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Occult Hepatitis B Demonstrated by Anti-HBc and HBV DNA in HIV-Positive Patients

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Abstract

Background: In patients who are hepatitis B virus (HBV) DNA-positive, but HBV surface antigen (HBsAg) -negative, the infection is referred to as occult hepatitis B infection (OBI). Occult HBV infection is harmful when other liver diseases are present, and can aggravate liver damage in in patients with chronic liver diseases. In human immunodeficiency virus (HIV) infection the suppression of viral replication by the immune system might be inactivated, and classical HBV infection in OBI patients may occur.

Health care professionals should be aware of OBI in HIV patients. The routine test for HBV infection in Iran is the enzyme-linked immunosorbent assay for the HBV surface antigen (ELISA HBsAg); therefore, the aim of this study was to evaluate the prevalence of OBI in Iranian HIV patients.

Methods: This cross-sectional study was conducted in 2012 on sera from all the known and accessible HIV patients in Jahrom and Fassa, two cities in southern Iran. All samples were tested for the HBsAg, HBV core antibody (HBcAb). All the results were analyzed using SPSS.

Results: Of the 91 patients, seven (7.7%) were HBsAg-positive and forty-five (49.5%) were HBcAb-positive. In patients with negative HBsAg (84 patients), 39 (46.4%) were HBcAb positive and 53 (63%) were positive for HBV DNA.

Conclusion: The prevalence of HBV infection is relatively high in HIV patients, and more accurate tests than those presently in use should be used for diagnosis.

Keywords: Hepatitis B, HIV infection, Occult hepatitis

Introduction

Hepatitis B virus (HBV) infection is prevalent worldwide; approximately 350 million individuals are infected by this virus and 75% of them live in Asia (about 262 million patients) (1, 2).

Liver cirrhosis, liver failure, and hepatic adenocarcinoma are three significant complications of chronic HBV infection (2-4).

In addition, patients with chronic HBV are the main carriers of infection in communities (5).

The incidence of HBV infection by blood transfusion has been reduced over the last four decades (6); however, it has been shown that transmission can occur via blood components negative for the HBV surface antigen (HBsAg), and HBV remains the most commonly transmitted viral infection by transfusion (7-9). In patients who are HBV DNA-positive, but HBsAg, the infection is referred to as occult hepatitis B infection (OBI) (10). In these patients the molecular basis of OBI is

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due to strong suppression of viral replication activity and gene expression. In addition, other influences such as host immune responses, co-infection with other infectious agents, and epigenetic factors may contribute to HBV inhibition (11, 12).

In some OBI patients, serum HBV DNA levels are similar to those with serologically-positive HBV infections. In these patients, because HBsAg is negative "false OBI" should be considered. In these patients HBV variants with mutations in the S gene are usually the cause of the negative serology (10).

OBI itself is harmless, but when other liver diseases such as fibrosis or other viral hepatitis infections are present, the virus might aggravate the course of liver damage. In addition, in patients with chronic liver disease, OBI might contribute to progression of liver fibrosis and hepatocellular carcinoma (HCC) (13-15).

Although in 50% of HBV infections the route of virus entry is not known, one important route for HBV transmission is shared injections (16, 17); therefore, Intravenous drug abusers (IDU) are at high risk for HBV infection. In Iran the main route for HIV, HCV, and HBV infections is reported to be shared injections (18).

In some clinical conditions, including HIV infection, the state of forceful suppression of viral replication by the immune system might be inhibited, and classical HBV infection may develop, often with a severe clinical course (5, 19, 20).

In addition, in HIV patients, T and B lymphocyte abnormalities can cause increased susceptibility to infections. Therefore, susceptibility to HBV is increased in HIV-positive patients due to impaired T and B cell immunity responses, and the likelihood of chronic HBV infection is increased in these patients (21).

Currently, effective anti-HIV drugs are available that prevent disease progression; however, HBV infection remains an important cause of morbidity and mortality in HIV patients (3).

In light of this information, it is predicted that the rate of HBV infection in HIV patients should be high. It has been demonstrated that the HBsAg test is not a sufficiently-sensitive diagnostic test for HBV infection. Azadmanesh et al. showed that HBV core antibody (HBcAb) is positive in about 21% of the Iranian HIV patients who tested negative for the HBsAg (1).

In Iran the mean frequency of HBV infection in the community is estimated to be about 2% (3) but the prevalence of HBV infection differs in different community sub-groups (22).

