ORIGINAL ARTICLE

SCREENING OF ANTI-HBc IN BLOOD DONORS FOR THE PRESENCE OF OCCULT HBV ACTIVITY

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ABSTRACT

OBJECTIVE: To study screening of antibody to hepatitis B core antigen (anti-HBc) in healthy blood donors negative for hepatitis B surface antigen (HBsAg) to find incidence of anti-HBc positive amongst blood donors and to highlight serious concerns regarding safety of blood supply still after donor screening for HBsAg.

MATERIALS AND METHODS: A total of 150 serum samples collected from HBsAg-negative healthy blood donors were tested for antibody to hepatitis B core antigen. RESULTS: Out of 150 samples tested, 12 (8.0%) blood samples were found to be reactive for anti-HBc. It was lower in voluntary (6.0%) as compared to replacement donors (9.0%, P = 0.52).

CONCLUSION: Keeping in view that 8% of our donor population was anti-HBc reactive and there is high reported incidence of post transfusion hepatitis B in our patients. We recommend that anti-HBc testing should be added to pre-transfusion screening and units reactive for anti-HBc should be tested for HBV DNA with highly sensitive techniques to improve safety from TAHBV.

Keywords: Anti-HBc, Blood donors, HBsAg, HBV, TAHBV

INTRODUCTION

Hepatitis B is a major public health problem worldwide. Approximately 30% of the world’s population or about 2 billion persons have serological evidence of either current or past infection with hepatitis B virus.1 HBV is highly infectious and can be transmitted covertly by percutaneous routes and overtly by blood transfusion. Blood transfusion service (BTS) is an integral and indispensable part of the healthcare system. The priority objective of BTS is to ensure safety, adequacy, accessibility, and efficiency of blood supply at all levels.2 It is well known that blood transfusion is associated with a large number of complications, some are only trivial and others are potentially life threatening, demanding for meticulous pre-transfusion testing and screening.

The use of unscreened blood transfusion keeps the patient at risk of acquiring many transfusion transmitted infections (TTI) like hepatitis viruses (HBV, HCV), human immunodeficiency viruses (HIV), syphilis, malaria, etc. Transfusion departments have always been a major portal to screen, monitor, and control infections transmitted by blood transfusion.3 Hepatitis B virus (HBV) presents a higher residual risk of transmission by transfusion than hepatitis C virus (HCV) or human immunodeficiency virus (HIV).4 Occult hepatitis B infection (OBI) is defined as the presence of HBV DNA in blood or tissues without detectable HBsAg, with or without anti-HBc or hepatitis B surface antigen (anti-HBs).5 Such occult hepatitis B infection may be detected in (1) individuals with resolving HBV infection reactive for both anti-HBc and anti-HBs, (2) “anti-HBc-only” carriers in a window period of infection who are seronegative for HBsAg, and anti HBs (3) carriers in whom HBsAg is not detectable due to presence of escape mutants.5 Despite mandatory screening for HBsAg by ELISA for over 20 years, TAHBV continues to be a major problem in India, more so in patients receiving repeated transfusions. The incidence of TAHBV in patients receiving multiple transfusions, such as Thalassemia, ranged from 17.9% in the first year to 69.2% by the third year. Patients on renal dialysis showed similar rates of infection with

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HBV.\(^6\) It has been demonstrated that some HBsAg negative donors who are anti-HBc positive continue to replicate hepatitis B virus.\(^7\) They may harbor and maintain HBV-DNA sequences in their liver and blood, thus, representing potential sources of HBV transmission. Thus the absence of HBsAg in the blood of apparently healthy individuals may not be sufficient to ensure lack of circulating HBV.\(^7\) Blood containing anti-HBc with or without detectable presence of HBsAg might be infectious; therefore, routine blood donor screening for anti-HBc has been implemented in some countries resulting in a decrease in the risk of post-transfusion HBV infection.\(^8\)

With the fairly high incidence of HBV in India, there is a definite risk of inadvertently transfusing HBV infected blood still after donor screening for HBsAg. It is therefore strongly felt that a marker must be utilized for screening of blood in the Indian population to detect the presence of hepatitis B during the window period. The aim of this study was to screen blood donors for antibodies to hepatitis B core antigen IgM type (anti HBc-IgM) and to find the incidence of anti-HBc IgM in our subject population of blood donors negative for HBsAg.

