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Original Article

HEPATITIS B VIRUS NUCLEIC ACID AMPLIFICATION TESTING ASSAY IN DETECTING WINDOW PERIOD AND OCCULT HEPATITIS B VIRUS INFECTIONS IN BLOOD DONORS

Mariya P. Georgieva-Sredkova, Neli S.Doseva[1], Vladislav M. Nankov, Pencho T. Tonchev, Aneta A. Surdzhyska[1]

Medical University-Pleven, Bulgaria, [1]Regional Center for Transfusion Hematology Pleven, Bulgaria

Summary

To reduce the residual risk of transfusion-transmitted infections, nucleic acid amplification testing (NAT) of donated blood with higher sensitivity for HBV, HCV, and HIV 1/2 was implemented in Bulgaria at the end of 2019. This study aimed to assess the clinical sensitivity of HBsAg testing and NAT testing of donated blood to detect all forms of HBV infection. A total of 9498 consecutive blood donations collected for six months, from February 10 to July 17, 2020, from first-time and repeat donors at the Regional Center of Transfusion Hematology Pleven, Bulgaria, were screened for HBsAg and HBV DNA. The detection of HBsAg was performed by enzyme-linked immunoassay and chemiluminescent immunoassay. Detection of HBV DNA was performed using the HIV1/2 /HCV / HBV multiplex Procleix Ultrio Elite assay in a fully automated and integrated Procleix Panther System. The overall HBsAg prevalence was 0.05%. HBV DNA was detected in 25 blood units (0.26%), but only 12 (0.13%) were found positive after repeat testing and were confirmed by a discriminatory test. The other 13 units were false positive, with the initial reactive result and negative results after repeat testing. HBV DNA‘s overall incidence was significantly higher in HBsAg-positive donors than the HBsAg- negative (Fisher exact p=0.0063). In our study, blood donations were not tested for anti-HBc and anti-HBs, so it is difficult to determine whether HBV DNA-positive/HBsAg-negative results were associated with the early phase of infection or persistent occult infection. There was no statistical difference in the incidence of HBV DNA between repeat-donors (0.16%) and first-time donors (0.06%) (Fisher exact test p=0.239 NS), and also between the incidence in female donors (0.12%) and male donors (0.13%) (Fisher exact test p=1.0 NS). The results of this study showed a low rate of detection of the hepatitis B virus in donated blood. NAT testing demonstrates higher sensitivity for the detection of HBV, as compare to HBsAg screening.

Keywords: hepatitis B virus, NAT testing, blood donation

Corresponding author:
Mariya P. Georgieva-Sredkova
Department of Microbiology, Virology and Medical Genetics,
Medical University – Pleven, Bulgaria,
1, St. Kl. Ohridski Str.,
5800 Pleven,
e-mail: microvir@abv.bg

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Introduction

Ensuring safe blood products is a crucial element in preventing the risk of developing transfusion-transmitted infections. Human immunodeficiency viruses 1 and 2 (HIV 1/2), hepatitis B virus (HBV), and

hepatitis C virus (HCV) are the most important viruses that can be transmitted by donated blood [1,2)]. Traditionally, various blood-screening serological assays, including enzyme-linked immunosorbent assays (ELISAs) and chemiluminescence immunoassays (CLIAs), are used to estimate the residual risk for transfusion of viral infections. These methods commonly include detecting antibodies to HIV 1/2, hepatitis B surface antigen (HBsAg), and antibodies to the hepatitis C virus.

Diagnostic sensitivity of screening assays is essential in reducing a residual risk of donated blood and blood components. The residual risk of HBV transmission by transfusion is associated mainly with a window period, occult hepatitis B infection (OBI), and genetic diversity among viral strains, classified into nine genotypes and subgenotypes [3,4,5]. Blood donors in the pre-seroconversion period, donors in the early convalescence phase of acute HBV infection, and those with chronic HBV infection have low HBsAg levels that cannot be detected by HBsAg assay [6)]. To reduce the residual risk of transfusion-transmitted infections, nucleic acid amplification testing (NAT) of blood products with higher sensitivity for HBV, HCV, and HIV 1/2 was first introduced in Germany in the 1990s [7], and later in other countries (8). In Bulgaria, NAT technology as a multiplex assay detecting HBV, HCV, and HIV1/2 was implemented at the end of 2019 for testing donated blood. According to Bulgaria‘s national testing guidelines, all blood units are screened for HBV by testing for HBsAg and HBV DNA.

