

Anti-HBc screening in Indian blood donors: Still an unresolved issue

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anti-HBc screening and discarding large number of blood units *versus* considering ID NAT (Individual donor nucleic acid testing) needs to be assessed.

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Abstract

AIM: To study the seroprevalence of antibody to hepatitis B core antigen (anti-HBc) in healthy blood donors negative for HBsAg and to evaluate whether anti-HBc detection could be adopted in India as a screening assay for HBV in addition to HBsAg.

METHODS: A total of 1700 serum samples collected from HBsAg-negative healthy blood donors were tested for the presence of anti-HBc antibody (IgM + IgG). All samples reactive for anti-HBc antibody were then investigated for presence of anti-HBs and for liver function tests (LFTs). One hundred serum samples reactive for anti-HBc were tested for HBV DNA by PCR method.

RESULTS: Out of 1700 samples tested, 142 (8.4%) blood samples were found to be reactive for anti-HBc. It was significantly lower in voluntary (6.9%) as compared to replacement donors (10.4%, $P = 0.011$). Seventy-two (50.7%) anti-HBc reactive samples were also reactive for anti-HBs with levels > 10 mIU/mL and 70 (49.3%) samples were non-reactive for anti-HBs, these units were labeled as anti-HBc-only. These 142 anti-HBc reactive units were also tested for liver function test. HBV DNA was detected in only 1 of 100 samples tested.

CONCLUSION: Keeping in view that 8%-18% of donor population in India is anti-HBc reactive, inclusion of anti-HBc testing will lead to high discard rate. Anti-HBs as proposed previously does not seem to predict clearance of the virus. Cost effectiveness of introducing universal

INTRODUCTION

Hepatitis B virus (HBV) infection is a serious global health problem affecting two billion people worldwide, and 350 million people suffer from chronic HBV infection^[1]. Despite mandatory screening for HBsAg by ELISA for over 20 years, transfusion-associated HBV (TAHBV) continues to be a major problem in India, more so in patients receiving repeated transfusions^[2]. The incidence of transfusion-associated hepatitis (TAH) after cardiac surgery (4 + 2.4 units transfusion) was estimated at 6.9%, of which TAHBV constituted 20%^[2]. 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 HBV^[2]. It has been demonstrated that some HBsAg-negative individuals and those reactive for anti-HBc continue to replicate HBV^[3,4]. Thus the absence of HBsAg in the blood of apparently healthy individuals may not be sufficient to ensure lack of circulating HBV. 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^[5].

These findings suggest that recovery from acute hepatitis B virus infection may not result in complete virus elimination, but rather the immune system keeps the virus at a very low level called occult hepatitis B infection. Occult hepatitis B infection (OBI) is defined as the presence of HBV DNA in blood or tissues without detectable HBsAg, with or without antibody to hepatitis B core antigen (anti-HBc) or hepatitis B surface antigen (anti-HBs)^[6]. 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 (3) carriers in whom HBsAg is not detectable due to presence of escape mutants^[7].

As of today, some countries have retained or adopted anti-HBc testing to decrease HBV transfusion risk, while others have not. Anti-HBc testing is still not mandatory in blood banks in India and only HBsAg testing by ELISA is used as screening test for HBV^[8]. In this study, we aimed to evaluate whether anti-HBc detection could be adopted in India as a screening assay for HBV in addition to HBsAg to improve the safety of blood transfusion.

MATERIALS AND METHODS

Materials

This study was conducted by the Department of Transfusion Medicine in collaboration with Department of Hepatology, PGIMER, Chandigarh, India from July 2005 to December 2006, after obtaining an approval from the Ethics Committee of the Institute. Seventeen hundred HBsAg ELISA non-reactive blood donors were included, 998 of them were voluntary donors and 702 were replacement donors. These samples were then tested for anti-HBc (IgM + IgG), anti-HBs, LFTs and HBV DNA in the following sequence: (1) Blood units non-reactive for HBsAg and reactive for anti-HBc were tested for antibody to surface antigen (anti-HBs) and for liver function tests (LFTs); (2) the blood units non-reactive for HBsAg but reactive for anti-HBc with or without anti-HBs were tested for HBV DNA by PCR method.

Serological tests

HBsAg was tested by using commercial ELISA kit SURASE B-96 (TMB) (GBC, Taiwan, ROC); anti-HBc antibodies were tested using commercial ELISA kit HBcAb two-step incubation (MBS-SRL, Milano, Italy); anti-HBs antibodies were tested using commercial ELISA kit MONOLISA anti-HBs 3.0 (BIORAD).

HBV DNA detection

The DNA from serum was extracted using QiaAmp (Qiagen, Hilden, Germany), followed by in-house nested PCR, amplifying two different regions of the HBV genome using two sets of primers shown in Table 1. The procedure was standardized by positive control obtained from National Institute of Virology Pune, India.

