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MAJOR ARTICLE

High Rates of Occult Hepatitis B Virus Infection in HIV-Positive Individuals Initiating Antiretroviral Therapy in Botswana

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Background. Hepatitis B surface antigen (HBsAg)–negative but hepatitis B virus (HBV) DNA-positive infection—known as *occult* hepatitis B infection (OBI)—occurs in 1% to >15% of HIV-positive individuals in the United States and South Africa, respectively. However, there are no data on OBI from Botswana, a country known to be hyperendemic for chronic HBV infection and to have a significant HIV burden.

Methods. Two hundred seventy-two adults enrolled in an HIV treatment study of tenofovir/emtricitabine as the nucleoside backbone who were previously determined to be HBsAg negative were tested for HBV DNA at baseline and 1 year after initiation of highly active antiretroviral therapy (HAART).

Results. HBV DNA was detected in 72 of 272 (26.5%). Six individuals (8.3%) had HBV DNA levels greater than 200 IU/mL, and the highest viral load was 3280 IU/mL. Of 65 participants with OBI evaluated at 12 months after initiating HAART, only 1 (1.5%) had detectable HBV DNA.

Conclusions. Occult HBV infection is quite common in HIV-infected patients in Botswana, although its impact on the course of HIV disease progression is unknown. The suppression of occult HBV DNA levels by tenofovir/emtricitabine suggests an effective therapeutic option, although the long-term suppressive abilities remain unstudied.

Keywords. Africa; Botswana; HBV; hepatitis B virus; HIV; HIV/HBV; occult; tenofovir.

Globally, 240 million people have chronic hepatitis B virus (HBV) infection, and HBV is the world's leading cause of cirrhosis and hepatocellular carcinoma (HCC) [1, 2]. In sub-Saharan Africa (SSA), both HIV and HBV are endemic, yet HBV remains understudied in this region. Historically, HBV infections have been diagnosed by the detection of hepatitis B surface antigen (HBsAg). More recently, nucleic acid amplification testing (NAT) has been used to monitor HBV viral loads to determine treatment options and risk of disease progression. However, with the increased utilization of highly sensitive NAT techniques, cases of HBsAg-negative but HBV DNA-positive infection (known as *occult* hepatitis B infection or OBI) have been discovered. OBI is frequently defined as the existence of HBV DNA (typically less than 200 IU/mL) in the blood and/or hepatic tissue with the absence of serum HBsAg [3], although this definition has not been consistently applied.

Transmission of OBI has been demonstrated through blood transfusion, organ donation, vertical transmission, and via household contacts of chronic HBV-infected individuals, and it can lead to the development of chronic HBV infection in the recipient [4–13]. Moreover, OBI has been associated with advanced liver fibrosis, reduced response to interferon (IFN) therapy, and liver enzyme elevations in some studies [14, 15]. Although chronic HBV is considered the primary cause of liver failure and HCC, OBI is also a risk factor for progression to end-stage liver disease and HCC (systematic review [16]). Without appropriate screening, OBI goes undiagnosed, resulting in long-term sequelae of viral infection, as well as transmission to others.

In South Africa, HIV co-infection is associated with increased risk of HBV infection, including OBI [17, 18]. The prevalence of OBI varies from 1% of HIV-positive individuals in the United States to >15% of HIV-positive individuals in countries such as South Africa [19, 20]. HBV vaccination policies and practices, utilization of HBV-active antiretroviral therapies for HIV, and success in implementing the Joint United Nations Programme on HIV/AIDS (**UNAIDS**) 90-90-90 target for HIV diagnosis

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and treatment differ across the countries of southern Africa. Thus, South Africa is not reflective of the entire region, and there are no data on OBI from Botswana, a country known to be hyperendemic for chronic HBV infection and to have a high HIV prevalence. We hypothesized that occult HBV infection would be high in HIV-positive individuals in Botswana and that HBV-active HIV regimens would suppress HBV replication in most individuals with OBI.

