

The detection of HBV DNA with polymerase chain reaction in blood donors with isolated hepatitis B core antibody

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ABSTRACT

Aim To analyze the presence of HBV DNA in blood donors admitted to blood banks with HBsAg negativity and isolated anti-HBc positivity.

Methods Sera samples of 2500 HBsAg negative donors were included in the study. HBsAg tests were assayed with VITROS analyzer. Anti-HBc total, anti-HBs, HBeAg, anti-HBe and anti-HBc IgM tests were manually studied with DIA PRO kit by employing ELISA method. HBV DNA test was evaluated with iQ5 Real Time PCR Detection System with real-time PCR method.

Results A total number of 401 (16.4%) HBsAg negative blood donors had anti-HBc positivity. Forty-five of 401 (1.8%) anti-HBc positive samples were anti-HBs negative. These 45 persons were evaluated for anti-HBc positivity. Thirty-six of 45 anti-HBc positive persons had only anti-HBc positivity, and other nine patients had Anti-HBe antibody. HBV DNA was detected in three (6.6 %) of 45 isolated Anti-HBc positive persons as in one of nine persons and two of 36 persons.

Conclusion The persons who have only HBs Ag negativity may not be appropriate to become blood donors. Anti-HBc test should be done for HBsAg negative persons. They should not be accepted as blood donors if Anti-HBc is found positive.

Key words: Anti-HBc alone, blood banks, DNA

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INTRODUCTION

HBV is a public health problem carrying significant mortality and morbidity worldwide (1). It is one of the causative agent in terms of acute and chronic hepatitis (1). Hepatitis B virus surface antigen (HBsAg) and core antibodies (Anti-HBc) are helpful for the diagnosis (1). HBsAg is positive in serum throughout the acute disease process. Antibodies against hepatitis B core antigen (anti-HBc) develop after HBsAg and remain positive for a long while (1). Recently, with the widespread use of screening tests, in instances where none of the serological markers of the HBV infection can be detected, anti-HBc IgG, whose positivity is quite rare, can be detected as the sole antibody (2). This positivity had been found to be in correlation with the passive transfer or non-specific false positivity of anti-HBc, the fact that the infection is in the incubation or the window period (2). The presence of chronic HBV infection which goes together with false negativity of other HBV markers as the expression of HBsAg or replication of HBV is suppressed, or during the late immune period when the levels of anti-HBs are below the detection threshold due to recovery from the infection (2).

Despite the fact that serological tests have higher sensitivities nowadays, hepatitis transmitted with transfusions still carry serious risks (3). HBsAg negative and anti-HBc positive blood and organ donors are reported to transmit the infection (4). That is why individuals not demonstrating any serological marker of a HBV infection or those with only anti-HBc positivity should be investigated (5). Several studies have shown that HBV transmission cannot be prevented by only screening for HBsAg (6). Hepatitis B virus deoxyribonucleic acid (HBV DNA) can be detected in individuals who are negative for HBsAg. Authors believe that isolated Anti-HBc total positivity is negative of HBsAg and Anti-HBs (7).

In this study, isolated Anti-HBc total positive patients who were negative as HBsAg, hepatitis C antibody (Anti-HCV) and human immunodeficiency virus antibody (Anti-HIV) were divided in two groups of patients only Anti-HBc total positive and positive together with Anti-HBe and Anti-HBc total. Later, all the patients were investigated for HBV-DNA.

PATIENTS AND METHOD

The study was performed on volunteer blood donors aged 18-65 years at Suleyman Demirel University (SDU) Hospital from February 2007 to May 2007. The study was approved by the Ethical Committee of SDU. The sera of 2500 blood donors, which were HBsAg negative, were collected and stored at -20°C until being analyzed.

Anti-HBc total was assayed in all serum samples and positive ones were further analyzed for the presence of anti-HBs. The samples, which were also negative for Anti-HCV, Anti-HIV, and HBsAg, were named as isolated Anti-HBc total positive. HBeAg, Anti-HBe, and Anti-HBc Ig-M were analyzed from isolated Anti-HBc total positive sera samples. All the sera samples were negative for Anti-HBc Ig-M. Isolated Anti HBc positive sera samples were evaluated for the presence of HBV-DNA by PCR.

Anti-HCV, anti-HIV and HBsAg tests were evaluated with VITROS (Johnson&Johnson Company, Brasil) analyzer while anti-HBc total, anti-HBs, anti-HBc IgM, HBeAg, anti-HBe and tests were studied manually with DIA PRO kit (Diagnostic Bioprobes, Milano-Italy) by using ELISA method.

For real-time PCR used to detect HBV DNA, DNA extraction was performed with HBV Real-TM Quant kit (Caserta, Italy) from Sacace Biotechnologies. Primers obtained from X-gene region of the HBV DNA and probes were used to prepare the reaction mix. HBV DNA was kept on the iQ5 Real Time PCR Detection System (BIO-RAD) for 15 minutes at 95°C and was amplified with 42 cycles consisting of 20 sec at 95°C followed by 1 min at 60°C . With the program on the equipment, amplification and detection were performed automatically. As a result of the amplification and detection, if the result was 0 copy/ml, it was evaluated as negative. The results below 500 copies/ml were weak positive, those between 500 copies/ml to 100.000.000 copies/ml were positive and those above 100.000.000 copies/ml were strong positive.

RESULTS

The mean age of 2500 blood donors who were negative for HBs Ag was 37.9 ± 21.35 years and 117 were females (4.68%).

