in HIV-infected patients is 16-52%, which is two to three times higher than the prevalence of HBsAg in the general population [13-15].

Unfortunately, HBsAg-positive patients are not routinely evaluated for serological markers of HBV replication and infectivity (*i.e.* HBV DNA or hepatitis B e antigen [HBeAg]) in Nigeria due to the extremely high cost of these investigations. This situation makes it even more difficult to accurately classify patients according to the phase of the natural history of HBV infection they have. So far, the impact of HIV on HBV-replication has largely been unexplored in Nigeria. Furthermore, with the wide use of antiretroviral therapy (ART) for HIV-infected individuals, antiretroviral drug-induced hepatotoxicity has been more frequently reported in HIV/HBV co-infected subjects with high HBV replication [16]. This further makes determinations of HBV replication markers a priority in co-infected patients in a country such as Nigeria where access to ART is being scaled up. In view of these potential implications for HIV/HBV co-infected patients, and the paucity of data on hepatitis B e antigenaemia for co-infected populations in our locality, we determined the prevalence of HBeAg seropositivity in HIV/HBV co-infected adult Nigerian patients in comparison with HBV mono-infected controls. We further focused on only the co-infected patients in order to investigate the relationship between HBeAg seropositivity and degree of HIV-induced immunosuppression.

Methodology

Study design and setting

This was a cross-sectional study conducted in the HIV and Gastroenterology Clinics of Jos University Teaching Hospital (JUTH) between May and November 2010. JUTH is a tertiary health institution located in Jos, Plateau State, north central Nigeria and serves as a referral centre to at least seven neighbouring states in the region.

Subjects

Confirmed antiretroviral treatment-naïve HIV-positive patients accessing care in the HIV clinic of JUTH who had HBV infection (defined as HBsAg seropositivity) were recruited. Age- and sex-matched HBV mono-infected controls were also recruited from the Gastroenterology Clinic of the hospital. Only individuals 18 years of age or older were considered eligible. Exclusion criteria included evidence of hepatitis C virus (HCV) infection and previous exposure to HBV or HIV therapy. A structured questionnaire was used to obtain information from each participant about demographics, hepatitis B vaccination status, history of alcohol consumption, and HBV risk factors. All subjects were tested for HBeAg. The participants were consecutively recruited.

Sample size and sampling technique

A minimum sample size of 98 subjects was calculated using the formula for comparing proportions in two groups [17]:

$$n = \left[\frac{Z_{\left(1-\frac{\alpha}{2}\right)}\sqrt{p_0(1-p_0)} - Z_{(1-\beta)}\sqrt{p_1(1-p_1)}}{p_1 - p_0}\right]^2$$

Where n = minimum sample size, Z = standard Z score, 1-α/2 = 95% confidence interval = 1.96, 1-β = power of 80% = 0.842, P_1 and P_0 = prevalence of HBeAg among HIV/HBV co-infected and HBV mono-infected subjects from a previous study in sub-Saharan Africa [18]= 25% and 8.5% respectively.

The sample size was rounded to 100.

Co-infected subjects were recruited. These were HIV seropositive patients who were newly referred to the HIV clinic for care and treatment but were still undergoing baseline evaluation and had yet to commence antiretroviral therapy. Hepatitis B surface antigen and anti-HCV tests using third-generation ELISA kits were part of the HIV clinic protocol for baseline evaluation before antiretroviral therapy was started. All the consenting HIV-positive patients who were found to have positive HBsAg and negative anti-HCV at baseline evaluation during the study period and who did not have any exclusion criteria were consecutively enrolled until a sample size of 100 was attained. Although some of these patients knew about their HBsAg seropositive status having done the test sometime in the past for various reasons, HBsAg seropositive status was based on the test done during the study period.