Table 1 Primers used for HBV PCR

| | |
|---------------------------------------------|--------------|
| First PCR | |
| GP1 5'-YCCTGCTGGTGGCTCCAGTTC-3': sense | nt 3144-3164 |
| GP2 5'-AAGCCANACARTGGGGGAAAGC-3': antisense | nt 583-604 |
| Second PCR (nt 120-604) | |
| GP3 5'-GTCTAGACTCGTGGTGGACTTCTCTC-3': sense | nt 120-145 |
| GP4 5'-AAGCCANACARTGGGGGAAAGC-3': antisense | nt 583-604 |

First PCR was carried out in 20 μ L volume containing 1 \times Taq buffer [100 mmol/L Tris HCL (pH 8.4), 25 mmol/L MgCl₂ and 500 mmol/L KCL], 20 pmol of reverse and forward primers (Table 1), 1 unit of Taq polymerase and 200 ng viral DNA. Thirty-five amplification cycles were performed in PCR machine (Stratagene, La Jolla, USA). Each cycle consisted of denaturation at 94°C for 30 s, annealing at 55°C for 30 s and extension at 72°C for 30 s. A second PCR was performed using the first PCR product as a template using the primers GP-3 and GP-4 which amplified a 485-bp product.

For the detection of PCR product, the second PCR product was run on 20 g/L agarose in TBE buffer containing 0.5 μ g/mL ethidium bromide at 50 V for about 1 h and finally visualized under a UV transilluminator (UVP, Upland, USA) and then photographed. A 100-bp DNA ladder (MBI Fermentas, Opelstrasse, Germany) was also run in parallel and the predicted size of the PCR product, which was found to be 485 bp.

Biochemical tests

Liver function tests, including serum bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), were carried out by enzymatic method.

RESULTS

A total of 1700 HBsAg non-reactive blood donors (998 voluntary and 702 replacement donors) were screened for anti-HBc (IgM + IgG) using competitive ELISA, of them 142 turned out to be reactive, giving an overall seropositivity of 8.4%. It was markedly lower in voluntary (6.9%) as compared to replacement donors (10.4%, $P = 0.011$). Donors with age 18-30 years had minimum seropositivity (6.1%) with 50% donors contributing from this group. No significant difference was found in the seropositivity of first time *versus* repeat donors, male *versus* female donors and student *versus* non-student donors, although the seropositivity was slightly less in female donors and student donors.

The anti-HBc reactive donors were tested for anti-HBs. Seventy-two (50.7%) anti-HBc reactive samples were also positive for anti-HBs with levels > 10 mIU/mL and 70 (49.3%) samples were non-reactive for anti-HBs, these units were labeled as anti-HBc-only. The anti-HBc-only reactivity was significantly lower in voluntary (34/998, 3.4%) than in replacement donors (36/702, 5.1%; $P = 0.38$) (Table 2). The anti-HBc reactive units were also tested for LFTs. All the samples had normal

Table 2 Prevalence of anti-HBc and anti-HBs in study population

| Donor category | Number | Anti-HBc total | Anti-HBc-only | Anti-HBc and anti-HBs |
|-----------------|------------|----------------|---------------|-----------------------|
| Voluntary (%) | 998 (58.7) | 69 (6.9) | 34 (3.4) | 35 (3.5) |
| Replacement (%) | 702 (41.3) | 73 (10.4) | 36 (5.1) | 37 (5.3) |
| Total (%) | 1700 | 142 (8.4) | 70 (4.1) | 72 (4.2) |
| P value | | 0.011 | 0.038 | 0.03 |

serum bilirubin levels, and 25 (18%) samples showed enzyme elevation. Out of these 25 samples with elevated enzyme levels, 14 (56%) were positive for anti-HBc-only and 11 (44%) were positive for anti-HBc + anti-HBs ($P = 0.460$).

One hundred samples from these 142 anti-HBc reactive (45 anti-HBc-only and 55 anti-HBc with anti-HBs) samples were tested for HBV DNA by using PCR. Only one sample was positive for HBV DNA. This sample had an anti-HBs level > 150 mIU/mL, LFTs for this sample were within normal limits. This was a 22-year-old male repeat replacement blood donor.

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^[2].

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^[2,4,6,9-12].

This study was conducted on 1700 HBsAg ELISA non-reactive blood donors. The study population belonged to Chandigarh and states of Punjab, Haryana, Himachal Pradesh, Jammu and Kashmir. The present study showed 8.4% anti-HBc positivity. Prevalence of anti-HBc was 6.9% in voluntary donors and 10.4% in replacement donors. A study reported from New Delhi (Northern India) by Chaudhuri *et al*^[2] revealed that the prevalence of anti-HBc was 10.82% with distribution of 6.92% in voluntary donors and 12.53% in replacement donors. In contrast, a study from West Bengal (Eastern India) by Bhattacharya *et al*^[7] showed an anti-HBc positivity as high as 18.3% in voluntary blood donors. Prevalence of anti-HBc reported by Behzad-Behbahani *et al*^[10] 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*^[6] 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^[6]. A study from Japan^[4] reported anti-HBc prevalence of as 1.1%. 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. In our study, a significantly low seropositivity (6.1%) was seen in donors with age 18-30 years as compared to donors with age 31-40 years (11.6%, $P = 0.003$). Donors with age 18-30 years were 50% of the study population and were largely composed of young college students. The results are comparable with study from our department in 2004 by Sharma *et al*^[13] that student donors have significantly lower incidence of all the markers for transfusion-transmitted infections. Therefore, efforts should be made to increase and retain these young motivated voluntary donors to maintain safe blood supply.

