Hepatitis B virus in HIV-infected patients in north-eastern South Africa: Prevalence, exposure, protection and response to HAART

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Objective. Hepatitis B virus (HBV) and HIV are endemic infections in many African countries. The objectives of this study were to determine the levels of exposure to, and protection from, HBV, as well as the prevalence of HIV/HBV co-infection and the response of HBV to highly active anti-retroviral therapy (HAART) in a cross-section of HIV-infected patients in north-eastern South Africa.
Study design. This was a laboratory-based, unmatched study. Three hundred and eighty patients were screened by ELISA for HBsAg, anti-HBc and anti-HBs. Samples non-reactive for HBsAg but reactive for anti-HBc were examined for occult HBV infection. Response to HAART was assessed by measuring HBV viral loads, seroconversion from HBeAg to anti-HBe, and levels of aminotransferase.
Results. Of the study population of 380, 60% (95% CI 54.8 - 64.9) were exposed to HBV based on HBsAg, anti-HBs or anti-HBc; 20% (95% CI 16.1 - 24.4) had active HBV infection, based on HBsAg serology, and 30% (95% CI 25.2 - 35.2) were protected, based on anti-HBs levels ≥10 IU/l. Of 181 HBsAg-negative individuals, 61 had HBV occult infection (33.7%, 95% CI 26.9 - 41.1). The differences in prevalence were not statistically significant when gender, marital status and CD4+ cell counts were considered. Of 21 patients analysed, 80% showed adequate response to the first-line HAART regimen (stavudine/lamivudine/efavirenz or nevirapine) after 12 months of use.
Conclusion. The study confirms the higher level (60%) of exposure to HBV in HIV patients in Limpopo Province, as well as the high (20%) prevalence of HBsAg positivity and occult hepatitis B (33.7%). However, further studies are warranted to corroborate the benefit of lamivudine-containing HAART regimens, as HIV/HBV co-infected patients have a higher liver-related mortality if hepatitis B is not treated.

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Human immunodeficiency virus (HIV) and hepatitis B virus (HBV) infections are endemic in sub-Saharan Africa,[1] and transmitted via similar routes.[2,3] Both infections are of high public health importance. Infection with HBV is not always considered in the management of HIV infection,[4] although co-infection has been associated with increased turnover rate of HIV RNA-increased liver morbidity, and complicated HIV pathology.[5-7] On the other hand, the use of highly active antiretroviral therapy (HAART) with a lamivudine component is used for HIV treatment in South Africa, and would be expected to be associated with the development of HBV drug-resistant strains, since lamivudine is the sole active drug (monotherapy) against HBV.

Several studies have reported the prevalence of HIV/HBV co-infection in South Africa. For example, Boyles and Cohen[8] in a hospital setting reported a prevalence of 7.1% in Eastern Cape Province. They reported male gender and alanine aminotransferase as important predictors of HBV infection in HIV-infected patients. Hoffmann and colleagues reported a prevalence of 20% among male mine workers in several regions of South Africa.[9] In Gauteng Province, exposure to HBV, considering HBsAg and anti-HBc, of 39% was documented in HIV-positive women.[10] An earlier prospective study in Johannesburg showed a 6% infection rate of HIV/HBV co-infection.[11]

The prevalence of HIV in Limpopo Province is estimated to be 17.5%. On the other hand, data are scarce for the prevalence of HBV in the general population, and more so in HIV-infected patients. Since patients with HIV/HBV co-infection are at higher risk for early decompensation of liver disease, liver cirrhosis and hepatocellular carcinoma, updating data on the prevalence of HIV/HBV co-infection is important to better understand the burden of co-infection relevant for patient management, particularly in regions where little data exist. The present study was carried out to establish the level of HBV exposure in HIV patients, assess the prevalence of HIV/HBV co-infection, evaluate the level of HIV patients protected against HBV, and assess the response of HBV to the first-line HAART regimen in HIV-infected patients in north-eastern South Africa.

