HBV/HIV co-infection: The dynamics of HBV in South African patients with AIDS

Simnikiwe H Mayaphi, Theresa M Rossouw, Difuro P Masemola, Steve A S Olorunju, M Jeffrey Mphahlele, Desmond J Martin

Objective. As sub-Saharan Africa is highly endemic for hepatitis B virus (HBV) and human immunodeficiency virus (HIV) infections, and their co-infection requires special management, we aimed to assess the serological and molecular characteristics of HBV in patients with AIDS.

Design. This was a cross-sectional, case control study, which enrolled 200 patients with AIDS and 200 HIV-negative controls. HBV serology was done in all participants and HCV serology in participants with a hepatitis B core antibody (anti-HBc) only serological pattern. Nested HBV polymerase chain reaction (PCR) and HBV viral load assays were used for HBV molecular detection.

Results. Hepatitis B surface antigen (HBsAg) prevalence was 3-fold higher while the ‘anti-HBc only’ pattern was 6-fold higher in the AIDS group compared with the controls. Mean HBV viral load was significantly higher in HBsAg-positive patients with CD4+ cell counts <100 cells/µl than in patients with CD4+ cell counts of 100-200 cells/µl (p = 0.019). There were markedly reduced hepatitis B surface antibody (anti-HBs) titres in the AIDS group compared with the controls (p = 0.002). A significant proportion of AIDS patients with an ‘anti-HBc only’ pattern had CD4+ cell counts <100 cells/µl (p = 0.004). Occult HBV prevalence was 3.5% in the AIDS group compared with 1% in the controls (p = 0.092). When occult HBV infection was taken into consideration, the overall HBV prevalence became 10% in the AIDS group and 3% in the control group.

Conclusion. We showed an increased HBV prevalence in patients with AIDS and identified a CD4+ cell count <100 cells/µl as a major risk factor for the ‘anti-HBc only’ pattern and increased HBV replication. These data have significant public health implications for HBV in developing countries, especially in areas where antiretroviral (ARV) guidelines do not cater for HBV/HIV co-infection.


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dilutions: 10^{-1}–10^{-7}) of samples with known HBV viral loads. The mean CD4+ cell count in the AIDS group was 92.9 cells/µl (Table 1).

Serological testing was done on serum samples and molecular testing on plasma samples. Architect (Abbott Diagnostics, Wiesbaden, Germany) and Modular Analytics E170 (Roche Diagnostics, Mannheim, Germany) immunoassays, which are fourth-generation HIV enzyme-linked immunosorbent assays (ELISA), were used for HIV diagnosis. Assyn immunoassays (Abbott Diagnostics, Wiesbaden, Germany) were used for HBV and hepatitis C virus (HCV) serological testing. The HBV screening included HBsAg, anti-HBs and anti-HBc. Hepatitis B e-antigen (HBeAg) and hepatitis B e-antibodies (anti-HBe) were done on all HBsAg-positive specimens as well as those found to be positive for anti-HBc alone during HBV screening. HCV serology was done in participants with an 'anti-HBc only' serology as HCV infection is often associated with this serological picture. Vol.

Viral nucleic acid was extracted from 100 µl of plasma using the MagNa Pure LC Total Nucleic Acid Isolation kit in the MagNa Pure LC instrument (Roche Diagnostics, Germany) according to manufacturer’s instructions. Nestled HBV DNA polymerase chain reaction (PCR) assay (qualitative in-house assay) was performed to detect occult HBV infections in participants who were HBsAg-negative after HBV serological screening. HBV core primers were used for nested HBV-PCR as previously described. The sensitivity of the nested HBV-PCR was determined by serial dilution (10-fold dilutions: 10^{-1}–10^{-7}) of samples with known HBV viral loads.

HBV viral loads were determined by Cobas TaqMan 48 HBV assay (Roche Molecular Systems, Inc., Branchburg, USA) for all HBsAg-positive participants, those with 'anti-HBc only' pattern and in those positive on nested HBV PCR. Epics and UniCel Dxc880i analysers (Beckman Coulter Diagnostics) were used for CD4 count measurements and liver function tests (LFTs), respectively.

Data analysis

Descriptive analysis was used to obtain summary statistics (mean, standard deviation, standard error, and 95% confidence intervals) for the parameters. This was followed by a comparison between the groups using two sample t-test for proportions. The mean HBV viral load and anti-HBs measurements between groups were compared using the Mann-Whitney or Wilcoxon rank sum test. The software used was STATA 11.2. A p-value of <0.05 was considered statistically significant.