METHODS

Study Participants and Samples

In 2008, Botswana adopted tenofovir plus emtricitabine (truvada) combined with either efavirenz or nevirapine as its firstline highly active antiretroviral therapy (HAART) regimen. The Botswana National Evaluation Models of HIV Care (*Bomolemo)* study was an observational cohort designed to demonstrate the tolerability and virologic and immunologic response of a truvada-containing regimen in HIV subtype C–infected adults conducted in Gaborone between November 2008 and July 2011. Participants were HIV infected, HIV treatment naïve, and age 18 years and older. Additional eligibility criteria included the presence of an AIDS-defining condition and a CD4 count <250 cells/uL, consistent with World Health Organization guidelines at the time. Female participants were excluded if they were pregnant or had received single-dose nevirapine for prevention of mother-to-child transmission within the 6 months preceding enrollment. After study entry and HAART initiation, participants were scheduled for evaluations at 1 month and then every 3 months until the final study visit at week 96. The current investigation represents a retrospective analysis of de-identified plasma samples from the *Bomolemo* study. The University of Botswana Institutional Review Board and the Human Research Development Committee at the Botswana Ministry of Health and Wellness approved the study.

Sample Screening

Two hundred seventy-two plasma samples from individuals who were previously determined to be HBsAg negative [21] were tested for HBV DNA using the COBAS AmpliPrep/TaqMan HBV Test, version 2.0 (Roche Diagnostic, Mannheim, Germany). Quantitative levels were recorded when ≥20 IU/mL, while samples with HBV DNA that were detectable but below this quantitative threshold were reported as <20 IU/mL. Antibody screening for hepatitis B core antibody (Monolisa Anti-HBC PLUS, Biorad, France) and hepatitis B surface antibody (Monolisa Anti-HBS PLUS, Biorad, France) was performed in triplicate per the manufacturer's instructions. Individuals with OBI at baseline and follow-up plasma samples obtained 1 year after initiation of HAART were evaluated for HBV DNA at 1 year using the COBAS AmpliPrep/TaqMan system, version 2.0.

Assessment of Liver Injury

Aspartate aminotransferase (AST) to platelet ratio index (APRI) and FIB-4 represent 2 distinct noninvasive indices of liver damage (reviewed in [22]). The APRI score is equal to 100 × (AST/40) / platelet, while the FIB-4 value is calculated as age [years] × AST [IU/L] / √ (PLT [10^9/L] × (ALT [IU/L]). Initially validated for hepatitis C virus, these scoring systems have been evaluated in other diseases of the liver [23–26]. Both FIB-4 and APRI were calculated for all subjects with available data.

Statistical Analysis

Sociodemographic and clinical data from baseline, 12-month follow-up, and 24-month follow-up visits were obtained from the *Bomolemo* study. Baseline OBI was defined as the detection of HBV DNA at any level in the absence of detectable HBsAg. HBV DNA levels were quantified at baseline and 12 months, with a value of 10 IU/mL assigned to all detectable HBV DNA levels <20 IU/mL. The chi-square test was used to evaluate the difference in proportions for dichotomous variables. Kruskal-Wallis and the Wilcoxon rank sum test were used for all continuous and ordinal nonparametric data. Analysis of variance with contrasting groups was utilized to compare antibody status among the HIV/OBI, HIV/chronic HBV, and HIV mono-infected groups. Multinomial regression models were used to evaluate baseline sociodemographic and clinical data as potential risk factors for OBI. All statistical analyses were performed using SAS 9.4.

RESULTS

Of the 309 participants enrolled in the *Bomolemo* study, 300 (97.1%) were screened for HBsAg as described elsewhere [21]. Twenty-eight individuals were HBsAg positive, giving a chronic HBV prevalence of 9.3% (95% confidence interval [CI], 6.3–13.2). Of the participants testing negative for HBsAg, HBV DNA was detected in 72 of 272 (26.5%; 95% CI, 21.3–32.1), denoting OBI. As shown in Table 1, baseline demographics and clinical variables—including age, gender, BMI, HIV viral load, CD4 cell count, platelet count, hemoglobin, AST, AST, alkaline phosphatase, and total bilirubin—were similar among the OBI/HIV, chronic HBV/HIV, and HIV mono-infected groups. APRI values were similar among all groups (0.29, 0.32, and 0.28, respectively), as were FIB-4 scores (1.02, 1.08, and 1.04, respectively). Among those individuals with OBI, HBV DNA levels were <20 IU/mL (lower limit of quantification) in 49 of 72 (68.1%). Only 6 individuals (8.3%) had HBV DNA levels greater than 200 IU/mL, and the highest viral load was 3280 IU/mL.

