OCCULT HEPATITIS B VIRUS INFECTION AMONG BLOOD DONORS WITH ANTIBODIES TO HEPATITIS B CORE ANTIGEN

A. Jafarzadeh[1*], M. Kazemi Arababadi[1], M. Mirzaee[1] and A. Pourazar[2]

1) Department of Immunology, School of Medicine, Rafsanjan University of Medical Sciences, Rafsanjan, Iran

2) Department of Immunology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran

Abstract- Diagnosis of hepatitis B is routinely based on of serological assay of hepatitis B surface antigen (HBsAg). Occult hepatitis B virus (HBV) infection is generally defined as the detection of HBV -DNA in the serum or tissues of subjects who have negative test for HBsAg. Transmission of HBV infection has been documented from HBsAg negative, anti-HBc positive blood and organ donors. The aim of this study was to determine the rate of occult HBV infection among HBsAg negative and anti-HBc positive blood donors of Rafsanjan blood transfusion center. Sera from 270 healthy blood donors who were negative for both HBsAg and anti-HCV, were tested for anti-HBc antibodies by use of ELISA technique. The samples that were negative for HBsAg but positive for anti-HBc markers also examined for the presence of HBV-DNA by polymerase chain reaction (PCR). Out of 270 HBsAg negative blood samples, 14 samples (5.18%) were positive for anti-HBc antibodies. HBV-DNA was detected in 4/14 (28.57%) of HBsAg negative and anti-HBc positive samples. Moreover, anti-HBs antibody was detected in 2/4 (50%) of HBV-DNA positive samples. These results indicated that HBV-DNA found in the majority of HBsAg negative and anti-HBc-positive donors. In addition, the present study recommend the incorporation of routine anti-HBc screening of blood as a surrogate marker of occult HBV infection to prevent some transfusion-transmitted HBV infections.

Acta Medica Iranica, 46(1): 27-32; 2008

Key words: Hepatitis B virus infection, blood donors, anti-HBc antibody, HBV-DNA

INTRODUCTION

The risk of transfusion-transmitted HBV infection has been reduced by screening all blood donations for HBV surface antigen (HBsAg) since 1970. Although this serologic method reduces transfusion-transmitted HBV infections, some HBsAg-negative blood samples can still induce post transfusional hepatitis in recipients (1).

The frequency of post transfusional HBV infection is apparently due to the fact that HBsAg is in circulating at very low and undetectable level for screening assays, nonetheless, anti-HBc antibody screening tests are able to eliminate some of these donor units (2). Occult hepatitis B infection is defined as the presence of HBV-DNA without detectable HBsAg with or without anti-HBc or anti-HBs, outside the pre-seroconversion window period (3,4). In most cases, occult HBV infection is related to low level HBV infection with sub-detectable levels of HBsAg and not infection with HBV variants that cannot express S proteins or produce S

Received: 18 Apr. 2006, Revised: 2 Nov. 2006 Accepted: 19 Nov. 2006

*** Corresponding Author:**
Abdollah Jafarzadeh, Department of Immunology, School of Medicine, Rafsanjan University of Medical Sciences, Rafsanjan, Iran
Tel: +98 391 5234003
Fax: +98 391 5225209
E-mail: jafarzadeh14@yahoo.com

proteins with aberrant epitopes wich are not detected by conventional serological assays (5). An important observation in subjects with occult HBV infection is the low HBV-DNA levels in the serum and in the liver cells (5,6). The frequency of detection of HBV-DNA is higher in liver tissue than in serum. Because of the low amounts of HBV-DNA, sensitive polymerase chain reaction (PCR) assays, increasingly used (7). Accordingly, many reports on positive findings for HBV-DNA in the liver and blood of HBsAg-negative individuals that were positive for anti-HBc have been published. Blum et al described, the presence of HBV-DNA in a patient with HBsAg-negative chronic hepatitis who was positive for anti-HBc (8). Michalak et al demonstrated the long-term persistence of HBV-DNA in serum and peripheral blood mononuclear cells of patients up to 70 months after complete clinical, biochemical, and serologic recovery from acute viral hepatitis (9). Rehermann et al showed that traces of HBV were often detectable in the blood for many years after clinical recovery from acute hepatitis (10). Furthermore, reactivation of apparently cured HBV infection has been described in patients under of chemotherapy or immunomodulating therapy after renal and bone marrow transplantation, and in some of these cases a reverse seroconversion from anti-HBs to HBsAg has been observed (11,12). These findings suggest that recovery from acute hepatitis B virus may not result to complete virus elimination, but rather the immune system keeps the virus at very low level.