The aim of this study was to assess the clinical sensitivity of HBsAg testing and NAT testing of donated blood to detect all forms of HBV infection.

Material and methods

Donations

A total of 9498 consecutive blood donations collected for six months (February 10 to July 17, 2020) from first-time and repeat donors at the Regional Center for Transfusion Hematology in Pleven, Bulgaria, were screened for HBsAg and HBV DNA. Of these donors, 2429 were female, and 7069 were male. Three thousand two hundred and sixty-nine (34.42%) of these donations were from first-time donors, and 6229 (65.58%) were from repeat-donors.

Testing

Each blood unit was tested for HBsAg and HBV DNA.

HBsAg screening

The detection of HBsAg was performed by enzyme-linked immunoassay and chemiluminescent immunoassay. Six thousand nine hundred and thirty-four (6934) of the donated blood units were serologically screened by enzyme-linked immunoassay using commercially available Monolisa™HBsAg ULTRA assay (Bio-Rad Marnes-la-Coquette, France) with analytical sensitivity was estimated by the manufacturer as less than 0.025 IU/ml. Blood units positive for HBsAg were retested in duplicate and confirmed by neutralization test using the Monolisa™ HBsAg ULTRA Confirmatory assay.

Two thousand five hundred and sixty-four (2564) of donated units were screened by chemiluminescent immunoassay ARCHITECT HBsAg Qualitative II (Abbot Ireland, Diagnostics Division, Finisklin Business Park, Sligo, Ireland) on an automated platform ARCHITECT System (ABBOT Laboratories). According to the manufacturer‘s instructions, the assay‘s analytical sensitivity ranged from 0.019 to 0.020 IU/ml. A reactive sample was retested in duplicate by ARCHITECT HBsAg Qualitative II. A repeatedly reactive sample was confirmed by a neutralizing ARCHITECT HBsAg Qualitative Confirmatory test.

NAT testing

Detection of HBV DNA was performed using the HIV1/2 /HCV / HBV multiplex Procleix Ultrio Elite assay in a fully automated and integrated Procleix Panther System (Grifols diagnostics Emeryville, California) according to the manufacturers’ recommendations. The automated assay consisted of three main steps in a single reaction tube on the Procleix Panther System: sample preparation/target capture; HBV DNA target amplification by Transcription-Mediated Amplification (TMA); detection of the amplification products by the Hybridisation Protection Assay. An Internal Control for monitoring assay performance was incorporated

in each individual reaction tube. The 95% lower limit of detection (LOD) for HBV DNA reported by the manufacturer is 3.4 IU/ml. Nucleic acid was extracted from 500 µl of individual plasma samples. Initial reactive samples were repeat-reactive in duplicate in the Ultrio Elite Assay and confirmed as true positive with the HBV discriminatory assay.

Statistical analysis

The Fisher exact test was used to analyze contingency tables, as some of the numbers were small. The risk ratio and confidence interval for RR were calculated using a Z-score. Free online calculators were used for calculations [https://www.scistat.com/statisticaltests/fisher.php]

Results

HBsAg testing of donated blood

ELISA and CLIA were used to test 9498 blood units. Five of them were found positive for HBsAg, as shown in Table 1. The overall HBsAg prevalence was 0.05%. There was no statistically significant difference in HBsAg prevalence among blood units screened by ELISA and CLIA. Out of the 6934 units screened by ELISA, two units (0.03%) were HBsAg-positive, and three (0.12%) of the units tested by CLIA were also identified as HBsAg-reactive (Fisher exact test, p=0.1256 NS).

