Research Article

Detection and identification of occult hepatitis B virus infection among blood donors

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Abstract

Background: Occult hepatitis B virus infection (OBI) is defined as the presence of circulating hepatitis B virus (HBV) DNA as detected by HBV nucleic acid test (NAT), in the absence of detectable HBV surface antigen (HBsAg), with or without antibodies to hepatitis B core antigen (anti-HBc) or hepatitis B surface antigen (anti-HBs). HBV infection is a continuing threat to transfusion safety, especially in developing counties like India where detection of HBV is primarily based on the screening for HBsAg as a marker of infection.

Objective: To know the prevalence of OBI among blood donors and their serological and molecular characterization of NAT yield samples.

Materials and Methods: A total of 41,090 blood donor's samples from February 2014 to August 2015 were tested by individual donor-NAT (ID-NAT) apart from routine serological screening for anti-HIV 1-2, P24 antigen, anti-HCV, and Hepatitis B surface antigen (HBsAg) by Biomerieux (Vironostika® HIV Ag-Ab, Hepanostika® HCV Ultra, and HBsAg Ultra, France). All the samples were tested individually by Procleix® Utrio Plus® Assay (Novartis Emeryville, CA). Blood units that were HBsAg nonreactive but ID-NAT reactive (NAT yield) were further worked up with anti-HBc, anti-HBsAg, viral DNA load, and viral genotyping.

Result: Of the 41,090 samples, 29 were reactive for HBV DNA (NAT yield). Among the 29 NAT yields, 24 were individual NAT yield, 4 were HBV–HCV NAT co-yield, and 1was HBV-HIV NAT co-yield. A total of 24 HBV individual NAT yield samples were further tested for OBI. A total of 11 samples were reactive for anti-HBc only, 3 samples carried both anti-HBc and anti-HBs, yielding a total of 14 (58.3%) samples that were classified as OBI and 1 (4.2%) sample was reactive for anti-HBs only. Nine samples (37.5%) did not have any serological marker owing to incomplete antibody, and they remained unclassified, as window period infection could not be excluded. Two HBV-NAT yield samples of genotype D, one with a window period and the second being an OBI had high viral load by qPCR.

Conclusion: Blood products from donors with OBI carry a high risk of HBV transmission by transfusion. Because of multiple antigens and antibodies present in blood in response to HBV infection, at present there is no single test to detect the infection. In developing country such as India with high seroprevalence of HBV infection, combination of at least two tests would help us to improve the transfusion safety.

KEY WORDS: Occult hepatitis B virus infection, nucleic acid testing, blood screening

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Introduction

Developing countries, such as India, which are endemic for hepatitis B pose a serious challenge for the blood banking community. The need of safe blood is of utmost importance for the repeat transfusion. Among the chronically transfused patients such as patients with thalassemia, the hepatitis B virus (HBV) prevalence as measured by HBV surface antigen (HBsAg) is 33%, whereas the prevalence in blood donor population is

1.2%[1] and posttransfusion prevalence of hepatitis has been observed to be 1%–1.8% in postsurgical transfusion.[2] Detection of HBV is primarily based on screening of hepatitis B surface antigen (HBsAg) as a serological marker of infection, which has been established globally for the diagnosis of acute or chronic HBV infections and for the screening of blood or organ donors in developing countries.[2,3] Emergence of mutant variants of Hepatitis B because of increased immunological pressure, by global immunization programs, or viral escapes in chronically infected patients have led to suboptimal performance of certain HBsAg assays. This is mainly because of natural variation and mutations in HBV S gene, which can induce HBsAg conformational changes.[4]

Occult HBV is the major contributor for transfusion transmitted HBV infection in highly endemic countries such as India. Hence, the need for individual donor nucleic acid test (ID-NAT) for HBV is necessary to circumvent the risk of missed positive cases during the window period, occult infections, and HBsAg assays that do not detect all known S mutants. Hepatitis B core antibody testing in countries endemic for HBV is also not suitable because of the high prevalence of individuals who harbor these antibodies. In addition, hepatitis B core antigen (anti-HBc) assay does distinguish between current and resolved HBV infections.[3]

Occult hepatitis B virus infection/carriage (OBI) is characterized by the presence of very low levels of HBV DNA in plasma and/or in liver, with undetectable HBsAg using the most sensitive commercial assays, with or without antibodies to anti-HBc or hepatitis B surface antigen (anti-HBs) outside the preseroconversion window period. OBI prevalence varies between different geographic areas and populations according to HBV endemicity and HBV genotype. In Southeast Asia, where genotype B and C are prevalent, up to 90% of the population has evidence of past exposure to HBV.[5] A study was conducted to know the prevalence of OBI among blood donors and their serological and molecular characterization of NAT yield samples.

