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Prevalence of hepatitis B virus (HBV) co-infection in HBV serologically-negative South African HIV patients and retrospective evaluation of the clinical course of mono- and co-infection

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SUMMARY

Objectives: Hepatitis B virus (HBV) infection with undetectable hepatitis B surface antigen (HBsAg) has been reported in HIV patients, but the clinical significance is unknown. This study presents the prevalence of HBV DNA in HIV-positive patients negative for all HBV serological markers and a retrospective evaluation of the clinical course of mono- and co-infection.

Methods: Of 502 HIV-positive patients, 222 tested negative for HBsAg, antibody to hepatitis B surface antigen (anti-HBs), and antibody to hepatitis B core antigen (anti-HBc). An in-house real-time PCR targeting the HBV S-region was used to quantify HBV DNA. HBV isolates were genotyped. Baseline demographic and clinical characteristics of HBV DNA-positive and HBV DNA-negative patients were described. Treatment outcomes of patients at 6, 12, and 24 months after initiation of antiretroviral therapy (ART) were summarized.

Results: HBV DNA was detected in 5.4% (12/222) of serologically negative patients. Mean HBV viral load was 5359.2 IU/ml (standard deviation (SD) ±12 768.27). Eleven HBV isolates belonged to genotype A and one to genotype C. There were no significant differences in baseline characteristics or clinical course between the HBV DNA-positive and HBV DNA-negative groups.

Conclusions: We found 5.4% of the HBV serologically-negative HIV-positive patients had low levels of HBV DNA. There were no significant differences in clinical outcome between the mono- and co-infected groups.

1. Introduction

Between 5% and 17% of HIV-infected patients in South Africa are co-infected with hepatitis B virus (HBV).[1–3] Co-infection with HBV has been shown to increase mortality rates from 1.7 per 1000 person-years to 14.2 per 1000 person-years as a result of liver disease in HIV-infected men.[4] Moreover, HBV infection can complicate the treatment of HIV patients on antiretroviral therapy (ART). The risk of ART toxicity is increased threefold,[4,5] and this risk further increases when patients are taking concomitant medications for tuberculosis (TB).[2]

In South Africa, HBV DNA has been detected in hepatitis B surface antigen (HBsAg)-negative HIV-infected patients.[6–8] In these studies, the HIV-infected patients were positive for isolated antibody to hepatitis B core antigen (anti-HBc). The clinical significance of HBV DNA in the setting of HBsAg-negative serology is controversial. Some studies have found that the presence of HBV DNA in HIV-infected patients, in the absence of HBsAg, does not increase the frequency of transaminitis and hepatic flares.[9,10] Others have shown only minimal hepatocellular inflammation;[11] and yet others have shown statistically significant increases in hepatitis flares.[12] Confounding factors including ethnicity and variation in the prevailing HBV genotypes may account for these differences.

At present, there is very little information regarding the clinical course of HBV DNA-positive, HBsAg-negative HIV-infected patients from Africa. It is important that this is studied because the strains of HBV[13] and HIV[14] prevailing in Africa differ from those found in other regions of the world. The classification of HBV into eight genotypes, A to H, with a distinct geographic distribution[15] is well established, and a ninth genotype, I, has recently been identified and characterized.[16–21] In Africa, genotypes A, D, and E have been isolated and a geographical distribution demonstrated, with genotype A found in southern eastern Africa, genotype D in

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northern Africa, and genotype E in western and central Africa.[13] Evidence in this field from non-African countries may thus not necessarily be extrapolated to Africa. The objective of this sub-study of previous work,[1,8] was to determine the prevalence of HBV DNA in HBV serologically-negative HIV-positive patients and to evaluate the clinical impact of co-infection with HBV in this population.