The controls were also recruited consecutively from the Gastroenterology Clinic. Initially, the clinic records were used to identify 117 patients who were reported to have positive HBsAg tests but negative HIV and anti-HCV tests. This group of patients included those who had been on follow-up at the clinic but were yet to receive HBV therapy and those who had just been referred to the clinic following a recent diagnosis of HBsAg seropositivity during medical evaluation for different reasons. All 117 patients had fresh blood samples collected from them, which were tested for HBsAg, anti-HCV, and HIV using the same ELISA kits that were used for the co-infected patients to ensure uniformity. Out of these 117 patients, only 115 remained eligible for the study. One patient was excluded because of a negative HBsAg test while another was excluded because of a positive anti-HCV test using the third generation ELISA kits. All of them were HIV negative. The 115 HBV mono-infected subjects were then matched with the recruited co-infected patients for sex and age using one-to-one matching until 100 pairs of matched subjects was obtained. The remaining 15 HBV mono-infected subjects who did not match the co-infected patients were not part of the study.

Laboratory investigations

For each subject, about five mL of blood was collected from the antecubital vein into plain bottles under aseptic conditions. The blood samples were centrifuged within six hours, and separated sera were transported to the laboraory and stored at -20°C in aliquots for subsequent analysis. HBsAg was detected from sera using a commercially available third-generation ETI-MAK-2 Plus ELISA kit (Dia Sorin S.P.A, Saluggia, Italy). HBeAg detection from sera was performed using a semi-quantitative ETI-EBK Plus ELISA kit (Dia Sorin S.P.A, Saluggia, Italy). In order to exclude those with presumed HCV infections, anti-HCV antibody test was performed on each participant using a commercially available third-generation ELISA kit (DIA PRO Diagnostic Bioprobes, Milan, Italy).

HIV screening was done using DIALAB HIV 1/2 ELISA kit (DIALAB Diagnostics, Vienna, Austria). For the HIV seropositive patients, this was followed by confirmatory test using the Western blot hybridization technique (QualiCode HIV-1/2 Immunetics Inc., Boston, USA). These two tests were basic protocols in the HIV clinic and had already been done for the HIV seropositive patients before study recruitment. Among the 100 HIV serpositive patients recruited, 91 (91%) had only HIV-1 infection while 9 (9%) patients had both HIV-1 and HIV-2 infections. None of them had only HIV-2 infection.

Another three mL of whole blood was collected from the co-infected patients into EDTA bottles for quantification of the CD4+ T-lymphocytes within two hours of sample collection using flow cytometry (Partec, Munster, Germany). HIV-RNA quantification for the HIV seropositive patients was done from plasma by reverse transcriptase polymerase chain reaction using Cobas Amplicor HIV-1 Monitor Test version 1.5 (Roche, Branchburg, USA). The Roche COBAS Amplicor analyser used has a lower detection limit of 400 copies/mL (i.e. 2.6 $\log_{10}$ copies/mL). Considering that the control group comprised apparently healthy HIV-negative subjects and by implication had neither HIV viraemia nor HIV-induced CD4+ lymphocyte depletion, CD4+ cell quantification and HIV viral load were not necessary tests for them.

CD4 cell count and HIV viral load stratification

The CD4+ cell counts of the HIV seropositive patients were categorised into three using the Centers for Disease Control and Prevention (CDC) classification as follows: ≥500 cells/μL, 200-499 cells/μL, and <200 cells/μL [19].

Starting from the lower detection limit of the Roche COBAS Amplicor test, the HIV viral load of the HIV seropositive patients was stratified into four, as proposed by Papastamopoulos *et al*. [20]: ≤ 2.6 $\log_{10}$ copies/mL, 2.7-4.0 $\log_{10}$ copies/mL, 4.1-5.0 $\log_{10}$ copies/mL and >5 $\log_{10}$ copies/mL.

Ethical considerations

The Research and Ethics Committee of JUTH approved the study protocol and the study conformed to the provisions of the Declaration of Helsinki. Each subject signed an informed consent form before enrolment. The HBeAg test results were disclosed to the participants and subsequently made available to the managing physicians to enable them make further decisions on the treatment/follow up of the patients in their respective clinics based on their individual eligibility.