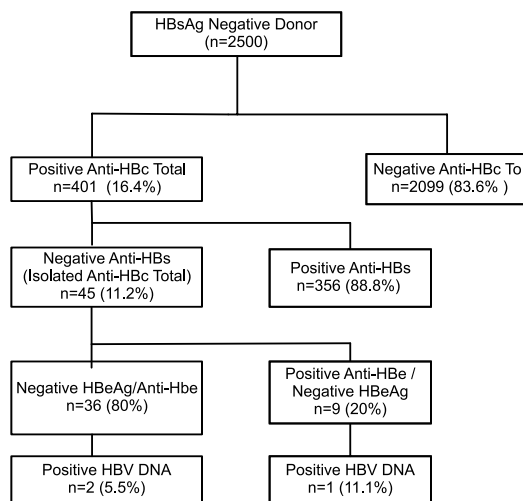


Figure 1. Results of the study and followed algorithm

Anti-HBc, Hepatitis B virus core antibody; Anti-Hbe, Hepatitis B virus infectivity antibody; HBe Ag, Hepatitis B virus infectivity antigen; HBs Ag, Hepatitis B virus surface antigen; HBV DNA, Hepatitis B virus deoxyribonucleic acid;

Anti-HBc total was found positive in 401 (16.4%) of 2500 donors. Forty five of the 401 serum samples, which were Anti-HBc positive, were negative for Anti-HBs and Anti-HBc Ig-M. Nine of these 45 samples were positive for Anti-HBe, but all samples were negative for HbeAg (Figure 1).

HBV DNA was found positive in three (6.6%) of the 45 isolated anti-HBc positive donors, including two (5.5%) of the 36 only anti-HBc positive donors and only one (11.1%) of the nine anti-HBe positive donors. HBV-DNA results were evaluated as weak positive in only one patient and positive in two patients (Figure 1).

DISCUSSION

Despite widespread use of serological tests in the diagnosis of HBV infections, they show different variations especially in HBsAg (8). When infections with mutant strains are also taken into consideration, the existing serological tests may be insufficient at diagnosis and follow-up of HBV infections (9).

Of the serological indicators of the hepatitis B virus, although anti-HBc is the most sensitive marker demonstrating the contact with the virus, the enzyme immunoassay (EIA) kits that are commercially available with a widespread use have high false positivity rates like 60–70% (1).

When anti-HBc positivity is identified, especially in countries where the prevalence of HBV is high, it is necessary to discriminate whether this relates

to contact with the cross reacting antibodies or to contact with the virus (1).

Interesting dimension of uncommon HBV serology are the cases that are called isolated anti-HBc positive (11); they have negative HBsAg and anti-HBs. Especially in HBsAg negative blood donors, if HBV DNA is detected, there arises the need to demonstrate isolated anti-HBc positivity (1). Thus, isolated anti-HBc positivity is among the donor screening criteria in several countries and those who are shown to be positive are not accepted as donors (1). HBV DNA levels of Isolated anti-HBc positive 45 cases in our study were tested, and in 6.6% cases HBV DNA had been detected. In another study of liver transplantation candidates, when needle biopsies were performed on the livers of HBsAg, anti-HBs negative and anti-HBc total positive patients, 8.2% had HBV DNA positivity and anti-HBc positivity was reported as a reason not to accept these patients (10).

In a certain proportion of HBV infections where anti-HBs disappears but only anti-HBc persists, HBV DNA also exists (11). Individuals with such a serological profile can transmit HBV infection both through transfusions and via maternal-fetal route (12). Altunay et al (7) have shown HBV-DNA positivity in 6 of 658 only Anti-HBc positive donors. For this reason, they suggest that it is not necessary to detect isolated Anti-HBc as a routine in Blood Banking. In another study, isolated anti-HBc positivity (HBsAg negative, anti-HBs negative and anti-HBc positive) was investigated in hemodialysis patients and was found in 18 of 289 hemodialysis patients. Nine of these 18 patients were positive for HBV-DNA though isolated anti-HBc positivity may show occult HBV infection in hemodialysis patients (13). The study of Ozkan et al reported that HBV-DNA PCR was found positive in six of 39 isolated Anti-HBc positivity (HBsAg and Anti-HBs negative) patients (14). A study reported that only one of 35 isolated Anti-HBc positive patients had positivity for HBV-DNA (15).

In a different study, in 30.5% of samples with all negative serological markers except for anti-HBcIgG positivity, HBV-DNA were detected (16). In our study, of 36 patients in whom all serological markers were negative except for anti-HBcIgG positivity (isolated anti-HBc positivity),

2 (5.5%) had HBV DNA detected. Another study reported acute and chronic hepatitis cases where HBV DNA was detected, while all hepatitis B serology was negative. These patients might have mutations of the region encoding the X gene of the virus, and they can have pre-C defective mutations and core gene mutations (17). Weber et al. (18) identified HBV DNA positivity in 14.4% of individuals with isolated anti-HBc positivity and in cases having false anti-HBc positivity there can be mutations in HBsAg.

In conclusion, serological tests may be insufficient in diagnosis of hepatitis B infection. In our

opinion, alone HBsAg negative persons may not be suitable to be blood donors. Also, Anti-HBc test should be done for all blood donors who are negative for HBsAg. HBV-DNA is assayed for all the isolated Anti-HBc positive persons. Isolated Anti-HBcAg positivity persons as HBV DNA negative may be blood donors.

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TRANSPARENCY DECLARATIONS

Competing interests: none to declare.

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