Occult hepatitis B virus infection in patients with isolated core antibody and HIV co-infection in an urban clinic in Johannesburg, South Africa

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Summary

Background—The prevalence of HIV/hepatitis B virus (HBV) co-infection in South Africa ranges from 4.8% to17% using the standard marker surface antigen (hepatitis B surface antigen, HBsAg) for chronic active HBV infection. However, sensitive molecular techniques for detecting HBV DNA in serum can detect occult HBV infection. We report the first observational prospective study of occult HBV infection in HIV-positive people in South Africa.

Methods—Five hundred and two patients attending an urban hospital were screened for HBV using serological testing for HBsAg, core antibody (anti-HBc), and surface antibody (anti-HBs). DNA was analyzed using real-time quantitative PCR to determine the HBV viral load.

Results—Of the 502 participants, 24 (4.8%) were HBsAg-positive and 53 (10.6%) were positive for anti-HBc alone. Of these 53, screening for occult disease was carried out in 43, of whom 38 (88.4%) were positive. The mean HBV viral load was 2.8×10^4 copies/ml (range 1×10^2 to 1×10^6 copies/ml).

Conclusions—Combining the participants with positive HBsAg and occult HBV DNA results, the prevalence of HBV increases from 4.8% (HBsAg alone) to 12.4%. While the clinical impact of occult HBV infection is unclear, consideration should be given to changing the guidelines to recommend dual HBV therapy for the treatment of co-infected patients in the developing world.

Introduction

Both HIV and hepatitis B virus (HBV) infections are endemic in sub-Saharan Africa.[1,2] Recent studies have shown the prevalence of HIV/HBV co-infection (using hepatitis B surface antigen (HBsAg) as a marker for HBV) in South Africa ranging from 4.8% to 17%,

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depending on the population studied.[3−5] HIV/HBV co-infection rates from the Themba Lethu Clinic cohort in Johannesburg have been reported previously.[5] Serological markers have been the standard method of diagnosis of HBV infection for more than 30 years. The wider availability during recent years of very sensitive molecular biology techniques for detecting HBV DNA in serum and liver tissue has increased attention to occult (silent) HBV infection. Occult HBV DNA is defined as HBV DNA found in liver or serum in HBsAg-negative patients. Occult HBV infection has important implications when considering the transmission of the virus and complications of chronic HBV infection such as cirrhosis and hepatocellular carcinoma. Further investigation of occult HBV DNA infection in HIV-seropositive patients is needed given the more rapid progress towards chronic liver disease in people with HIV/HBV co-infection, the possible complications of antiretroviral therapy (ART), perinatal transmission of HBV from mother to child, and the risk of HBV transmission through liver transplants.[6]

Recent advances in antiviral therapy directed at both HIV and HBV have led to the development of the World Health Organization (WHO) guidelines for standardized regimens to treat both diseases. These include the recommendation that HIV/HBV co-infected patients be treated with an antiretroviral (ARV) regimen that contains three drugs, two of which are effective against HBV as well as HIV.[7]

Occult HBV infection needs to be considered when establishing the prevalence of HIV/HBV co-infection in different populations. This information is already available in some countries.[8−10] However, there has only been a single study evaluating occult HBV in sub-Saharan Africa. This was a retrospective laboratory-based analysis on stored serum, which found that one third (5/15) of HIV-positive patients whose serum was negative for HBsAg but positive for antibody to the core antigen (anti-HBc) were also positive for HBV DNA (occult HBV infection was present). In contrast, none of the 31 HIV-negative patients with anti-HBc alone tested positive for occult HBV DNA.[11] We report the first observational prospective study of HIV-seropositive people in sub-Saharan Africa. Our study used volunteers from the US President's Emergency Plan for AIDS Relief (PEPFAR)-supported Themba Lethu Clinic in Johannesburg, South Africa.