Materials and methods

Ethical considerations

Ethical clearance was obtained from the University of Venda's Health, Safety and Research Ethics Committee to use archived confirmed HIV-positive samples from the specimen bank in the Department of Microbiology, University of Venda. The samples had been stored at -80°C for less than 2 years.

Study population and sample collection

Initial blood samples were collected in 2008 from antiretroviral-naïve and -experienced patients (n=380) in Bela Bela (Waterberg district), Mankweng (Capricorn district), Musina and Madimbo (Vhembe district) in the Limpopo Province of South Africa. A subset of 105 patients of the 380 who presented samples were drug-experienced. Samples from this group were collected for evaluation before treatment initiation and subsequently at 3-month intervals for 1 year. All the 380 samples were used to determine the degree of exposure to HBV; the prevalence of

HIV/HBV co-infection; occult HBV infection; and the level of protection against HBV. The full group comprised 104 males and 276 females, aged between 19 and 65 years old, with varying CD4 counts and stages of HIV disease. The only exclusion criterion was inadequate specimen volume for the anticipated assays. The subset of samples (n=105) determined to be HIV/HBV co-infected, and under D4T/3TC/EFV treatment, were followed as a cohort for a year in assessing the efficacy of HIV treatment regimen on HBV infection.

Determination of the level of exposure and prevalence of HBV in HIV patients

A total of 380 plasma specimens were screened and confirmed for HBsAg (a marker for active HBV infection) using the Murex HBsAg Version 3 and Murex HBsAg Confirmatory Version 3 ELISA protocols, respectively (Abbott-Murex, USA) according to the manufacturer's instructions. Exposure to HBV was determined by screening for the presence of at least one of the serological markers HBsAg, anti-HBc or anti-HBs by means of ELISA.

Determination of occult HBV infections in HIV-infected patients

The determination of occult HBV infection was carried out by screening HBsAg-negative plasma samples (n=304) for anti-HBc using Murex anti-HBc ELISA (Abbott-Murex, USA) according to the manufacturer's instructions. This was followed by amplification of HBV core gene using an in-house nested polymerase chain reaction (PCR) protocol as previously described.[12] The nested PCR amplification product was verified for expected fragment size by a 1% ethidium bromide-stained agarose gel electrophoresis at a constant voltage of 100 V for 40 minutes, and viewed by UV transillumination.

Determination of the level of protection against HBV in HIV patients

Protection against HBV was done by the detection and quantification of anti-HBs. Screening was performed on 343 HIV-positive plasma samples of sufficient volume using Murex anti-HBs ELISA (Abbott-Murex, USA), according to the manufacturer's instructions. Samples with antibodies ≥10 mIU/ml were considered positive and protective against HBV, while samples <10 mIU/ml were considered non-protective.

Determination of the efficacy of HAART on HBV infection in HIV/HBV co-infected patients

This objective was to assess 3 parameters of clinical responses to therapy: serological, viral and biochemical responses. Each clinical parameter was assessed independently and their results compared for consistency of patients' response to antiretroviral drugs at one year of therapy. The same samples were subjected to viral and biochemical responses, but biochemical response had some new inclusions for the non-consistent samples (reasons for the independent assessment of each parameter). Measurements for viral loads and serological responses were done before the initiation of therapy and at 44 weeks, while biochemical response was assessed before therapy and at 12 and 24 weeks respectively. Twenty-one HIV/HBV co-infected patients who had been on a 4DT/3TC//EFV treatment regimen were used for this assessment.

Quantification of viral DNA (viral response)

Quantification of HBV viral load was done using the Cobas Taqman 48 analyzer (Roche) following DNA extraction using the Cobas Taqman Pure Viral Nucleic Acid (Roche) before treatment and at 44 weeks of treatment.

Serological and biochemical responses

The Elecsys automated platform (Roche) was used to screen for HBeAg and anti-HBe before the onset of treatment and at 44 weeks after the initiation of treatment to evaluate seroconversion from HBeAg to anti-HBe. Biochemical response to therapy was determined by comparing aminotransferase levels in plasma samples before treatment and at 24 weeks into therapy. Aminotransferase values were obtained from available HIV treatment evaluation data from the patients. A response was characterised by a return of abnormally high aminotransferase to the normal range (3 - 35 IU/l).