**MATERIALS AND METHODS**

This study is being conducted in the Department of Pathology and blood transfusion SMS Medical College Jaipur from July 2007 to January 2009 after obtaining an approval from the Ethics Committee of the Institute. Annually there are approximately 43-45000 donations. 150 blood donors are included in the present study.

The blood donors are selected from two groups: 1) Replacement donors: 100, 2) Voluntary donors: 50. As per Drugs and Cosmetics (3\(^{rd}\) amendment 2001) rules, Govt. of India, all the units were tested for HIV, HBsAg, HCV, VDRL and Malaria. Testing for additional markers of HBV infection: Blood units negative for HBsAg were tested for antibody to hepatitis B core antigen (anti HBc). Serological tests: HBsAg was tested by using commercial ELISA kit SURASE B-96 (TMB) (GBC, Taiwan, and ROC); anti-HBc antibodies were tested using commercial ELISA kit HBcAb two-step incubation (MBS-SRL, Milano, Italy).

**RESULTS**

A total of 150 HBsAg non-reactive blood donors (50 voluntary and 100 replacement donors) were screened for anti-HBc (IgM) using competitive ELISA, of them 12 turned out to be reactive, giving an overall seropositivity of 8.0%. It was lower in voluntary (6.0%) as compared to replacement donors (9.0%, \(P = 0.52\)).

**Table 1: Frequency of anti HBc in VD/ RD**

<table>
<thead>
<tr>
<th>Type of donor</th>
<th>Anti HBc positive donor</th>
<th>Total donor tested in group</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Voluntary donor</td>
<td>3</td>
<td>50</td>
<td>6</td>
</tr>
<tr>
<td>Replacement donor</td>
<td>9</td>
<td>100</td>
<td>9</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>12</strong></td>
<td><strong>150</strong></td>
<td><strong>8.0</strong></td>
</tr>
</tbody>
</table>

Donors with age 18-30 years had minimum seropositivity (6.67%) with 50% donors contributing from this group.

**Table 2: Frequency of anti HBc in four age groups**

<table>
<thead>
<tr>
<th>Age group (Yrs)</th>
<th>Anti HBc +ve</th>
<th>Total donor</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>18 – 30</td>
<td>5</td>
<td>75</td>
<td>6.67</td>
</tr>
<tr>
<td>31 – 40</td>
<td>4</td>
<td>45</td>
<td>8.89</td>
</tr>
<tr>
<td>41 – 50</td>
<td>2</td>
<td>24</td>
<td>8.33</td>
</tr>
<tr>
<td>51 – 60</td>
<td>1</td>
<td>6</td>
<td>16.67</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>12</strong></td>
<td><strong>150</strong></td>
<td><strong>8.0</strong></td>
</tr>
</tbody>
</table>

In our study student donors have (4.16%) anti HBc reactivity which is lower than nonstudent donors (8.73%).

**Table 3: Frequency of anti HBc in student and non-student population**

<table>
<thead>
<tr>
<th>Type of donor</th>
<th>Anti HBc positive donor</th>
<th>Total donor tested in group</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Student donor</td>
<td>1</td>
<td>24</td>
<td>4.16</td>
</tr>
<tr>
<td>Non-student donor</td>
<td>11</td>
<td>126</td>
<td>8.73</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>12</strong></td>
<td><strong>150</strong></td>
<td><strong>8.0</strong></td>
</tr>
</tbody>
</table>

No significant difference was found in the seropositivity of first time versus repeat donors, male versus female donors, although the seropositivity was less in female donors.