2. Methods

2.1. Patient selection

Between 2006 and 2007, eligible HIV-positive patients initiating ART at the Themba Lethu Clinic (located in a secondary-level government academic hospital in Johannesburg) were invited to participate in the study. The study participants were educated about HBV/HIV co-infection and ART. Enrolment criteria included treatment-naïve patients who were aged 18 years and older and who were about to initiate lamivudine (3TC)-containing ART in accordance with public sector guidelines (i.e., a CD4 count <200 cells/mm^3 and/or World Health Organization (WHO) clinical stage 4).[22] None of these patients had received vaccination against HBV, because universal HBV vaccination at 6, 10, and 14 weeks of age was only introduced into the South African Expanded Programme on Immunization (EPI) in 1995 and therefore none of the participants would have received any vaccination. A total of 502 people agreed to participate in this study and signed informed consent. The parent study and cohort characteristics are described elsewhere.[1]

The study was approved by the Human Research Ethics Committees (Medical) of the University of the Witwatersrand and Saint Louis University Internal Review Board. Reasons for non-participation included additional time needed for HBV education, informed consent, poor understanding of the study, refusal to have extra blood drawn, and feeling too ill to participate.

2.2. Laboratory tests for HBV

2.2.1. HBV serology

This included qualitative testing for HBsAg, hepatitis B e antigen (HBeAg), antibody to hepatitis B surface antigen (anti-HBs), antibody to hepatitis B e antigen (anti-HBe), and anti-HBc, using the Axsym assays (Abbott Laboratories, IL, USA).

2.2.2. DNA extraction

DNA was extracted from 222 of the 502 serum samples that tested negative for HBsAg, HBeAg, anti-HBs, anti-HBe, and anti-HBc. DNA was extracted from 200 μl of serum using the QIAamp MinElute Virus Spin Kit (Qiagen GmbH, Hilden, Germany), in accordance with the manufacturer's instructions, and eluted into 200 μl elution buffer.

2.2.3. Real-time PCR quantification of HBV DNA

PCR primers, HBV-Taq1 and HBV-Taq2, as well as the FAM/TAMRA labeled TaqMan BS-1 probe were used,[23] and real-time PCR quantification of HBV DNA performed as described previously.[7] A serial dilution of cloned plasmid DNA containing a single genome of HBV DNA, ranging from 5×10^2 to 5×10^7 IU/ml in concentration, was used as template to generate the standard curve. The linear standard curved obtained was in agreement with the previous reports for this primer/probe set. The second WHO International Standard for HBV Nucleic Acid Amplification Techniques (product code 97/750 National Institute for Biological Standards and Controls (NIBSC); Hertfordshire, UK), which has a final concentration of 1×10^6 IU/ml, was used as the internal standard. The standard curve, blank, positive and negative controls, and samples were all tested in duplicate. The measured IU/ml for each reaction was calculated using the Ct (cycle threshold) value of each PCR interpolated against the linear regression of the standard curve. The dynamic range of the in-house real-time PCR was 5×10^2 to 5×10^7 IU/ml (2 logs to 7 logs of linear dynamic range).

2.2.4. HBV genotyping

Only samples that were HBV-DNA-positive for two separate real-time PCR reactions were genotyped. A restriction fragment length polymorphism (RFLP) assay was used to genotype HBV isolates.[24] Primers P7 and P8 were used to amplify nucleotides 256 to 796 (from the *Eco*RI site) in the S region. The amplicon was cleaved using restriction enzymes *Hinf*I and *Tsp*509I, in separate reactions, to give the characteristic RFLP patterns for the different genotypes.[24]

2.3. Clinical course

The 2-year clinical course of 211 of 222 HBV serologically-negative HIV-positive patients was evaluated retrospectively by file review through the TherapyEdge-HIV (TE)^{TM} electronic patient management system. The evaluation reviewed HIV clinical adverse events (i.e., TB, ART side-effects), HIV monitoring, and routine safety laboratory parameters (CD4 count, HIV viral load, liver transaminases, and full blood count). Hepatic flare was defined as any elevation of liver transaminases with clinical signs of hepatic injury or any asymptomatic elevation greater than two times the upper limit of the normal range.

2.3.1. Statistical analysis

Baseline demographic and clinical characteristics of HIV-positive patients with and without HBV DNA were described. Differences in clinical and laboratory responses at three time-points after ART initiation (6, 12, and 24 months) were estimated using the Student's *t*-test (parametric), the Kruskal–Wallis test (non-parametric), and the Wilcoxon test (non-parametric) for continuous variables, and the Chi-square test for proportions. Mortality was ascertained via South Africa's National vital registration system, and lost to follow-up (LTFU) was defined as having missed a clinic appointment (clinical assessment, anti-retrovirals pickup, counselor visit) by at least 3 months after the scheduled visit date.[25] All analyses were performed with SAS version 9.1 (SAS Institute Inc., Cary, NC, USA).