Methods

Study design and subjects

HBV serology is not standard of care in the evaluation of HIV-seropositive people in the South Africa National Department of Health's (DoH) Comprehensive Care, Management and Treatment Program for HIV/AIDS.[12] Patients from the HIV Themba Lethu Clinic, which is located in a secondary government academic hospital in Johannesburg, South Africa and is affiliated to the University of the Witwatersrand, were invited to participate in this study. The study participants, who had already been diagnosed with HIV, were attending an educational session regarding HIV and ART. In this session, HBV infection was discussed and inclusion in the seroprevalence study was offered. The patients who decided to participate in the study signed a written consent and most had their hepatitis B serology done after the educational session. These patients were about to initiate ART in accordance with public sector guidelines (i.e., with a CD4 count <200 cells/ mm^3 and/or WHO stage 4).[12] Participants needed to be treatment-naive and able to initiate an ART regimen that included lamivudine. If the patients were found to be HBsAg-positive, they were asked to return for initiation of ARVs and followed for 6 months. HBsAg-negative participants were referred to the Themba Lethu Clinic or another clinic of their choice for ART. The study was approved by the University of Witwatersrand Ethics Committee and the Saint Louis University Internal Review Board.

HBV serology

Routine laboratory investigations for HIV monitoring were performed according to the public sector guidelines. Hepatitis B serology included HBsAg, anti-HBc, and antibody to the surface antigen (anti-HBs) using the Axsym assay from Abbott, which uses microparticle enzyme immunoassay (MEIA) methodology. The hepatitis B serology testing was conducted as part of the primary study, which has been reported previously.[5]

Real-time PCR quantification of HBV DNA

HBV DNA from only the 43 HIV-positive samples with isolated anti-HBc were analyzed by real-time quantitative PCR using the ABI Prism 7500 (Applied Biosystems, USA) to determine the HBV viral load. DNA was extracted from 50 μl of serum using the OlAamp MinElute Virus Spin kit (Qiagen, Germany) according to the manufacturer's instructions, and eluted into 50 μl of nuclease free water. PCR primers, HBV-Taq1 and HBV-Taq2 as well as the FAM/TAMRA labeled TaqMan BS-1 probe were used as described previously.[13] PCR reactions were performed in a 50 μl total volume using 25 μl of $2 \times$ TaqMan Universal PCR Master Mix (Applied Biosystems, USA), 300 nM HBV-Taq1 forward primer, 300 nM HBV-Taq2 reverse primer, 200 nM TaqMan BS-1 probe, and 2 μl of DNA. PCR conditions for HBV detection were as follows: 1 cycle of 2 min at 50 °C and 10 min at 95 °C, followed by 45 cycles of 95 °C for 15 s and 60 °C for 1 min. A plasmid encoding a single genome of HBV DNA was run to obtain a linear standard curve with an observed range of 10^3 to 10^8 copies in agreement with previous reports for this primer/probe set.[13] The second WHO International Standard for HBV Nucleic Acid Amplification Techniques, product code 97/750, which has a final concentration of 1×10^6 IU/ml, was obtained from the National Institute for Biological Standards and Controls (NIBSC; Hertfordshire, UK) and used as a positive control, as well as to calibrate and align the standard curve. 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. For each sample the duplicate average value was multiplied by 4.7 to convert from IU/ml to copies/ ml. The detection limit was found to be 10 IU/ml or 50 copies/ml. All the samples underwent PCR assay testing for hepatitis C virus (HCV) RNA using the Cobas Amplicor HCV Monitor (Roche) methodology.[14]

Data collection

A complete demographic history was obtained from each participant. The medical history was obtained from both the participant and the medical chart held in Therapy edge-HIV, an electronic medical data system. Laboratory data were collected from the medical chart. The data collected were entered/stored on site in an Access database by the medical research staff at the Clinical HIV Research Unit (CHRU). The seroprevalence database was also designed by CHRU staff.

Statistical analysis

All statistical analysis was done using Stata 9 statistical software. Descriptive statistics were done, calculating means and standard deviations for normally distributed data, median and interquartile ranges for data that were not normally distributed. The Wilcoxon rank sum test was used to test the association between HBV viral load and the categorical variables age group, CD4 count, and gender. The relationship between the log of HBV viral load and gender, CD4 count, and age was investigated using linear regression. In this analysis of 38 individuals, assuming an alpha of 0.05, we had 80% power to detect a 3 log difference in HBV viral load between CD4 cell count groups, a 1.75 log difference in HBV viral load between age groups, and a 1.73 log difference in HBV viral load between the genders.

Results

A total of 502 people agreed to the HBV screening and signed a written informed consent, after the educational session. Common reasons for not participating included additional time needed to listen to the education on hepatitis B, poor understanding of the study, refusal to have extra blood drawn, and feeling too ill to participate in the study.