Results

Demographics

Black subjects comprised 98% of participants in this study and there were more males in the control group than in the AIDS group. The mean age was higher in the AIDS group. Only a small proportion of participants were vaccinated (0.5% in the AIDS group v. 3% in controls). Intravenous (IV) drug use and the number of lifetime sexual partners were significantly higher in the controls and the AIDS group, respectively. The mean CD4+ cell count in the AIDS group was 92.9 cells/µl (Table 1).

HBV serology results

HBsAg prevalence of 6.5% in the AIDS group was significantly higher than that of 2% in the control group. The total HBV exposure was significantly higher in the AIDS group compared with the control group, and the same trend was also noticed for both HBeAg and ‘anti-HBc only’ prevalence (Table 1). When stratifying the HBsAg-positive AIDS participants by CD4+ cell counts, the group with CD4+ cell counts of <100 cells/µl had a higher HBsAg prevalence of 83.0% (5 of 6) compared with 57.1% (4 of 7) in the group with CD4+ cell counts of 100 - 200 cells/µl; however, this difference was not statistically significant (p = 0.680). HBeAg prevalence was 25% (1 of 4) in HBsAg-positive participants in the control group (Table 2). Two HBsAg-positive patients with AIDS had negative anti-HBc (Table 2).

The ‘anti-HBc only’ serological pattern was 6-fold higher in the AIDS group compared with the control group (Table 1). When patients with an ‘anti-HBc only’ serology in the AIDS group were stratified according to CD4+ cell counts, 73.7% (14 of 19) had CD4+ cell counts of <100 cells/µl compared with 26.3% (5 of 19) in the group with CD4+ cell counts of 100 - 200 cells/µl (p = 0.004) (Table 3). HCV serology results were negative in all patients with an ‘anti-HBc only’ pattern (Table 3).

In the AIDS and control groups 60.5% and 76.0%, respectively, had no immunity to HBV (Table 1). AIDS patients had significantly decreased anti-HBs titres; mean titre of 235.17 mIU/ml compared to 514.29 mIU/ml in the control group (p = 0.002). No difference was found in mean anti-HBs titres when the AIDS group was stratified by CD4+ cell counts.

HBV viral load results

The lower detection limit of the HBV viral load assay was 6 IU/ml (~30 copies/ml). Mean HBV viral loads were significantly higher in HBsAg-positive patients with CD4+ cell counts of <100 cells/µl compared with the group with CD4+ cell counts of 100 - 200 cells/µl (p = 0.019) (Table 2). HBV viral load values >110 000 IU/ml were excluded from the comparison between these groups as they are outliers (fall outside the reference range). Interestingly, even the HBeAg-negative (but anti-HBe-positive) patient (3 658) with a CD4+ cell count of <100 cells/µl (Table 2) had a very high HBV viral load compared with counterparts (3 464 and 3 321) with CD4+ cell counts of 100 - 200 cells/µl.

Nested HBV-PCR results

Nested HBV-PCR was used for detection of occult HBV infection, and its performance was assessed in all HBsAg-positive samples; it was positive in all these samples except for one that had a ~6 IU/ml viral load (with an HBsAg titre of 217.04 S/N) (Table 2). Its lower detection limit was 0.2 IU/µl (~1 copy/µl) on serial dilution of HBsAg-positive samples with known viral loads (Fig. 1). This high sensitivity of nested HBV-PCR was noticed in some samples where HBV viral load was ~6 IU/ml, but nested PCR was clearly positive (Table 3). Occult HBV infection was detected in most participants with ‘anti-HBc only’ serological picture (Table 3) and in one participant who was positive for both anti-HBc and anti-HBs (confirmed on repeat serology). The latter had an anti-HBs titre of 15 mIU/ml, a CD4+ cell count of 72 cells/µl, positive nested HBV-PCR, HBV viral load of 290 IU/ml, and alanine aminotransferase (ALT) of 16 IU/L. In total, occult HBV prevalence was 3.5% in the AIDS group compared with 1% in the control group (p-value = 0.092). Only one patient with occult HBV infection had an HBV viral load of >200 IU/ml (mentioned above). Contamination was successfully avoided in the nested PCR runs as all negative controls used at the end of each run or in between
samples were negative (e.g. Fig. 1). Subsequently, all positive samples on nested PCR were sequenced, and the phylogenetic tree also did not show any indication of contamination (data not shown). When occult HBV infection was taken into consideration, the overall HBV prevalence in this study became 10% in the AIDS group and 3% in the control group.