Our study revealed similar prevalence of anti-HBc positivity in first time donors (8.4%) and repeat donors (8.3%, $P = 0.94$), suggesting that lack of education among both our donor groups regarding minor modes of HBV transmission like tattooing, ear/nose piercing, sharing of shaving kits or a visit to a road side barber. It highlights the uniformity of donor behavior between the two groups. Our study is in accordance with recent three studies by Retrovirus Epidemiology Donor Study (REDS)^[14-16] groups which showed that the incidence rate of viral infection is not lower in repeat blood donors than first time donors and abbreviated screening for repeat blood donors is not advisable.

Prevalence of anti-HBc-only was 4.1% in our study, out of which 3.2% was in voluntary and 5.1% in the replacement donors ($P = 0.038$). In a study from New Delhi (Northern India)^[2], the reported prevalence of anti-HBc-only was 4.2%, out of which 2.72% was in voluntary and 4.85% in replacement donors^[2]. Similarly, a study from Iran^[10] reported anti-HBc-only prevalence of 2.3%.

Anti-HBc reactive samples were tested for LFTs. All the samples had normal serum bilirubin levels, and 25 (18%) samples showed elevation of liver enzymes. Elevated levels of liver enzymes in our donors indicates underlying hepatitis or some kind of liver injury which can be ruled out by additional investigations, such as hepatic ultrasound, liver biopsy and genetic testing which were not an objective of the present study.

HBV DNA was detected in 1 of 100 anti-HBc reactive donors tested. None of the anti-HBc-only samples were positive for HBV DNA. One sample was positive for HBV DNA and sample also contained anti-HBs levels > 150 mIU/mL, LFTs for this sample were within normal limits. Other studies from India^[2,7] reported HBV DNA positivity of 20.87% in New Delhi (Northern India) and 21% in West Bengal (Eastern India). HBV DNA was detected among 12.2% of anti-HBc reactive donors in Iran, 2.8% in Lebanon, 2.9% in Pakistan^[7]. A study from Japan^[4] reported that HBV DNA was detected in 19 (38%) of 50 anti-HBc reactive samples. The viral load in these samples is usually low and their detection requires sensitive DNA amplification techniques.

In our study, low incidence of HBV DNA in anti-HBc reactive samples may be due to limitation of sensitivity of HBV DNA amplification technique. Another reason of low HBV DNA positivity in our study could be due to type of blood donors and the endemicity of disease in the study population, in our study 50% of donors were between 18-30 years, largely belong to young college students. This group being better educated can understand and cooperate in answering to the risk factors in acquiring HBV infection during pre-donation questionnaire. Another study from Chandigarh (Northwestern India) by Duseja *et al*^[17] showed 0% prevalence of HBV DNA in 100 adult healthy blood donors, non-reactive for HBsAg.

Keeping in view that 8% to 18% of donor population in India is anti-HBc reactive, inclusion of anti-HBc testing will lead to high discard rate. Anti-HBs as proposed previously does not seem to predict clearance of the virus as the single donor, who tested reactive for HBV DNA in our study, had high anti-HBs titers. Cost effectiveness of introducing universal anti-HBc screening and discarding large number of blood units *versus* considering ID NAT (Individual donor nucleic acid testing) needs to be assessed. Awareness and education of donors is required regarding minor modes of HBV transmission, modification of the donor questionnaire to eliminate all donors with a history of jaundice in adult life and more stringent one-to-one donor screening to elicit such information should be implemented.

COMMENTS

Background

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.

Research frontiers

The present study was undertaken to assess the seroprevalence of antibody to hepatitis B core antigen (anti-HBc) in serum samples of healthy blood donors negative for HBsAg and to evaluate whether anti-HBc detection could be adopted in India as a screening assay for HBV in addition to HBsAg.

Innovations and breakthroughs

There is high prevalence of anti-HBc in Indian blood donors. Voluntary donors have lower incidence of anti-HBc than replacement donors. Anti-HBs does not seem to predict clearance of the virus in the blood donors.

Applications

Keeping in view that 8%-18% of donor population in India is anti-HBc reactive, inclusion of anti-HBc testing will lead to high discard rate. Cost effectiveness of introducing universal anti-HBc screening and discarding large number of blood units *versus* considering ID NAT needs to be assessed.

Peer review

This is an important and timely paper assessing the role of anti-HBc testing to detect occult HBV infectivity for addressing the important issue of TAHBV in Indian blood donors. The research was done at PGIMER Chandigarh, India, one of the premier institutes in the country. This paper deserves publication.

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