Of the parent cohort, 24 (4.8%) were positive for HBsAg with no association to age, sex, or CD4 count.[5] The complete serological results of this study are shown in Table 1. A total of 53 out of 502 (10.6%) participants were found to have isolated anti-HBc. These specimens were eligible for inclusion in the DNA assessment for occult infection. Seven patients had signed consent in the parent study with the proviso that they did not want to store blood for further testing, and three serum samples were not available for further testing. A final total of 43 serum samples were evaluated for occult HBV DNA. Of these 43 samples, 38 (88.4%) were positive for HBV DNA on real-time PCR (see Table 2). The mean HBV viral load was 2.8×10^4 copies/ml (range 1×10^2 to 1×10^6 copies/ml). The viral copy number was 1×10^2 copies/ml in 7% of the patients, 1×10^3 copies/ml in 62%, 1×10^4 copies/ml in 12%, 1×10^5 copies/ml in 5%, and 1×10^6 copies/ml in 2%. Females constituted 56% ($n = 24$) of the cohort. The average age of these patients was 39.98 years (SD 11.64); males were significantly older than females (44.32 ± 14.14 vs. 36.55 ± 7.95 years, respectively; $p = 0.0229$). The mean CD4 count of participants was 49 cells/mm^3 (SD 77.21). These results were consistent with results from the larger cohort.[5]

Five patients (12%) had undetectable HBV viral loads and were not included in further analysis, hence the final cohort for analysis consisted of 38 patients. The median HBV viral load of these patients was 2285 copies/ml (IQR = 1580, 8930). If the HBsAg-positive patients were added to the occult HBV DNA results, a prevalence rate of 12.4% was found (i.e., 24 HBsAg-positive patients+ 38 occult HBV DNA =62; 62/ 502 = 12.4%).

Univariate analysis of the associations between HBV viral load and categorical variables gender, CD4 count (CD4 count <50 cells/mm^3 vs. CD4 count ≥;50 cells/mm^3), and age group (<40 years vs. ≥40 years) using Wilcoxon rank sum tests showed no significant association between HBV viral load and the exposure variables considered. The distribution of HBV viral load was improved by log transformation. Multivariate linear regression showed there was no statistically significant association between log HBV viral load and gender, CD4 count, or age.

Only one out of the 43 serum samples was positive for HCV on PCR, which is consistent with previous prevalence studies of HCV co-infection in South Africa.[4,15]

Discussion

To-date, this is the largest outpatient prospective study of occult HBV infection in HIV-seropositive patients in South Africa. The only previous report was a retrospective review from the University of Limpopo of stored samples from HIV-positive and negative people where 5/15 samples were found to be positive for HBV DNA.[11] A less sensitive assay, the High Pure Viral Nucleic Acid Kit from Roche, was used in that study. In contrast, we prospectively studied a cohort of outpatients from an HIV urban clinic. Of the parent cohort, 4.8% (24/502) were HBsAg-positive and therefore traditionally considered to have chronic active hepatitis B. In the same cohort, 53/502 (10.6%) were found to be isolated anti-HBc-positive. We used real-time PCR to evaluate the 43 available samples in which HBsAg was negative but isolated anti-HBc was present. A significant proportion (12.4%) of patients in this South African urban ARV clinic were found to be co-infected with HBV, and serology

tests alone may not be adequate to identify HBV co-infection in HIV-1-infected patients initiating ART.

The clinical impact of occult HBV in HIV-seropositive patients has not been described in Africa, but a retrospective analysis on this cohort is planned. The clinical significance of occult hepatitis reported from Europe and North America has been conflicting. In a cohort from Philadelphia no significant increase in liver transaminases was seen after controlling for alcohol exposure and hepatitis C.[16] An Italian cohort found an increase in liver complications measured by elevated liver transaminases in HIV-seropositive patients who were co-infected with occult HBV. A statistically significant increase in hepatitic flares during the treatment and after the discontinuation of lamivudine was noted in these co-infected patients when compared to HIV-seropositive patients who did not have occult HBV DNA (64.7% vs. 24.6%, $p < 0.005$).[17] If occult HBV DNA in HIV is found to be clinically significant, the overall prevalence of HIV/HBV co-infection has significant consequences for the ARV treatment program in resource-poor settings such as southern Africa.

