SHORT COMMUNICATION

Occult Hepatitis B virus Infection among Anti-HBc only Positive Individuals in the Southeast of Iran in high prevalence of HBV Infection Region

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Abstract

Background: The persistence of HBV-DNA in the serum of hepatitis B surface antigen negative individuals with or without the presence of HBV antibodies is termed occult HBV infection.

Methods: From April 2005 to November 2006**,** we evaluated 110 patients who had only a positive test for anti-HBc.

Results: Out of 110 anti-HBc positive samples, HBV-DNA was detected in three cases. Positive samples for HBV-DNA had a level normal of ALT.

Conclusion: HBV-DNA can be detected among anti-HBc only positive samples. Therefore, further testing for detection of HBV-DNA is recommended on each anti-HBc only positive individual.

Keywords: Hepatitis B Virus infection; HBV-DNA; Anti-HBc; Iran

Introduction

The persistence of hepatitis B virus (HBV) genome in hepatitis B surface antigen (HBsAg) negative individuals with or without the presence of HBV antibodies is termed occult HBV infection.[1-3] Antibody to hepatitis B core antigen (anti-HBc) is the most sensitive marker of previous hepatitis B virus infection.[4] It appears in acute phase of HBV infection and usually persists after the virus disappears. Diagnostic problems may be observed when anti-HBc is found without any HBsAg or anti-HBs seropositivity.[4,5] Isolated anti-HBc may be associated with: (a) a chronic carrier state in individuals who have an undetectable HBsAg; (b) during window period; (c) past infection with loss of measurable anti-HBs and (d) cross-reacting antibody.[6] Occult HBV can cause the transmission of the infection by organ transplantation or blood transfusion and also reactivation of infection when an immunosuppressive status occurs.[1-3] Post-transfusion hepatitis B is still a problem in many countries. Regardless of all efforts to guarantee safety of blood, hepatitis B residual risk is the highest among transfusional transmitted infections, accounting for 1 in 60,000 transfused units.[3] Blood donations in Iran are collected from healthy donors.[3] All donors are tested for only HBsAg as a marker of HBV infection and this may affect the safety of blood supply.[3,7] In Iran, the rate of hepatitis B carriers varies from 1.7 to 5.4% with an average of 3.5%.[7] Salehi et al. performed a study on total population in Zahedan (Sistan and Baluchistan Province in southeast of Iran) and found that 5.4% of this group were carriers and 34% were only positive for anti-HBc.[8] Based on the results which have emerged from previous studies in Iran, the rate of hepatitis B carriers in Zahedan is higher than that in other provinces (5.4%).[8,9] Therefore, the rate of occult HBV can be high. The present study assessed the presence of HBV-DNA in serum samples of individuals with isolated anti-HBc.

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Materials and Methods

In this cross-sectional and descriptive study from April 2005 to November 2006, all family members (with age>16years) of patients with acute and chronic HBV infection, who had been registered to Zahedan Hepatitis Center, were evaluated. Those who had only a positive test for anti-HBc, were selected and underwent clinical evaluation and retesting of HBV markers (HBsAg, anti-HBc**)** by ELISA (Kit Diasorin BioMedica, Saluugia, *Italy*). Informed and written consent was obtained. Blood samples from all anti-HBc only positive individuals were collected and screened for HBV-DNA by a qualitative PCR-Kit (Cinna Gen, Tehran, Iran). Liver function test including aspartate aminotransferase (AST) and alanine aminotransferase (ALT) also were performed for all anti-HBc only positive individuals. All tests were carried out in the laboratory of Research Center of Blood Transfusion Organization. The specimens were stored at –20^{o}C until use. Preparation of DNA samples from the sera was made according to the guidelines elaborated by Kowk and Higushi[14] to prevent any contamination. Each three steps - denaturation, annealing and extension- were done at 93, 61 and 72^{o}C, respectively. These steps were reapeted for 35 cycles. The cycles were done on an automated cycler, which rapidly heats and cools the test tubes containing the reaction mixture. PCR amplification was performed using a published oligonucleotide primer set selected from a highly conserved HBV surface gene. The PCR sensitivity assay was equal to about 100 copies of HBV genome in PCR mixture.

