Reappraisal of screening strategy in hepatitis B surface antigen-negative blood donors: Correlation with hepatitis B virus-DNA quantification

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Abstract:

BACKGROUND: In spite of screening for hepatitis B surface antigen (HBsAg), transfusion-associated hepatitis B virus (TAHBV) infection remains a serious public health problem due to transmission of HBV in window period and occult HBV infection. To avoid TAHBV infection, some health-care facilities have started Hepatitis B core antibody test along with HBsAg, but this leads to a lot of potential donor rejection who are not HBV infected. Our aim is to find a new protocol of donor screening to prevent TAHBV without compromising blood availability.

MATERIALS AND METHODS: A total of 88 HBsAg-negative anti-HBc total positive blood donors were included in this study. All samples were also tested for anti-HBs by enzyme immunoassay and for the presence of HBV-DNA viral load by real-time polymerase chain reaction.

RESULTS: A total of 88 HBsAg negative and anti-HBc, total positive blood donors were tested for anti-HBs and HBV-DNA (Qn.). Among them, 76 donors (86.4%) (males 73 and females 3) were found to be positive for anti-HBs, while rest 12 (13.6%) showed no detectable antibody against HBsAg. HBV-DNA was found to be positive in 4 (7.7%) donor samples among 52 (60%) who have anti-HBs level <100 mIU/ml, while 36 (40%) donor samples were found to have >100 mIU/ml anti-HBs antibody with no detectable HBV-DNA.

CONCLUSION: HBV-DNA should be implemented as a screening test of the blood donors to prevent TAHBV infection without potential donor rejection.

Keywords:
Anti-hepatitis B core antigen total, hepatitis B surface antigen, hepatitis B virus-DNA

Introduction

More than 350 million people are chronic carriers of hepatitis B virus (HBV), and this infection represents a worldwide public health problem.[1] Many patients chronically infected with HBV, as defined by the persistence over 6 months of hepatitis B surface antigen (HBsAg), will develop life-threatening diseases such as liver cirrhosis and hepatocellular carcinoma. It has been estimated that up to 30% of them will die from the consequences of their infection.[2-4] Infected blood and blood product is the main mode of infection for HBV. Presence of HBV-DNA in the serum represents the gold standard of HBV infection. For practical purposes, however, HBsAg is estimated in the sera to assess if someone is infected with the HBV or not. Assessment of HBsAg is comparatively cheaper, and this can be accomplished in most, if not all, medical facilities of developing countries. However, it is now evident that a group of HBV-infected persons may not express
HBsAg will be negative in window period, occult hepatitis B and during the recovery phase of acute HBV infection, whereas anti-HBc total can be detected and remains positive for lifelong. Therefore, anti-HBc may be present in the absence of HBsAg and anti-HBs. In addition, anti-HBc screening having value in the detection of HBV-infected donors who have mutation in HBsAg not detectable by some HBsAg assay. It has also been demonstrated that some HBsAg-negative, but anti-HBc-reactive individuals continue to replicate HBV. Hence, the absence of HBsAg in the blood of apparently healthy individuals may not be sufficient to ensure the absence of HBV infection. Blood containing anti-HBc with or without the detectable presence of HBsAg might be infectious; therefore, routine blood donor screening for anti-HBc along with HBsAg has been recommended by the World Health Organization (WHO). However, this protocol, if implemented, can result in loss of significant number of potential donors as anti-HBc total cannot distinguish between resolved cases from the current infection. The situation has been worsened by the fact that about 5%-8% apparently healthy people of Bangladesh are chronically infected with the HBV and high prevalence of anti-HBc (11.7%-22.6%) has been reported among healthy individuals. Therefore, it is essential to establish a new protocol for blood screening to ensure the prevention of TAHBV infection without hampering blood availability, which is the aim of the present study.

