Clin. Lab. 2019;65:XXX-XXX

ORIGINAL ARTICLE

Safer Blood Supply for Transfusion: Which Algorithm Should Be Used to Determine Occult Hepatitis B Infection in Blood Donors?

Rukiye Berkem and Ayşe E. Karakoç

University of Health Sciences, Ankara Training and Research Hospital, Department of Medical Microbiology, Ankara, Turkey

SUMMARY

Background: **Transfusion-transmitted hepatitis B virus continues to be a problem despite its significantly reduced prevalence. In this study, in addition to screening for the presence of HBsAg in donors' blood, anti-HBc and anti-HBs markers were investigated using the chemiluminescence immunoassay (CLIA) method, and real-time PCR was used to detect HBV DNA.**

Methods: **The study's material involved serum samples of 4,073 blood donors. HBsAg, anti-HBs, anti-HBc tests were undertaken using the CLIA method, and HBV DNA's presence was investigated using the real-time PCR method.**

Results: **HBsAg and anti-HBc tests were negative in 3,331 (81.78%) and positive in 37 (0.90%). For the remaining 705 (17.30%), HBsAg was negative and anti-HBc was positive. According to the results of the anti-HBs test for these samples, HBsAg negativity and anti-HBc and anti-HBs positivity were found in 619 samples (15.19%), while 86 samples (2.11%) were negative for HBsAg and anti-HBs but positive for anti-HBc (isolated anti-HBc positivity). ID-HBV DNA real-time PCR tests were performed on 86 samples. None of the samples was positive for HBV DNA.**

Conclusions: **Recommended tests for screening occult HBV infection include anti-HBc, anti-HBs, and/or HBV DNA. Anti-HBc screening may result in loss of donors and blood products, particularly in countries with moderate endemicity of HBV, such as Turkey.**

(Clin. Lab. 2019;65:xx-xx. DOI: 10.7754/Clin.Lab.2018.180920)

Correspondence:
Rukiye Berkem, MD
University of Health Sciences
Ankara Training and Research Hospital
Department of Medical Microbiology
Ankara, 06110
Turkey
Phone: +90 3125953571
Mobile: +90 5337113708
Email: rukiyeberkem@yahoo.com

Manuscript accepted October 22, 2018

KEY WORDS

Hepatitis B virus, safe transfusion, blood donor, occult HBV infection, anti-HBc, NAT

INTRODUCTION

Hepatitis B virus (HBV) infection is a serious global public health problem that affects more than two billion people worldwide despite the availability of vaccination and antiviral treatment [1]. Over the last few years, the risk of transfusion-transmitted HBV (TT-HBV) has been steadily reduced by voluntary blood donors, donor selection based on risk-behavior assessment, and the development of increasingly more sensitive and specific HBsAg screening tests [2,1]. Although anti-HBc screening and recent introduction of HBV nucleic acid

testing (NAT) have increased blood and patient safety measures in some endemic countries, the desired zero risk target has not yet been achieved [3]. The risk of TT-HBV, absence of detectable HBsAg, and extremely low HBV DNA levels are associated with the pre-seroconversion window period and occult HBV infection (OBI) [4]. TT-HBV has a residual risk that varies according to HBV epidemiology, donor populations, and screening strategies. The residual risk estimate calculated for HBV ranges from 1 to 1.4 per million in low-endemic countries and from 16 to 100 in high-endemic countries, depending on the mathematical models used [1,5]. The risk of TT-HBV is primarily associated with blood donation testing negative for HBsAg and/or HBV DNA and the blood being collected in the early or late phase of infection [6]. The success or failure in preventing potentially infectious donations relies on the screening strategy and the performance of the serological and molecular methods used. Despite all the measures taken today, TT-HBV is still reported, albeit rarely. All these measures and increases in cost have led to serious debates concerning how to reduce the cost of HBV screening. In particular, accumulating data suggests that screening for HBV NAT and anti-HBc cannot be performed without compromising the supply of donated blood in high-endemic regions [7].

