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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