Results

Level of exposure, and prevalence of HBV infection

The level of exposure to HBV (presence of any or all of HBsAg, anti-HBc and anti-HBs) in the studied population was 60% (95% CI 54.8 - 64.9). The level of exposure to HBV in married persons was 75% (87/116). Although this value was higher than that for the unmarried of 56.4% (149/264), the difference was not statistically significant (p=0.14). Males (67.3% (70/104)) were insignificantly (p=0.2) more exposed than females (59.8% (165/276)). The prevalence of HBV based on HBsAg was 20% (95% CI 16.1 - 24.4). The proportions of infection in the population between males and females were 19.2% (20/104) and 20.3% (56/276) respectively (p=0.82). Prevalence (based on HBsAg) in women of child-bearing age was 19.6% (53/270). The prevalences in married and unmarried patients were 23.3% (27/116) and 18.6% (49/264) respectively (p=0.74). In the 86 patients with available CD4+ cell count measurements, an insignificant difference (p=0.63) in HBV prevalence was seen in HIV patients with CD4+ cell counts ≤200 cells/µl (27% (17/63)) compared with 21.7% (5/23) in patients with CD4+ cell counts >200 cells/µl (p=0.63).

The distribution of HBV infection among the age groups was (*i*) <20 years old: 16.7% (2/12); (*ii*) 21 - 30 years old: 16.8% (17/101); (*iii*) 31 - 40 years old: 19.0% (27/142); (*iv*) 41 - 50 years old: 26.7% (23/86); and (*v*) >50 years old: 18% (7/39). The prevalence of HBV differed across the various geographical areas of the province, ranging from 10.4% (11/106) in Mankweng to 28.5 % (55/193) in Bela Bela (p=0.000).

Occult hepatitis B infection in HIV/HBV co-infected patients

Occult HBV infection was assessed based on the presence of detectable HBV-DNA in reactive anti-HBc samples with negative HBsAg serology. Out of 380 patients, 304 were non-reactive to HBsAg; of the 304, 181 were serologically reactive for anti-HBc, and 61 of the 181 reactive anti-HBc samples had detectable HBV-DNA identified by PCR targeting the core gene. Consequently, the prevalence of occult HBV infection in the study population was 33.7% (95% CI 26.9 - 41.1).

Level of protection against HBV in HIV-infected patients

The level of protection against HBV was defined by a plasma anti-HBs concentration of ≥10 mIU/ml. An overall protection rate of 30% (95% CI 25.2 - 35.2) was observed. Protection did not differ significantly (p=0.2) between the genders – 28% (95% CI 22.4 - 34.0) in females and 35.1% in males (95% CI 31.9 -5 4.7). The level of protection in married patients of 35.6% (95% CI 26.4 - 45.6) was insignificantly higher (p=0.14) than that in unmarried patients 27.6% (95% CI 22.0 - 33.7). Protection in patients with CD4 counts

≤200 cells/μl (19.7%, 95% CI 10.69 - 31.8) was also not significantly different (p=0.6) from patients with CD4 counts >200 cells/μl (15%, 95% CI 3.2 - 37.9) (p=0.6).

The effects of DT4/3TC/EFV treatment on HBV infection in co-infected patients

HBV viral load outcome

Most of the patients entered therapy with low HBV viral loads, which were sustained during the first year of therapy. Twenty-one HIV/HBV co-infected patients on HAART were involved in this aspect of the study. Nine of the 21 patients (42.9%) had undetectable viral loads before entering therapy, and 12/21 (57.1%) had detectable viral loads ranging from <6 IU/ml to 110 million IU/ml. Of the 9 subjects with undetectable viral load, 7 maintained undetectable levels after 44 weeks on HAART and 2 reverted to detectable viral load within 44 weeks of treatment. At the end of 44 weeks, the 12 patients with detectable viral loads prior to therapy had the following profile: 7/12 (58.3%) had undetectable DNA levels, 2/12 (16.7%) had a marginal decrease in viral loads lower than their initial values prior to therapy, 1/12 (8.3%) had maintained low DNA levels <6 IU/ml as at the beginning of therapy, and 2/12 (16.7%) had increased DNA levels over their starting values. The virological response in terms of reduction in viral loads between pretreatment and during treatment was 75% (9/12), and virological response based on reduction as well as maintenance of viral loads relative to pretreatment values in those with detectable viral loads was 83.3% (10/12). The average reduction of viral loads in samples with detectable differences in DNA viral loads (patients responding to therapy) between pretreatment and treatment at 44 weeks was 3.6 $\log_{10}$.