Results

We evaluated 512 cases of family members of patients with acute and chronic HBV infection. Among this group, 110 cases (21.5%) had only a positive test for anti-HBc. Out of 110 only anti-HBc positive samples (69 males, 41 females; mean age=32 years; age range=16-64 years), HBV-DNA was detected in three cases (2 males, 1 female). Rate of hepatitis B carriers (only HBsAg positive) among family members of patients with HBV infection was 3.3%. The liver function test results were all in normal range except in 9 of HBV-DNA negative subjects. Positive samples for HBV-DNA had a level normal of ALT. Hepatitis C virus infection was confirmed by Anti-HCV and a qualitative RT- PCR-Kit (Cinna Gen, Tehran, Iran), in two cases who had an elevated level of ALT. Anti-HCV (third generation assay) was measured by enzyme immunoassay (EIA). There were no signs or symptoms in our subjects except, in 11 cases who had mild fatigue.

Discussion

Our study showed that the overall prevalence of occult HBV infection (DNA in serum) in family members of patients with acute and chronic HBV in Zahedan was 2.27%. Since, anti-HBc detection is not routinely used in Iran**,** especially in sera of healthy blood donors, we evaluated whether this test (PCR) could be adopted as a screening assay in anti-HBc only positive individuals. Most studies on occult HBV infection have reported higher rates of HBV-DNA detection.[3,10,11] Behzad-Behbahani et al. reported an overall prevalence of occult HBV in healthy blood donors in Shiraz, Southern Iran to be 12.2%.[3] It is interesting to note that the prevalence of chronic HBV infection (healthy carries) in Zahedan is higher than Shiraz (5.4% versus 1.7%).[9-11] Furthermore, the prevalence of anti-HBc only positive individuals in Zahedan is high (34%)[9,12] but the prevalence of occult HBV infection in our study population was lower than that in Shiraz study. This difference may be due to different methods and kits used for evaluation and detection of DNA**.** The sensitivity of PCR assay in Shiraz study was equal to about 80 copies of HBV genome in PCR mixture, but in our study, PCR sensitivity assay was equal to about 100 copies of HBV genome in PCR mixture. In Behbahani's study, there was an association between anti-HBc titration and the intensity of expected PCR product. Nevertheless, no association was found between the presence of anti-HBc and positivity of HBV-DNA.[3] In the present study, there was no anti-HBc titration, and we could not find any association between anti-HBc titration and positivity of HBV-DNA. Amini et al. performed a study on 4930 healthy blood donors in Iran, and found that 5.1% were only positive for anti-HBc without having any detectable HBsAg. However, they did not determine the presence of HBV-DNA.[13] Occult HBV infection is an entity with world-wide diffusion, and the available data show that its prevalence varies in different countries because of different prevalence of HBV infection and different sensitivity and specificity of the methods used for its detection in many studies.[3,10,12] Prevalence of occult hepatitis B virus infection in Zekri study from Saudi Arabia in blood units with isolated anti-HBc was 1.25%.[14] One report from Germany showed that 1.6% of first-time blood donors with antibodies to hepatitis B core

antigen had occult hepatitis.[15] Higher rate of occult hepatitis B among anti-HBc only positive individuals has been repoted.[3,16] A Swedian study showed that 10% of isolated anti-HBc individuals were HBV-DNA positive.[16] Although in our study population, the rate of anti-HBc only positive individuals was lower than that in the total population (21.4% vs 34%), it is still high. Detection of HBV-DNA in the sera of individuals with anti-HBc may be due to chronic and persistent HBV infection. Everybody with any marker for past exposure to HBV should be considered potentially infectious.[17] At present, HBsAg detection is the only diagnostic screening test for HBV infection in blood transfusion centers in Iran and using other tests is not practical because they are not cost effective. Therefore, we think anti-HBc antibody should be tested routinely on blood donor volunteers and if the sera are positive, the blood should be discarded.

HBV-DNA can be detected among anti-HBc only positive individuals. A screening strategy that tests only for HBsAg, especially in blood donor volunteers, will miss a large number of individuals with isolated anti-HBc and it can lead to transfusion-transmitted HBV infections. Therefore, further testing for detection of HBV-DNA is recommended on each anti-HBc only positive individual.

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Conflict of interest: None declared.

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