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Are isolated anti-HBc blood donors in high risk group? The detection of HBV DNA in isolated anti-HBc cases with nucleic acid amplification test (NAT) based on transcription-mediated amplification (TMA) and HBV discrimination

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ABSTRACT

Aim: Hepatitis B virus (HBV) can be transmitted by blood transfusions even so using serological tests having high sensitivity and specificity. We aimed to detect HBV DNA in isolated Anti-HBc donors and show if they have transfusion risk or not.
Method: We investigated Anti-Hbc and Anti-HBs in serum samples of 12858 HBs-Ag negative blood donors who were applied to the Turkish Redcrescent between June 2007 and October 2008 by the Micro ELISA kit (Hepanostica ultra HBsAg, Bio Meriux, France). Anti-HBc and Anti-Hbs positive cases were omitted. We used Procleix ultrio (Chiro, USA) test kit (Chiron Tigris automated instrument was used) based TMA (Transcription-Mediated Amplification) for NAT study in Anti-HBc positive and Anti-HBs negative plasma samples. The discrimination of HBV in NAT positive samples were performed by Procleix Discrimination (Chiro, USA) test.
Results: 2748 (21.4%) Anti-HBc seropositivity were detected in 12852 HBsAg(−) serum samples. 23.5% Anti-HBs negativity was detected in 2748 Anti-HBc positive serum samples. On the other hand, 5.1% isolated Anti-Hbc positivity [HBsAg(−), Anti-HBc(+), Anti-HBs(−)] were detected in 12852 HBsAg(−) serum samples. 0.091% and 0.047% HBV-DNA positivity were detected in isolated Anti-HBc positive plasma samples and HBsAg(−) plasma samples, respectively.
Conclusion: As a result, even we have detected one HBV transmission in every 2142 blood transfusion by HBsAg screening tests; we suggest that it is not necessary to add additional tests to detect isolated Anti-HBc for routine purposes in Blood Banking. The reasons are higher negativity rates (99%) of isolated Anti-HBc serum samples and the rejection of blood donors with Anti-HBc positivity.

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1. Introduction

The infection risks caused by viruses such as Hepatitis B Virus (HBV) during blood transfusion and using blood products are still high. Although there are highly sensitive

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analytical techniques utilizing Hepatitis B surface antigen (HBsAg), are available for detection, HBV transmissions cannot be prevented easily in post-transfusion. There is a significant increase in studies and research to determine factors associated with the lowest decreasing rates of HBV transmission via blood and blood products. There are also efforts to solve the problems related to these factors. Most studies focus only on infectiosity of Anti-HBc positive; however, anti- HBsAg and anti- HBs negative blood patterns are most likely caused by different reasons and there is a discussion to use the blood supplies in safe transfusions [1–3].

In this study, we investigated whether only anti-HBc donors pose a risk for HBV infection. For that purpose, we used NAT and discrimination tests to detect HBV DNA viremia levels of isolated anti-HBc positive donors from 12858 HBs-Ag negative donors. All blood donations were given by volunteers at the Turkish Red Crescent Society – Capa Blood Center, Istanbul, Turkey.

2. Material and methods

2.1. Study group

The study was performed between July 2007 and October 2008, with 12858 out of total 13084 volunteers who applied for blood donations at the Turkish Red Crescent Society – Capa Blood Center. Following donation, blood was screened for HBsAg. Of the 12,858 HBs-Ag negative donors, 643 (5%) were female and 12,215 (95%) were male ages between 18 and 60 (the mean: 40) in this sectional study.

2.2. Collection of samples and methods

2.2.1. Collection of samples

Volunteer donors were accepted for donation after physical examination of vital. Ten milliliters of blood without anticoagulant was collected from each donor to screen Anti-HIV/1–2, Anti-HCV, HBsAg and TPHA serologic tests. Ten milliliters of blood without anticoagulant was collected from each donor. The same serum samples were also used for both Anti-HBc and Anti-HBs tests. Blood samples from the same donors were collected into 9 ml sterile tubes containing tetra-acetic acid (EDTA) for NAT studies and plasma samples were obtained by centrifugation at 2500 rpm.