Table 1. Characteristics of the study participants

Characteristic	HIV/HBV co-infected N=100 n (%)	HBV mono-infected N=100 n (%)	p-value
Age (yrs), mean ±SD	34 ± 8	34 ± 8	0.83
Sex (F/M)	61/39	61/39	1.00
Marital status			
Married	47 (47)	59 (59)	0.09
Single/Separated/divorced/Widowed	53 (53)	41 (41)	
Residence			
Urban	59 (59)	60 (60)	0.86
Rural	41 (41)	40 (40)	
Occupation			
Civil servant	12 (12)	16 (16)	
Farmer	12 (12)	14 (14)	
Health care worker	3 (3)	7 (7)	
Student	14 (14)	13 (13)	
Trader	23 (23)	22 (22)	0.64
Others	36 (36)	28 (28)	
Ethnicity			
Plateau indigenous tribes	53 (53)	56 (56)	0.38
Hausa/Fulani	11 (11)	17 (17)	
Igbo	4 (4)	2 (2)	
Yoruba	3 (3)	5 (5)	
Others†	29 (29)	20 (20)	
Hepatitis B Vaccination	1 (1)	1 (1)	1.00
On-going HBV risk factors			
MSP‡	58 (58)	35 (35)	0.001
Sharing of sharps	73 (73)	68 (68)	0.44
CD4+ cell count (cells/μL)			
<200	46 (46.0)	------	------
200-499	43 (43.0)	------	------
≥500	11 (11.0)	------	------
Log_{10} HIV viral load (copies/mL)			
≤2.6	13 (13.0)	------	------
2.7-4.0	17 (17.0)	------	------
4.1-5.0	43 (43.0)	------	------
>5.0	27 (27.0)	------	------
HBeAg seropositivity	28 (28)	15 (15)	0.03

†Others included minority tribes in northern and southern Nigeria; ‡MSP= multiple sex partners

Statistical analysis

Data analysis was performed using Epi Info version 3.5.1 statistical software (CDC, Atlanta, GA, USA). Continuous variables were expressed as means ± standard deviation (SD) for uniformly distributed data and median (interquartile range, IQR) for non-uniformly distributed variables. Proportions were used to describe categorical variables. The Chi square test was used to assess differences in frequencies for categorical variables. Differences were considered statistically significant for p-values < 0.05. Multivariate logistic regression analysis was performed to assess the independent association of variables with HBeAg seropositivity using variables that had a p-value of < 0.25 on univariate analysis. HIV viral load was log_{10}-converted.

Results

Characteristics of the study participants

The study included 100 HIV/HBV co-infected patients and 100 age and sex-matched HBV mono-infected controls. The characteristics of the study participants are shown in Table 1. The mean age of the co-infected patients and controls were 34 ± 8 years and 34 ± 8 years, respectively (p = 0.83). The majority of the participants in each group were females (61%). Male subjects were significantly older than female subjects independent of HIV status (37 ± 8 years vs.

Table 2. Univariate analysis for factors associated with HBeAg seropositivity

Variables	HBeAg positive N=43 n (%)	HBeAg negative N=157 n (%)	OR (95% CI)	p-value
HIV status				
Negative	15 (34.9)	85 (54.1)	1	
Positive	28 (65.1)	72 (45.9)	2.2 (1.04-4.72)	0.03
Sex				
Female	22 (51.2)	100 (63.7)	1	
Male	21 (48.8)	57 (36.3)	1.67 (0.84-3.32)	0.14
Age (yrs)				
≥ 40	11 (25.6)	67 (42.7)	1	
< 40	32 (74.4)	90 (57.3)	2.17 (0.96-4.95)	0.04
MSP†				
No	18 (41.9)	89 (56.7)	1	
Yes	25 (58.1)	68 (43.3)	1.82 (0.87-3.81)	0.08
Sharing of sharps				
No	9 (20.9)	50 (31.8)	1	
Yes	34 (79.1)	107 (68.2)	1.76 (0.80-4.15)	0.16
Blood transfusion				
No	39 (90.7)	141(89.8)	1	
Yes	4 (9.3)	16 (10.2)	0.90 (0.25-2.74)	0.86
Past history of jaundice				
No	35 (81.4)	139 (88.5)	1	
Yes	8 (18.6)	18 (11.5)	1.77 (0.80-4.15)	0.22
Alcohol consumption				
No	27 (62.8)	113 (72.0)	1	
Yes	16 (37.2)	44 (28.0)	1.52 (0.73-3.10)	0.24
Residence				
Rural	25 (58.1)	94 (59.9)	1	
Urban	18 (41.9)	63 (40.1)	0.93 (0.47-1.87)	0.83