HBsAg is the first serological marker that emerges during an HBV infection and is used to screen HBV in blood donors. HBsAg screening generally requires optimal analytical sensitivity to limit the 'window period', defined as the time between infection and detection of the viral antigen, and to increase the ability to detect the smallest amount of HBsAg in the asymptomatic late phase of chronic infection. The sensitivity and specificity of the HBsAg test are under constant improvement. Recently, chronic HBV infection accompanied by antigen levels below the detection threshold of HBsAg tests has been increasingly reported in donors and called OBI [1,2,6].

Anti-HBc antibodies usually appear 6 to 12 weeks after the onset of infection. They are the most sensitive and life-time serological markers of exposure to HBV. In cases where anti-HBs are reduced to undetectable levels or in OBI where HBsAg detection is not possible, anti-HBc antibodies may be a single marker. Even in low-endemic countries, anti-HBc screening is still limited. The specificity of the anti-HBc test is not optimal, with false reactivity rates being reported as 16 - 75% according to tests and screening algorithms. Due to the absence of recombinant/peptide antigen-based validation tests, secondary testing with an alternative EIA is required to distinguish true and false positives and validate limit-reactive results that may be associated with low avidity or low-titer antibodies. Additional tests, such as anti-HBs, anti-HBe and/or HBeAg are considered to provide validating values for anti-HBc. Although these complex validation algorithms introduce economic and organizational constraints on blood services, they are useful in terms of preventing the permanent rejection of a donor due to false positive results. Another limitation is that anti-HBc screening is unable to identify window period infections [7,1].

The ID-HBV DNA test has a 95 - 99.9% detection limit (2 - 4 IU/mL). This high sensitivity results in significant shortening (by approximately 15 days) of the window period of the HBV infection, which cannot be detected with the HBsAg test. HBV NAT revealed many cases of HBsAg-negative OBI among anti-HBc- and/or anti-HBs-positive blood donors. In the majority of donors with OBI, the viral load is < 50 IU/mL [8].

The HBV screening strategy should be based on local epidemiological data, HBV transmission risk estimates, and the financial resources of a country [9].

The aim of this study is to generate data for HBV screening strategies that can be implemented in our country.

MATERIALS AND METHODS

The material of the study consisted of serum samples collected from blood donors at the Ankara Training and Research Hospital, Nilgün Sönmez Acar Blood Center for microbiological screening tests. The study had a prospective design. In the first stage of the study, HBsAg and anti-HBc tests were simultaneously performed with CLIA; then, in the second stage, the anti-HBs test was performed on the HBsAg-negative and anti-HBc-positive samples. Commercial kits (Ortho-Clinical Diagnostics, Vitros) were used to test HBsAg (sensitivity < 0.10 IU/mL, specificity 99.98%), anti-HBc (sensitivity < 1 U/mL, specificity 99.6%), and anti-HBs (sensitivity 10 mIU/mL, specificity 100%) according to the manufacturer's recommendations.

In samples that were negative for HBsAg and anti-HBs but positive for anti-HBc, the tests were repeated twice using the same kit to investigate whether anti-HBc positivity was recurring reactive. HBsAg negativity was analyzed again using the following two test kits capable of detecting HBV escape mutants; HBsAg ES (sensitivity < 0.10 IU/mL, specificity 99.88%, Ortho-Clinical Diagnostics, Vitros) and HBsAg (sensitivity ≤ 0.050 IU/mL, specificity 99.96%, Radim, Italy) according to the manufacturer's recommendations. In these samples, commercial test kits (Ortho-Clinical Diagnostics, Vitros) were used to perform tests related to the other markers of HBV infection; anti-HBcIgM (sensitivity 98.2%, specificity 100%), HBeAg (sensitivity < 0.35 U/mL, specificity 99.88%), and anti-HBe (sensitivity < 0.25 U/mL, specificity 100%). The presence of HBV DNA was investigated for each individual donor (individual donation, ID) using the real-time PCR method and kit (Abbott Real Time HBV, ABD) (sensitivity 10 IU/mL, specificity 100%) according to the manufacturer's recommendations. Co-administration of HBsAg, anti-HBc, and HBV NAT in endemic countries with low HBV prevalence allows the detection of both the early phase of acute infection and occult infection, thereby in-

