

# Detection of HBV DNA in HBsAg Negative Normal Blood Donors

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## ABSTRACT

**Background:** The risk of infection by transfusion-transmitted viruses has been reduced remarkably. However, a zero-risk blood supply is still desirable. The screening for antibody to HBc (anti-HBc) has been shown as an alternative test for the detection of HBV infection. **Objective:** The main aim of this study was to evaluate HBV infection markers and the potential value of anti-HBc testing of blood donors to detect HBV infection. **Methods:** In this descriptive cross-sectional study, 545 blood samples were collected and tested for HbsAg using ELISA method. Then all HBsAg negative samples were tested for anti-HBc by the same method. To detect HBV infection, all HBsAg negative and anti-HBc positive samples were tested by PCR for HBV DNA. **Results:** All blood samples were HBsAg negative of which, 43 (8%) were anti-HBc positive. From those which were positive for anti-HBc, five samples were also positive for HBV DNA. **Conclusion:** Occult HBV infection is a clinical form of HBV infection in which HBsAg is not expressed by HBV and blood samples cannot be screened by ELISA method, therefore more sensitive techniques are needed. Our results demonstrate that a complementary test such as PCR, for detecting HBV DNA, is essential to ensure safety of blood samples.

**Keywords:** Occult HBV, HBsAg, Anti-HBc, PCR, ELISA

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## INTRODUCTION

Blood transfusion and component therapies are well-established and essential medical practices. However, blood collected from large populations is associated with the risk of pathogen transfusion, such as HBV (1). Currently HBsAg screening test is a method used to reduce transfusion-transmitted HBV (2). It effectively decreases the viral infection rate to approximately 7 to 32 per million units (3). Although this serologic method reduces transfusion-transmitted infections, some HBsAg-negative blood samples can still induce post hepatic transfusion in recipients (1, 4). This is probably related to HBsAg seronegative window period and Occult HBV Infection (OHI) (1). OHI is one of the several clinical features that are characterized by a low level of HBV-DNA remaining detectable in serum and liver tissue in some patients who have cleared HBsAg from either acute self-limited or chronic HBV infection, or even after a successful anti-HBV treatment (5). Therefore to further reduce the risk of transfusion-related HBV, more sensitive methods should be used to detect HBV genome.

In addition, anti-HBc is produced in a person exposed to HBV and may be a good marker of past HBV infection (1,7), but using anti-HBc as a screening test to find infectious blood samples is still controversial (1,8-12). For example in a study by Steven H. Kleinman et al. the rate of positive anti-HBc samples was reported to be 8% but no HBV DNA was detected by PCR in these samples (10). In another study of 26492 consecutive blood donations from United States with a low prevalence of hepatitis B serologic markers, HBV DNA was not detected by PCR (11). A recent study of 9238 East Anglian blood donors screening for anti-HBc showed that all anti-HBc positive samples were negative for HBV DNA, tested with nested PCR (12). In contrast, in Germany, it has been shown that 1.59% of negative HBsAg and positive anti-HBc blood samples have HBV DNA and are probably infectious and may transmit HBV. This study proposed that routine anti-HBc screening of blood could probably prevent some transfusion-transmitted HBV infections (8). Moreover, in a study of endemic area in Taiwan, the rate of HBV DNA was relatively high (7%) in negative HBsAg and positive anti-HBc samples (1). Considering these controversial results and with the knowledge that clinical features of HBV infection are dependent on race, geographical area and subtype of HBV (2, 6, 7, 13), this study was carried out to detect HBV DNA in HBsAg negative/anti-HBc positive blood donors.

## MATERIALS AND METHODS

### Subjects

Peripheral blood samples were collected from 545 volunteers referred to Isfahan Blood Transfusion Organization in 5.5 ml tubes containing potassium-EDTA (ethylene diamine tetraacetic acid) at a concentration of 1.6 mg EDTA per ml of blood. The samples were centrifuged at 3500×g for 4 minutes, and plasma was separated within 24 hours. Plasma specimens were stored at -20°C for a maximum of 2 months or at -70°C until further processed.