Occult HBV infection has also been reported in populations without symptomatic liver disease, such as blood donors, individuals with normal liver tests or general populations (13,14). It has been shown that blood donations from HBV carriers with of HBsAg-negative but anti-HBc-positive can cause post transfusion hepatitis B (15). PCR assay has demonstrated that some HBsAg-negative individuals and those positives for anti-HBc continue HBV replication (16). Thus, Mosley et al suggested that anti-HBc screening of blood donations might prevent HBV transmission from HBsAg-negative blood donors and the donors that are positive for anti-HBs should be considered noninfectious for HBV (17). The feasibility of routine PCR screening of blood donations in a blood bank setting has been shown by Roth et al (18).

In Iran, Amini et al. performed a study on 4930 healthy blood donors and found that 5.1% were only positive for anti-HBc without having any detectable HBsAg, however, they did not determine the presence of HBV-DNA (19). In our previous study on 545 Iranian blood donors from Isfahan city, it had been found that 8% of individuals were positive for anti-HBc antibody only, and from these group 11.62% were also positive for HBV-DNA (20). The aim of the present study was to assess the rate of anti-HBc antibody among blood donors of Rafsanjan city and to determine the frequency of HBV-DNA in HBsAg negative and anti-HBc positive samples. We evaluated to determining whether routine anti-HBc screening of blood donations could prevent some transfusion-transmitted HBY infections.

MATERIALS AND METHODS

Subjects

Between December 2004 to December 2005, blood samples were collected from 270 healthy volunteers (age range 17-59 years) referred to Blood Transfusion Center of Rafsanjan. Of them 230 were male and 40 were female. All samples were negative for Treponemal infection, HBsAg, anti-HCV, anti-HTLV-1 and anti-HIV antibodies. Before transfusion, donors were interviewed and medically examined and those with high-risk behaviors, such as intravenous abusers, had medical problems, and those received HBV vaccination were excluded from the study population. All participants were basically health, with no acute or chronic illnesses. Indeed each individual with history of chronic or acute disease and use of any drug were excluded from the study. The plasma samples from each participant were obtained and stored at -70 ºC until use. Every sample that was HBsAg-negative but anti-HBc-positive was further investigated for the presence of HBV-DNA by use of PCR technique.

Detection of HBV markers

HBs antigen, anti-HBs and anti-HBc IgG antibodies were detected by enzyme-linked immunosorbent assay (ELISA) Using commercial

kits (Radim, Italy). All serological tests were performed as instructed by the manufacturers. Anti-HBs antibody was measured by using standard samples with known concentrations of anti-HBs antibody expressed as IU/L, provided by the manufacturer.

The presence or absence of anti-HBc antibodies were determined by using the recorded absorbance and comparison with the calculated cut-off values. Samples with an optical density (OD) less than the cut-off values were considered as negative. Samples with OD higher than, or equal to, the cut-off value were also considered as positive and retested in duplicate before the final interpretation. All samples that were negative for HBsAg but positive for anti-HBc also examined for the presence of HBV-DNA by PCR assay. Detection of anti-HBs antibody also performed on all anti-HBc positive samples.

DNA extraction from plasma samples

DNA extraction performed according to the guidelines elaborated by Kowk and Higushi (21). DNA was extracted from HBsAg negative and anti-HBc positive samples and then PCR was used for detection of HBV-DNA. DNA was purified from 100 µl of plasma samples. Briefly, each serum sample was incubated at 72 ºC for 10 minutes and then cooled at 4°C for 5 minutes in 100 µl proteinase K (200 µg/ml). 100 µl of phenol-chloroform (1:1) mixed and centrifuged for 10 minutes at 12000 g. The upper phase transferred to a new tube and 1/10 volume of sodium acetate (3M) and 3 volume of 96% ethanol was added and put on ice for 30 minutes. Then the tubes were centrifuged at 12000 g for another 15 minutes. Supernatant was removed and 500 µl of 70% ethanol added to the pellets. Then the tubes were centrifuged at 12000 g for another 5 minutes. Supernatant was removed and the pellet dried for 5 minutes at 65 ºC. Finally, 30 µl autoclaved and deionized water added and stored at -20°C.

PCR amplification

PCR amplification was performed by use of a primer set selected from a highly conserved HBV surface gene (20,21). The sequence of forward primer was 5-TAT GTT TCC CTC CTG CTG CT -3 and the sequence of reverse primer was 5-CCC CCA ACT CCC AA T TCT A T-3. These primers amplify a 354 bp of the HBV genome. Five µl of extracted DNA sample was added to 25 µl of reaction mixture containing 5 units Taq polymerase, 0.01% gelatin, 0.6 µM of each primer, 200 µM of each deoxynucleotid triphosphate, 5 µl of reaction buffer (50 mM KC1, 10 mM tris-HCI, pH = 8.3) and 1.5 mM $MgCl_2$. PCR amplification was done using Touch method (20) including one cycle of 93°C for 60 sec, 60°C for 20 sec and 72°C for 40 sec. Then followed by 35 cycles of 20 sec at 93 ºC, 20 sec at 61°C and 40 sec at 72°C in an Eppendorf thermal cycler (Master cycler 5330). HBV genome provided by Sinagen company was used as positive control.