Materials and Methods

Study Design

All voluntary and replacement blood donors donating between February 2013 to August 2014 in the Department of Immunohematology and Blood Transfusion, Dayanand Medical College and Hospital, Ludhiana, Punjab were included in the study.

A total of 41,090 blood donor's samples were tested by ID-NAT apart from routine mandatory screening by ELISA (for HBsAg and anti-HIV, anti-HCV). Blood units that were HBsAg nonreactive but ID-NAT reactive (NAT yield) were further worked up with anti-HBc, anti-HBsAg, hepatitis "e" antigen (anti-HBe), viral load, and viral genotyping.

Donor Samples Studies

Serology

Anti-HBs screening was done by Biomerieux (HBsAg Ultra, France). Screening for antibody against anti-HBc total, anti-HBc IgM, anti-HBs, and anti-HBe was done by chemiluminescence immunoassays (CLIA).

ID-NAT

All the samples were tested individually by Procleix® Utrio Plus® Assay (Novartis Emeryville, CA). It is a transcription-mediated amplification (TMA)-based screening for the simultaneous, single tube detection. The entire test takes place in a single tube and involves three steps (1) target capture, (2) target amplification by TMA, (3) detection of the amplification products with chemiluminescent probes by the hybridization protection assay. Finally, the dual kinetic assay simultaneously detects the internal control and the viral RNA or DNA.

All three assays incorporate internal control to validate each reaction. ID-NAT is a multiplex assay that provides simultaneous detection of HIV, HCV RNA, and HBV DNA. Samples found reactive initially in the Utrio plus Assay were later retested by using the multiplexed protocol.

Individual Donor Procleix® Ultrio Plus® Assay is a sensitive screening assay available. The analytic sensitivity of Procleix® Ultrio Plus® Assay (95% detection limit for routine testing) for HIV-1 is 27.6 IU/mL, for HCV is 3.1 IU/mL, and for HBV is 2.1 IU/mL.[6]

Viral Load and Genotyping

HBV DNA quantification, amplification, and sequencing were determined by quantitative real-time PCR (polymerase chain reaction) by Roche Cobas Ampliprep-TaqMan Assay. Linear reporting range of the assay was 20–1.7 × 10^8 IU/mL. Conversion factor used for 1 IU/mL was 5.82 copies/mL. HBV genotyping was done on reactive cases if the value was >2000 copies/mL and distinguishes A–H genotypes.

Result

From the initial screening of 41,090 samples, 29 were found reactive for HBV DNA (NAT yield). Among these 29 NAT yields, 24 were individual NAT yield, 4 were HBV–HCV NAT co-yield, and 1 was HBV–HIV NAT co-yield; therefore, HBV NAT yield rate is 1 in 1,417.

A total of 24 HBV individual NAT yield samples were further tested for OBI and 5 samples of NAT co-yield could not be further classified because CLIA data were not available because of insufficient quantity of sample. Mean age distribution of donors of these 24 NAT yield was 39.2 years (25 to 58 years). All donors were male, 10 of 24 (41.6%) were voluntary and 14 of 24 (58.4%) were replacement.

A total of 11 samples were reactive for anti-HBc only, 3 samples carried both anti-HBc and anti-HBs, yielding a total of 14 samples that were classified as OBI and one sample was reactive for anti-HBs only. Nine samples did not have any serological marker owing to incomplete antibody, and they remained unclassified, as window period infection could not be excluded. Two HBV-NAT yield samples of genotype D, one with a window period and the second being an OBI had a

Table 1: Triplicate repeat NAT and supplemental test results in 24 HBV NAT yields (classified in different infection stages)