We should be aware of HBV infection in HIV patients. The routine test for HBV infection in Iran is the ELISA HBsAg test. Because the actual HBV infection rate is not known in Iranian HIV patients, we conducted this study in Jahrom and Fassa, two cities in southern Iran, to determine the actual HBV infection rate in Iranian HIV patients by evaluating HBV DNA and HBcAb.

Materials and Methods

This cross-sectional study was conducted in 2012 on all of the known and accessible HIV patients who had attended the health centers for routine periodic laboratory tests in Jahrom and Fassa, two cities in southern Iran.

This study complies with current ethical considerations and conforms to the ethical guidelines of the 1975 Declaration of Helsinki. The proposal of the study was read and approved by the ethical committee of Jahrom University of Medical Sciences.

Ninety-one HIV-positive were evaluated. After receiving informed consent, five cc's of blood were obtained from each patient and coded. Sera were then isolated and stored at -20 °C until analyzed.

All sera were tested for HBsAg by ELISA (Radim, Italy), and the results were categorized as positive or negative using the manufacturer's guidelines. The samples were then analyzed for HBcAb by ELISA, and HBV DNA by nested PCR. Results were analyzed using SPSS.

Results

Ninety-one HIV patients were enrolled in the study. Their ages ranged from three to 55 years and their mean age was 34.3 ± 9.8 years. Six patients (6.5%) were less than 20 and four (4.4%) were less than 10 years of age. Sixty-nine patients (75.8%) were male and 22 (24.2%) were female.

Eighty-four patients (92.3%) were HBsAg-negative and seven (7.7%) were HBsAg-positive. Forty-five patients (49.5%) were HBcAb-positive. Fifty-three patients (58.7%) were positive for HBV DNA. (Fig. 1).

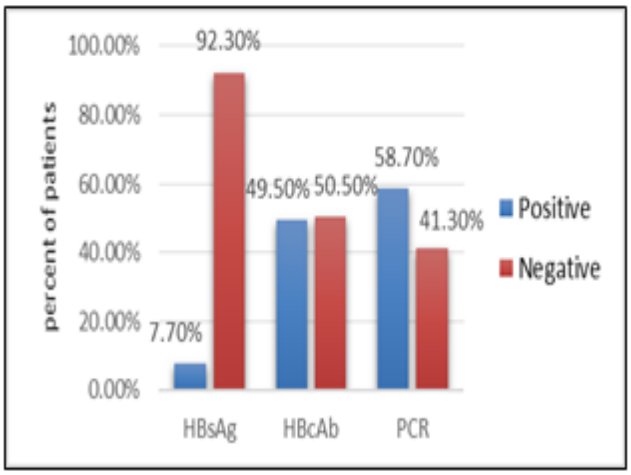

Fig. 1. HBsAg, HBcAb and HBV DNA in HIV positive patients.

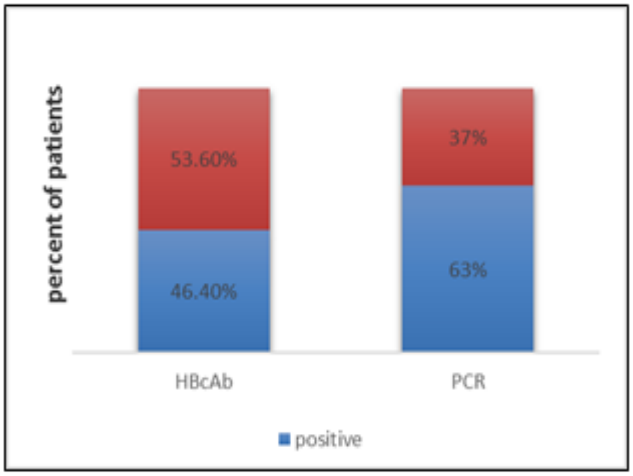

Fig. 2. HBcAb and HBV DNA positivity in HBsAg-negative patients.

In patients who were HBsAg-negative and HBcAb-positive, seven (17%) were negative for HBV DNA by nested PCR (Table 1).

Based on the results shown in Table 1, if we consider HBV DNA by nested PCR as the gold standard for diagnosis of HBV infection, the sensitivity and specificity of the HBcAb test for the diagnosis of OBI are 69.8 and 82%, respectively.

Table 1. HBcAb serology and PCR results in HIV infected patients

Test	HBcAb positive	HBcAb negative	Total
HBV DNA positive	37 (69.8%)	16 (30.2%)	53 (58.2%)
HBV DNA negative	8 (21.1%)	30 (78.9%)	38 (41.8%)
Total	45 (49.5%)	46 (50.5%)	91 (100%)

Discussion

In Iran about 1.5 million people are infected with HBV and it is estimated that 15 to 40 percent of them may develop HCC (23).