There were significant differences in surface and core antibody status among all 3 groups. For instance, core antibody was detected in 22 of 27 (81.5%) chronic HBV-infected individuals evaluated, 47 of 70 (65.3%) individuals with OBI, and 88 of 195 (45.1%) HIV mono-infected individuals (Table 2). Surface antibody was detected in 3 (11.1%) chronic HBV-infected individuals, 26 (37.1%) individuals with OBI, and 69 (35.4%) HIV mono-infected individuals. Multiple social and demographic data were evaluated for their potential association with occult

Table 1. Baseline Demographic and Clinical Data From HIV-Infected Individuals Enrolled in the *Bomolemo* Study

	Occult HBV (n = 72)	Chronic HBV (n = 28)	No HBV (n = 200)	*P* Value[a]
Age, y	35.5 (31.5–41)	35.5 (31.5–42.5)	37 (32–43.5)	.70
Male gender, n (%)	28 (38.9)	10 (35.7)	70 (35.2)	.89
BMI, kg/m^2	21.6 (19.5–24.7)	20.9 (18.9–23.6)	21.4 (19.0–25.0)	.87
HIV viral load, $\log_{10}$ copies/mL	5.17 (4.69–5.64)	4.91 (4.48–5.73)	5.12 (4.63–5.58)	.62
CD4 count, cells/uL	157 (71–226)	172 (93–239)	160 (80–229)	.84
Platelet	262 (204–327)	245 (204–300)	256 (205–314)	.84
Hemoglobin, g/dL	11.2 (9.5–12.5)	11.7 (9.6–13.1)	11.4 (10.1–13.0)	.26
ALT, U/L	19.1 (14.7–25.7)	23.3 (16.3–36.7)	21.4 (15.0–29.1)	.22
AST, U/L	28.2 (24.5–37.9)	33.5 (24.4–43.7)	28.5 (22.9–36.4)	.31
Alkaline phosphatase, U/L	73.6 (61.2–92.6)	78.1 (62.7–104.6)	69.9 (58.7–89.1)	.56
Total bilirubin, mmol/L	6.14 (4.23–7.89)	4.96 (4.26–6.07)	5.81 (4.25–7.73)	.41
FIB-4 score	1.02 (0.84–1.50)	1.08 (0.86–1.44)	1.04 (0.75–1.38)	.47
APRI	0.29 (0.19–0.44)	0.32 (0.28–0.46)	0.28 (0.21–0.38)	.41

Data represent medians (interquartile ranges in parentheses), except as noted.

Abbreviations: ALT, alanine transaminase; APRI, AST to platelet ratio index; AST, aspartate transaminase; BMI, body mass index; HBV, hepatitis B virus; HIV, human immunodeficiency virus.

[a]Comparisons were made using the Kruskal-Wallis test from Wilcoxon score, with the exception of male gender (chi-square test used).

HBV infection. No variables were statistically associated with the presence of OBI in univariate analyses (data not shown). Only the difference between isolated core antibody and combined core and surface antibody positivity remained significant, as demonstrated in Table 2.

Sixty-five of the 72 subjects with OBI had samples available to evaluate HBV viral load after 12 months of HAART containing tenofovir/emtricitabine. Only 1 of these 65 individuals with OBI (1.5%) had detectable HBV DNA at <20 IU/mL at 1-year post-HAART initiation (Table 3). There was no difference in mortality (8.3% in the OBI group, 14% in the chronic HBV group, and 12% in the no-HBV group; $P = .65$) or medication adjustments (4.2% in the OBI group, 0% in the chronic HBV group, and 4% in the no-HBV group; $P = .96$) among the 3 groups during the 2-year study duration. No differences were noted among baseline HBV status when evaluated for HIV viral load and CD4 counts at baseline, 1 year of follow-up, or 2 years of follow-up (Table 4).