creasing blood and patient safety. It is recommended that ID-NAT should be preferred, rather than MP-NAT since the former was found to be more effective in detecting both the window-period and occult HBV infections and further reducing the residual risk compared to the MP-NAT. However, there is still an ongoing debate around the world on how to reduce the costs of HBV blood screening without compromising blood safety. It is stated that in moderate- and high-endemic countries, the anti-HBc test cannot be applied without reducing blood availability and therefore, if there are enough resources, the HBsAg test combined with NAT can be undertaken. The cost limitation of NAT affects decisions about the screening strategy. Such decisions may also vary from one country to another depending on the sensitivity of the serologic tests and NAT analyses used, as well as HBV epidemiology. Furthermore, there is a known residual risk when HBsAg and anti-HBc serology are used in combination with ID-NAT. For all these reasons, it is recommended that each country decide on the HBV screening strategy based on local epidemiological data, transmission-risk estimates, cost-effectiveness-based cost analyses, and financial resources.

As a result of our study, anti-HBc screening may result in donor and product loss for countries, such as Turkey, that are moderately endemic. Undertaking an additional anti-HBs test, which is recommended for anti-HBc-positive samples, would further increase the cost of microbiological screening tests. In addition, the implementation of these tests may also affect decisions concerning the acceptance or rejection of donors on a local level. Other indicators of HBV infection, namely anti-HBcIgM, HBeAg, and anti-HBe, should only be used to identify infectious conditions in blood donors whose HBsAg positivity has already been confirmed.

Routine applicability of ID-HBV DNA screening in Turkey seems to be very difficult due to the challenges of inapplicability, high cost, requirement of experienced personnel, and lack of infrastructure. To prevent transfusion-transmitted HBV and identify blood donors with OBI, it is necessary to establish screening strategies based on more epidemiological data, transmission-risk estimates, cost efficiency analyses, and financial resources.

RESULTS

In this study, HBsAg and anti-HBc tests were performed on 4,073 samples, of which 3,331 (81.78%) were found to be negative for HBsAg and anti-HBc, 37 (0.90%) were positive for HBsAg and anti-HBc, and the remaining 705 (17.30%) were negative for HBsAg but positive for anti-HBc. When the anti-HBs test was undertaken for 705 samples, 619 (15.19%) were HBsAg-negative and anti-HBc and anti-HBc-positive, whereas 86 samples (2.11%) had HBsAg and anti-HBs negativity and anti-HBc positivity (isolated anti-HBc positivity) (Table 1).

For the 619 of the 705 anti-HBc-positive samples (87.80%), the amount of anti-HBs was found to be ≥ 10 mIU/mL. Among these samples, the amount of anti-HBs was ≥ 100 mIU/mL in 437 samples (10.73%) and < 100 mIU/mL in 182 samples (4.46%) (Figure 1). HBsAg presence, anti-HBc recurrent reactivity, anti-HBcIgM, HBeAg and anti-HBe secondary tests and ID-HBV DNA were examined again in 86 samples with isolated anti-HBc positivity using two different test kits. None of the samples was found positive for HBsAg, anti-HBcIgM, HBeAg or HBV DNA, but Anti-HBe positivity was detected in eight samples in which the amount of anti-HBs ranged from 0.1 to 9.3 mIU/mL (Figure 2).