There was a reduction in viral load by a factor of as much as 7 $\log_{10}$ in one patient. Of the 21 patients on therapy, 17 (81%) showed adequate response to therapy after 44 weeks with reduced or sustained low viral loads, and 4 (19%) showed clinical evidence (increased viral loads) of therapeutic failure.

Serological response

Serological response to HAART was 85.7% (95% CI 63.6 - 96.9) in terms of the number of patients with positive anti-HBe at 44 weeks of therapy. One sample (4.8%) had neither HBeAg nor anti-HBe detected, and 2/21 (9.5%) had failed to seroconvert.

Biochemical response

Biochemical response involved reverting abnormally high level of aminotransferase to its normal range (3 - 35 IU/l). Biochemical drug response was analysed on 24-weeks drug-experienced patients owing to unavailability of 44-week aminotransferase results. Twenty-one HAART-experienced patients started therapy; at 24 weeks, 8 had dropped out for various reasons, and 13 patients were available for analysis. The average level of aminotransferase in drug-experienced HIV/HBV co-infected patients prior to onset of therapy was 38.5 IU/l. This level dropped to 34.5 IU/l and 31.9 IU/l at 12 and 24 weeks respectively in response to HAART therapy. At the onset of treatment with D4T/3TC/EFV, 7 (33.3%) of the 21 HIV/HBV co-infected patients had abnormally high ALT levels (39 - 189 IU/l) and 14/21 (66.7%) had normal aminotransferase levels (10 - 35 IU/l). Of the 8 patients without aminotransferase values at 24 weeks, 1 had abnormal levels (59 IU/l) and 7 had normal levels at the onset of therapy. Therefore, the 13 patients analysed at 24 weeks comprised 6 with abnormal and 7 with normal aminotransferase levels prior to therapy initiation. Four (66.7%) of the 6 with abnormally elevated levels of aminotransferase had adequate biochemical response reverting to normal levels after 24 weeks of therapy, 1/6 (16.7%) showed significant reduction of aminotransferase to a level just above the normal range, and 1/6 (16.7%) showed an elevated level above the starting value. Hence, 5/6 (83.3%) of the patients had reductions of abnormal aminotransferase level during HAART therapy. Five (71.4%) of the 7 patients with normal aminotransferase levels prior to therapy initiation maintained normal levels, and 2 (28.6%) had abnormally elevated levels at 24 weeks on treatment.

Discussion and conclusion

The current study assessed the prevalence, exposure and protection to HBV infection, and the efficacy of a D4T/3TC/EFV treatment regimen on HBV infection in a cross-sectional population of HIV-infected patients in Limpopo Province. The study population comprised black, adult South Africans at various stages of HIV disease.

The prevalence of HBV in the studied population, as defined by HBsAg serology, was 20%, which is twice as high according to the WHO classification of more than 8% for a high-prevalence region. Although the prevalence of HIV/HBV co-infection was observed to differ significantly among various sites in Limpopo Province, Bela Bela was noted as having the highest (28.5%). The HBV prevalence of 20% in HIV-infected patients is much higher than previously reported figures from different study settings in Gauteng and Eastern Cape provinces.[8,11,13] However, high prevalence rates have also been documented; for example, Hoffmann *et al.* reported a prevalence of 20% among male mine workers in several regions of South Africa.[9] In Gauteng Province, exposure to HBV, based on HBsAg and anti-HBc, of 39% was documented in HIV-infected women.[10]