Table 2. HBV Antibody Status for HIV-Infected Individuals Enrolled in the *Bomolemo* Study

	Occult HBV (n = 70)[a], n (%)	No HBV (n = 195)[a], n (%)	Chronic HBV (n = 27)[a], n (%)	*P* Value[b]
Core + surface +	24 (34.3)	59 (30.3)	3 (11.1)	.06
Core + surface -	23 (32.9)	29 (14.9)	19 (70.4)	<.001
Core - surface +	2 (2.9)	10 (5.1)	0 (0)	.59
Core - surface -	21 (30.0)	93 (47.7)	5 (18.5)	<.001
Total core +	47 (67.1)	88 (45.1)	22 (81.5)	<.001
Total surface +	26 (37.1)	69 (35.4)	3 (11.1)	.03

Abbreviation: HBV, hepatitis B virus.

[a]Data were available for 292 individuals. Total values (core positive or surface positive) represent percentage of occult hepatitis B infection cases with specific antibody status positive.

[b]Comparisons made using Fisher's exact test for antibody status among the occult hepatitis B infection, chronic HBV, and non-infected HBV groups.

DISCUSSION

Chronic HBV infection rates between 3.8% and 13.6% have been reported in HIV-positive individuals in Botswana [21, 27–30]. However, this analysis represents the first evidence of a high rate of OBI infection in HIV-infected patients in Botswana. A rate of 26.5% is higher than anticipated, although this may be in part due to techniques permitting increased detection at very low viral loads. As 49 of 72 (68%) OBI samples identified had HBV DNA levels <20 IU/mL, utilization of viral load assays with different limits of detection may influence the overall OBI detection rate.

Prospective evaluations of viral suppression during OBI are rare. Thus, to date, there are limited data available to guide the development of specific OBI treatment guidelines, except for

Table 3. Post-HAART HBV Viral Loads in Occult and Chronic HBV Individuals

	HBV Viral Load Category	Occult HBV (n = 72)	Chronic HBV (n = 28)
Baseline HBV viral load	TND, n (%)	—	9 (32)
	<20 copies/mL, n (%)	49 (68.1)	5 (18)
	≥20 copies/mL, n (%)	23 (31.9)	14 (50)
	Median copies/mL (range)[a]	57.4 (22.9–3930)	31 600 (59–>1.7 × 10^8)
HBV DNA positive at 12 mo		(n = 65)	(n = 24)
	TND, n (%)	64 (98.5)	16 (66.7)
	<20 copies/mL, n (%)	1 (1.5)	6 (25.0)
	≥20 copies/mL, n (%)	—	2 (8.3)
	Median copies/mL (range)[a]	—	—

Abbreviations: HAART, highly active antiretroviral therapy; HBV, hepatitis B virus; TND, target DNA not detected.

[a]Median calculated for HBV loads ≥20 copies/mL (above lower limit of quantification of the assay).

Table 4. Baseline, 1-Year, and 2-Year Post-HAART Initiation HIV Viral Load and CD4 Count Categorized by HBV Status

	Occult HBV (n = 72)	Chronic HBV (n = 28)	No HBV (n = 200)	*P* Value
CD4 at baseline, cells/uL	169 (79–229)	172 (93–238)	159 (77–229)	.98
CD4 at year 1, cells/uL	339 (193–400)	315 (244–391)	323 (221–401)	.45
CD4 at year 2, cells/uL	386 (262–506)	376 (320–480)	372 (290–284)	.40
HIV viral load, baseline, copies/mL	144 000 (46 000–421 500)	80 800 (30 625–505 750)	131 000 (43 100–382 000)	.70
HIV viral load, year 1, copies/mL	All <400	All <400	All <400	—
HIV viral load, year 2, copies/mL	All <400	All <400	All <400	—

Data represent median (interquartile range).

Abbreviations: HAART, highly active antiretroviral therapy; HBV, hepatitis B virus.

instances of reactivation caused by immunosuppressive agents. In a multicenter cohort of HIV-infected adults in Zambia and South Africa, occult HBV infection was present in 13.3% before HIV therapy [31]. By 12 months post–treatment initiation, HBV DNA levels were below the limit of detection in 19 of 21 lamivudine-treated and 18 of 18 tenofovir-treated participants. In a pilot study of 29 HIV-infected individuals, 9 occult HBV infections were observed during the 100-week study period [32]. Three individuals had intermittent HBV DNA levels, while 6 were HBV DNA positive only at week 16 post-HAART; DNA levels subsequently decreased below the level of assay detection. In Thailand, 5 HIV-positive women with occult HBV infection achieved HBV suppression on a 3TC-containing HIV regimen [33]. Similarly, Cohen Stuart et al. stated that when HAART (including 3TC) was initiated in patients with occult HBV, HBV DNA was no longer detectable during 3 years of follow-up [34].