3. Results

Of the 502 HIV-positive patients enrolled into the parent study, 222 were negative for HBsAg, HBeAg, anti-HBs, anti-HBe, and anti-HBc. Thus 44% were HBV serologically-negative. Baseline demographics and characteristics of the HBV DNA-positive patients were similar to patients who were positive for anti-HBc from the larger study[7] in terms of age (mean ± SD, 34.2 ± 5.43 years vs. 39.98 ± 11.64 years), gender (female 60% vs. 56%), and CD4 count (mean ± SD, 91 ± 73.2 cells/mm^3 vs. 49 ± 77.2 cells/mm^3) (all *p*-values >0.05).

Of the 222 patients who were HBV serologically-negative, 12 (5.4%) tested positive for HBV DNA in duplicate real-time PCR HBV DNA quantification assays. The mean HBV viral load before initiating ART was 5359.2 (SD ± 12 768.27) IU/ml. Eleven HBV isolates belonged to genotype A and one to genotype C.

The clinical course of 211 of the 222 HBV-serologically-negative patients was evaluated retrospectively. The files of 11 patients could not be located on the TE management system and were declared LTFU. Two of these missing files were from patients with positive HBV DNA, thus leaving 10 HBV-positive patients evaluable

Table 1
Baseline demographic and clinical characteristics of HBV DNA-negative and HBV DNA-positive patients ($N = 211$)

	HBV DNA-negative	HBV DNA-positive	*p*-Value
Patients	$n = 201$ (95.3%)	$n = 10$ (4.7%)	
Age at initiation (years), median (IQR)	35.1 (31.8–42.2)	33.9 (28.8–34.9)	$p = 0.201$[a]
Sex, female	149 (74%)	6 (60%)	$p = 0.323$[b]
ALT (IU/l), median (IQR)	22 (16–33)	26 (20–47)	$p = 0.245$[a]
>40 IU/l	24/163 (15%)	2/8 (25%)	$p = 0.429$[b]
AST (IU/l), median (IQR)	32 (25–42)	43 (30–55)	$p = 0.137$[a]
> 40 IU/l	44/160 (28%)	4/8 (50%)	$p = 0.169$[b]
CD4 cell count (cells/mm^3), median (IQR)	108 (50–149)	89 (37–187)	$p = 0.796$[a]
<100 cell/mm^3	78/162 (48%)	4/8 (50%)	$p = 0.916$[b]
TB at ART initiation	13 (6%)	1 (10%)	$p = 0.178$[b]
First ART regimen			
d4T/3TC/EFV	138 (80%)	8 (89%)	$p = 0.448$[b]
d4T/3TC/NVP	11 (6%)	1 (11%)	$p = 0.546$[b]
Other regimen	25 (14%)	-	$p = 0.240$[b]

HBV, hepatitis B virus; IQR, interquartile range; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TB, tuberculosis; ART, antiretroviral therapy; d4T, stavudine; 3TC, lamivudine; EFV, efavirenz; NVP, nevirapine.
Data are *n* (%) unless otherwise indicated.
[a] *p*-Value obtained from *t*-test or Kruskal–Wallis.
[b] *p*-Value obtained from Chi-square test for associations.

for retrospective clinical analysis. At baseline, prior to the initiation of ART, the demographic characteristics of the participants with HBV DNA ($n = 10$) did not differ from those without HBV DNA ($n = 201$) (Table 1). Both groups were similar in terms of age, gender, liver function tests, and first ART regimen. The baseline median CD4 cell count was 89 cells/mm^3 (IQR 37–187) in the HBV DNA-positive group and 108 cells/mm^3 (IQR 50–149) in the HBV DNA-negative group; the difference was not statistically significant. All participants were negative for HCV by PCR assay.[1]

Out of the 211 patients, 183 (87%) initiated ART. Of the 28 patients who did not start ART, 19 were LTFU, one died of unknown causes, and one transferred to another clinic. Seven started ART in late 2009 to 2010 after the study period. The majority of patients, 80% (146/183), who started ART were on regimen 1a: stavudine (d4T), lamivudine (3TC), and efavirenz (EFV). The majority (89%) of patients with isolated HBV started regimen 1a. No patients received tenofovir as it was not available in government sector care during the time of this study. By the end of study follow-up, 84% ($n = 154/183$) of all study participants were alive and in care (Table 2). There were no events reported that were clinically consistent with hepatic flare in either group. No deaths were recorded among the HBV DNA-positive group, while 4% ($n = 7$) of those without HBV DNA had died over the course of 24 months of follow-up. The proportion lost to follow-up was not statistically significant.