HBV DNA was detected in 25 blood units (0.26%), but only 12 (0.13%) were true-positive after repeat testing and were confirmed by a discriminatory test. The other 13 units were false-positive, with an initially reactive result and negative results after repeat testing. The overall incidence of HBV DNA was significantly higher in HBsAg-positive donors than those that were HBsAg-negative (Fisher exact p=0.0063). Out of the 12 HBV DNA-positive blood units, one was HBsAg-positive, i.e., 20% of all HBsAg+cases), and 11 units were HBsAg-negative as presented in Table 2. One HBsAg-positive/HBV DNA-positive blood unit was also HCV-positive by NAT. The other four HBsAg-positive blood units gave HBV NAT-nonreactive results.

The Risk Ratio (RR) and RR confidence interval (CI) were calculated using the calculator available at https://www.scistat.com/statisticaltests/relative_risk.php, RR =172 CI (27-1097) z- statistic=5.457 p<0.0001. So, the relative risk to diagnose a positive HBV DNA blood unit is 172 times higher in units with positive HBsAg.

Twelve donors (0.13%) were found to be HBV DNA-positive. (Table 3). Of these donors, three were women, and nine were men. Donors‘ ages ranged from 38 to 65 years, and half were under 45 years old. Only two HBV DNA-positive blood units were from first-time donors (3269), and 10 were from repeat donors with 3 to 32 donations (6229). A statistically significant difference in the incidence of HBV DNA between repeat-donors (0.16%) and first-time donors (0.06%) was not found (Fisher exact test p=0.239 NS). Also, there was no statistical difference between the incidence in female donors (0.12%) and male donors (0.13%) (Fisher exact test p=1.0 NS).

Table 1. HBsAg Screening of 9498 Blood Donations by ELISA and CLIA

Screening Assay	Monolisa HBsAg ULTRA	Architect HBsAg Qualitative II	Total
Number Screened	6934	2564	9498
Number (%) positive	2(0.03%)	3(0.12%)	5(0.05%)
Number (%) negative	6932(99,97%)	2561(99,88%)	9493(99,95%)

Table 2. NAT Reactive and Confirmed Results for HBV in 9498 Blood Donations

	HBsAg (+) Units	HBsAg (-) Units	Total
HBV DNA (+) Units(%)	1(20%)	11(0,116%)	12 (0.126%)
HBV DNA (-) Units (%)	4(80%)	9482(99.884%)	9486(99.874%)
N screened for HBV DNA	5	9493	9498

Table 3. Demographic characteristics of HBV DNA-positive blood donors

Donors	Age	Gender	Firs-time donors	Repeat-donors	Number of donations
№XXXXXXX15308	65	female	–	+	10
№XXXXXXX16183	40	male	–	+	17
№XXXXXXX42211	45	male	–	+	4
№XXXXXXX16636	44	male	–	+	3
№XXXXXXX44471	58	male	–	+	3
№XXXXXXX44472	44	male	–	+	10
№XXXXXXX42341	42	male	+	–	1
№XXXXXXX17851	64	male	–	+	32
№XXXXXXX18392	38	male	–	+	20
№XXXXXXX91303	38	male	–	+	20
№XXXXXXX18815	58	female	–	+	21
№XXXXXXX90227	46	female	+	–	1

Discussion

The residual risk of HBV transfusion-transmitted infection is associated with blood donations collected in the early phase of infection or during the late stages of infection that show negative results for HBsAg and HBV DNA.

HBsAg testing is the initial step of blood screening for HBV, as the hepatitis B surface antigen is the key marker in the course of HBV infection. HBsAg-negative results were found in donors during the window period and in chronic HBV carriers, who did not have significant levels of HBsAg in the serum [9]. Decreased HBsAg detection sensitivity was also observed in donors infected with mutant HBsAg HBV strains [10,11]. Different immunoassays, including enzyme-linked immunosorbent assays and chemiluminescence immunoassays, are currently used for detecting HBsAg. Scheiblauer et al. evaluated comparatively clinical sensitivity, analytical sensitivity, and sensitivity to the major genotypes A-F and HBsAg subtypes of 70 HBsAg test kits (12). The sensitivity of these immunoassays ranges widely, between 0.013 and 1 IU/ml.

