Original Article

Occult Hepatitis B Virus Infection among Iranian Blood Donors: A Preliminary Study

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Abstract

Background: Although serological screening tests for blood-borne hepatitis viruses have effectively reduced the risk of HBV transmission through transfusion of infected blood, there is still a possibility that infected blood units from occult carriers being released into the blood supply.

The aim of this study was to determine the prevalence of anti-HBc among Iranian blood donors and evaluate the presence of HBV DNA in HBsAg negative plasma samples.

Methods: In the present study, 5000 HBsAg negative samples were collected from donors in blood transfusion centers in Tehran. All HBsAg negative samples were tested for the presence of anti-HBc antibody and anti-HBs antibody (HBsAb) using ELISA method. Also, all HBsAg negative samples were tested for the presence of HBV DNA by real-time PCR.

Results: Four hundred ninety nine (9.98%) out of the 5000 HBsAg negative blood donors were anti-HBc positive. Out of 499 anti-HBc positive samples that were tested for anti-HBs, 394 (78.4 %) were anti-HBs positive, and 275 (62.7%) had an antibody titer greater than 100 IU/mL. HBV DNA was detected in two samples.

Conclusion: In countries with intermediate rate of HBV infection like Iran, the prevalence of anti-HBc antibody in HBsAg negative blood donors is found to be high. As a result, routine anti-HBc screening of HBsAg-negative blood donors without complementary tests (anti-HBs / HBV-DNA) can limit the number of blood transfusions. Therefore, it might be better to include the detection of HBV DNA along with the routine tests.

Keywords: Anti-HBc, blood donor, HBsAg negative, HBV-DNA, Hepatitis B Virus

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Introduction

Despite effective vaccination, hepatitis B still is an important worldwide public health issue. Serological screening tests, high precision selection of blood donors and an effective vaccination against hepatitis B infection have clearly decreased the risk of HBV transmission via blood transfusion. However, there is still a residual risk for transfusion transmitted HBV.[1]

In Iran, screening blood donors for HBsAg became mandatory in 1974.[2] The presence of HBV DNA in serum and/or liver of HBsAg negative individuals with or without antibodies against HBV is defined as occult HBV.[3] In many developed countries, successful implementation of Nucleic Amplification Technique (NAT) for detection of HIV or HCV in plasma has reduced the risk of post transfusion infection at window period when the viral proteins could not be detected[1]. Primary data using NAT from volunteers showed that the HBV window period has decreased from 38 days to 6 – 15 days.[4,5] HBV NAT has been performed in some countries such as Germany, Austria, and Japan routinely.[1]

However, each country for assurance of blood safety should develop its blood screening strategies based on HBV endemicity, prevalence of HBV and Cost-effectiveness screening tests.[1]

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In this study, we determined additional anti-HBc or HBV NAT tests which should be used to decrease the residual risk of HBV infection thorough blood transfusion.

Material and Methods

Study design

This cross-sectional study was carried out among blood donors from May 2008 to March 2009 in Tehran province. Also a questionnaire was completed by blood donors (asking for their age, sex, the number of blood donations, and prior history of hepatitis B vaccination).

A total of 5000 blood donors who were negative for hepatitis B surface antigen (HBsAg), hepatitis C virus antibody (HCV Ab) and human immunodeficiency virus antigen-antibody (HIV Ag-Ab) were selected.

ELISA tests

Serum samples were tested by ELISA method using monoclonal antibody against IgM-IgG-HBc Ab (DADE Behring, Germany) and antibody against hepatitis B surface antigen (HBsAb) (DADE Behring, Germany) according to the manufacturer's instructions.

Real-Time PCR

All Serum samples were examined by Real time PCR and their viral load was measured. HBV-DNA was extracted using a QIAamp Viral DNA Mini kit (QIAGEN) according to the manufacturer's instructions. For the detection of HBV-DNA, Real-

Table1. Demographic, serological and molecular markers of the blood donors.

Characteristics	
Gender No (%)	*n* = 5000 (100%)
Male	4655 (93.1%)
Female	345 (6.9%)
Number of donations	
First- time	835 (16.7%)
Periodic	4170 (83.4%)
Serological markers	
Anti-HBc-Positive	499 (9.98%)
First- time donors	65 (13%)
Periodic donors	434 (87%)
Anti-HBs-Positive	394 (78.4%)
HBV-DNA-positive	
First- time	2 (0.04%)
Periodic	0 (0%)

Time PCR was performed by using Real ART HBV LC PCR kit (Artus GmbH, Hamburg, and Germany). The lower detection limit for the HBV Real-time PCR in this study was 50 IU/mL. All data were analyzed statistically with SPSS 13.5 software with $P < 0.05$ using the Chi-Square test.

Results

Of 5000 blood donors, 4655 (93.1%) were male and 345 (6.9%) were female. Their mean age was 36.8 ± 11.1 years (range 17 to 65). About 1365 (27.3%) donors had been vaccinated against HBV. Considering the number of blood donations, 4170 (83.4%) were periodic blood donors and 835 (16.7%) were first-time donors.

Of 5000 blood samples, 499 (9.98%) were positive for anti-HBc. Serum samples which were positive for anti-HBc were tested for the presence of anti-HBs. One hundred and five (21.7%) were negative for this antibody. Among the remaining 394 (78.4%) HBsAb positive samples, HBsAb titer in 275 (62.7%) samples was higher than 100 IU/L ($P < 0.001$). HBV DNA was detected in two samples with viral load level 248 and 235 IU/mL. There was a significant difference between the number of blood donations (first- time or periodic) and presence of HBsAb: most of the HBsAb positive blood donors were periodic donors ($P < 0.001$).

Discussion

The residual risk of HBV infection in endemic regions is mainly associated with chronic HBV carrier blood donors with occult hepatitis and undetectable HBsAg level.[6] In a study, the prevalence of anti- HBc in the general population in Tehran was 14.2%.[7] In our study, 499 (9.98%) blood donors were positive for anti-HBc. Among them 394 (78.4%) were positive for anti-HBs antibody. Assessing the risk of HBV transmission from anti-HBc positive donors depends on detection of HBV DNA and presence or absence of anti-HBs antibody. The presence of high titer HBV virions in peripheral blood makes it possible for anti-HBs antibodies to neutralize the infectivity of the viruses.

In this study 105 samples (21%) of isolated anti-HBc antibody in HBsAg-negative blood donors could be a marker of resolved HBV infection or low-level chronic infection. Two samples obtained from first-time blood donors were PCR positive. To confirm the presence of HBV DNA in these two positive samples, another sensitive HBV NAT method was used. These individuals were first-time blood donors, negative for HBsAg and anti-HBs and finally none of them had prior history of hepatitis B vaccination. Thus, increasing the number of blood donors and the retention of first-time donors for continuing blood donation (as repeat donors) is necessary.

The risk of transfusion-transmitted HBV infection from blood products obtained from Occult hepatitis B infection (OBI) donors depends on the presence of anti-HBs and viral load.[7] Considering the high prevalence of anti-HBc (9.98%) in our study, performing anti-HBc tests without additional tests (anti-HBs / HBV-DNA) in HBsAg-negative blood donors can make about 10% of donated blood unusable. Under such condition, in our country with high anti-HBc prevalence, screening of blood donors with HBV-NAT would be more effective than anti-HBc screening. In conclusion, decrease in the prevalence of anti-HBc due to hepatitis B vaccination in Iran and participation of younger people in blood donation and also performing more multicenter studies of transfusion of the occult HBV infection, decision on anti-HBc screening methods should be revisited in coming years.

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