†MSP= multiple sex partners; OR = odds ratio

Table 3. Multivariate analysis for factors associated with HBeAg seropositivity

Variable	AOR	95% C.I	p-value
Age (<40/≥40 yrs)	2.83	1.29-6.17	0.01
Alcohol consumption (Yes/No)	1.49	0.66-3.35	0.33
HIV status (Positive/Negative)	2.44	1.17-5.07	0.02
Multiple sex partners (Yes/No)	0.89	0.39-2.05	0.80
Past history of jaundice (Yes/No)	2.00	0.75-5.29	0.16
Sex (Male/Female)	1.91	0.85-4.30	0.10
Sharing of sharps (Yes/No)	1.45	0.63-3.40	0.38

AOR= adjusted odds ratio; C.I= confidence interval

32 ± 8 years, $p < 0.0001$). There was no statistically significant difference between the co-infected patients and the control group in terms of marital status ($p = 0.09$), place of residence ($p = 0.86$), occupation ($p = 0.64$), and ethnicity ($p = 0.38$). Only one subject (1%) in each group had prior hepatitis B vaccination. In terms of ongoing HBV risk factors, having multiple sex partners (MSP) was more common among the co-infected patients (58%) than the controls (35%), $p = 0.001$. There was a high rate of sharing unsterilized sharp instruments in the study population, but the difference between the patients (73%) and controls (68%) was not significant ($p = 0.44$) (Table 1).

The various categories of CD4+ cell count and HIV viral load of the HIV seropositive patients are shown in Table 1. The median (IQR) CD4+ cell count of the HIV seropositive patients was 222.0 cells/μL (107-320 cells/μL). The median (IQR) $\log_{10}$ HIV viral load of the HIV seropositive patients was 4.6 $\log_{10}$ copies/mL (3.8-5.1 $\log_{10}$ copies/mL).

Prevalence of HBeAg seropositivity

Of the entire 200 subjects, 43 (21.5%) had a positive HBeAg serology. The prevalence of HBeAg seropositivity was significantly higher among

HIV/HBV co-infected patients than the controls, 28 (28%) vs. 15 (15%), p = 0.03 (Table 1).

HIV/HBV co-infected patients were compared to determine whether degree of HIV-induced immunosuppression (reflected by the CD4+ cell count) and degree of HIV viral replication (reflected by HIV viral load) affected the prevalence of HBeAg seropositivity.

The prevalence of HBeAg was significantly higher in co-infected patients with CD4 cell count < 200 cell/μL (n=19, 41.3%) than in co-infected patients who had CD4 cell count of 200-499 cell/μL (n=8, 18.6%) and ≥ 500 cell/μL (n=1, 9.1%), p = 0.006.

On the other hand, there was no significant difference in the prevalence of HBeAg among co-infected patients according to various levels of HIV viral load: 5 (38.5%) for those with HIV viral load of ≤ 2.6 $\log_{10}$ copies/mL, 4 (23.5%) for 2.7-4.0 $\log_{10}$ copies/mL, 12 (27.9%) for 4.1-5.0 $\log_{10}$ copies/mL and 7 (25.9%) for > 5 $\log_{10}$ copies/mL, p = 0.82.

Factors associated with HBeAg seropositivity

On univariate analysis (Table 2), HBeAg seropositivity was associated with age < 40 years (odds ratio (OR) = 2.17, p = 0.04), and HIV seropositive status (OR = 2.20, p = 0.03). History of multiple sex partners tended to be associated with HBeAg seropositivity on univariate analysis (OR = 1.82, p = 0.08). Other socio-demographic variables and HBV transmission factors had no significant association with HBeAg seropositivity: male sex (OR = 1.67, p = 0.14), urban residence (OR = 0.93, p = 0.83), alcohol consumption (OR = 1.52, p = 0.24), sharing sharp objects (OR = 1.76, p = 0.16), previous blood transfusion (OR = 0.90, p = 0.86), and past history of jaundice (OR = 1.77, p = 0.22).

After adjusting for potential confounding effects in the logistic regression analysis (Table 3), age < 40 years (adjusted odds ratio (AOR) = 2.83, 95% CI =1.29-6.17, p = 0.01) and HIV seropositive status (AOR = 2.44, 95% C.I = 1.17-5.07, p = 0.02) remained independent determinants of HBeAg seropositivity.