In a study conducted by Merat et al. in Iran, the prevalences of HBsAg and HBcAb in the general population were estimated to be 2.6 and 16.4%, respectively (24). The prevalence of HBV infection differs between populations with different risk factors; for example, in intravenous drug abusers in Iran, the reported prevalence of chronic HBV infection ranged from 1.5 to 3.7% (25).

HBV co-infection may exacerbate symptoms in HIV patients; therefore, evaluation of this group is important. Patients with OBI are negative on the routine serologic HBsAg test, and this may cause misdiagnoses; therefore, the more sensitive HBcAb and HBV PCR tests should be used to better evaluate these patients. In our study 92.3% of the HIV patients were HBsAg-negative, similar to the result reported by Gupta et al. in India, in which 92.7% of HIV patients were HBsAg-negative (26). Also, in the Gupta et al study, 45.3% of patients with negative HBsAg were HBV DNA-positive (26), compared with 63% in our study. It should be noted that in our study all the HIV patients were analyzed by nested PCR, while in the Gupta study only 53 of 776 HBsAg-negative patients were tested.

In a study conducted by Azadmanesh in Iran, 22 of 106 HIV patients (20.75%) were HBcAb-positive but HBsAg- and HBsAb- negative (1).

In another study by Santos et al., 68% of HIV patients were positive for HBcAb (27), greater than the 49.5% we observed in our study. In the Santos et al. study, 20% of the HBcAb-positive samples were positive for HBV DNA, less than the 82% observed in our study. These differences may be due to lower sensitivity of the HBcAb test or higher sensitivity of the nested PCR in our study. The result of our study is similar to the Frinhaber et al. study in Africa, in which 88.4% of

HBcAb-positive patients were HBV DNA-positive (4).

It is known that patients with OBI may be seronegative for HBs and/or HBc antibodies (28); therefore, analyses for HBV DNA from blood and/or liver samples by PCR should be the gold standard for OBI diagnosis (10).

The sensitivity of the HBcAb test in our study was only 49.5%: however, this test is less costly than PCR and can be used to screen HBsAg-negative HIV patients to diagnose OBI. Occult HBV infection is a possible contributor to the development of HCC and liver fibrosis in HIV patients with chronic liver diseases.

Decreasing the carrier pool is the best program for reducing the rate of HBV infection (23). This aim could be achieved by vaccination programs. but more accurate evaluations for the prevalence of HBV infection than are currently in use are needed (29).

To accurately diagnose chronic HBV infections, more sensitive diagnostic tests than those currently in use are needed.

In addition, OBI should be considered in HIV patients. However, further studies are necessary to evaluate the cost-effectiveness and usefulness of OBI management in HIV patients.

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References

1. Azadmanesh K, Mohraz M, Aghakhani A, Edalat R, Jam S, Eslamifar A, et al. Occult hepatitis B Virus infection in HIV-Infected Patients With Isolated Hepatitis B Core Antibody. Inter virology 2005; 51:270-4.

2. Martin C. Cytokine expression during chronic versus occult hepatitis B virus infection in HIV co-infected individuals. Cytokine 2009; 47:194-8.

3. Ramazani A. Occult hepatitis B Virus infection: A major concern in HIV-infected patients. Hepat Mon 2011; 11(1):7-10.

4. Firnhaber C. Occult hepatitis B virus infection in patients with isolated core antibody and HIV co-infection in an urban clinic in Johannesburg, South Africa. International Journal of Infectious Diseases 2009; 13:488-92.

5. Raimond G. Occult hepatitis B virus infection. Journal of Hepatology 2007; 46: 160-70.

6. Liu Y, Li p, Li C, Zhou J, Wu C, Zhou YH. Detection of hepatitis B virus DNA among accepted blood donors in Nanjing, China. Virol J 2010; 7:193.

7. Kafi-abad SA, Rezvan H, Abolghasemi H, Talebian A. Prevalence and trends of human immunodeficiency virus, hepatitis B virus, and hepatitis C virus among blood donors in Iran, 2004 through 2007. Transfusion 2009; 49:2214-20.

8. Calderon GM, Gonzalez-velazquez F, Gonzalez-Bonilla CR, Novelo-Garza B, Terrazas JJ, Martinez-Rodriguez ML, et al. Prevalence and risk factors of hepatitis C virus, hepatitis B virus and human immunodeficiency virus in multiply transfused recipients in Mexico. Transfusion 2009; 49:2200-7.