### Methods

**Enzyme Linked Immunosorbent Assay (ELISA).** HBsAg screening test were performed using HBsAg ELISA kit (RADIM, Italy). Reactive samples were retested

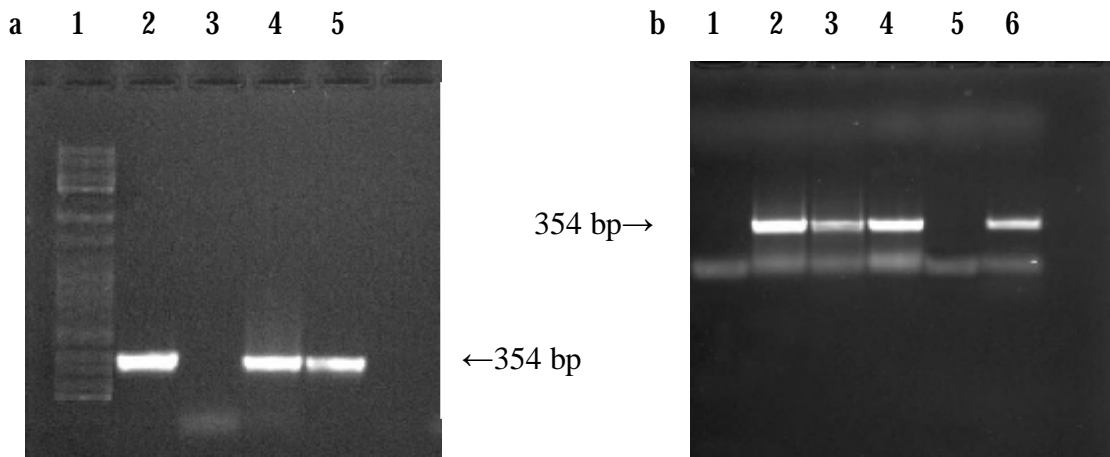
in duplicate and considered to be reactive if at least 1 of the 2 repetitions also gave a positive result. This test was performed using sandwich ELISA method.

Anti-HBc screening test was also performed by a manual microplate enzyme immunoassay using anti-HBc kit provided by RADIM (Italy). The present method is based on a competitive enzyme immunoassay (EIA).

**DNA Extraction.** Viral DNA was purified from 100 µl of plasma samples. Briefly, each serum sample was incubated at 72°C for 10 minutes and then cooled at 4°C for 5 minutes in 100µl proteinase K (200 µg/ml). After phenol/chloroform extraction (1:1), the viral DNA was precipitated with ethanol and the pellet was redissolved in DNase free, deionized water and stored at -20°C.

**PCR and Gel Electrophoresis.** PCR was carried out in a 25 µl mixture containing 10 mM tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl<sub>2</sub>, 0.01 % gelatin, 5 units recombinant Taq DNA polymerase, 200 µM of each dNTPs, 0.6 µM of each primer, and 5 µL of the DNA extracted from 100 µl of plasma. The sequence of forward primer was 5'-TAT GTT TCC CTC CTG CTG CT-3' and the sequence of reverse primer was 5'-CCC CCA ACT CCC AAT TCT AT-3'. These primers amplify a 354bp of the HBV genome. A fast temperature cycling was performed. PCR amplification was done using Touch down method including one cycle of 93°C for 60 sec, 60°C for 20 sec and 72°C for 40 sec, then 5 cycles of 93°C for 20 sec, 60°C to 56°C for 20 sec and 72°C for 40 sec followed by 30 cycles of 93°C for 20 sec, 55°C for 20 sec and 72°C for 40 sec. HBV genome provided by Sinagen company was used as positive control.

For the analysis of PCR amplification, 10 µl of the amplified DNA were run on a 2% agarose gel after adding 4 µl loading dye. The presence of a 354 bp fragment indicated positive result. Ladder was also run on the gels to estimate the molecular weights of DNA fragments in the gel.