2.2.2. Serologic methods

Each serum was tested for Anti-HIV/1–2, Anti-HCV and HBsAg using Vironostica, HIV Uniform II Ag/Ab (Biomerux-France), Innotest HCV Ab III (Innogenetics-Belgium), Hepanostica Ultra HBsAg (Biomerux-France) kits, respectively using Da-Vinci automatic micro ELISA instrument (Biomerux-France). For the TPHA test, THPA kit (Randox-ABD) was used.

In addition, the same serum samples from 12858 Hbs-Ag negative cases were tested for Anti-HBc and Anti-HBs using Hepanostica HBc Uniform Total test kit (Biomerux-France) and Anti-HBs kit (Bio-Kit-Spain), respectively.

The serum samples considered non-reactive for HBsAg in the first study and the serum samples reactive in the first study but found non-reactive in a two-wells system, were both considered negative for HBsAg.

The serum samples tested positive for both Anti-HBc and Anti-HBs in the first study were considered reactive after being confirmed in two-wells study. For the quantitative evaluation of Anti-HBs $\geqslant$10 IU/ml value was considered as the cut-off value according to World Health Organization (WHO) [4]. For identification of Anti-HBc only, Anti-HBc positivity and HBsAg, Anti-HBs negativity were considered [5].

2.2.3. NAT studies

In the NAT studies, the Procleix Nitro Kit (Chiron-ABD) was used for the TMA (Transcription-Mediated Amplification) method which is based on RNA amplification of both RNA and DNA targets with RNA polymerase and reverse transcriptase enzymes (100–1000 copies/mL amplified in each cycle, Chiron Tigris automated system [Chiron-ABD]). A discrimination test kit (Chiron-ABD) was used for detecting HBV-DNA.

The same plasma samples were studied in a two-well system after found reactive using the Procleix Ultrio kit. The plasma sample was considered NAT positive when found reactive at least using one of the two tests. This reactive sample was also separately studied with Procleix Discrimination HBV, HCV, HIV/1 kits, in order to discriminate which virus was responsible for the NAT positivity. Plasma samples were considered HBV-DNA positive using HBV discrimination assay. When the plasma samples were detected reactive in the first NAT assay and found negative in two-wells study, the first reactive results were considered false-positives.

3. Results

We detected 2748 (21.4%) Anti-HBc positive and 10110(78.6%) Anti-HBc negative cases in 12858 HBs-Ag negative serum samples. Of the 2748 Anti-HBc positive serum samples, 658 (24%) were Anti-HBs negative and 2090 (76%) were Anti-HBc positive. Based on these results, 5.1% (658/12,858) of the total 12858 HBs-Ag negative serum samples were Anti-HBC positive, HBS-Ag negative and Anti-HBs negative .

Of these 12858 HBs-Ag negative plasma samples, 9 (0007%) were detected positive while 12849 (99.93%) were detected negative according to the NAT study. Of these 9 NAT positive plasma samples, 6 (0.91%) were HBV-DNA positive.

HBV-DNA positivity ratios were 6/12858 (0.046%) and 6/658(0.91%) for 12858 HBs-Ag negative and 658 only anti-HBc positive plasma samples, respectively (Table 1).

4. Discussion

Chronic HBV infection can cause serious clinical diseases. Hepatitis antigens and antibodies (Anti-HBsAg, Anti-HBc and Anti-HBe) are commonly used as markers for laboratory diagnosis of HBV infection. The detection

Table 1
Serologic and NAT test results of the 12,852 HBs-Ag negative serum samples.

Serologic tests	*n*	%
HBs-Ag negative	12,858	
Total Anti-HBc positive	2748	21.4
Both Anti-HBs positive	2090	76
Only Anti-HBc positive	658	24
Total Anti-HBc negative	10,110	78.6
Only Anti-HBs positive	1274	9.9
NAT		
-Positive	9	0.070
HBV-DNA	6	0.047
-Negative	12,849	99.93
Number of only Anti-HBc positive:	658	
HBV-DNA positive	6	0.91

of Anti-HBc is sometimes useful as a blood screening method as well as for diagnosing chronic hepatitis B, inactive HBsAg carriers and resolved hepatitis. A meeting was held in 2000 (it was the first-ever workshop sponsored by the National Institute of Diabetes and Digestive and Kidney Diseases of the National Institutes of Health (NIH) in Bethesda, Maryland USA, September 8–10, 2000) which standardized the terminology and diagnostic criteria related to HBV [5]. When Anti-HBc is the only positive marker found positive.

