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Lack of occult hepatitis B virus infection among blood donors with isolated hepatitis B core antibody living in an HBV low prevalence region of Iran

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ABSTRACT

Background: Occult hepatitis B virus (HBV) infection in blood donors is considered a potential threat for the safety of the blood supply, however conclusive studies on this issue are lacking. The aim of this study was to assess the occult HBV infection in blood donors with isolated hepatitis B core antibody (anti-HBc) living in the city of Arak, in the Central Province of Iran, as a low prevalence region for HBV.
Methods: A total of 531 voluntary blood donors in Arak, Iran were included in this study. Hepatitis B surface antigen (HBsAg), hepatitis B surface antibody (anti-HBs), anti-HBc, and hepatitis C antibody (anti-HCV) were tested in all subjects. The presence of HBV-DNA was determined quantitatively in plasma samples of cases with isolated anti-HBc (HBsAg-negative, anti-HBs-negative, and anti-HBc-positive) by real-time PCR using the artus HBV RG PCR kit on the Rotor-Gene 3000 real-time thermal cycler.
Results: Of 531 subjects enrolled in this study, 11 (2.1%, 95% confidence interval 0.8–3.2%) had isolated anti-HBc. HBV-DNA was not detected in any of the cases with isolated anti-HBc.
Conclusions: Our study showed that all the blood donors with isolated anti-HBc were negative for HBV-DNA, and occult HBV infection did not occur in the blood donors of this low prevalence region for HBV infection.
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1. Introduction

Infection with the hepatitis B virus (HBV) is a serious global health problem, with two billion people infected worldwide and 350 million suffering from chronic HBV infection. Of these, 75% are Asians.[1] Among the many transmission routes, transfusion is the one that could be prevented. The first major success in enhancing transfusion safety came with the implementation of hepatitis B surface antigen (HBsAg) detection in the early 1970 s. However, studies have demonstrated that transmission by HBsAg-negative blood components can still occur in the acute phase of infection during the seronegative window period, or during chronic stages of infection with undetectable HBsAg.[2]

With the development of sensitive assays to detect HBV-DNA it has been shown that healthy HBsAg-negative donors who have antibodies to HBV core antigen (anti-HBc) may harbor an occult HBV infection and maintain HBV-DNA sequences in their liver and blood, thus representing potential sources of HBV transmission.[3–5]

The serological pattern defined as isolated anti-HBc (HBsAg-negative, hepatitis B surface antibody (anti-HBs)-negative, and anti-HBc-positive) is observed in 10–20% of individuals from areas of low endemicity for HBV.[6] The significance of this serological pattern is unclear. It may reflect past infection with HBV, after which anti-HBs either did not develop[7] or decreased to an undetectable level.[6] Also, this serological pattern can be observed in the window phase of a resolving case of acute hepatitis B. Finally it may represent occult chronic HBV infection, with levels of the HBsAg below the limits of detection.[8]

Detection of HBV-DNA without detectable HBsAg is defined as occult HBV infection.[9,10] The frequency of detection of occult HBV infection depends on the relative sensitivity of both HBsAg and HBV-DNA assays. It also depends on the prevalence of HBV infection in the population.[11] The prevalence of occult HBV infection is higher in areas in which HBV infection itself is more frequent. Iran is in the low endemic area for HBV.[12] The prevalence of HBsAg in Iran ranges from 1.7% to over 5% in the different

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provinces.[13,14] In the Central Province of Iran the prevalence of HBV is estimated to be 0.5%. It is estimated that 7500–15 000 chronic HBV individuals live in this province, which has a population of 1.5 million.[15]

Anti-HBc screening of blood donations is controversial and variably performed in different countries.[16] With regard to transfusion, anti-HBc screening has been used initially as a surrogate marker for non-A, non-B hepatitis, and its screening can potentially prevent occult HBV transmission.[17] Occult HBV infection in blood donors is considered a potential threat for the safety of the blood supply, however conclusive studies on this issue are lacking.[11]

The aim of the present study was to assess the occult HBV infection in blood donors presenting with the serological pattern of isolated anti-HBc, living in Arak City, in the Central Province of Iran, as a low prevalence region for HBV.

2. Patients and methods

The blood samples used in this study were collected from the Arak Blood Transfusion Organization in Arak City, in the Central Province of Iran between October and December 2008. A total of 531 blood donors out of the 7200 were randomly enrolled in this study. A questionnaire was used to collect data such as age and sex. This project was approved by the Iranian Society for the Support of Patients with Infectious Diseases ethics committee, and informed consent was obtained from patients prior to their enrollment.

Blood samples were collected from all patients and plasma was stored at −80 °C. All patients were tested for HBsAg, anti-HBs, anti-HBc, and hepatitis C antibody (anti-HCV) by ELISA. The commercial enzyme immunoassay kits used were as follows: HBsAg, anti-HBs and anti-HCV (Enzygnost, Dade Behring, Marburg GmbH, Germany) and anti-HBc (Enzywell, Diesse.Tec Diagnostica, Monteriggioni (Siena), Italy). All anti-HBc-positive samples were re-tested with the same assay.

HBV-DNA was extracted using the High Pure Viral Nucleic Acid kit (Roche Diagnostics GmbH, Mannheim, Germany) following the manufacturer's instructions. HBV-DNA was determined quantitatively by real-time PCR using the artus HBV RG PCR kit (Qiagen, Hamburg, Germany) on the Rotor-Gene 3000 real-time thermal cycler (Corbett Research, Sydney, Australia). According to the user manual, the analytical detection limit of the kit is 0.02 IU/μl.