Discussion

This study determined the prevalence of HBeAg in treatment-naïve HIV/HBV co-infected patients compared with HBV mono-infected controls. A high prevalence of HBeAg in both groups was found; co-infected patients had a significantly higher value than the mono-infected controls. Higher HBeAg prevalence in HIV/HBV co-infected patients than in HBV mono-infected subjects has also been observed in both developed nations and sub-Saharan Africa [4,18,21-23].

The prevalence of HBeAg seropositivity of 28% in the co-infected patients in this study is consistent with the rates of 25-27% reported in other studies in sub-Saharan Africa [18,24-26]. Some studies in Europe and Asia have documented much higher HBeAg prevalence (50-78%) in their co-infected populations [23,27,28]. There are some reasons to explain the disparity. The majority of the participants in the current study were women (61%), while those other studies either had a male preponderance (86-89%) [27,28], or an entirely male population [23]. The predominance of women in the current study is reflective of the higher female HIV burden in Nigeria as well as the observation that men find it more difficult to disclose their HIV seropositive status, leading to poorer health-seeking behaviour in attending public HIV treatment facilities [12,29]. However, the role of gender in the disparity of HBeAg burden is not fully known. While female gender has been reported to be an independent predictor of HBeAg clearance following HBV therapy [30], some studies that focused on spontaneous HBeAg seroconversion in the absence of HBV therapy found that male gender was more likely to be associated with HBeAg clearance [31,32]. It is not clear if these contrasting observations are actually due to gender differences or differences in infecting genotypes. Although HBV genotypes were not determined in the studies that documented higher rates of HBeAg than those found in the current study, the fact that the predominant genotype sub-type A_2 in Europe and genotype C in Asia are associated with slower HBeAg clearance than the African genotype sub-type A_1 could partly explain the higher HBeAg prevalence in those areas [33].

On the other hand, the HBeAg prevalence of 15% in the HBV mono-infected control was higher than the rate of 8-10% generally reported in HBV mono-infected individuals in sub-Saharan Africa [18,22,34].This may be due to differences in the characteristics of the study subjects. For example, a study in Enugu, southeastern Nigeria [34] excluded symptomatic patients, compared to the current study, in which symptomatic patients were included.

The finding that HIV seropositivity leads to increased prevalence of HBeAg compared to HIV negative individuals can be explained by the role of HIV-induced immunosuppression. In order to create a better understanding of the role of HIV seropositivity in increased rates of HBeAg, the prevalence of HBeAg

across various categories of CD4+ cell count in the co-infected patients was determined. The prevalence of HBeAg seropositivity increased with deteriorating immune status reflected by lower CD4+ cell counts. Increasing prevalence of HBeAg with worsening HIV-induced immunosuppression has also been reported by most of the studies that have investigated this relationship in co-infected subjects [21,24,28]. Cellular immunity plays a central role in the pathogenesis of HBV infection. Effective immunological control of HBV replication requires an intact immune system and involves the targeting of HBV antigens on infected hepatocytes through polyclonal, multi-specific CD8+ T-lymphocyte activity supported by CD4+ T-cells [35,36].In the early stages of HIV infection, CD4+ cell decline is often gradual, while CD8+ cells are relatively preserved, which could still maintain significant immunological control of HBV replication [36].However, with advanced HIV-induced immunosuppression, both CD4+ and CD8+ cells are depleted, leading to severe impairment in HBeAg clearance [36]. Beyond defective HBeAg clearance attributed to T-cell lymphopaenia, it has also been shown that HIV-induced immunosuppression favours HBV re-activation, which would make individuals who had previously experienced HBeAg seroconversion (*i.e.,* had developed anti-HBe antibody and lost HBeAg) to become HBeAg seropositive again, a phenomenon known as reverse-seroconversion [37,38].

On the other hand, the prevalence of HBeAg did not differ in the co-infected patients according to the various levels of HIV viral load. This finding is similar to other reports from Romania [21] and South Africa [38] that found no relationship between HBV replication markers and HIV viral load among co-infected patients. The reason for the lack of association between HIV viral load and HBV replication markers cannot readily be explained, especially because only a few studies have investigated this relationship. Large population-based cohort studies would probably be needed for a better understanding of any interaction between HIV viral load and HBV replication markers in the setting of HIV-HBV co-infection.