The current study also examined the effect of declining CD4+ cell count on the prevalence of HBV, as reactivation is expected to be proportional to the level of immune depreciation.[14] The prevalence of HBV in HIV-infected patients with CD4+ count ≤200 cells/μl was shown to be insignificantly higher in patients with CD4+ count >200 cells/μl. However, a recent South African study showed a higher HBV prevalence in AIDS patients with CD4+ cell count <100 cells/μl.[15]

The study also showed that as much as 60% of HIV patients were exposed to HBV. All the patients analysed for the study were born at a time when vaccination against HBV was not part of the childhood vaccination programme. Therefore the anti-HBs results are probably due primarily to infection rather than to vaccination which is not routinely provided to adults. The high prevalence of HIV/HBV co-infection could be due to both high transmission of HBV in the general population early in life and HBV reactivation associated with HIV infection.

Occult HBV infection in HIV patients was found to be 33.7%. The implication is that negativity to HBsAg serology may not always indicate the absence of infection. Previous studies in South Africa have shown that HBV DNA is significantly higher in HIV-infected than in HIV-negative patients, suggesting that HIV infection may be a risk factor for occult HBV infections.[12,16] This finding highlights the level of undetected active HBV infections that have the potential to affect antiretroviral therapy (ART) as their presence increases hepatotoxicity of ART with consequent limitation of their use.[17,18] Anti-HBs are known to confer protection against HBV at plasma levels ≥10 IU/ml. In the current investigation, 30% of HIV-infected patients were protected against HBV disease. A low level (28%) of protection against HBV in women, especially those of child-bearing age, predisposes most of them and their unborn/newborn children during pregnancy, labour and the postnatal period to HBV infection. The level of protection against HBV in the absence of vaccination is a reflection of (or consistent with) the high level of exposure and active HBV infection existing in this group of individuals, as reflected by the prevalence data.

At the time of the study, first-line ART in South Africa was a combination of stavudine, lamivudine, and efavirenz. Lamivudine is recommended for the treatment of HBV infection. The efficacy of HAART against HBV was measured by means of viral, serological and biochemical responses. Adequate drug response against HBV was observed for more than 80% of the 21 patients analysed within a year of therapy based on these parameters. This limited observation, in terms of the sample size, is in contrast to the outcomes of a randomised study that showed little benefit in the use of a lamivudine-containing HAART for HIV/HBV-infected patients.[19] Although hepatotoxicity of HAART is increased by the presence of HBV, studies have shown that patients with high HBV-DNA baseline levels have a higher risk of hepatotoxicity than non-HBV-infected HIV patients.[9] Most of the patients in the current study had undetectable and low viral loads prior to therapy initiation and within a year on HAART, which may explain why the average level of aminotransferase was not significantly higher in these patients, concurring with the observation of Boyles and Cohen[8] that aminotransferase may be an insensitive marker for HBV infection. It could be assumed that most of the patients on HAART in the studied population had a lower risk of developing hepatotoxicity within the first year of treatment.

The findings presented here are apparently the first set of data from Limpopo Province on HIV/HBV co-infection. Nevertheless, it is important to interpret the results with some caution for the following reasons: (*i*) the effect of a small sample on the statistical differences in the exposure, prevalence and protection in terms of gender, marital status and CD4+ cell counts of the study population; (*ii*) viral load and biochemical response data were available for a small number of patients, which precludes preliminary conclusions on virological and biochemical outcomes based on the treatment regimen; (*iii*) consecutive samples for the assessment of drug efficacy were not consistent within the follow-up period and, coupled with lack of resistance data, makes it difficult to obtain a broader picture of response to lamivudine-containing HAART regimens on HBV disease; and (*iv*) the study was limited to HIV patients without a matched control group, and comprised individuals who might not have benefited from the inclusion of hepatitis B vaccine in the expanded programme on immunisation during their childhood, which might have contributed to higher prevalence values.

In conclusion: High levels of exposure and active HBV infection, and a moderate level of protection, were observed. Future studies employing a large sample size should look at mother-to-child transmission of HBV, and the impact of lamivudine-containing HAART on clinical outcomes and resistance development to lamivudine.

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