ORIGINAL ARTICLE

Prevalence of occult hepatitis B amongst Indian human immunodeficiency virus type 1 infected individuals—a pilot study

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ABSTRACT

BACKGROUND

The diagnosis of hepatitis B is routinely based on the detection of hepatitis B surface antigen (HBsAg) only. However, occult hepatitis B virus (HBV) infection (OBI), which is defined as infection with positive hepatitis B core antibody (anti-HBc) antibodies, positive DNA (deoxyribonucleic acid) PCR (polymerase chain reaction), and undetectable HBsAg, as well as anti-HBs antibodies in serum or plasma of HBV infected individuals, will remain undetected using this screening diagnostic approach of detecting HBsAg. The current study aims in studying the prevalence of the OBI amongst human immunodeficiency virus type 1 (HIV-1) infected individuals who have not been exposed to anti-retroviral therapy.

METHOD

Estimation of HBsAg, anti-HBs, and anti-HBc total antibody status amongst 100 HIV-1 infected study participants was carried out using enzyme-linked immunosorbent assay (ELISA) kits. Detection of HBV-DNA was carried out by in-house qualitative PCR. CD4+T lymphocyte counts were analysed using Becton Dickinson's (BD) FACSCount™ system.

RESULTS

The median age of the HIV-1 infected study population was 35 years (range: 22–67), with the gender distribution being 53 males and 47 females. The mean CD4 T lymphocyte count of the study participants was 210/mm^3. Overall, serological evidence of HBV infection was observed in 28% of the HIV-1 infected study participants. There was 5% seropositivity for HBsAg, of which 2% were additionally positive for HBV-DNA-PCR. 'Anti-HBc alone' status was seen in 18% of study participants, this being statistically higher in those with CD4 T lymphocyte counts <200/mm^3. While there was a single specimen with co-positivity for anti-HBc total antibodies and HBV-DNA, 5% of the in the study population exhibited anti-HBs antibodies positivity, with one sample exhibiting dual positivity for HBsAg and anti-HBs antibodies.

CONCLUSION

Occult HBV infections may contribute to chronic liver damage, and associated reactivation amongst immunocompromised individuals, HIV-1 infected being a subset of them. 'Anti-HBc' testing followed by HBV-DNA detection by PCR can be utilised for such populations to detect OBIs. Early detection of hepatitis B viraemia will be important for deciding the antiviral therapeutic protocol so as to avoid evolution of antiviral resistance in the circulating HBV strains in HIV-1 infected individuals harbouring OBIs.

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Key Words: human immunodeficiency virus; occult hepatitis B

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INTRODUCTION

Infections caused by hepatitis B virus (HBV) are of a major health concern globally. It is estimated that about 2 billion people worldwide are infected with HBV, of which approximately 400 million are suffering from chronic hepatitis B, more so in the developing countries. Complications, including liver cirrhosis and hepatocellular carcinoma (HCC), related to chronic hepatitis B leads to 500,000–1,200,000 deaths annually.[1] In India, the HBsAg positivity in the healthy donor population has been reported to be around 4.7%, with the HBV carrier pool in India being around 43 million.[2]

Occult HBV infection (OBI) is defined as infection with positive anti-HBc antibodies and undetectable HBsAg as well as anti-HBs antibodies in serum or plasma of HBV infected individuals. A portion of such individuals may have detectable HBV-DNA (deoxyribonucleic acid).[3] Recently, this definition has further been revised by the requirement of presence of HBV-DNA in the liver, with detectable or undetectable HBV-DNA in serum of individuals with negative hepatitis B surface antigen (HBsAg), and a cut-off value for serum HBV-DNA as <200 IU/mL.[4]

Though the clinical significance of OBI remains largely unknown, there are possibilities of associated morbidities and mortalities as a result of this otherwise 'occult' entity. Firstly, OBI is associated with the potential risk of HBV transmission through blood transfusion, haemodialysis, and organ transplantation. Secondly, it may lead to fulminant hepatitis, chronic hepatitis B, and HCC. Finally, immunosuppression has been shown to play a considerable role in triggering reactivation of the infection.[5]