This study also revealed that age below 40 years was independently associated with HBeAg seropositivity. This is in agreement with other studies that found a strong association between younger age and HBeAg seropositivity in Taiwan [38] and the Gambia [39], both of which have similar HBV transmission epidemiology to Nigeria. In areas of high endemicity such as sub-Saharan Africa and Asia, horizontal and perinatal mechanisms are the most important means of HBV transmission, with most HBV-infected individuals acquiring the infection in early childhood, leading to high rates of chronic HBsAg carriage [8,40]. The natural history of HBV infection consists of three main chronological phases: immune tolerance, immune clearance, and low replicative phases. A cohort study in sub-Saharan Africa showed that about one-third of young HBV carriers cleared HBsAg and progressed to the immune clearance phase within 10 years of infection [39]. The remaining two-thirds persistently tested positive for HBsAg and continued to tolerate the virus [39]. In contrast to HBsAg, clearance of HBeAg occurred at steady rates over the years, such that the majority of patients had undetectable HBeAg in adulthood [39]. It was shown that 86% of the HBsAg carriers who were recruited between one and four years of age had lost HBeAg by the age of 19, and by the age of 24, only 13.6% of HBsAg carriers remained positive for HBeAg [39]. In natural history studies of Asian HBsAg-positive children, the rate of spontaneous HBeAg seroconversion was less than 2% per year among children three years of age or younger and between 4% and 5% per year in older children [40]. Approximately 90% of HBsAg carriers who acquired HBV in early life remained HBeAg-positive at age 15–20 years, but HBeAg clearance increased with increasing age, such that the prevalence of HBeAg was less than 10% in patients older than 40 years [31,38]. These observations therefore explain our finding that age below 40 years was independently associated with HBeAg seopositivity.

Surprisingly, we did not find a significant association between gender and HBeAg seropositivity. Ijoma *et al.* [34] in Enugu, southeastern Nigeria also did not find gender to be a determinant of HBeAg seropositivity. A number of studies that investigated spontaneous HBeAg seroconversion found that men were significantly more likely to achieve HBeAg clearance than women, even though the mechanisms are not completely understood [31,32]. While further studies are required in Nigeria to explore this relationship, it is also possible that the smaller number of male subjects in our study affected our findings.

Although history of multiple sex partners and sharing unsterilized sharp instruments had no significant relationship with HBeAg seropositivity in this study, the high rates of these risky behaviour in both co-infected and mono-infected groups remains a

serious source of concern in Nigeria, where both HIV and HBV are already endemic and resources for their management are largely sub-optimal.

There are some limitations of this study that should be noted. Assessment of HBV replication using HBeAg will miss the few individuals who may have a mutation in the pre-core or basic core promoter region of the viral genome, leading to inability to synthesize HBeAg despite high rates of HBV replication [41]. HBV DNA is considered a superior marker of replication and would identify such people. However, the limited resources available for this study could not support the extremely high cost of HBV DNA determination. Concerning the major limitation of the HBsAg, HBeAg, and anti-HCV antibody ELISA test kits, the manufacturers stated that borderline results should be cautiously interpreted, especially in severely immunosuppressed patients. Considering the cross-sectional design of this study, we were unable to determine whether the subjects were HBV chronic carriers since the duration of their hepatitis B surface antigenaemia could not be ascertained. The cross-sectional study design also made it impossible to ascertain the duration of their hepatitis B e antigenaemia. Regarding our findings about the factors associated with hepatitis B e antigenaemia, it should be pointed out that the use of logistic regression for a cross-sectional study with a prevalent outcome can overestimate the odds ratio. Although the immunological status of the HBV mono-infected subjects was not determined, the authors do not readily consider this a limitation in this study, considering that the control subjects were HIV-negative and, by implication, neither had HIV viraemia nor HIV-induced immunosuppression.

In conclusion, the prevalence of HBeAg in HIV/HBV co-infected patients is significantly higher than in HBV mono-infected individuals in north central Nigeria. This observation is of immense public health concern because HBeAg seropositivity is associated with high infectivity and also has a direct relationship with progression of liver disease. It is important to include HBeAg testing in the routine evaluation of HBV-infected patients in sub-Saharan Africa, especially if they are co-infected with HIV.

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