Liver function tests
ALT levels were normal or slightly raised in HBsAg-positive patients with AIDS (Table 2).

Discussion
This study demonstrates a 3-fold increase in HBsAg prevalence in patients with AIDS (6.5%) compared with the control group (2%). This impact would have been difficult to observe without using a control group, as the average South African HBsAg prevalence is considered to be 10%. Therefore, HBV/HIV co-infection studies without a control group are more likely to underestimate the impact HIV/AIDS has on HBV prevalence, especially in areas of high HBV endemicity where most of the population are exposed to HBV during childhood. A study in South African patients with AIDS at the Chris Hani Baragwanath Hospital noted a similar HBsAg prevalence of 6% but could not conclude whether this was high or not, probably because of the lack of a control group.13 The reason for low HBV prevalence (<10%) in both groups of this study could be that the HBsAg prevalence of 10% comes from studies conducted in populations at high risk for chronic HBV (adult males or children only) and, therefore, is not always applicable for assessment of HBV prevalence in the general adult population. For instance, Tsebe et al.14 noticed a 3.3% HBsAg prevalence in the general adult population of Limpopo (formerly Northern Province). Despite the number of lifetime sexual partners being significantly higher in the AIDS group, this is unlikely to have influenced the high HBV prevalence in this group as other HBV risk factors such as male gender and IV drug use were significantly higher in the controls.
HBV prevalence varies considerably in South Africa owing to gender, age, race and geographic location of a studied population, e.g. blacks, males, and residence in the rural or coastal areas such as Eastern Cape and KwaZulu-Natal (KZN) are risk factors associated with high HBV prevalence. This prevalence could be as high as 10% or more in those at high risk for HBV and as low as 1% in low risk groups as noted in children born into an urban environment. There was a lower HBV prevalence in the control group despite more risk factors for chronic HBV prevalence such as higher number of males and IV drug users (Table 1). South African studies have also shown an increased HBV prevalence in adult patients infected with HIV; one reported a 22.9% and the other a 5% HBsAg prevalence. However, the latter study used an HBsAg prevalence of 1% as a reference point even though this prevalence rate was only noticed among a group of children born in an urban area, and would therefore be inappropriate for a diverse adult population in terms of risk factors for HBV.

Mean HBV viral load of HBsAg-positive patients was significantly higher in the AIDS group with CD4+ cell counts of <100 cells/µl, indicating an increased HBV replication with profound immunosuppression. This in turn results in an increased risk of HBV transmission to patients’ close contacts (e.g. family members and friends) and susceptible health care workers. Immunosuppression could also account for a very high HBV viral load in an HBeAg-negative (but anti-HBe-positive) patient with a CD4+ cell count of <100 cells/µl, and for failure of some HBsAg-positive patients to produce anti-HBe (Table 2). The undetectable HBV DNA in an HBsAg-positive participant in the control group was surprising; however, this phenomenon has been described in chronic HBV infection especially after HBeAg seroclearance.

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Liver damage is mediated by cytotoxic immune responses in immunocompetent individuals. However, HBV/HIV co-infected

<table>
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<tr>
<th>Table 2. Characteristics of HBsAg-positive individuals in the AIDS group v. control group</th>
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<td>CD4 category</td>
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<td>CD4+ cell count &lt;100 cells/µl</td>
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<td>Control group (HIV-negative)</td>
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Pt ID = participant identity; HBsAg = hepatitis B surface antigen; anti-HBs = hepatitis B surface antibody; anti-HBc = hepatitis B core antibody; HBeAg = hepatitis B virus e antigen; anti-HBe = antibody to HBVe antigen; ALT = alanine aminotransferase; HBV-PCR = HBV polymerase chain reaction; F = female; M = male; ND = not done.