DISCUSSION

Hepatitis B continues to be a transfusion-transmitted viral infection with a residual risk varying according to HBV epidemiology, donor populations, and screening strategies [1,2,8]. HBV genotypes, sub-genotypes, and HBV chronic carrier state and prevalence have different geographical distributions changing from one area to another. The seroprevalence of HBsAg is more than 8% in endemic areas, such as sub-Saharan Africa, South East Asia, China, and the Amazon region, lower (2 to 4.99%) in the Mediterranean region, Eastern Europe, Middle East, and the North West countries of South America, and less than 2% in Western and Northern Europe, North America, South America, India, and parts of Australia [1]. Turkey has moderate endemicity in terms of the prevalence of HBV infections, and there are significant differences between different regions in the country, with the seroprevalence ranging from 2 to 7% as reported in previous studies [10,11]. In a meta-analysis of studies conducted in Turkey between 1999 and 2009, the prevalence of HBsAg was determined as 4.57% [11]. Among anti-HBc carriers, the frequency of those with anti-HBs varies according to HBV epidemiology and vaccination coverage. Studies in Europe, Japan, and North America reported that approximately 90% of anti-HBc-reactive blood donors were positive for anti-HBs, and of these donors, 63 - 70% had an antibody level greater than 100 IU/mL [1,2]. In contrast, in many countries with higher HBV endemicity, anti-HBs were only detected in 24.5% of anti-HBc-reactive donors [12,13]. In studies conducted with blood donors in Turkey, anti-HBc positivity varies from region to region, ranging from 26.3 to 44.7% [14]. However, in recent years, studies have shown a decrease in these rates. For example, 10% anti-HBc positivity and 1.3% isolated anti-HBc positivity were reported in a study by Yilmaz et al., 21.1% anti-HBc positivity was found by Köten et al., and 18% anti-HBc positivity determined in research by Bal et al. [9,14,15]. In the current study, anti-HBc positivity was found to be 17.30% and isolated anti-HBc positivity was 2.11%. Of the anti-HBc positive samples, 12.20% were negative and 87.80% were

Table 1. Results of serologic screening tests.

	Number of donors	%
HBsAg (-) anti-HBc (-)	3,331	81.78
HBsAg (+) anti-HBc (+)	37	0.90
HBsAg (-) anti-HBs (+) anti-HBc (+)	619	15.19
HBsAg (-) anti-HBs (-) anti-HBc (+) isolated anti-HBc positivity	86	2.11
Total number of donors	4,073	100

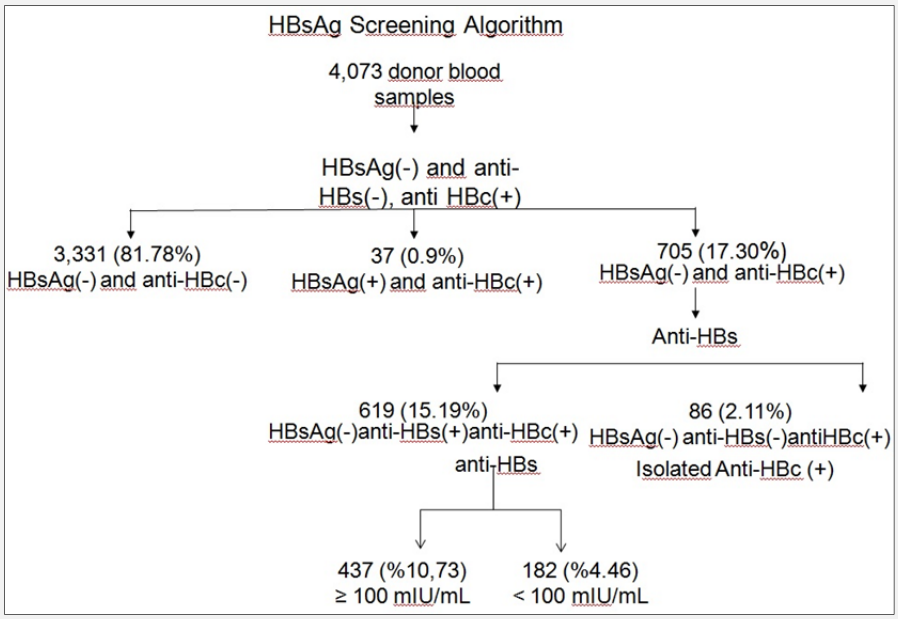

Figure 1. The algorithm used in HBsAg screening and the results obtained.