In this study, HBsAg was detected in five (0.05%) blood donors. One of these donors was HBV DNA-positive and HCV RNA-positive. This prevalence of HBsAg was lower than the data reported in the literature [13, 14] but in concordance with European countries' prevalence, varying from 0% to 5.2% [15).

We also found four blood units that were HBsAg-positive but HBV DNA negative. Over four years, the prevalence of HBsAg among 22.4 million donations in the USA was 13.4 per hundred thousand [16]. One hundred and forty-four blood units were HBsAg-positive without any serologic markers for HBV, including HBV DNA. Six of them were interpreted as HBsAg-positive with HBV DNA levels below the detection level of the NAT procedure we used (Procleix Ultrio Plus).

Our data showed that 25 (0.26%) of donations were reactive in the multiplex Procleix-Ultrio Elite assay, and 13 (52%) were non-reactive by HBV discriminatory assay. Data from Gou, H. et al. have shown that initially reactive by multiplex Ultrio assay were 0.17% of HBsAg-negative donations, and only one-third of them were HBV discriminatory assay-reactive [17]. Similar data have been reported by other authors [18,19,20]. They found that 0.09-0.29% of tested donations that were initially reactive might be non-repeat reactive in the discriminatory assay. These discrepancies are probably due to the Poisson distribution statistics of HBV DNA levels around the limit of detection (LOD) of the assay used, especially in donors with OBI. The Poison

Distribution law represented the probability of detecting low viral load in the window period or OBI. We used the Procleix Ultrio Elite assay with a 95% LOD of 3.4 IU/ml for HBV DNA reported by the manufacturer. When the viral load is very low, the virus might be present or not in the 500 µl of a sample used in the assay. These lead to discrepant results in a repeat test, as either positive or negative are obtained.

In our study, 12 (0.13%) of all donations HBV DNA-were positive. This incidence of HBV DNA detected donations was in concordance with data from the multi-regional study on 10 981 776 donations from different regions of the world screened by Ultrio assay for HBV DNA [21]. In first-time donors, regional rates of HBV detection ranged from 0.08% to 1.07%. Window period NAT rates varied from 1:7 700 to 1 294 000, and OBI NAT rates were between 1:3 900 and 1:59 000.

Among our 12 HBV DNA-positive donations, only one was with detectable HBsAg. It is known that HBV is characterized by long doubling time (approximately 2.56 days) of viral load during replication with the persistence of low-level of viremia without detectable HBsAg [22]. Implementation of nucleic acid testing assays reduced the serological window period from 32 to 15 days in acute infection and improved blood safety by detecting donors with occult HBV infection [22,23)]. OBI was defined as the long-lasting persisting of the HBV genome in the liver or blood and absence of detectable HBsAg in the serum [24)]. In most OBI donors, the viral load is less than 50 IU/ml, and the high sensitivity of HBV NAT allows detecting occult HBV infection in donors with anti-HBc and/or anti-HBs-positive results [24]. In Japan, the implementation of sensitive NAT methods increased the OBI detection rate from 3.9 to 15.2 per million, and the confirmed OBI transmission rate also increased from 0.67 to 1.49 per million [25]. As in our study, blood donations were not tested for anti-HBc and anti-HBs, it is difficult to say whether HBV DNA-positive/HBsAg-negative results were associated with the early phase of infection or persistent occult infection.

Conclusion

The results of this study showed a rare rate of detection of the hepatitis B virus in donated blood. NAT testing demonstrates higher sensitivity for the detection of HBV, compare to HBsAg screening. To increase the detection rate of occult hepatitis B infection, it is appropriate to include anti-HBc and anti-HBs as supplemental serologic markers for HBV in HBV DNA-positive blood units.

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