Sr. no.	Case Donor status			Blood bank NAT result					Supplemental viral load and serology (CLIA) results							
	Sex	Type	Tube IR	3X Replicate					VL qPCR (IU/mL)	Geno-type	HBsAg	a-HBc (Total)	a-HBc (IgM)	a-HBs	a-HBe	Classification HBV infection
				Tube	Bag	dHIV	dHCV	dHBV								
1	M	R	R	1/3	2/3	0/3	0/3	3/3	2161	D	NR	NR	NR	NR	NR	
2	M	V	R	3/3	3/3	0/3	0/3	3/3	39		NR	NR	NR	NR	NR	
3	M	V	R	3/3	3/3	0/3	0/3	3/3	94		NR	NR	NR	NR	NR	
4	M	R	R	3/3	3/3	0/3	0/3	3/3	ND		NR	NR	NR	NR	NR	Window period
5	M	R	R	3/3	3/3	0/3	0/3	3/3	ND		NR	NR	NR	NR	NR	
6	M	V	R	2/3	1/3	0/3	0/3	2/3	ND		NR	NR	NR	NR	NR	
7	M	R	R	2/3	2/3	0/3	0/3	2/3	14		NR	NR	NR	NR	NR	
8	M	R	R	2/3	3/3	0/3	0/3	3/3	ND		NR	NR	NR	NR	NR	
9	M	V	R	3/3	3/3	0/3	0/3	3/3	ND		NR	R	R	NR	R	Late acute
10	M	V	R	1/3	1/3	0/3	0/3	1/3	ND		NR	NR	NR	R	NR	Anti-HBs breakthrough
11	M	R	R	1/3	1/3	0/3	0/3	1/3	ND		NR	R	NR	NR	R	
12	M	R	R	3/3	3/3	0/3	0/3	3/3	21		NR	R	NR	NR	R	OBI with a-Hbe and without a-HBs (low level carrier, early convales-cent period, chronic; potentially infectious)
13	M	R	R	3/3	3/3	0/1	0/1	1/3	<10		NR	R	NR	NR	R	
14	M	V	R	3/3	0/3	0/3	0/3	2/3	ND		NR	R	NR	NR	R	
15	M	V	R	1/3	3/3	0/3	0/3	3/3	ND		NR	R	NR	NR	R	
16	M	R	R	2/3	2/3	0/3	0/3	2/3	31		NR	R	NR	NR	R	
17	M	R	R	2/3	1/3	0/3	0/3	1/3	ND		NR	R	NR	NR	NR	
18	M	V	R	2/3	2/3	0/3	0/3	2/3	17		NR	R	NR	NR	NR	OBI without a-Hbe and a-HBs (chronic; potentially infectious)
19	M	R	R	1/3	2/3	0/3	0/3	2/3	NA		NR	R	NR	NR	NR	
20	M	V	R	3/3	3/3	0/3	0/3	3/3	10		NR	R	NR	NR	NR	
21	M	R	R	3/3	3/3	0/3	0/3	3/3	2,434	D	NR	R	NR	NR	NR	
22	M	R	R	2/3	1/3	0/3	0/3	1/3	ND		NR	R	NR	R	NR	OBI with a-HBs (late phase) previous infection with immunity
23	M	V	R	1/3	3/3	0/3	0/3	3/3	ND		NR	R	NR	R	NR	
24	M	R	R	2/3	2/3	0/3	0/3	2/3	<10		NR	R	NR	R	NR	

NAT, nucleic acid test; CLIA, chemiluminescence immunoassays; HBsAg, hepatitis B surface antigen; HBe, hepatitis “e” antigen; HBV, hepatitis B virus; HIV, human immunodefi-ciency virus; NA, sample not available; D, genotype D; ND, not detectable; OBI, occult HBV infection; M, male; a, anti; V/R, voluntary/replacement.

Table 2: HBV infection rate

HBV yield rates			
Classification	***n***	**Rate**	**% Age**
Pre-HBsAg WP	09	1/4,565	37.5
aHBs breakthrough	01	1/41,090	4.2
OBI aHBs (negative)	11	1/3,735	45.8
OBI aHBs (positive)	3	1/13,696	12.5
Total classified HBV NAT yield	24	1/1,712	100

HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; OBI, occult HBV infection; NAT, nucleic acid test.

high viral load that was detected by qPCR. Twenty-four of the HBV-NAT yields could be grouped in different stages of infections according to the supplement HBV serology results as shown in Table 1. Of the residual confirmed HBV-NAT yields, 8 (33.3%) were classified as window period, 1 (4.2%) as late acute window period or early recovery stage, 1 (4.2%) as anti-HBs breakthrough or abortive infection, and 14 (58.3%) as OBI. Table 2 summarized the proportion and rate of different acute and chronic HBV infection stages.

Discussion

To assess the safety of the blood supply as it pertains to the risk of transmitting HBV to the recipients requires comprehensive understanding of virus–host relationship, the natural history of this disease, and the serological changes that follow exposure to the virus.