The median CD4 cell counts were similar at each time-point evaluated for both groups (Table 2). The proportion of patients who switched antiretrovirals was the same in both groups (43% vs. 39%) and these switches occurred earlier for the HBV DNA-positive group (33% vs. 13% by 6 months), but this was not statistically significant. Among all patients who experienced a treatment switch, one of the most common reasons was peripheral neuropathy (Table 2). HBV viral loads and genotypes were not determined in the follow-up samples.

Table 2
Clinical and laboratory responses at 6, 12 and 24 months after initiation of ART

Variable	Clinical and laboratory responses					
	HBV DNA-negative			HBV DNA-positive		
	Baseline to 6 months	6 to 12 months	12 to 24 months	Baseline to 6 months	6 to 12 months	12 to 24 months
Number with follow-up available	174	162	147	9	8	7
AST (IU/l), median (IQR)	28 (22–40)	27 (21–35)	22 (19–30)	26 (24–33)	24 (19–25)[a]	38 (25–43)[b]
AST >40 IU/l	35/144 (24%)	15/100 (15%)	14/77 (18%)	0	0	1/3 (33%)
ALT (IU/l), median (IQR)	24 (16–35)	21 (15–35)	21 (16–27)	23 (10–37)	19 (16–21)[a]	26 (12–36)
ALT >40 IU/l	28/148 (19%)	18/98 (18%)	7/77 (9%)	2/8 (25%)	0	0
CD4 cell count (cells/mm^3), median (IQR)	227 (153–324)	243 (188–333)	329 (204–428)	167 (78–399)	150 (62–312)	78 (68–399)
Number of ART substitutions or regimen switch, yes	23 (13%)	31 (19%)	58 (39%)	3 (33%)	3 (38%)	3 (43%)
Reason for ART change known						
Peripheral neuropathy	1	2	0	0	0	1
Lactic acidosis/hyperlactatemia	1	0	1	0	0	0
Toxicity (not specified)	1	0	1	1	0	0
Virological failure	0	2	2	0	0	1
Pregnancy	0	0	3	0	0	0
Outcomes of ART patients (NS)						
Alive, on treatment	162 (93%)	147 (84%)	140 (81%)	8 (89%)	8 (89%)	7 (78%)
Dead	1 (0.6%)	6 (3%)	7 (4%)	0 (0%)	0 (0%)	0 (0%)
Lost to follow-up	6 (3.4%)	13 (8%)	18 (10%)	1 (11%)	1 (11%)	2 (22%)
Transferred to another facility	5 (3%)	8 (5%)	9 (5%)	0 (0%)	0 (0%)	0 (0%)

ART, antiretroviral therapy; HBV, hepatitis B virus; IQR, interquartile range; AST, aspartate aminotransferase; ALT, alanine aminotransferase; NS, results not statistically significant.
Data are *n* (%) unless otherwise indicated.
[a] $p < 0.001$ by *t*-test (parametric).
[b] $p < 0.001$ by Wilcoxon test (non-parametric).

4. Discussion

Three South African studies have previously demonstrated HBV DNA in 33%,[6] 88.5%,[7] and 10%[8] of isolated anti-HBc-positive HIV-infected patients. To our knowledge, this is the first African study to evaluate HBV DNA prevalence and the clinical course in HIV-infected patients without any serological markers for HBV. Of the 502 original serum samples screened, 222 (44%) had no HBsAg, HBeAg, anti-HBs, anti-HBe, and anti-HBc. A subset of 12 samples were considered HBV DNA-positive only when they tested positive in duplicate re-extracted real-time PCR assays, as well as in the subgenomic qualitative PCR used for genotyping.[24] The use of this stringent criterion as well as strict control measures in the laboratory,[26] preclude the possibility that the detection of HBV DNA was the result of contamination.[27]