positive for anti-HBs. Among the positive samples, the level of anti-HBs was detected as ≥ 100 mIU/mL in 70.60% and < 100 mIU/mL 29.40%. Bal et al. reported anti-HBs positivity in 85% of the 1,679 anti-HBc-positive samples [14]. Several investigations have indicated that utmost care should be taken when comparing the seroprevalence between studies due to the differences in screening algorithms and methodology [1,2]. In moderate- and high-endemic regions, where the prevalence of anti-HBc in blood donors varies between 8% and 50%, anti-HBc screening is usually not cost-efficient and causes serious donor loss, resulting in problems in supplying blood for transfusions, whereas in moderate- or low-endemic countries, this screening test is considered to be sustainable [16,17]. In order to limit the potential donor loss associated with approximately 5% of anti-HBc prevalence, Japan has implemented a complex screening algorithm to determine the amount of anti-HBs in anti-HBc-positive donors and prevent donor loss [18,19]. Thus, Japan uses donations with an anti-HBs level of ≥ 200 mIU/mL while some countries use anti-HBc-positive donations with ≥ 100 mIU/mL anti-HBs. The anti-HBc test has the potential to further increase blood safety, but may also jeopardize the availability of blood in countries with moderate to high HBV prevalence [20,21].

HBV NAT application is limited in low- or middle-income countries due to the high cost of efficient, fully automated commercial systems and reagents [22,23]. In high-income countries with generally low prevalence of HBV, the benefit of NAT in reducing clinical risk is affordable and cost efficient [23,19]. In many countries,

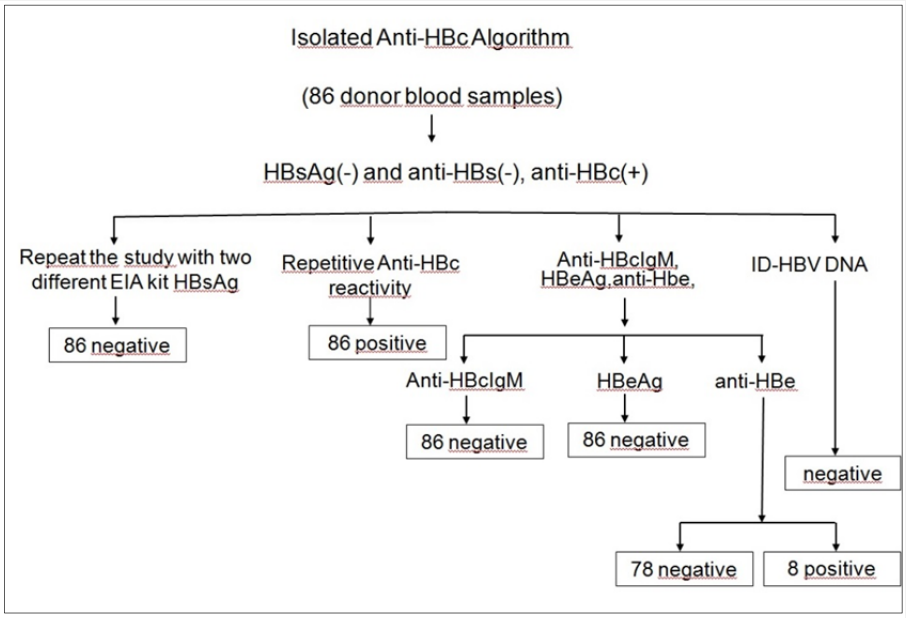

Figure 2. The algorithm used for the confirmation of isolated anti-HBc and the results obtained.