3. Statistical analysis

Chi-square and t^2 tests were used with SPSS v. 16 for statistical analysis (SPSS Inc., Chicago, IL, USA). Data are presented as mean ± standard deviation (SD) or, when indicated, as an absolute number and percentage. The 95% confidence interval (95% CI) was calculated.

4. Results

A total of 531 voluntary blood donors living in the city of Arak, in the Central Province of Iran, with a mean age of 36 ± 10.18 years (range 16–60 years) were enrolled in the study. Ninety-three percent of patients were male and 7% were female. HBsAg, anti-HBs, anti-HBc, and anti-HCV were found in 0.4%, 31.8%, 11.5%, and 0.2% of subjects, respectively. Of the 531 cases, 11 subjects (2.1%, 95% CI 0.8–3.2%) had isolated anti-HBc.

HBV-DNA was not detected in any of the cases with isolated anti-HBc. All HBV-DNA-negative samples underwent confirmatory testing with additional real-time PCR on the same nucleic acid extracted material, but all of the samples were repeatedly negative for HBV-DNA.

5. Discussion

In this study we investigated the frequency of HBV-DNA in HBsAg- and anti-HBs-negative, anti-HBc-positive blood samples collected from voluntary blood donors living in the city of Arak in the Central Province of Iran, where HBV prevalence is low. This survey showed that the prevalence of isolated anti-HBc and HBV-DNA was negligible in blood donors in this region.

In published studies, the prevalence of anti-HBc in HBsAg-negative blood donors ranges from 0.56% in the UK,[18] 0.84% in the USA,[19] 1.4% in Germany,[4] 15.03% in Greece,[20] and 16.4% in Saudi Arabia,[21] to 76% in Ghana.[18]

Occult HBV infection is most frequently seen in patients with anti-HBc as the only HBV serological marker.[22] When detectable, the amount of HBV-DNA in the serum is usually very low (<200 IU/ml).[23] Occult HBV infection in blood donors is considered a potential threat for the safety of the blood supply. A number of explanations for the persistence of HBV-DNA in HBsAg-negative samples have been proposed, including the presence of HBV-DNA at a low copy number,[9] genetic variations in the S gene,[24,25] and the presence of immune complexes in which HBsAg may be hidden.[26,27] Occult hepatitis B may also be due to the window period following acute HBV infection, poor laboratory detection of HBsAg due to low levels of HBs antigenemia, underlying HCV co-infection, immunosuppression, or other host factors.[6,28] Moreover, it has been suggested recently that HIV infection may be a risk factor for occult hepatitis B.[29,30]

The relative proportion of occult HBV is likely to be related to local epidemiology, composition of the study populations, and the level of sensitivity of the HBV-DNA assay.

The percentage of samples containing HBV-DNA from either blood donors or the general population ranges between 0% and 7.7% in areas in which the prevalence of HBV is low.[4,19,31] In areas of higher HBV infection prevalence, such as Greece (15.8%), China (70.0%), and Ghana (83.6%), the frequency of DNA-positive isolated anti-HBc cases increases to 1.9%, 2.7%, and 12.7%, respectively.[18,20,32]

One large study investigated 6313 consecutive blood donors and found that the prevalence of anti-HBc-positive subjects was 4.85%, and 4.86% of subjects were confirmed to have circulating HBV-DNA at a low level. The sensitivity of the assay for HBV-DNA was 4.9 IU/ml.[33] A second study investigated 244 blood donors with isolated anti-HBc and reported that all samples were negative for HBV-DNA.[34] In a study of 760 healthy Egyptian blood donors, HBV-DNA was found in 11.54% of the anti-HBc-positive units.[35] García-Montalvo et al.[36] investigated the sera of 158 volunteer blood donors, negative for HBsAg and anti-HBs, but positive for anti-HBc, using a nested PCR. Occult HBV infection was observed in approximately 8% of anti-HBc only donors. The lower limit of detection for the nested PCR assay was 30–300 copies of the viral genome per ml. Duseja et al.[37] studied 100 healthy adult blood donors, negative for HBsAg, anti-HCV, HIV-1, and other risk factors for HBV-DNA by PCR. All the healthy blood donors were negative for HBV-DNA.

Kaminski et al.[38] reported that 20.5% of HBsAg-negative blood donors had anti-HBc only pattern and HBV-DNA was not detected in any of these cases. Our results regarding occult HBV infection are compatible with the results of the studies of Kaminski et al.,[38] Duseja et al.,[37] and Kupski et al.[34] and are comparable to the rates (0–7.7%) reported in regions in which the HBV prevalence among blood donors is low.[4,19,31]

In a study by Behzad-Behbahani et al. in Fars Province of Iran, on 2000 healthy blood donors, 6.55% of blood samples were found to be positive for anti-HBc. HBV-DNA was detected in 12.2% of anti-HBc-positive specimens.[39] In the Central Province of Iran, the prevalence of HBV is estimated to be 0.5%.[15] In Fars Province, in the

southwest of Iran, the prevalence of HBV carriers in asymptomatic healthy blood donors was about 1%.[13] The discrepancy between the study of Behzad-Behbahani et al. and our survey may be due to local epidemiology, prevalence of HBV infection, and composition of the study populations.

In conclusion, in this study all the blood donors with isolated anti-HBc were negative for HBV-DNA, and occult HBV infection did not occur in the blood donors in this low prevalence region for HBV infection. However, due to the limited number of cases in the present study, a definite conclusion cannot be reached regarding the frequency of occult HBV infection in blood donors. Because of the relationship of occult HBV with local epidemiology, further studies on more cases in different populations are suggested to determine whether anti-HBc screening should be implemented in screening programs for HBV infection among blood donors in HBV low-endemic areas.

Conflict of interest: No conflict of interest to declare.

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