Detection of PCR product

For analyses of the PCR amplification 10 µl of the amplified samples was electrophoresed on a 2% agarose gel made in Tris acetated EDTA buffer (pH=8.0-8.5) and visualized by UV illumination after ethidium bromide (10 µg/ml) staining. Positive and negative controls were also treated as samples.

RESULTS

Serological results of HBV

A total of 270 blood donors were included in study. The serological results of HBV markers in blood donors summarized in Table 1. All participants were negative for HBsAg. 14/270 (5.18%) of blood donors were negative for HBsAg and positive for anti-HBc. Anti-HBs antibody was detected in 6/14 (42.85%) of HBsAg negative and anti-HBc positive samples with serum levels of 85, 1100**,** 530, 950, 40 and 18 IU/L.

Molecular findings

Detection of HBV-DNA performed on all samples that were negative for HBsAg and positive for anti-HBc antibody by use of PCR technique. HBV-DNA was detected in 4 out of 14 anti-HBc positive specimens (28.57%). Anti-HBs antibody was detected in serum samples obtained from 2 out 4 individuals (50%) who had HBV -DNA positive test with levels of 40 and 1100 IU/L (Table 1).

Table 1. The rates of serological HBV markers and HBV-DNA in blood donors

Total No. of blood donors	HBsAg positive rate	HBsAg negative rate	HBsAg- anti-HBc$^+$ rate	HBV-DVA positive rate	Anti-HBs positive rate*	Anti-HBs positive rate**
270	0/270	270/270	14/270	4/14	6/14	2/4
	0%	100%	5.18%	28.57%	42.85%	50%

* represent the rate of anti-HBs antibody in HBsAg- anti-HBc$^+$ samples

** represent the rate of anti-HBs antibody in HBV-DNA positive samples.

DISCUSSION

At present, HBsAg detection is the only diagnostic screening test for HBV infection in blood transfusion centers in Iran. We examined 270 HBsAg negative sera obtained from healthy blood donors and found that 5.18% of them were positive for anti-HBc, which is slightly lower than those found in Iranian population from Shiraz (22) and Isfahan (20). In transfusion, anti-HBc screening has been used initially as a surrogate marker for non-A, non-B hepatitis. Since the implementation of anti-HCV screening, this usage of the test is no longer relevant but its potential to prevent occult HBV transmission has re-evaluated (23).

The prevalence of anti-HBc only in Europe and North America is overall quite low. A prevalence of 0.07% in the UK and 1.5% in Germany was reported (24, 25) In areas of higher HBV infection prevalence about 20%-70% of subjects are positive for anti-HBc antibody (26). The prevalence of anti-HBc in our blood donor population was relatively low. This finding may have been due to the blood donor selection prior to blood collection and to regional differences in the prevalence of HBV infection. It should be noted that all of our samples were negative for anti-HIV, anti-HCV and anti-HTLV antibodies. In our study the overall prevalence of occult HBV infection (DNA in serum) in healthy blood donors was 28.57% among HBsAg negative and anti-HBc positive individuals. Regarding to the rate of samples containing HBV-DNA from blood donors, different results have been obtained in some studies. These differences in the occult HBV prevalence may be attributed to race and ethnicity, geographical area and the HBV subtypes. The frequency of HBV-DNA detected in HBsAg negative samples also varies considerably according to the prevalence of the infection. In Northern countries where the prevalence of chronic infection is less than 1%, no more than 5% of HBsAg-/anti-HBc+ blood donor samples contain HBV-DNA (25,27).

Moreover, The prevalence of HBV-DNA in only anti-HBc positive blood donors has been reported to be 0% in brazil (28), 0.3% in china (29), 1.1 % in Japan (30), 3.2% in Saudi Arabia (31) and 12.7% in Ghana (32).

In high prevalence areas, HBV-DNA detected by hybridisation or PCR is found in 4-24% of the population in India, Taiwan, Japan, and Sardinia (14). In West Africa, approximately 5% of total HBV-DNA carriers are HBsAg negative (32). The presence of anti-HBc without anti-HBs is usually called anti-HBc only. The main interpretations of anti-HBc only seropositivity is that after many years of HBV chronic carriage with non-productive infection, the level of HBsAg in the circulation becomes too low to be detected. Therefore, it might be worth considering the merits of anti-HBc screening for identification of occult HBV by testing deferred donations for HBV-DNA. However, detection of HBV-DNA in sera of individuals anti-HBc positive may be due to chronic and persistent HBV infection (33).