9. Liu CJ, Chen DS, Chen PJ. Epidemiology of HBV infection in Asian blood donors: emphasis on occult HBV infection and the role of NAT. J Clin Virol 2006; 36(1):S33-S44.

10. Liedo JL, Fernandez C, Gutierrez ML, Ocana S. Management of occult hepatitis B virus infection: an update for the clinician. World Gastroenterol 2011; 17(12):1583-8.

11. Raimondo G, Pollicino T, cacciola I, Squadrito G. Occult hepatitis B virus infection. J Hepatol 2007; 46:160-70.
12. Levrero M, Pollicino T, Petersen J, Belloni L, Raimondo G, Dandri M. Control of CCCDNA function in hepatitis B virus infection. J Hepatol 2009; 51: 581-92.
13. Chemin I, Guillaud O, Queyron PC, Trepo C. Close monitoring of serum HBV DNA levels and liver enzymes levels is most useful in the management of patients with occult HBV infection. J Hepatol 2009; 51:824-5.
14. Hu KU. Occult hepatitis B virus infection and its clinical implications. J Viral Hepat 2002; 9:243-57.
15. Marrero JA, Lok AS. Occult hepatitis B virus infection in patients with hepatocellular carcinoma: Innocent bystanded, cofactor, or culprit? Gastoenterology 2004; 126:347-50.
16. Azadmanesh K. Occult hepatitis B Virus infection in HIV-Infected Patients With Isolated Hepatitis B Core Antibody. 2005; 51:270-4.
17. Torbenson M, Thomas D. Occult hepatitis B. lancet infect dis2002; 2:479-86.
18. Pourahmad M, Javady A, Karimi I, Ataei B, Kassaeian N. Seroprevalence of and risk factors associated with hepatitis B, hepatitis C, and human immunodeficiency virus among prisoners in Iran. Infect Dis Clin Pract 2007; 15(6):368-72.
19. Altfeld M, Rockstroh JK, Addo M, Kupfer B, Pult I, Will H, et al. Reactivation of hepatitis B in a long term anti HBs-positive patient with AIDS following lamivudine withdrawal. J Hepatol 1998; 29:306-9.
20. Puoti M, Torti C, Bruno R, Filice G, Carosi G. Natural history of chronic hepatitis B in co- infected patients. J Hepatol 2006; 44:S65-S70.
21. Argani H, Akhtarishojaie E. Levamizole enhances immune responsiveness to intra-dermal and intra-muscular hepatitis B vaccination in chronic hemodialysis patients. Journal of Immune Based Therapies and Vaccines 2006; 4(3):1-5.
22. Quddus A, Luby SP, Jamal Z, Jafar T. prevalence of hepatitis B among Afghan refugees living in Balochistan, Pakistan. International Journal of Infectious Diseases 2006; 10:242-7.
23. Alavian SM, Hajarizadeh B, Ahmadzad-Asl M, Kabir A, Bagheri-Lankarani K. Hepatitis B virus infection in Iran: a systematic review. Hepat Mon 2008; 8(4):281-94.
24. Merat S, Rezvan H, Nouraie M, Jamali A, Assari S, Abolghasemi H, et al. The prevalence of hepatitis B surface antigen and anti-hepatitis B core antibody in Iran: a Population-based study. Arch Iranian Med 2009; 12(3):225-31.
25. Poorolajal J, Majdzadeh R. Prevalence of chronic hepatitis B infection in Iran: a review article. J Res Med Sci2009; 14(4):249-58.
26. Gupta S, Singh S. Occult Hepatitis B Virus infection in ART-Naïve HIV-Infected patients seen at a tertiary care center in North India. BMC Infection Diseases 2010; 10:53.
27. Santos EA, Yoshida CF, Rolla VC, Mendes JM, Vieira IF, Arabe J, et al. Frequent occult hepatitis B virus infection in patients infected with human immunodeficiency virus type 1. Eur J Clin Microbiol Infect Dis 2003 Feb; 22(2):92-8.
28. Raimondo G, Allain JP, Brunetto MR, Buendia MA, Chen DS, Colombo M, et al. Statements from the Taormina expert meeting on occult hepatitis B virus infection. J Hepatol. 2008; 49: 652-7.
29. Alavian SM. Ministry of health in Iran is serious about controlling hepatitis B. Hepat Mon 2007; 7(1):3-5.