Low frequency of occult hepatitis B infection in anti-HBc seropositive blood donors: experience from a tertiary care centre in South India

Ashrafali M. Ismail[1], Seetha Devakumar[1], Raghavendran Anantharam[1], Gnanadurai J. Fletcher[1], Thenmozhi Subramani[1], George T. John[2], Dolly Daniel[3], Priya Abraham[1]

[1]Department of Clinical Virology, [2]Department Nephrology, [3]Department Immunohaematology and Transfusion Medicine, Christian Medical College, Vellore, India

Dear Sir,

Historically, transfusion-associated transmission was the major cause of hepatitis B virus (HBV) disease burden worldwide. Increased awareness of donor screening for hepatitis B surface antigen (HBsAg) has greatly reduced the rate of transmission of HBV. However, blood transfusion in the early acute phase of infection and HBV surface gene variants have justified the need for HBV core antibody (anti-HBc) screening and implementation of nucleic acid testing in donor populations. The residual risk of HBV transmission through blood transfusion is estimated to be 1 in 200,000 to 500,000 and is comparatively higher than that for hepatitis C virus (HCV) and human immunodeficiency virus (HIV)[1]. Occult infection could be one of the reasons for the relatively high risk of HBV infection.

The clinical impact of occult HBV infection among recipients of blood and blood products is not completely known. The majority of anti-HBc positive individuals acquire HBV surface antibody (anti-HBs) through natural clearance of infection or as a result of HBV vaccination. However, an anti-HBs level that clearly precludes the circulation of HBV DNA and disease transmission has not yet been clearly identified. We attempted to address these issues by analysing the seroprevalence of HBV DNA in HBsAg negative, anti-HBc positive healthy blood donors with varying levels of anti-HBs antibody.

Blood donors in our tertiary care hospital in south India were recruited in October 2008. Serum samples from these donors were tested for HBsAg, HIV and HCV antibody using Vitros ECI (Ortho-Clinical Diagnostics, Raritan, NJ, USA). A total of 1,300 replacement blood donors negative for these serological markers were investigated for the presence of anti-HBc antibody (Diasorin S.p.A. Saluggia, Italy). All the samples that were confirmed to be anti-HBc positive in Architect Anti-HBc II (Abbott, Weisbaden, Germany) were further tested for anti-HBs levels (AxSYM, Abbott, Weisbaden, Germany) and categorised into three groups according to these levels: anti-HBs negative, anti-HBs <100 mIU/mL and anti-HBs >100 mIU/mL.

DNA was extracted from 200 µL of plasma using the QIAamp DNA blood MiniKit (Qiagen GmbH, Hilden, Germany) and HBV DNA was quantified using a CE-marked artus® HBV RG real-time polymerase chain reaction (PCR) (Qiagen GmbH, Hilden, Germany) in the Rotor-Gene 3,000 or 6,000 platform (Corbett Research, Mortlake, Australia). The assay targets the 134 bp region of the HBV core gene and the lower limit of detection (LLD) stated by the manufacturer is 20 IU/mL (system 1). Samples were further tested by another sensitive, FDA approved automated nucleic acid system (Abbott RealTime HBV, Weisbaden, Germany) targeting the surface region of the HBV genome. The LLD in this case is 10 IU/mL with a sample input of 500 µL (system 2). The study was approved by the Institutional Review Board of the hospital.

The median age of the study donors was 32 (range, 14-65) years and most of the donor were male (89%). Of 1300 samples tested for anti-HBc, 217 (16.7%) were confirmed to be positive. When these anti-HBc positive blood donors were tested for anti-HBs, 86 (39.6%) were anti-HBs negative, 58 (26.7%) had an anti-HBs titre <100 mIU/mL and 73 (33.6%) donors had an anti-HBs titre of >100 mIU/mL. The available 184 samples from the anti-HBc seropositive blood donors were tested for HBV DNA and all were negative on testing in system 1 (artus® HBV RG PCR). When all these samples were further tested in the automated nucleic acid system 2 (Abbott RealTime HBV), two samples were found to be positive for HBV DNA with a viral load of <10 IU/mL (Figure 1). The overall prevalence of occult HBV in anti-HBc seropositive individuals was thus 1.1%.

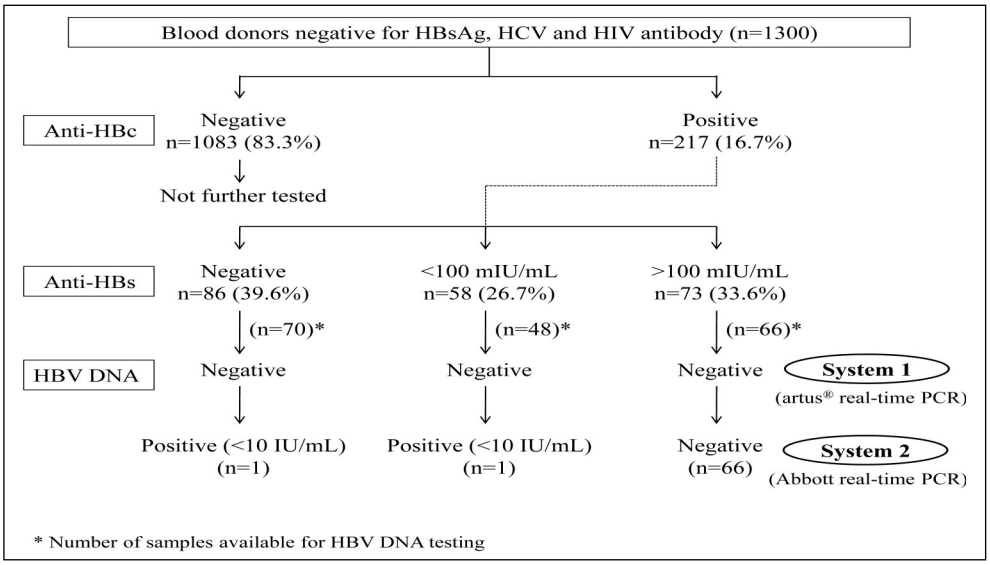

Figure 1 - Flow Chart of the Study.