attempts have been made to test viral genomes in mini-pool (MP) plasma pools of various sizes (usually 6 - 8) to reduce the cost of NAT. The dilution factor resulting from pooling reduces HBV NAT sensitivity and its ability to detect low levels of HBV DNA observed in the majority of OBI donors [24,25]. The positive HBV DNA yield obtained from MP-NAT is lower than expected, and therefore many researchers have emphasized that anti-HBc should be incorporated into screening strategies to detect low-level HBV DNA-positive donors by employing HBV MP-NAT [23,19]. However, even ID-NAT has been reported as not being sufficiently sensitive to detect potentially infectious blood products with extremely low levels of HBV DNA [20,19]. Recently, increasing evidence for HBV transmission by ID-NAT-negative, anti-HBc-reactive donors of HBsAg, and HBV has been demonstrated with the most sensitive methods available [10,16]. In a study conducted in Japan using ID-NAT and anti-HBc for screening blood donations for HBV, TT-HBV infection was reported in 4 - 13 cases/year. The researchers found that ID-NAT prevented 75 - 85% of TT-HBV cases caused by the window period and OBI donations, 1.94% of donations with low anti-HBc and anti-HBs titers were viremic, and anti-HBc titers and the frequency of viremia were not correlated. As a result of that study, the Japanese Red Cross chose maximum blood safety by not using any blood units with low anti-HBc and anti-HBs titers, which constituted 1.3% of total donations [18].

In a study undertaken in China [4], it was stated that the anti-HBc test was applied in a few countries with low prevalence of HBV. HBsAg was not detected, but it could be used as a marker for HBV carriers in cases where post-transfusion could reduce the risk of HBV infection. However, the authors also suggested that it would not be practical to reject anti-HBc-positive donors in areas with an anti-HBc prevalence of > 2 - 5%. It was also emphasized that the blood donation gap resulting from the rejection of anti-HBc positive donors was too large to provide an adequate blood supply, and therefore many countries with moderate to high HBV endemicity, such as Italy, Greece, Spain and various countries in Asia, choose not to test donors for anti-HBc. It was also reported that when anti-HBc screening is not undertaken, the risk of TT-HBV is caused by HBV DNA or undetectable HBsAg in blood, as well as OBI carriers with or without anti-HBc and anti-HBs antibodies. Therefore, HBV DNA screening has become the main complementary option to the HBsAg test in these areas, including China. In the same study, it was noted that Shenzhen blood donors in South China had higher exposure to HBV, 47.5% of blood donors were HBsAg-negative/anti-HBc-positive, and 2.86% of anti-HBc-positive donors had OBI, suggesting that a higher NAT sensitivity should be adopted and anti-HBc could be an alternative screening parameter. The authors explained that including the anti-HBc test in screening strategies would lead to the rejection of many donor

units, but would, at the same time, eliminate HBV-infected donations, and both strategies, with their potential consequences, would reduce OBI-associated TT-HBV infections, especially in the immune-deprived patient populations in resource-poor environments. It was concluded that models based on clinical and experimental evidence demonstrated a further 3 - 14% risk associated with OBI donations by testing non-reactive HBsAg and ID-NAT; thus, in addition to having the potential to increase blood safety, the anti-HBc test might also jeopardize the availability of blood in countries with a moderate/high HBV prevalence, but it was still possible to improve the screening of donors in Chinese blood banks through the collection of more data.

In studies investigating the presence of HBV DNA in anti-HBc-positive blood donors in Turkey, very low rates ranging from 0 to 0.45% have been reported. Researchers suggested that the anti-HBc test could be integrated into screening strategies depending on the anti-HBc prevalence, but this application could lead to donor loss. The MP-NAT application had more false positives and lower sensitivity, and ID-NAT procedures were very costly; thus, more research was required before these techniques could be incorporated into routine applications [8]. In the current study, ID-HBV NAT positivity was not detected in 86 samples with isolated anti-HBc positivity.