**Figure 1.** The result of PCR amplification of HBV DNA in HBsAg<sup>-</sup> and Anti-HBc<sup>+</sup> blood units. The bands demonstrate presence of HBV infection. a: Lane 1: ladder. Lane 2: positive control. Lane 3: negative control. Lane 4 and 5: two positive samples. b: Lane 1: negative samples. Lane 2, 3 and 4: three positive samples. Lane 5: negative control. Lane 6: positive control.

## RESULTS

This study was performed on 545 samples collected from Isfahan blood transfusion

organization. The detection of HBsAg showed that all (100%) samples were negative for this antigen. Testing for Anti-HBc ELISA showed that forty-three (8%) of samples were positive for anti-HBc.

These 43 samples that were positive for anti-HBc and negative for HBsAg were selected for detection of HBV DNA by PCR method. As a result, five samples were positive for HBV DNA. These results showed that 5 out of 545 samples were infected with HBV virus and donors of these samples had occult HBV infection. Figure 1 demonstrates the results of gel electrophoresis of PCR amplification products of these samples. The size of amplified DNA fragment was about 354 bp.

## DISCUSSION

Viral hepatitis is a serious disease produced by hepatitis B virus (HBV), which is transmitted through transplantation and transfusion (13). Therefore diagnosis of this infection in blood and organ donors is of great importance (14). Screening of blood and organ donors for HBV surface antigen (HBsAg) has reduced the risk of transfusion-transmitted hepatitis B virus infection since 1970 (8). In spite of such measures, infection with hepatitis B virus is the major challenge in transfusion (8). Regardless of all efforts to guarantee blood safety, hepatitis B residual risk is still the highest among transfusion-transmitted diseases (15). Studies are performed in different countries to discover the mechanism of this transmission. In recent years, these studies are mostly on the role of anti-HBc as a marker of the infectivity of blood units (15). Anti-HBc was selected because it is the first antibody produced during HBV infection and usually persists after virus clearance (16). Detecting anti-HBc in serum shows that, the donor has been exposed to HBV in the past (17). If HBsAg is negative and anti-HBc is positive, several conditions may prevail: (a) Chronic carrier state in which HBsAg is not detectable (occult HBV infection); (b) Remote infection with loss of measurable HBsAg (occult HBV infection); (c) Passive transfer of anti-HBc (d) Non specific, cross-reacting antibody and (e) The period when HBsAg has disappeared (18,19).

545 samples of HBsAg-negative blood units from "Isfahan Blood Transfusion Organization" were tested for anti-HBc by ELISA method. All of these samples were tested for Anti-HIV, Anti-HCV, and Anti-HTLV 1 and 2 and were found to be negative for all of these infections. 43 samples were positive for anti-HBc that is 8% of HBsAg negative blood donors in Isfahan. In order to evaluate the infectivity of samples, all anti-HBc positive samples were tested for the presence of HBV DNA using a PCR method. Five out of 43 samples were positive for HBV DNA that is about 11.6% of the donors that were anti-HBc positive and 0.92% of all blood samples and could be considered as HBV carrier. These five samples had occult HBV infection. Therefore our results demonstrate that the occult HBV infection is relatively of high or intermediate prevalence in Iran. This might be either because of ethical or geographical characteristics of Iran or because of specific prevalent subtypes of HBV in our country (7). Therefore it might be worth considering routine testing for anti-HBc to be able to eliminate the risk of HBV transmission by HBsAg negative, HBV DNA positive blood samples in Iran. Our results demonstrate that routine anti-HBc screening of blood donations is needed to prevent probable transfusion-transmitted HBV infection. This test should be performed on blood samples in Iran because: 1) HBV infection has intermediate prevalence in Iran, 2) HBV DNA is

detected in some HBsAg negative, anti-HBc positive blood samples and 3) anti-HBc by itself is an important risk factor. It also should be noted that anti-HBc is produced against other antigens that cross-react with HBsAg. Therefore it is a risk factor for other infectious diseases such as HIV and HCV as well (18, 19). The results of our study suggest that Blood Transfusion Organization should consider HBV PCR test to detect HBV DNA in order to reduce post-hepatic transfusion transmitted infection.

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