**DISCUSSION**

Despite mandatory screening of donor blood for HBsAg, transfusion-associated HBV (TAHBV) continues to be a major problem in India, more so in patients receiving repeated transfusions.\(^6\) The HBsAg test is at present the only mandatory HBV screening tool for blood donations in India. However, donations from seroconverting donors
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...and from chronic HBV carriers with low HBsAg levels, and from donors infected with rarely occurring mutant HBsAg HBV strains, may be not detected by the currently implemented HBsAg HBV assays and therefore represent the most frequent residual risks for HBV transmission to recipients of blood products. Hence additional HBV markers must be evaluated to reduce this risk. Literature worldwide shows presence of anti-HBc in HBsAg-negative blood donors. The incidence of anti-HBc in blood donors varies from 0.07% to 18% and 0.3%-38% of these donors show presence of HBV DNA in their blood, depending on the type of blood donors and the endemicity of disease in the study population. This study was conducted on 150 HBsAg ELISA non-reactive blood donors. The study population belonged to Jaipur and nearby areas of Rajasthan state. The present study showed 8.0% anti-HBc positivity. Prevalence of anti-HBc was 6.0% in voluntary donors and 9.0% in replacement donors. A study reported from PGI, Chandigarh by Dhawan H.K. et al revealed that prevalence of anti HBc was 8.4% of which 6.9% in voluntary donors and 10.4% in replacement donors. A study reported from PGI, Chandigarh by Dhawan H.K. et al revealed that prevalence of anti HBc was 8.4% of which 6.9% in voluntary donors and 10.4% in replacement donors. A study reported from New Delhi (Northern India) by Chaudhuri et al revealed that the prevalence of anti-HBc was 10.82% with distribution of 6.92% in voluntary donors and 12.53% in replacement donors. Makroo et al pegged the prevalence of anti-HBc total amongst blood donors in New Delhi at 11.6%. Another study from New Delhi by Mohammad Asim et al revealed 18.9% of donor population was reactive for anti HBc. A study from West Bengal (Eastern India) by Bhattacharya et al showed anti-HBc positivity as high as 18.3% in voluntary blood donors. Prevalence of anti-HBc reported by Behzad-Bebahan et al in Iran was 6.55%, in this study only voluntary donors were included. High prevalence of anti-HBc (17.28%) was reported by Bhatti et al from Pakistan, and all the donors in this study were replacement donors. The prevalence of anti-HBc in Europe and North America is quite low, an anti-HBc prevalence of 0.07% in the UK and 1.5% in Germany has been reported. In our study seroprevalence of anti HBc in voluntary donors was less as compared to replacement donors (6.0% vs. 9.0%). All these results suggest voluntary donors are safer than the replacement donors, also in India because they invariably are more educated and can better understand the implication of donor questionnaire. Replacement donors on the other hand are compelled to donate blood within a given time frame (admission and therapy). This lays importance to enlarge the voluntary donor base to 100%.

In our study, a low seropositivity (6.67%) was seen in donors with age 18-30 years as compared to donors with age 31-40 years (8.89%), (8.33%) in 41-50 years age group and (16.67%) in 51-60 years age. In our study student donors have (4.16%) anti HBc reactivity which is lower than nonstudent donors (8.73%). Therefore, efforts should be made to increase and retain these young motivated voluntary donors to maintain safe blood supply. The enormous variation in global seroprevalence of anti-HBc among blood donors is a reflection of difference in the type of blood donors and HBV endemicity of the study population. The low seroprevalence in US, UK and German blood donors may be due to 100% voluntary donor base, stringent donor screening, high literacy rates and self exclusion by high-risk donors.

The most widely used marker for diagnosing HBV infection in donated blood is by screening it for HBsAg. However, HBsAg is not detected during the window period of the infection. Therefore, transfusion of blood collected from a donor who is in the window period may lead to post transfusion 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 has been found to be an excellent indicator of occult HBV infection during the window period. Anti-HBc IgM is a marker of recent infection, whereas, anti-HBc IgG positivity indicates a past infection. Anti-HBc IgM may remain positive for life in an affected individual although the individual has protective levels of anti-HBs and therefore, this does not necessarily mean that blood of such a donor is infectious. So, anti-HBc IgM is considered to be a more specific marker for HBV infection during the window period.

CONCLUSION

Keeping in view that 8% of our donor population was anti HBC positive and there is reported high incidence of post transfusion hepatitis B in our patients. We recommend that anti HBC testing should be added to pre-transfusion screening and units reactive for anti HBC should be further tested for anti HBs and HBV DNA with sensitive techniques and units positive for HBV DNA and low anti HBs titers should be discarded. However, the usefulness of screening for anti HBc as an additional screening test to improve the safety of the blood supply in India deserve further studies from different regions to establish sensitive screening modalities for blood transfusion.
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REFERENCES