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The serological pattern, 'anti-HBc alone', characterised by the presence of anti-HBc antibodies as the only marker of hepatitis B, has been reported in various diagnostic settings. Depending on the prevalence of HBV infection and the patient group investigated, anti-HBc alone positivity has ranged from 1% to 31%. Anti-HBc alone has frequently been reported in intravenous drug addicts, human immunodeficiency virus (HIV)-infected individuals, patients who are co-infected with HBV and hepatitis C virus, and pregnant women.[6] Occult hepatitis B virus infections carrying detectable anti-HBs, reported in approximately 50% blood transfusion units, may essentially be not infectious by transfusion.[7] The prevalence of OBIs in haemodialysis patients has varied from 0.9% in Greece,[8] to about 6% in Iran,[9] to 26.6% in Italy.[10]

While the overall prevalence of HBV co-infection in HIV-infected from different parts of the world, including India, has ranged from 6% to 11%,[11–14] the prevalence of OBIs in HIV-infected, reported from different parts of the world have been widely divergent. Studies have reported markedly varying rates of OBI amongst HIV-positive populations from as low as nil to as high as 89.5%.[15–18]

In India, Rai et al have reported the prevalence of OBI by demonstrating HBV-DNA in 12.2% of HBsAg-negative and IgG anti-HBc positive subjects, with an overall prevalence of HBV-DNA of 13.7% in a study population of 58 sexually acquired HIV-positive study participants from Jaipur.[19] Similarly, Gupta et al have reported anti-HBc alone seroprevalence of 11.3% subjects from North India after analysing 53 HIV-positive, HBsAg-negative serum samples.[20]

The aim of this study was to assess the prevalence of occult hepatitis B amongst anti-retroviral treatment naïve HIV-1 infected individuals attending the anti-retroviral treatment centre affiliated with the Armed Forces Medical College, Pune.

MATERIALS AND METHOD

This study was a cross-sectional study, wherein the study participants were anti-retroviral treatment naïve, HIV-1 infected individuals ($n=100$) reporting to the anti-retroviral treatment centre affiliated with the Armed Forces Medical College, Pune between June 2010 and August 2010.

Collection of samples from the study participants was done, after obtaining informed consent, by venipuncture into sterile tubes as well tubes containing anti-coagulant (EDTA). Serum samples were stored at –70°C until assayed.

Estimation of HBsAg, anti-HBs, and anti-HBc antibody status amongst the study participants was carried out using ELISA kits, namely Hepalisa (J Mitra Co. Ltd., India), ETI-AB-AUK-3 (DiaSorin, Italy), and ImmunoLISA (Orgenics Ltd., Israel), respectively. Detection of HBV-DNA was carried out by in-house qualitative polymerase chain reaction (PCR) amplifying the 5-prime end of pre-core and core regions of the HBV genome (nucleotide positions 1730–2388) using assay steps described previously by Lahiri et al.[21] Briefly, the PCR primers used were primer 1 (forward) 5′-CTG-GGA-GGA-GTT-GGG GGA-GGAGAT-T-3′ and primer 2 (reverse) 5′-GGC-GAG-GGA-GTT-CTT-CTT-CTAGGG-G-3′. The PCR cycling conditions were as follows: Initial denaturation at 94°C, followed by 30 cycles of denaturation (94°C × 1 minute), primer annealing (50°C × 1 minute), extension (72°C × 2 minutes), and final extension at 72°C × 7 minutes. CD4 T lymphocyte counts were analysed with Becton Dickson's (BD) FACSCount™ flow cytometer.

Fisher exact test of significance was used to analyse the association of the frequency of anti-HBc alone seropositivity with the CD4+ T lymphocyte counts in the study participants. Cut-off P value of ≤ 0.05 was considered significant.

RESULTS

The median age of the HIV-1 infected study population was 35 years (range 22–67), and the gender distribution was 53 males and 47 females. The mean CD4 T lymphocyte count of the study participants was 210/mm^3, while the median value was 142/mm^3. The distribution of the study participants as per the 1993 Revised Classification System for HIV Infection and Expanded Surveillance Case Definition for AIDS among Adolescents and Adults was as shown in Table 1.[22]

The 1.5% agarose gel electrophoresis picture showing 658 base pairs amplicon of HBV-DNA is shown in Figure 1.