The US Department of Health and Human Services (DHHS) guidelines and WHO recommendations for the treatment of HBV include the use of more than one medication active against HBV in combination therapy for HIV infection. The consequences of the high prevalence of HBV co-infection need to be considered when resource-poor countries decide on the antiviral therapy regimens to be used.[7,18] Lamivudine, a nucleoside analogue reverse transcriptase inhibitor, is active against both HIV and HBV. Lamivudine is currently one of the three medicines in the highly active antiretroviral therapy (HAART) regimen prescribed by clinicians in resource-limited settings. However, while HBV can be treated with lamivudine, there are reports of treatment complications in cases of HIV/ HBV co-infection. In particular, the addition and/or removal of lamivudine can cause liver inflammation and, on rare occasions, liver failure.[17,19] Another complication of using lamivudine as the only anti-HBV agent in regimens given to HIV/HBV co-infected patients is the development of resistance. The incidence of lamivudine resistance in this situation is approximately 20% per year. The implication is that during the first year of ARV treatment using lamivudine, most patients will receive adequate treatment of their hepatitis B. However, by the second year approximately 40% of these patients will have developed resistance. These figures have led to changes in the DHHS/WHO guidelines to recommend dual therapy for HIV/ HBV co-infection using either tenofovir/lamivudine or Truvada (tenofovir/emtricitabine).[7,18]

Other possible aspects of clinical significance of occult HBV DNA in HIV-seropositive patients besides ART treatment considerations is in pregnancy. In many countries where the prevalence of hepatitis B is high, hepatitis B serology testing is not standard of care in pregnancy; this is the case in South Africa.[12] Perinatal transmission of HBV is well described.[20] How occult HBV DNA in the HIV pregnant woman might affect transmission of HBV and HIV to the baby and how to treat occult HBV DNA in HIV-seropositive pregnant women are unknown.

In addition, it has been shown that HCV RNA is found in HIV patients who are positive for occult HBV.[19] In this cohort, only one sample was positive for HCV RNA. Our findings are consistent with previous reports that HCV is seen in only 0.5–1% of the HIV population in South Africa,[4,15] Co-infection with HCV is not a marker or a risk factor for occult HBV in isolated anti-HBc positive patients, as seen in other studies.[16]

Limitations of this study may include potential selection bias, in that the average CD4 count of all patients recruited into the parent study was below 200 cells/mm^3. This is due to current government guidelines on initiating ART in HIV-infected persons in South Africa,[12] so it is unclear what the occult hepatitis B rate might be in patients who are more immune

competent. The lack of statistical significance in the multivariate linear regression analysis between log HBV viral load and gender, CD4 count, or age may be partly due to the small sample size and consequent lack of statistical power. Also, HBV DNA was only measured in the isolated anti-HBc-positive sera, as this has been reported most commonly in HIV patients. If the entire cohort were tested for HBV DNA, a more accurate picture of occult DNA would be obtained. Plans are underway for this testing in the future.

Conclusions

This study highlights the fact that HBsAg may not be an effective tool for diagnosis of HIV/ HBV co-infection in our study population. It also demonstrates that the HIV/HBV co-infection rate is significantly underestimated with the current serology testing methods alone.

The WHO and the South African HIV Clinician's Society have recommended the use of tenofovir in combination with either lamivudine or emtricitabine as first-line therapy in ARV clinics in resource-limited countries due to the toxicity of stavudine. We would like to add further support to these recommendations. With the high prevalence of HBV co-infection in the HIV population in South Africa, treatment with tenofovir/lamivudine as first-line therapy would not only improve the safety and simplify HIV treatment, but would also appropriately treat the patients co-infected with a carcinogenic virus, HBV.

Acknowledgments

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Table 1

Hepatitis B seroprevalence study ($N = 502$)

	Number	%
HBsAg-positive	24	4.8
anti-HBc isolated[a]	53	10.6
anti-HBs + anti-HBc	124	24.7
anti-HBs isolated[b]	13	2.6
Hepatitis B serology negative	288	57.4

HBsAg, hepatitis B surface antigen; anti-HBc, hepatitis B core antibody; anti-HBs, hepatitis B surface antibody. No hepatitis e antigen was obtained on these screening samples.

[a] Isolated anti-HBc describes serology that is positive for anti-HBc only and negative for anti-HBs and HBsAg.

[b] Isolated anti-HBs describes serology that is positive for anti-HBs only and negative for anti-HBc and HBsAg.

Table 2

Sample viral load results.

Viral load	%
Below detectable	12%
1×10^2	7%
1×10^3	62%
1×10^4	12%
1×10^5	5%
1×10^6	2%