Of the two HBV DNA positive donor samples, the anti-HBs titer of one sample was 63 mIU/mL and the other sample was anti-HBs negative.

Previous reports have documented a prevalence of occult HBV of 7.5 to 30% in India[2-5]. In contrast, here we found a prevalence of 1.1% occult HBV in anti-HBc seropositive healthy blood donors. Such a low rate of occult HBV infection from a region of intermediate endemicity for HBV is noteworthy. The differences in rates of occult HBV seen in this population questions the uniformity of our screening practices. Though most studies have used sensitive nested PCR, HBsAg assays used in the diagnosis of occult HBV are highly varied (Table I). The difference in rates of occult HBV reported across the country may be due to the varying sensitivity of HBsAg assays used for donor screening. Donors may well be HBsAg positive when screened by more sensitive HBsAg assays, hence eliminating them from the category of donors with so-called occult infection. Furthermore, contamination in the nested PCR approach for nucleic acid detection is possible and replicate testing to discriminate true and false positive HBV DNA results is required.

In a context of limited resources, anti-HBc can be an affordable marker for diagnosing occult HBV infection. However, there are reports of occult infection in anti-HBc seronegative individuals. There is, therefore, a residual risk of HBV transmission in transfusion settings that employ anti-HBc alone as a supplementary marker for occult HBV screening. Moreover, the use of anti-HBc screening relies on the

Table I - Occult HBV infection in Indian anti-HBc seropositve blood donors.

References	Study population	Anti-HBc %	Anti-HBc seropositive		HBV DNA System	HBsAg Assay
			Anti- HBs %	HBV DNA %		
2	Blood donors, Delhi (n=2,175)	18.9	63	7.5	In-house nested PCR (core and surface region)	Qualisa, Qualpro Diagnostics, India
5	Blood donors, Orissa (n=729)	30.1	18.2	30	In-house nested PCR (pre-core and surface region)	Biomerieux, Boxtel, The Netherlands
3	Blood donors, Kolkata (n=1,027)	18.3	-	21.3	In-house nested PCR (core and surface region)	Span Diagnostics, India
4	Blood donors, Delhi (n=24,694 and 6,159)	10.8 and 3.7	5.3 and 1	27.2 and 20.9	PCR (core and surface region)	Organon Teknika, Boxtel, The Netherlands

geographic endemicity of HBV. Screening for anti-HBc and excluding about 20% of anti-HBc positive blood donations without knowing the HBV DNA status will result in a higher discard rate of blood units.

Anti-HBs is the antibody protecting against HBV and might serve to neutralise the infectivity of these virions. Earlier studies suggested that blood units with >100 mIU/mL of anti-HBs antibody were safe for transfusion. The two HBV DNA positive donors in our study had anti-HBs titers <100 mIU/mL. This suggests that the circulation of HBV DNA in donors with anti-HBs titers >100 mIU/mL is not common and may not require further HBV DNA testing. However, different assays used for anti-HBs testing may also account for the variability of anti-HBs titres. Moreover, transfusions cannot be considered solely on anti-HBs levels as there are studies that have shown the presence of HBV DNA in individuals with >100 mIU/mL of anti-HBs antibody. Anti-HBs as a screening assay in blood donors does, therefore, need further evaluation as additional testing of anti-HBs in this setting will add to further cost and delay to the release of blood units.

Our attempt to rule out false negative HBV DNA results by using a second real-time system with enhanced sensitivity picked up two samples with a viral load of <10 IU/mL. The estimated LLD (95% detection limit) of this assay as determined by the WHO International Standard for HBV was 1.43 IU/mL (*unpublished data*). This ensures the reliability of the HBV DNA status reported in this study. The presence of very low HBV DNA levels in blood donors necessitates the need for highly sensitive assays with a LLD of less than 10 IU/mL for the correct diagnosis of occult HBV infection. The lower prevalence of HBV DNA seen in anti-HBc seropositive donors provides indirect evidence that the preponderance of HBV DNA in anti-HBc seronegative donors will be much lower. Although we did not see high rates of occult infection in our setting, nucleic acid testing is still recommended as the residual risk of HBV transmission from healthy donors remains. Follow-up of our two HBV DNA positive blood donations could have helped to understand the clinical significance of these occult infections.

In summary, the presence of HBV DNA in anti-HBc seropositive blood donations was low in our setting. The different rates of occult HBV infection reported across the country may be due to the varying sensitivity of HBsAg assays used for donor screening. The implementation of anti-HBc screening in donor populations is limited as excluding isolated anti-HBc blood donations will result in higher discard rates of blood units especially in higher endemicity regions. Nucleic acid testing cannot be exempted as there is a residual risk of HBV transmission in healthy blood donors. Anti-HBc and nucleic acid testing in transfusion settings can be minimised by the use of very sensitive HBsAg screening assays. Larger, multicentre studies are required to understand the actual burden of occult HBV infection in transfusion settings.

The Authors declare no conflicts of interest.

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Correspondence: Priya Abraham
Department of Clinical Virology
Christian Medical College
Ida Scudder Road
632 004 Vellore, India
e-mail: priyaabraham@cmcvellore.ac.in