Materials and Methods

This observational study was conducted from June 2017 to July 2018. All serological tests except HBV-DNA were done at the Department of Laboratory Medicine of Asgar Ali Hospital. Test for HBV-DNA was done at Molecular diagnostic laboratory of Popular Diagnostic Centre Limited.

Patients and samples

Serum samples positive for anti-HBc total and negative for HBsAg, treponemal infection, malarial infection, anti-hepatitis C virus (HCV), and anti-HIV I and II antibodies were collected from 88 healthy blood donor, who came to donate blood in Asgar Ali Hospital blood bank. Of them, 85 were male (age range 18–54 years) and 3 were female (age range 22–36 years). Donors were interviewed and medically examined before transfusion. Those with high-risk behavior, including intravenous drugs abusers or with any medical problem, were excluded from the study population. These selected donor samples were collected in a red-capped vacationer, and the serum samples were separated and kept in Eppendorf tube and stored at −30°C until HBV-DNA test. Asgar Ali Hospital Ethical Committee granted ethical approval for the study. Informed written consents were also obtained from all the participants.

Test for hepatitis B surface antigen

HBsAg was tested by ELISA (enzyme immunoassay [EIA]) method using commercial reagents of Vitros 5600 HBsAg ES (Ortho Clinical Diagnostics, Pencoed Bridgend, PZ, UK). The presence or absence of HBsAg was determined by comparing for each sample of the recorded absorbance with that of the calculated cutoff value. Samples with an optical density less than the cutoff values were considered to be negative. Samples with optical density higher than, or equal to, the cut-off value was considered to be positive.

Test for anti-hepatitis B core total

Commercially available Vitros 5600 anti-Hbc, total, a competitive EIA kit (Ortho Clinical Diagnostics, Pencoed Bridgend, UK) was used for the detection and/or of antibody to HBc antigen.

Test for antibody to hepatitis B surface

Commercially available Vitros 5600 Anti-Hbs (OrthoClinical Diagnostics, Pencoed Bridgend, CF35 5PZ, UK) was used for the detection and/or quantitative determination of antibody to HBsAg (ad and ay subtypes). Samples showed anti-Hbs titer below 4.23 mIU/ml considered to be negative (i.e., as not having anti-HBS). The lowest amount of anti-HBs that can be detected with the VITROS Anti-HBs Quantitative test was determined in accordance with NCCLS EP17. Every sample that tested negative for HBsAg but positive for anti-HBc, total alone or in combination with other serological markers was also examined for the presence of HBV-DNA assay.

Polymerase chain reaction for hepatitis B virus-DNA

DNA extraction

Roche high pure viral nucleic acid (manual) extraction kits were used to extract HBV-viral DNA from 500 μl of
blood serum, and during the extraction, a known number of HBV quantitation standard (QS) DNA molecules are introduced in each specimen.\(^\text{[22]}\)

**Amplification and detection**

The real-time polymerase chain reaction (RT-PCR) was performed by the COBAS TaqMan 48 HBV assay (Roche, USA), simultaneously amplifying two targets: HBV-DNA (HBV genotypes A-H including precore mutation and the HBV-QS. Results were expressed as international unit per milliliter (IU/mL). The limit of detection of the assay was set as 6 IU/mL with the dynamic range from about 29 to 1 \(\times\) 10\(^8\) IU/mL. It gives a relative quantification of the virus DNA by competing with known copy number of HBV genome.

**Results**

A total of 88 HBsAg negative and anti-HBc, total positive blood donors (2 voluntary, 3 replacement, and 83 directed donors) were tested for anti-HBs and HBV-DNA. HBsAg and anti-HBs were tested using enhanced chemiluminescence-sandwich ELISA, while anti-HBc, total was tested using enhanced chemiluminescence-competitive ELISA. The donors were mostly males (\(n = 85\)) with an age range (18–56 years) and only three female (18–36 years) [Table 1]. Only 14 males were vaccinated against HBV.

All blood samples were tested for quantitative anti-HBs. Among them, 76 donors (male 73, female 3) were found to be positive for anti-HBs, while rest 12 showed no detectable antibody against HBsAg [Table 2].