Fig. 1. Serial dilution of a sample that had an HBV viral load of 33 839 3 IU/µl. Nuclease-free water served as a template for negative controls (NC) used between dilutions. Analysis of nested HBV-DNA PCR products was done on 2% agarose gel electrophoresis. Molecular weight (MW) marker = 100 bp DNA marker.
patients are often asymptomatic and usually have normal or slightly raised ALT levels (Table 2), possibly because of weak cytotoxic immune responses as a result of immunosuppression. Paradoxically, the risk of developing cirrhosis is much greater in HBV/HIV co-infected patients despite having less necro-inflammatory activity in the liver. HBV replication in the liver is non-cytopathic; however, fibrosing cholestatic hepatitis, a severe and rapidly progressive form of HBV infection thought to be due to a direct cytopathic effect of the virus, has been anecdotally described in patients infected with HIV and other groups of immunosuppressed patients.\textsuperscript{18} HBV/HIV co-infected patients with AIDS are also at a high risk of developing immune reconstitution inflammatory syndrome (IRIS) after initiation of highly active antiretroviral therapy (HAART). The accelerated HBV progression (Table 2) and higher likelihood of IRIS in patients with AIDS clearly argue for earlier initiation of HAART in HBV co-infected patients before their CD4+ cell counts drop below 200 cells/µl as advocated by the 2010 WHO ARV guidelines.\textsuperscript{19}

HBV/HIV co-infection requires special management as some ARV drugs (tenofovir, lamivudine and emtricitabine) are active against HBV. Dual therapy for HBV is recommended in HIV co-infected patients in order to avoid HBV drug resistance, particularly to lamivudine, which has a low resistance barrier. As a result, the use of tenofovir and lamivudine/emtricitabine is recommended as part of a HAART regimen for HBV/HIV co-infected patients.\textsuperscript{20}

Isolated anti-HBc is commonly associated with occult HBV infection. Mphahlele et al.\textsuperscript{11} reported an occult HBV prevalence of 33.3% in South Africans infected with HIV who had an ‘anti-HBc only’ serological picture. In our study, there was a significantly high proportion of patients with an ‘anti-HBc only’ serological picture in the AIDS group, and occult HBV infection was detected in 31.6% of these patients. The finding of positive nested HBV PCR in some samples with HBV viral load <6 IU/ml shows that a more sensitive test is needed for the diagnosis of occult HBV (Table 3). Other South African studies have also reported a high prevalence of occult HBV in patients with AIDS.\textsuperscript{5,6,11} The finding of negative HCV serology in all patients with an ‘anti-HBc only’ serological picture was not surprising, given that South Africa has an HCV prevalence of less than 2%.\textsuperscript{21}

Although there is uncertainty regarding the clinical significance of occult HBV infection, a clear association of occult HBV infection and

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**Table 3. Characteristics of patients with ‘anti-HBc only’ serological pattern in the AIDS group versus control group**

<table>
<thead>
<tr>
<th>CD4 category</th>
<th>Pt ID</th>
<th>Age</th>
<th>Gender</th>
<th>CD4+ count (cells/µl)</th>
<th>ALT (UI/l)</th>
<th>HCV serology</th>
<th>HBV viral load (IU/ml)</th>
<th>Nested HBV PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4+ cell count &lt;100 cells/µl</td>
<td>3 267</td>
<td>26</td>
<td>F</td>
<td>6</td>
<td>13</td>
<td>ND*</td>
<td>TND</td>
<td>-</td>
</tr>
<tr>
<td>CD4+ cell count &gt;100 - 200 cells/µl</td>
<td>3 712</td>
<td>31</td>
<td>F</td>
<td>2</td>
<td>41</td>
<td>-</td>
<td>-6</td>
<td>-</td>
</tr>
<tr>
<td>CD4+ cell count &gt;200 cells/µl</td>
<td>3 734</td>
<td>39</td>
<td>M</td>
<td>20</td>
<td>49</td>
<td>-</td>
<td>36</td>
<td>+</td>
</tr>
<tr>
<td>Control group (HIV-negative)</td>
<td>N012</td>
<td>29</td>
<td>M</td>
<td>ND</td>
<td>ND</td>
<td>-</td>
<td>&lt;6</td>
<td>+</td>
</tr>
<tr>
<td>Control group (HIV-negative)</td>
<td>N068</td>
<td>28</td>
<td>M</td>
<td>ND</td>
<td>ND</td>
<td>-</td>
<td>&lt;6</td>
<td>-</td>
</tr>
<tr>
<td>Control group (HIV-negative)</td>
<td>N199</td>
<td>39</td>
<td>M</td>
<td>ND</td>
<td>ND</td>
<td>-</td>
<td>99</td>
<td>+</td>
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Pt ID = participant identity; ALT = alanine aminotransferase; HCV = hepatitis C virus; HBV = hepatitis B virus; HBV-PCR = HBV polymerase chain reaction; F = female, M = male; ND = not done; TND = target not detected;*Insufficient sample.
liver disease in the absence of other causes such as HCV infection or alcohol abuse has been described.\textsuperscript{10} The prevalence of occult HBV ranges from 0% to 10% among individuals without liver disease, 11% to 19% in patients with chronic hepatitis, 12% to 61% in patients with hepatocellular carcinoma, and 1% to 95% in patients with HCV infection.\textsuperscript{21} It would be difficult to diagnose occult HBV infection in patients with AIDS as some guidelines utilise only HBsAg for HBV screening.\textsuperscript{16,22} More data are needed on occult HBV infection to elucidate its role in clinical disease in HIV co-infected patients, especially in areas of high HBV endemicity.

