

Anti-HBc Screening of Blood Donors in Bangladesh: Relevance to Containment of HBV Propagation

Munira Jahan^{*}, Md Asadul Islam[†], Sheikh Mohammad Fazle Akbar[‡], Kazuaki Takahashi[‡], Shahina Tabassum^{*},
Atiar Rahman^{*}, Md Atiqul Haque^{*}, Joly Biswas^{*}, Shunji Mishiro[‡], Mamun Al-Mahtab[§]

^{*}Department of Virology, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh, [†]Department of Transfusion Medicine, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh, [‡]Department of Medical Sciences, Toshiba General Hospital, Tokyo, Japan and [§]Department of Hepatology, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh

Objectives: To avoid further transmission of hepatitis B virus (HBV) infection, blood is tested for hepatitis B surface antigen (HBsAg) before transfusion. However, post-transfusion hepatitis B has been detected in clinics after transfusion of HBsAg-negative blood. The study presented here was undertaken to assess if HBsAg-negative blood is free from HBV or not. **Methods:** Sera were collected from 398 blood donors who were negative for HBsAg. Out of 398 blood samples, antibody to hepatitis B core antigen (anti-HBc) was detected in 82 sera samples. HBV DNA was evaluated in HBsAg-negative, anti-HBc-positive sera. HBsAg, hepatitis B e antigen (HBeAg), antibody to HBeAg (anti-HBe), and anti-HBc in the sera were measured by an enzyme-linked immunosorbent assay (ELISA). HBV DNA was quantified by a real time polymerase chain reaction (PCR). **Results:** Out of 82 HBsAg-negative, anti-HBc-positive sera samples, HBV DNA were detected in the sera of 7 voluntary blood donors. Out of these 7 subjects, all were negative for HBeAg. The levels of ALT were more than 30 IU/L in 6 of 7 HBV DNA-positive subjects and it was above upper limit of normal (>42 IU/ml) in one subject. **Conclusions:** The present recommendation about blood transfusion of HBsAg-negative blood system is not capable of blocking HBV transmission to blood recipients. Although advanced countries have adopted nucleic acid testing (NAT) for preventing HBV transmission, developing countries may apply anti-HBc testing and ALT estimation before blood transmission. (J CLIN EXP HEPATOL 2016;6:115–118)

Important insights have been developed about epidemiology, virology, molecular biology, immunology, and pathogenesis, mode of transmission, prevention and treatment of hepatitis B virus (HBV) during last three decades.¹ Although satisfactory treatment modalities are yet to be surfaced,² significant developments have been achieved regarding prevention of HBV infection.^{3,4} Potent vaccines against HBV are widely used as part of expanded program of immunization around the world. Public health measures have been accentuated to ensure HBV-free safe childbirth. Also, attention has been given for safe

transfusion of blood. Taken together, it is now clear that HBV prevention is an achievable goal. In fact, new HBV infection has been a rare entity in most developed and advanced countries with improved health care delivery system. However, the beneficial effects of different HBV prevention programs have not been properly implemented in developing and resource-constrained countries of the world as several millions of new HBV infection emerges every year in developing countries of the world.

Bangladesh, a developing country of South-East Asia, has a population of 160 million. HBV is the most common cause of chronic liver diseases including cirrhosis of liver, hepatic failure, and hepatocellular carcinoma. The risk of acquiring new HBV infection has been reduced in Bangladesh with the introduction of hepatitis B surface antigen (HBsAg) screening in blood donors. However, with the advent of 'occult HBV infection', it is now clear that many HBV-infected subjects may not express HBsAg but may harbor HBV DNA. In fact, the blood transfusion system of Bangladesh has not been optimized to tackle these situations. In fact, studies have been conducted in neighboring countries of Bangladesh to develop insights about limitations of HBsAg-based blood screening system. A study from West Bengal, India, a neighboring Indian province of Bangladesh has reported that 21.3% HBsAg-negative and anti-HBc-positive blood donors were

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Address for correspondence: Mamun Al-Mahtab, Associate Professor, Department of Hepatology, Bangabandhu Sheikh Mujib Medical University, Shahbagh, Dhaka 1000, Bangladesh. Tel.: +880 1711567275; fax: +880 28826840.