In Turkey, the legal obligation for HBV screening in blood donors is to test for the presence of HBsAg using EIA or CLIA. The Guide to the Preparation, Use and Quality Assurance of Blood Components published 2016 in Turkey states that the anti-HBc test can be applied as a "supportive" test in the presence of repetitive reactivity of the HBsAg screening test, demonstrating that the blood donor has previously been introduced to HBV, and if the test finding indicates reactivity, then the donor should be permanently rejected with the suspicion of past or ongoing HBV infection. However, currently, there is no legal regulation or algorithm specific to the determination of OBI. To date, in this area, only a limited number of studies have been undertaken with a limited number of samples. More extensive research, epidemiological data, risk-cost analyses, and decisions involving a wide range of stakeholders are required to introduce efficient legal regulations in Turkey.

CONCLUSION

Blood donors with an occult hepatitis B infection can promote the risk of releasing potentially infectious blood products with extremely low levels of HBV DNA. In Turkey, the legal obligation for HBV screening in blood donors is to test for the presence of HBsAg by EIA or CLIA. Considering the costs and the epidemiological data of HBV infections, we recommend the Turkish Health authorities to implement anti-HBc screening in Turkish blood guidelines in order to improve screening strategy to efficiently exclude OBI donors from blood donation. Based on international scientific studies and our actual evaluation; it would be of benefit to regulate Turkish HBV screening in blood donors according to the related EU protocols.

Funding:
There was no funding.

Declaration of Interest:
There are no conflicts of interest.

References:

1. Candotti D, Laperche S. Hepatitis B Virus Blood Screening: Need for Reappraisal of Blood Safety Measures? Front Med (Lausanne). 2018;5:29 (PMID: 29515997).

2. Candotti D, Boizeau L, Laperche S. Occult hepatitis B infection and transfusion-transmission risk. Transfus Clin Biol. 2017;24(3): 189-95 (PMID: 28673499).

3. Esposito A, Sabia C, Iannone C, Nicoletti GF, Sommese L, Napoli C. Occult hepatitis infection in transfusion medicine: screening policy and assessment of current use of anti-HBc testing. Transfus Med Hemother. 2017;44(4):263-72 (PMID: 289244 31).

4. Ye X, Li T, Xu X, et al. Characterisation and follow-up study of occult hepatitis B virus infection in anti-HBc-positive qualified blood donors in southern China. Blood Transfus. 2017;15(1):6-12 (PMID: 27416568).

5. Somda K, Sermé A, Coulibaly A, et al. Hepatitis B Surface Antigen Should Not Be the Only Sought Marker to Distinguish Blood Donors towards Hepatitis B Virus Infection in High Prevalence Area. Open Journal of Gastroenterology. 2016;6(11):362. http://file.scirp.org/Html/6-1900358_72458.htm

6. La Torre G, Saulle R. Risk factors from HBV infection among blood donors: A systematic review. Asian Pacific Journal of Tropical Biomedicine. 2016;6(4):344-9. https://www.sciencedirect.com/science/article/pii/S222116911530 8960

7. Alshayea AI, Eid GE, El-Hazmi MM, Alhetheel AF. Prevalence and characterization of occult hepatitis B infection among blood donors in central Saudi Arabia. Saudi Med J. 2016;37(10):1114-9 (PMID: 27652363).

8. Seo DH, Whang DH, Song EY, Han KS. Occult hepatitis B virus infection and blood transfusion. World J Hepatol. 2015;7(3):600-6 (PMID: 25848484).

9. Yilmaz S, Unlu A, Cetinkaya RA, et al. A strategical re-thinking on National Blood Donor Pool: Anti-HBc positivity related re-entry mechanisms. Transfus Apher Sci. 2016;54(2):271-5 (PMID: 26928919).

10. Lieshout□Krikke RW, van Kraaij MG, Danovic F, Zaaijer HL. Rare transmission of hepatitis B virus by D utch donors with occult infection. Transfusion. 2016;56(3):691-8 (PMID: 26559665).