Markedly reduced anti-HBs titres could explain the high HBsAg prevalence and 'anti-HBc only' presentation in patients with AIDS, as some of these individuals eventually lose protective antibodies. This loss of HBV protective immunity may lead to reactivation of HBV, or exposure to new HBV infections. As patients infected with HIV lose protective antibody levels more quickly (40% loss in 1 year vs 5% loss in HBV-negative individuals),\textsuperscript{23,24} it may be a good practice to administer HBV vaccine booster doses to those with relatively low anti-HBs titres prior to commencement of HAART. Some experts use anti-HBs levels of less than 100 IU/l as a cut-off point for HBV vaccine booster doses in HBV-infected individuals,\textsuperscript{25} and our findings support this practice and reinforce its value in HIV endemic countries. The lack of HBV immunity in 60.5% of patients with AIDS could be explained by the fact that HBV vaccine was only implemented in the South African Expanded Programme on Immunisation (EPI) in 1995 and then not routinely offered to adults. Patients infected with HIV but with no HBV immunity should be considered for HBV vaccination to prevent HBV co-infection. Hepatitis B vaccine response in patients infected with HIV correlates with the CD4+ cell count (87%, 33% and 25% in CD4 count >500, 200-500 and <200 cells/µl, respectively).\textsuperscript{26} Therefore, HBV vaccination of patients infected with HIV may be most cost-effective in patients with CD4 count >500 cells/µl. In addition to using HBsAg in the HBV screening protocol, HIV management guidelines for developing countries should also incorporate anti-HBs to identify patients with low or no HBV immunity.

HIV/AIDS increased HBV prevalence and has enhanced other transmission risk patterns for HBV, which were previously uncommon in sub-Saharan Africa. For instance, horizontal transmission in childhood predominates in this region, but, with high HBV viral loads in HIV co-infected patients, there is now a high risk for both horizontal transmission among adults and vertical transmission of HBV. This undermines the HBV vaccination efforts which are targeted at reducing the HBV prevalence, and warrants changes in HBV screening and vaccination policies in sub-Saharan Africa in order to curb the spread of HBV. For example, in the past HBV screening of pregnant South African women was not considered to be cost-effective as HBV mono-infected carriers in South Africa are usually HBsAg-negative and therefore less infectious.\textsuperscript{27} This practice may need to be changed for HIV/HBV co-infected patients with AIDS as they tend to be HBsAg-positive with high HBV viral loads.

Conclusions

This study demonstrated a significantly increased HBV prevalence and decreased anti-HBs titres in patients with AIDS, and also identified a CD4+ cell count of <100 cells/µl as a major risk factor for the 'anti-HBc only' serological pattern and increased HBV replication. There was an increased prevalence of occult HBV infection in patients with AIDS. These findings have important diagnostic and treatment implications for HBV in patients with AIDS, especially in areas with a high burden of HIV and HBV infections. Therefore, a more comprehensive HBV screening programme is recommended for adequate management of HBV in HIV/AIDS. This should include HBsAg and anti-HBs, to identify HBsAg-positive individuals and also those with no HBV immunity or low anti-HBs titres. Although this comprehensive HBV screening has cost implications, it could be cost-effective even in resource-constrained settings when HBV vaccine is made available for prevention of HBV in patients infected with HIV. Early initiation of HAART should be considered in HBV/HIV co-infection to avoid HBV disease progression and IRIS, which are more likely to occur at low CD4+ cell counts.

The limitations of this study include the small sample size, and the inability to exclude other factors associated with isolated anti-HBc such as the 'HBsAg window period' of acute HBV infection.

Acknowledgements

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