E-mail: shwapnil@agni.com

Abbreviations: CHB: chronic hepatitis B; ETV: entecavir; ELISA: enzyme-linked immunosorbent assay; HBeAg: hepatitis B virus e antigen; HBsAg: hepatitis B virus surface antigen; HBV: hepatitis B virus; HBV DNA: hepatitis B virus deoxyribonucleic acid; NA: nucleoside analog; PCR: polymerase chain reaction; Peg IFN: pegylated interferon

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harboring HBV DNA in their blood.⁵ Another study from India also showed that 7.5% of HBsAg-negative, anti-HBc-positive blood was expressing HBV DNA.⁶ Surprisingly, 4.6% people expressing both anti-HBc and anti-HBs were also expressing HBV DNA in their blood.⁶ Presence of HBV DNA in HBsAg-negative blood donors has also been reported from other Asian countries, such as Iran that showed HBV DNA among 12.2% HBsAg-negative, anti-HBc positive donors.⁷ Studies from Lebanon and Pakistan have revealed HBV DNA among 2.8% and 2.9% HBsAg-negative, anti-HBc-positive blood donors, respectively.^{8,9} Based on these observations, policy makers and professional organizations of these countries have been trying to optimize their blood transfusion system.

However, these scientific developments have not influenced the ongoing blood transfusion program of Bangladesh, even though high prevalence of anti-HBc (22.6%) has been reported among healthy subjects of Bangladesh.¹⁰ Additionally, anti-HBc-positivity is extremely high even among children below the age of 5 years (16.3%) and increased to 30% among family members.¹⁰ In this dangerous situation, almost nothing is known about the real extent of problem with anti-HBc positivity in this country. These facts have also indirectly induced the policy making authority of Bangladesh to adopt 'nothing to do' approaches about occult HBV infection in the context of blood transfusion.

The study presented here was accomplished at the Department of Blood Transfusion of Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, the only medical university of Bangladesh that harbors most of the departments with super-specialty and sub-specialty of this country. Also, BSMMU provides directions to the Govt. of Bangladesh for designing health care delivery system. Our study reveals that a serious situation is prevailing at Bangladesh regarding blood transfusion and the so-called safe blood that was transfused to many recipients of BSMMU was in fact contaminated with HBV. This study is supposed to act as an eye opener to the policy maker of Bangladesh and also in developing countries of Asia and Africa.

METHODS

Subjects

The study was conducted at the Department of Transfusion Medicine of Bangabandhu of Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh. As our primary aim was to develop insights about safety of transfused blood at the blood center of BSMMU, a total of 398 serum samples were collected from 398 blood donors who were HBsAg-negative. The bloods of these donors have been transfused to different recipients as safe blood according to the standard criteria of Bangladesh. All blood donors

were basically voluntary blood donors. Among them, 31% were voluntary blood donors with no relation to the recipient and 69% were relatives of recipients. In addition to HBsAg negativity, they were also negative for antibody to hepatitis C virus (anti-HCV) and antibody to human immune deficiency virus-1 (anti-HIV). The study was conducted with the ethical approval of Institutional Review Board (IRB) of BSMMU and all procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008.

Study design

Although all blood donors were HBsAg-negative, anti-HBc was checked in sera of all of them by enzyme-linked immunosorbent assay (ELISA). HBV DNA was quantified with in all blood donors expressing anti-HBc by a polymerase chain reaction (PCR), as described.¹¹ HBsAg-negative patients expressing anti-HBc and HBV DNA were further checked for hepatitis B e antigen and antibody to HBeAg (anti-HBe) by ELISA using a commercial kit (Abbott Laboratories, Chicago, IL, USA).^{12,13} Serum levels of alanine aminotransferase (ALT) were determined using commercial kit at BSMMU, Dhaka, Bangladesh.¹²

RESULTS

Out of 398 subjects negative for HBsAg, anti-HBc was detected in 82 donors (20.6%). HBV DNA was checked in all these subjects by real time PCR. Out of 82 subjects, HBV DNA was detected in 7 donors (8.5%). The levels of HBV DNA have been shown in Table 1. Assessment of HBeAg revealed that all of them were HBeAg-negative. However, anti-HBe was detected in 3 of 7 subjects (Table 1). One of them had ALT above upper limit of normal (ULN) (patient no. 3) (Table 1). Although the levels of ALT were below ULN in other 6 donors, the levels of ALT were more than 30 IU/L in 5 of 6 donors.

DISCUSSION

After the introduction of serologic screening of blood donations by assessment of HBsAg, post transfusion hepatitis has been reduced substantially. With the advent of new technologies with high sensitivity and specificity for detection of HBV DNA, it became evident that a group of people do not express HBsAg, but harbor HBV DNA.^{14,15} Most of the developed and advanced countries of the world modified blood transfusion procedures on the basis of new information and donor blood have been checked for anti-HBc and ALT. Recently, nucleic acid testing (NAT) has been adopted by many of those countries to avoid transfusion-induced hepatitis.¹⁶

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