**Table 1: Blood donors’ demographic profile**

| Gender       | Male, \(n\) (%) | Female, \(n\) (%) |
|--------------|----------------|------------------|
| Age (years) |                |                  |
|             | 18–52          | 20–36            |
| \(n\)       | 85 (96.6)      | 3 (3.4)          |
| Vaccination status | 14 (16.5) | 0                |

**Table 2: Hepatitis B virus serological profile of blood donors**

| Gender       | HBsAg | Anti-Hbc total | Anti-HBs |
|--------------|-------|----------------|----------|
|              |       | Detected       | Not detected* |
| Male (85)    | Negative | 73              | 12       |
| Female (3)   | Negative | 3               | 0        |

*Anti-HBs level <4.23 mIU/ml should be interpreted as not having detectable anti-HBs

**Table 3: Comparison between hepatitis B surface antigen 3.0 and hepatitis B virus DNA levels of blood donors (\(n=88\))**

| Anti-HBs level (mIU/ml) | Number of donors (%) | HBV-DNA |
|-------------------------|----------------------|---------|
|                         | Detected (%) | Not detected (%) | DNA level (copies) |
| \(\geq 100\)           | 36 (41)      | 0          | 36               |
| \(< 100\)              | 52 (59)      | 4***       | 48               | Up to 1.67\(\times\)10\(^7\) |
| Total                   | 88            | 4 (4.5)    | 84 (95.5)        |

\(^{***}P=0.001\)

All samples were tested by quantitative RT-PCR for estimating the viral load. Donors were categorized according to the level of anti-HBs (mIU/ml). Among them, 36 were found to have more than 100 mIU/ml anti-HBs antibody with no detectable HBV DNA. While, among the other 52 donors, who have <100 mIU/ml of anti-HBs, four were found to have HBV-DNA [Table 3].

There seems to be a significant difference in HBV-DNA positivity among the donors within these two groups (\(P = 0.001\)) (which is significant – \(P\) value should be given).

**Discussion**

Since its introduction in 1970, HBsAg has remained the first line of HBV screening in blood donors, as the sensitivity and specificity of the test has steadily been improving with the development of the EIAs.\(^\text{[23]}\) Apart from the window periods (period between infection and appearance of HBsAg, during HBsAg to HBs antibody seroconversion), HBsAg screening may fail to identify (i) donors infected with HBV variants, (ii) drug-induced mutations, (iii) the formation of immune complexes in the presence of HBV surface antibodies (anti-HBs), (iv) chronic HBV infection with antigen levels below the detection limit of HBsAg assay.\(^\text{[24]}\) Therefore, transfusion of blood collected from a donor who is in the window period may lead to posttransfusion hepatitis B in the recipient. A marker which would be indicative of hepatitis B infection during the window period, is, therefore, of paramount importance in blood banking. Anti-HBc, total has been found to be an excellent indicator of occult HBV infection during the window period. It usually appears 6–12 weeks after infection and remains detectable lifelong in immunocompetent patients constituting the most sensitive marker for exposure to HBV infection irrespective of the current infection state. Anti-HBc total may be the only serological marker of HBV infection at the end of a resolving episode when anti-HBs decline to undetectable levels or in Occult HBV infection, where HBsAg may be undetectable and HBV-DNA is only intermittently detectable.\(^\text{[25-28]}\) However, if only anti-HBc total is done for screening without HBsAg, there is a probability of missing HBV infection which usually manifests by the presence of HBsAg, as it becomes detectable at approximately 4 weeks after infection. Whereas, anti-HBc immunoglobulin M
becomes detectable after 6–8 weeks. This problem can be resolved if anti-HBc, total screening is introduced along with HBsAg for routine use. Nevertheless, the combination cannot distinguish between individuals who are anti-HBc reactive because of past, resolved HBV infection and are thus noninfectious, from those who have unresolved HBV infection and are thus potentially infectious.