The majority of HBV isolates belonged to genotype A, which is consistent with the genotypes found in South Africa.[13,28] The presence of HBV genotype C in one individual is unusual, but could be a result of the increased influx into South Africa of emigrants from Southeast Asia, where HBV genotypes B and C circulate.[15] HBsAg-negativity despite detectable HBV DNA can be as a result of the presence of mutants that affect expression and hence detection of HBsAg;[29–31] low HBV replication leading to low levels of HBsAg,[29] below the detection limit of the serological assays; and the formation of immune complexes, which mask the HBsAg epitopes preventing detection.[32] Following immune reconstitution as a result of ART, one study found 4/65 (6.2%) patients regained HBsAg.[33] The absence of anti-HBs may be a result of the immunocompromised state of the patients studied.[34,35] Increased CD4 counts following ART have been shown to be associated with the reappearance of anti-HBs.[33,34] The absence of anti-HBs has previously been shown to be a risk factor for the detection of HBV DNA in isolated anti-HBc-/HIV-positive patients.[35]

In the present study, the median CD4 count among the HBV DNA-positive group was 20 cells/mm^3, lower than that of the HBV DNA-negative patients. Although these low CD4 counts were not significantly associated with the detection of HBV DNA in the present study and another published study,[35] a significant association was found between lower CD4 counts in HBsAg-negative/isolated anti-HBc-positive HIV-infected patients in another study.[36] Detectable HBV may be as a result of reactivation of HBV infection following immunosuppression,[36] or a functional T cell defect.[35] Given this possible dynamic relationship between immune status, HBV DNA and HBV serology, repeat serological and HBV DNA measurements should be considered (if feasible and clinically indicated) to accurately assess the patient's current HBV/HIV co-infection status.

We demonstrated little difference in clinical outcomes between the HBV DNA-positive and HBV DNA-negative groups, although the small sample size may be a limitation in our ability to detect statistically significant differences. These findings are however consistent with a previous work by Shire and colleagues, who also failed to identify any factors that distinguish HBV DNA-positive HBV serologically-negative HIV patients from other HBV DNA-positive HBsAg-negative patients with isolated anti-HBc.[27] There may be a number of reasons for this in the present study. Firstly, the mean HBV viral load was quite low at 5359.2 IU/ml and may not have been a high enough burden to cause complications. Secondly, all these patients were started on an ART regimen containing lamivudine, which could have easily suppressed the HBV quickly, thus preventing complications as a result of the viral infection. Thirdly, while there is an increased risk of ART toxicity in HIV patients co-infected with HBV, severe hepatotoxicity (alanine aminotransferase more than five times normal) is rare.[37] In this population, the numbers may have been too small to demonstrate a serious adverse event.

Our results should be interpreted in light of the possible limitations of this study. Firstly, the small sample size may have limited our ability to detect differences. Secondly, this study retrospectively analyzed observational data and so we cannot exclude the possibility of bias. Finally, this sample is from one academic facility in Johannesburg, South Africa, and as such, results may not be generalizable to other settings, particularly settings in the developed world. Despite these limitations, our results add evidence to a limited body of knowledge in the field. Additionally, this study was carried out in the setting of an implementation government HIV treatment clinic and not the strict environment of a clinical trial. As such, these results may possibly reflect the practical clinical experience of HBV serology-negative DNA-positive individuals more closely.

Although there appears to be a lack of clinical significance of HBV DNA in HBV serologically-negative HIV patients in our setting, there are a number of other considerations that need to be taken into account in clinical practice. HBV DNA can be transmitted by blood donation and liver transplantation, can reactivate, and has been implicated in the development of hepatocellular carcinoma.[38] As has been shown in this and other studies,[27,33,39] HBV serological tests alone may not be sufficient to detect HBV infection in HIV-infected individuals. Sensitive PCR assays are needed to detect HBV DNA, but these are often not available or feasible in resource-limited environments. Implementation of alternative measures such comprehensive HBV vaccination and early initiation of ART should be considered to prevent possible opportunistic reactivation of HBV infection, transmission of HBV to uninfected individuals, and the potential development of HBV-related liver disease.

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Conflict of interest: No conflict of interest to declare.

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