11. Yılmaz S, Yilmaz S, Çetinkaya RA, Türker T, Avcı İY, Beşirbellioğlu BA et al. An epidemiological overview of anti-hbc total test positivity in blood banking practice. GULHANE MEDICAL JOURNAL.58(2). http://gulhanemedj.org/uploads/pdf/pdf_GMJ_1013.pdf

12. Abdelrazik A, Abdelaziz H. The Role of Hepatitis B Core Antibody Testing in Improving Blood Safety in Resource-Limited Countries Study on Voluntary Blood Donors Fayoum, Egypt. J Blood Disord Transfus. 2015;6:315. https://www.omicsonline.org/open-access/the-role-of-hepatitis-b-core-antibody-testing-in-improving-blood-safety-in-resourcelimited-countries-study-on-voluntary-blood-donors-fayoum-egypt-2155-9864-1000315.pdf

13. Oluyinka OO, Van Tong H, Tien SB, et al. Occult Hepatitis B Virus Infection in Nigerian Blood Donors and Hepatitis B Virus Transmission Risks. PLoS ONE. 2015;10(7):e0131912 (PMID: 26148052).

14. Bal SH, Heper Y, Kumaş L, Mistik R, Töre O. [Investigation of the presence of HBV-DNA in isolated anti-HBc positive cases and their importance in blood banking]. Mikrobiyol Bul. 2009; 43(2):243-50 (PMID: 19621609).

15. Köten F, Öztop Ay, Engin A. Investigation of the HBV DNA in isolated hepatitis B core antibody positive blood donors. Scientific Research and Essays. 2011;6(13):2720-3. https://academicjournals.org/article/article1380713441_Koten%20et%20al.pdf

16. Stramer SL, Zou S, Notari EP, Foster GA, Krysztof DE, Musavi F et al. Blood donation screening for hepatitis B virus markers in the era of nucleic acid testing: are all tests of value? Transfusion. 2012;52(2):440-6 (PMID: 21810101).

17. Arababadi MK, Hassanshahi G, Pourfathollah AA, Zarandi ER, Kennedy D. Post-transfusion occult hepatitis B (OBI): a global challenge for blood recipients and health authorities. Hepat Mon. 2011;11(9):714-8 (PMID: 22235213).

18. Taira R, Satake M, Momose Sy, et al. Residual risk of transfusion□transmitted hepatitis B virus (HBV) infection caused by blood components derived from donors with occult HBV infection in Japan. Transfusion. 2013;53(7):1393-404 (PMID: 23033944).

19. Yugi H, Mizui M, Tanaka J, Yoshizawa H. Hepatitis B virus (HBV) screening strategy to ensure the safety of blood for transfusion through a combination of immunological testing and nucleic acid amplification testing - Japanese experience. J Clin Virol. 2006;36:S56-64 (PMID: 16831695).

20. Candotti D, Allain JP. Transfusion-transmitted hepatitis B virus infection. J Hepatol. 2009;51(4):798-809 (PMID: 19615780).

21. Niederhauser C, Mansouri TB, Graziani M, Stolz M, Tinguely C, Schneider P. Blood donor screening: how to decrease the risk of transfusion-transmitted hepatitis B virus? Swiss Med Wkly. 2008; 138(9-10):134-41 (PMID: 18330733).

22. Sofian M, Aghakhani A, Izadi N, et al. 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. Int J Infect Dis. 2010;14(4):e308-e10 (PMID: 19656713).

23. Kuhns MC, Busch MP. New strategies for blood donor screening for hepatitis B virus: nucleic acid testing versus immunoassay methods. Mol Diagn Ther. 2006;10(2):77-91 (PMID: 16669606).

24. Karakoç AE, Berkem R, Beyaz E. Detection of hepatitis B virus deoxyribonucleic acid using real-time polymerase chain reaction method in pooled-plasma samples of Turkish blood donors. Clin Lab. 2009;55(5-6):229-34 (PMID: 19728557).

25. Karakadıoğlu Bali S, Mutlu B, M. S. Survey Of Hepatıtıs B Virus DNA In Healthy Blood Donors By Usıng Nucleıc Acıd Amplıfıcatıon Technology. Turkish Journal of Infection. 2008;3(22):131-4. http://infeksiyon.dergisi.org/pdf/pdf_INF_279.pdf