The distribution pattern of positivity amongst the study population for HBsAg, anti-HBc total antibodies, anti-HBs antibodies, and qualitative DNA-PCR positivity are shown in Table 2.

The distribution of anti-HBc total antibody positivity in the HIV-1 infected study participants ($n=100$) as per the as per the Centres for Disease Control and Prevention (CDC) classification system for HIV-infected adults and adolescents[22] is shown in Table 3.

DISCUSSION

In this study, after analysis of data on the various serologic and molecular markers for HBV infection amongst 100 study

Table 1 Distribution of the human immunodeficiency virus (HIV)-1 infected study participants ($n=100$) as per the centres for disease control classification system for HIV-infected adults and adolescents[22] with the mean CD4 T lymphocyte counts in each category.

CDC classification category	No. of participants	Mean CD4 T lymphocyte count (per mm^3)
Category 1: CD4 T cells > 500 cells/mm^3	11	660
Category 2: CD4 T cells 200–499 cells/mm^3	30	297
Category 3: CD4 T cells < 200 cells/mm^3	59	82

CDC: Centres for Disease Control.

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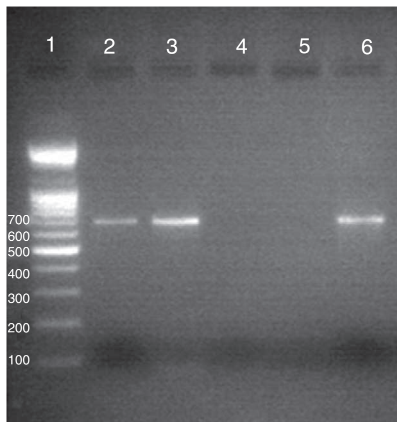

Figure 1 The 1.5% agarose gel electrophoresis picture demonstrating 658 base pairs amplicon of hepatitis B virus-deoxyribonucleic acid (nucleotide positions 1730–2388). Lane 1 shows 100 base pair ladder, lanes 2 and 3 show positive patient samples, lane 4 shows negative patient sample, and lanes 5 and 6 show negative and positive controls, respectively.

Table 2 Distribution pattern of positivity amongst the study population ($n=100$) for HBsAg, anti-HBc total antibodies, anti-HBs antibodies, and qualitative DNA-PCR amongst the HIV-1 infected study population.

Laboratory parameter	No. of positive samples
Anti-HBc total alone	18
Anti-HBc total+HBsAg	1
Anti-HBc total+HBV-DNA	1
HBsAg alone	1
HBsAg+Anti-HBs	1
HBsAg+HBV-DNA	2
Anti-HBs alone	4
Total	28

Anti-HBc: hepatitis B core antibody, Anti-HBs: hepatitis B surface antibody, DNA: deoxyribonucleic acid, HBsAg: hepatitis B surface antigen, HIV: human immunodeficiency virus, PCR: polymerase chain reaction.

participants, the evidence of past or current hepatitis B infection (HBsAg/anti-HBs/anti-HBc positivity) was detectable in 28% of the study participants. This was in agreement with the data on HIV-HBV co-infection from the West as well as India which has been reported to be between 6% and 11%.[11–14]

As stated earlier, published literature on OBIs in HIV-infected have ranged from nil to 89.5%.[15–18] In the Indian context, Rai et al reported HBsAg, anti-HBs, and anti-HBc IgG seropositivity of 1.7%, 10.3%, and 17.2%, respectively.[19] On studying HIV-infected individuals, Gupta et al observed that the prevalence of OBIs with detectable HBV-DNA was 24.5% of patients positive for anti-HBc antibodies, and 45.5% in HBsAg-negative patients. In addition, 20.7% showed anti-HBs antibodies and 35.8% were positive for anti-HBc antibodies.[20]

Analysis of the HIV-1 infected study population data revealed 5% seropositivity for HBsAg, of which 2% were additionally positive for HBV-DNA-PCR, and 1% with anti-HBc positivity as well. While only anti-HBc positivity anti-HBc alone status was observed in 18% of the study participants,

Table 3 Distribution of anti-HBc total antibody positivity as per the as per the CDC classification system for HIV-infected adults and adolescents[22] amongst the HIV-1 infected study participants ($n=100$).