Isolated Anti-HBc is profiled according to following criteria [6,7]:

(a) HBV infection was resolved, HBs-Ag negative but no detectable Anti-HBs
(b) Very low that can not be detectable HBsAg (10–100 genome/ml)
(c) The presence of mutant HBV
(d) False-positivity,

There are many studies on Anti-HBc only positivity for the diagnosis of HBV infections and particularly to determine the relationship Anti-HBc only and post-transfusion HBV infections [1,2,6–9]. These include a study of 26,492 blood donors with 6.1% isolated Anti-HBc by Douglas et al. [10], 103,883 donors with 0.07% isolated Anti-HBc by Allain et al. [2], 4885,732 donors with 0.15% isolated Anti-HBc by Kleinman et al. [12], 30,853 donors with 4.1% isolated Anti-HBc by Chaudhuri et al. [13], 6019 donors with 3.23% isolated Anti-HBc by Ozacar et al. [14], and 9282 donors with 2.7% isolated Anti-HBc by Bal et al. [15]. We detected 5.1% isolated Anti-HBc from 12,858 donors.

Most of these studies, evaluating the role of isolated anti-HBc individuals for HBV infectiosity and whether it is possible to use them as an eligible blood donors for blood banking were based on HBV-DNA molecular methods especially such as PCR, TAS, 2CR, NASBA, TMA, b DNA for screening HBV-DNA.

We detected 6(0.047%) HBV-DNA positive subjects among 12858 Hbs-Ag negative subjects. In other words 0.91% out of the total 658 anti-HBc only cases were detected HBV-DNA positive by NAT(RNA targeted) which is based on transcription-mediated amplification(TMA) method via using HBV-DNA discrimination kits.

Douglas et al. [10] reported no HBV-DNA positivity among 26492 donors similar to our results. Liang et al. [11] reported 30% HBV-DNA positivity of the 3% isolated Anti-HBc group out of the total 1407 donors.

Kleinman et al.[12] reported that 0.032% HBV-DNA positivity was found out of 1231 anti-HBc only cases whereas Chaudhuri et al. [13] observed 6.9% (88) of the total 1268 anti-HBc only cases. In Turkey, Bal et al. reported 0.45% [15] HBV-DNA positivity from 225 anti-HBc only cases out of 9282 donors.

In this and other studies, there are various rates of HVB DNA-positive donors among Anti-HBc positive, HBs-Ag negative Anti-HBs negative donors. So the presence of HBV-DNA suggests the donors with this profile could pose a risk factor for HBV infection and may cause some risk factors with various degrees for safe blood transfusion. In a study by Satake et al. [16], only 1 of 33 patients with HBV positive DNA and low Anti-HBc titers proved to be infectious by lookback studies. Likewise, whether in our study or Kleinman et al. [12] or Chaudri et al. [13], or Bal et al. [15] there is a transmission risk of 1 out of the total transfusions 2143, 49000, 350 and 8333, respectively.

In conclusion, according to our data, taking into account the 5% reduction of blood donors due to Anti-HBc positivity in the blood banks, the presence of higher HBV-DNA negativity (99.09%) in isolated anti-HBc cases. Therefore we recommend that there is no need to add Anti-HBc test to routine serological screenings even it was shown that there may be a risk about 1 per out of 2142 transfusion, in the biggest national blood center of Turkey.

Conflict of interest

On behalf of all authors as the correspondence author Bekir Kocazeybek, I confirm that there is any financial and personal relationships with other people or organizations that could inappropriately influence (bias) our work. Examples of potential conflicts of interest include employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registrations, and grants or other funding.

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This work has been approved by the Cerrahpasa Medical Faculty ethical committees.

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