Another important consideration is that anti-HBc assays often demonstrate a high level of nonspecificity. This, together with the problems associated with the confirmation of anti-HBc reactivity, often results in a situation where anti-HBc reactivity is identified in the absence of any other markers of HBV infection and majority of this reactivity is actually nonspecific and does not reflect HBV infection.

In a population with a high prevalence of infection, the number of blood donors with evidence of natural, resolved infection is likely to be significant, resulting in the potentially unnecessary discard of many blood donations. The donor loss caused by universal anti-HBc screening was considered sustainable in low-endemic countries, though, it is generally admitted that, rejecting anti-HBc reactive blood would severely affect blood supply in medium-to-high endemic areas like Bangladesh where the prevalence of anti-HBc is high (11.7%–22.6%).[18,20] Moreover, it gives inaccurate information on HBV infection among rejected donors.

To limit potential donor loss associated with high anti-HBc prevalence, the WHO suggested a complex screening algorithm that includes anti-HBs testing of anti-HBc, total positive donations. Donors with anti-HBc, total reactive, who contain anti-HBs levels >100 mIU/mL are considered eligible for transfusion and donations with anti-HBs levels <100 mIU/mL are rejected.[16] In our study, we found that, anti-HBc total along with anti-HBs assays cannot prevent unnecessary donor rejection. For the selection of the prospective blood donors in a cost-efficient way, we would like to propose the alternative algorithmic approaches until HBV-DNA tests become available widely [Figure 1]. HBV nucleic acid test (NAT) is very efficient in reducing transmission of HBV in the window period because of its high sensitivity and detectability before HBsAg becomes detectable. In addition, HBV-NAT uncovered a relatively large number of HBsAg-negative occult HBV infection (OBI) among blood donors who tested anti-HBc and/or anti-HBs positive. OBI is broadly defined as the presence of HBV-DNA in the liver of an individual who is HBsAg-negative, HBsAg status unknown, or HBsAg-negative with unknown HBsAg status. HBV-DNA tests are available in most countries and are widely used in the diagnosis and management of HBV infection.

Figure 1: (a) Percentage of positive and negative figures were taken from our donor archive (unpublished data), (b) Percentage of the prevalence of anti-hepatitis B core antigen, total, (c) Hepatitis B virus-DNA quantification, (d) HBV-DNA qualitative test in multiplex polymerase chain reaction.
individual testing negative for HBsAg with a cutoff value for serum HBV-DNA (<200 IU/ml). The probability of detecting HBV-DNA is highest in patients who are anti-HBc positive and anti-HBs negative. In low-endemic affluent countries, the implementation of both HBsAg, anti-HBc, and HBV-NAT provides the (additional) level of blood safety by allowing detection of both the early phase of acute infection, persistent occult infection with potential transient detectable viremia, and genetic and/or antigenic viral variants.

**Conclusion**

Considering the pitfalls of HBsAg, anti-HBc total and anti-Hbs, HBV-NAT would be an alternative with additional layer of safety because it will be positive in the early phase of acute infection, window period, and persistent occult infection along with preventing unnecessary donor rejection. Anti-HBc screening is not an option due to the high discard rates to detect window period infections. Larger studies are needed to determine the actual burden of window period infections in donor population so as to implement the use of HBV-DNA screening for all HBsAg-negative blood donations.

Although it is expensive, the cost can be lowered by high-throughput commercial multiplexed NAT assays that include HBV-DNA detection in addition to HIV and HCV RNAs. In this respect, cost and time are important.

While automated EIA takes less than an hour to test, the time for NAT has also been decreased to a couple of hours. Moreover, the cost of HBsAg, anti-HBc, anti-HBs, anti-HCV, and anti-HIV EIA combined will not be much different than multiplex HBV, HCV, and HIV-NAT with significant advantage of safe blood transfusion.

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**Conflicts of interest**

There are no conflicts of interest.

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