Category	No. of 'anti-HBc alone' seropositive participants	Fisher exact test result
Category 1: CD4 T cells = 500 cells/mm^3 ($n=11$)	3	$P=0.048$
Category 2: CD4 T cells 200–499 cells/mm^3 ($n=30$)	5	
Category 3: CD4 T cells < 200 cells/mm^3 ($n=59$)	10	

anti-HBc: hepatitis B core antibody, CDC: Centres for Disease Control, HIV: human immunodeficiency virus.

a single case alone qualified as OBI with anti-HBc total antibodies and HBV-DNA co-positivity. Although our finding of occult hepatitis B with co-positivity for both anti-HBc antibodies and HBV-DNA is in disagreement with a previous Indian study,[20] similar low positivity of 0–1% for HBV-DNA-PCR in individuals with anti-HBc alone status has been reported in both immunocompromised as well as immunocompetent populations.[23,24] The anti-HBs seropositivity was 5% in the study population, with one sample exhibiting dual positivity for HBsAg and anti-HBs antibodies. The coexistence of HBsAg and anti-HBs Ab appears to be associated with an increase of 'a' determinant variability, suggesting a selection of HBV immune escape mutants during chronic carriage, although the coexistence of HBsAg and anti-HBs does not seem to affect disease evolution.[25]

A statistically significant correlation between of the frequency of anti-HBc alone seropositivity with the CD4+T lymphocyte counts in the study participants was seen, with most of the anti-HBc alone positivity observed in those with CD4+ T lymphocyte counts <200 cells/mm^3, a finding which has been documented previously.[20]

The limitation of our cross-sectional study includes the small sample size of the study population wherein point prevalence of various laboratory markers for HBV infection was tested without any prospective follow-up. In addition, the use of real time PCR would have been superior for HBV-DNA detection, as compared to qualitative PCR method that was used in this study. The use of research based, invasive procedure such as detection of HBV-DNA in liver was not included in our study.

In conclusion, we studied 100 anti-retroviral treatment naïve HIV-1 infected individuals to assess the prevalence of OBIs. Overall, there was a high seropositivity for hepatitis B infection, detected in 28% of the study population. The anti-HBc alone seropositivity profile was exhibited by 18% of the study participants, being statistically higher in those with CD4 T lymphocyte counts <200/mm^3. There was one study participant sample where dual positivity of HBsAg and anti-HBs antibodies was found, thus highlighting the possibility of selection of HBV immune escape mutants during chronic carriage.

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Occult HBV infections may contribute to chronic liver damage, and associated reactivation amongst immunocompromised individuals, HIV-1 infected being a subset of them. Thus, anti-HBc followed by HBV-DNA testing is important in HIV-1 infected individuals for detection of OBIs. Early detection of hepatitis B viraemia will help in planning the therapeutic protocol so as to prevent evolution of antiviral resistance in the circulating HBV strains in HIV-1 infected individuals harbouring OBIs. Occult HBV infection continues to be poorly characterised because of the technical issues arising from extremely low viral load. However, advanced nucleic acid detection techniques will aid in characterising OBIs and bringing down the possibility of reactivation of HBV disease amongst HIV-infected individuals.

Intellectual Contributions of Authors

Study concept: Col Sourav Sen, Col K Shanmuganandan
Drafting and manuscript revision: Col Sourav Sen, Med Cdt Shakti Prasad Panda, Col RM Gupta, Col K Shanmuganandan, Surg Cmde AK Praharaj
Statistical analysis: Col Sourav Sen

CONFLICTS OF INTEREST

Part of this research work was submitted as an Indian Council of Medical Research sponsored short-term studentship (2010) project report by Med Cdt Shakti Prasad Panda under the discipline of Microbiology under the guidance of Col Sourav Sen.

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