In Botswana, HAART-containing tenofovir/emtricitabine demonstrated excellent OBI viral suppression at 1 year of follow-up. Interestingly, the 1 subject with a persistent HBV viral load started at a low HBV DNA level (less than 20 IU/mL). This subject's HIV viral load at 1 year was less than 400 copies/uL, with good improvement of the CD4 count from 257.5 to 482.6 cells/uL, suggesting that persistent HBV was not due to a lack of medication adherence. This subject had no change of therapy or other explanation for the persistent HBV viral load, although the genome of this virus was not sequenced due to its low viral load. Thus, it is uncertain if persistence was related to the virus itself (i.e., tenofovir resistance) or host immune system factors.

While some studies have reported a trend toward increased ALT and AST in OBI/HIV-infected individuals, this has not been found consistently [35–41]. One study found a transient rise in ALT and AST in OBI/HIV co-infected individuals when initiating HIV therapy [42]. Others have suggested a correlation between OBI and age or CD4 count in HIV co-infected individuals [34, 36, 42]. However, none of these factors was found to have any significant correlation with OBI/HIV co-infection in Botswana. The antibody status of HIV/OBI co-infected individuals varied widely with respect to HBV core and surface antibody status. Although core antibody positivity (either alone or in combination with surface antibody positivity) is a marker of previous exposure to HBV, this criterion is inappropriate in HIV-positive individuals in areas of high endemicity due to the significant (almost 30%) rate of core antibody-negative OBI. The results of this study are consistent with others conducted in South Africa [20, 36, 43]. Using multinomial logistic regression, OBI was significantly associated with core antibody status. There were unique differences in isolated core antibody-positive subjects between HIV/OBI and HIV mono-infected subjects, as well as between HIV/OBI and HIV/chronic HBV-infected subjects. HIV+ without OBI and HIV/HBV were used as references, respectively. However, this correlation with core antibody status cannot distinguish among OBI, chronic HBV, and noninfected individuals at the population level. Thus, core antibody status is not an appropriate screening test for OBI. As NAT is cost-prohibitive in many parts of the world as a screening technique, more studies will need to be conducted to find better ways to identify individuals with occult hepatitis B.

Several potential imitations exist. First, the original study included only truvada-based antiretroviral regimens for HIV; thus, no direct comparison between different HBV-active (or inactive) HAART regimens on HBV suppression was possible. Second, different HBV vaccination policies and strategies, distinct HAART regimens, and possible differences in the circulating HBV genotypes suggest that the data presented here may not reflect OBI in other African countries. Third, follow-up samples were not available for all individuals, as some individuals missed the study visit or the samples were depleted for previous studies. Fourth, HBV sequence data were not available to determine if lack of viral suppression during OBI was associated with drug resistance. Finally, given the modest number of individuals in each study group, we may have limited power to detect small differences that exist.

Occult hepatitis B is a significant public health concern in Botswana, as in several other African countries. Currently, methods to reduce chronic HBV (including vaccination) are also effective at reducing the incidence of OBI in healthy populations. Studies conducted in several countries, including South Africa, reported OBI despite HBV vaccination, suggesting that vaccination protection may not be as effective as previously thought [6, 44–48]. Risk factors for OBI include concomitant HCV, HIV, or other immunosuppression in addition to blood product exposure. As described above in other studies, OBI is associated with liver fibrosis, reduced IFN response, and liver enzyme elevations, and it is a risk factor for HCC [14–16]. In the absence of nucleic acid testing for HBV DNA, OBI may go

undiagnosed, can have a deleterious impact on the liver, and can result in viral transmission to other individuals.

This study, consistent with other previous studies, did not identify other epidemiological factors that could predict OBI. Without any additional modifiable risk factors, improved screening on blood products is of critical importance. Future research should focus on factors that contribute to the development of OBI in previously vaccinated individuals. Risk reduction strategies targeted at slowing the progression of liver disease to cirrhosis or preventing hepatocellular carcinoma will also be important considerations in the future.

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