HBsAg is the first hepatitis B serological marker to be detected in the blood following the HBV infection. Screening of blood for HBsAg has reduced the incidence of posttransfusion hepatitis but HBV still remains the major source of TTIs in India. HBV has a slow doubling time of 2.7 days resulting in slow increase in the viral load, hence the longest window period from infection to detection and even NAT testing.[7] The introduction of routine NAT testing of blood donation has resulted in a decrease in the rate of transfusion-transmitted viral infection by the interdiction of donors in serological window period; although NAT testing is being adopted more widely, it has become clear that challenges still remain.[8] In India, because of relatively high HBsAg positivity of 1%–2% in donor population, 10%–30% anti-HBc prevalence, the major HBV type being genotype D followed by A and C, with genotype D and A documented to remain at low viral load, and association of HBV genotype D with high OBI, which is characterized by fluctuating low viral load necessitate the need of NAT testing.[9,10]

It is generally accepted that the diagnosis of infection by HBV is based on the presence of the HBsAg in the blood stream. However, screening of blood bank donors for HBsAg does not totally eliminate the risk of HBV infection through blood transfusion, as the absence of this marker in the serum does not exclude the presence of HBV DNA. It is possible that, donors with OBI, who lacked detectable HBsAg but whose exposure to HBV infection was indicated by a positive anti-HBc and HBV DNA, are a potential source of HBV infection.[11,12]

In this study, additional testing, both NAT and serology and where appropriate, testing of follow-up samples, indicated that the 14 NAT-only yield samples were from donors with an OBI, 9 samples were from donors in a window period, and 1 with abortive infection. HBV activity might be impaired by other infectious agents in cases with coinfection such as HCV. HCV "core" protein strongly inhibits HBV replication, and OBI shows the highest prevalence in patients infected with HCV.[13] Even in our study, four samples were NAT co-yield with HCV and one with HIV; therefore, there is a requirement for ID-NAT testing in such region that is very sensitive for the detection and identification of HBV DNA.

Several recent studies have shown that blood products from donors with an OBI can result in transfusion-transmitted HBV.[14,15] HBV transmission can occur even in the presence of low levels of anti-HBs (<200 IU/L).[16] The prevalence of OBI previously reported among blood donors in Asia is 1:570–1:8,628 in China, 1:3,248 in Hong Kong, 1:894–1:1,029 in Taiwan, and 1:832 in Thailand.[5] A previous study from Delhi, tried to assess the ability of ID-NAT to detect OBI in the blood donors. Of the 18 NAT yields, anti-HBc was nonreactive in 6 out of 18 NAT yields, reflecting potential infectious donations that would escape detection by HBsAg and anti-HBc screening.[17]

Many studies were conducted in HBsAg-negative samples among blood donors in our country but almost all have studied[18,19] the seroprevalance of antibody to core antigen (HBc). The incidence of anti-HBc is being reported from 30.2% to 8.4%. Anti-HBc is long-lasting, which is detectable later than HBsAg but associated with lower rates of transfusion transmission in the absence of HBsAg. Application of anti-HBc as a screening modality in blood donors is largely debated because of its association with high discard rate.

Transfusion-associated HBV continues to be a significant problem in our country. Its prevalence is estimated at approximately 1.5% in postsurgical blood transfusion recipients or more in patients who had multiple transfusions in India.[20] Our study raises serious concerns regarding the safety of the blood supply in our community, even after donor screening for HBsAg and NAT. In conclusion, transfusion-transmitted infections including HBV have been a major concern in transfusion medicine. Screening of blood for HBsAg has reduced the incidence of posttransfusion hepatitis, but HBV still remains the major source of transfusion-transmitted infection in India.

HBV transmission by blood and blood products despite HBsAg screening is mainly because of prevalence of occult hepatitis B infection among donors. In a developing country like India with a high seroprevalence of HBV infection, combination of at least two tests would help us to improve the transfusion safety. The introduction of the NAT test in regions would add an extra layer of safety to the blood supply by interdicting samples from donors with an OBI.

Conclusion

Blood products from donors with OBI carry a high risk of HBV transmission by transfusion. Because of multiple antigens and antibodies present in blood in response to HBV infection, at present there is no single test to detect the infection. In a developing country like India, with a high seroprevalence of HBV infection, combination of at least two tests would help us to improve the transfusion safety.

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