Some information is available regarding the infectivity of anti-HBc-only blood products or organs. The infectivity of blood donations containing anti-HBc as the only marker of HBV infection has been known for several decades and indicated that no more than 4% of recipients of anti-HBc-only blood developed HBV infection post-transfusion (1) However, Mosley reported 17% infectivity of anti-HBc-only blood products (17), although, the immune status of the recipients was not indicated.

To determine the rate of HBV transmissions via anti-HBc-positive and HBsAg-negative blood donations, retrospective studies on regular blood donors and their respective recipients are necessary. However, anti-HBc screening has the potential of excluding the vast majority of occult HBV infection. The exclusion of anti-HBc positive donors is impractical in countries where HBV infection is prevalent and greater than 20% of the populations are anti-HBc positive (34). HBV is not highly endemic in Iran and it may be practical to introduce anti-HBc screening in blood banks (22). This means that routine anti-HBc screening of blood donations would lead to a low loss of blood donors.

It has been also reported that blood components containing anti-HBc with anti-HBs, do not appear to transmit HBV and there is clearly an inverse correlation between anti-HBs level and infectivity (17). In our study HBsAg negative and anti-HBc positive individuals was further divided into two groups: with anti-HBs antibody (50%) and without anti-HBs (50%) individuals. It has been reported that HBV-DNA is found in HBsAg negative, anti-HBc positive and anti-HBs positive donors (22). In this study 2/4 of DNA detection was found in subjects who were positive for anti-HBc but negative for anti-HBs antibody. However, the rest of them (2/4) were positive for both anti-HBc and anti-HBs. It seems that anti-HBc antibody should be tested routinely on blood donor volunteers and if the sera become positive regardless of anti-HBs titer, the blood should be discarding. Further testing for HBV-DNA is appropriate to follow up the blood donor patient for HBV infection.

In conclusion, the results of present study show that 5.18% of blood donors were positive for anti-HBc antibodies. HBV-DNA found in the majority of HBsAg-negative, anti-HBc-positive donors. Routine anti-HBc screening of blood donations could probably prevent some transfusion-transmitted HBV infections.

Acknowledgements

The authors are grateful to Ebrahim Rezazadeh, Mohammad Taghi Rezayati, Maryam Nemati and authorities of the Rafsanjan blood Transfusion Center for invaluable help.

REFERENCES

1. Allain JP. Occult hepatitis B virus infection: Implication in Transfusion. Vox Sanguinis. 2004 Feb; 86(2): 83-91.
2. Saraswat S, Banerjee K, Chaudhury N, Mahant T, Khandekar P, Gupta RK, Naik S. Post-transfusion hepatitis type B following multiple transfusions of HBsAg-negative blood. J Hepatol. 1996 Nov; 25(5); 25: 639-643.
3. Hu KQ. Occult hepatitis B virus infection and its clinical implications. J Viral Hepatitis. 2002 Jul; 9(4): 243-257.
4. Torbenson M, Thomas DL. Occult hepatitis B infection. Lancet Infect Dis. 2002 Aug; 2(8); 2:479-486.
5. Lok AS. Occult hepatitis B Virus infection: diagnosis, implications and management? J Gastroenterol and Hepatol. 2004; 19:519-522.
6. Cacciola I, Pollicino T, Squandrito G, Cerenzia G, Villari D, de Franchis R, Santantonio T, Brancatelli S, Colucci G, Raimondo G. Quantification of intrahepatic hepatitis B virus (HBV) DNA in patients with chronic HBV infection. Hepatology. 2000 Feb; 31(2): 507-512.
7. Matsumoto C, Nishioka K, Oguchi T, Mitsunaga S, Nojiri N, Tadokoro K, Juji T. Detection and quantitation of HBV DNA by semi-nested PCR in donated blood: comparison with HBV serological markers. J Virol Meth. 1997 Jun; 66(1): 61-69.
8. Blum HE, Offensperger WB, Walter E, Offensperger S, Gerok W. Latent hepatitis B virus infection with full-length viral genome in a patient serologically immune to hepatitis B virus infection. Liver. 1988 Oct; 8(5):307-316.
9. Michalak TL Pasquinelli C, Guilhot S, Chisari FV. Hepatitis B virus persistence after recovery from acute viral hepatitis. J Clin Invest. 1994 Aug; 94(2):907.
10. Rehermann B, Ferrari C, Pasquinelli C, Chisari FV. The hepatitis B virus persists for decades after patients' recovery from acute viral hepatitis despite active maintenance of a cytotoxic T -lymphocyte response. Nat Med. 1996 Oct; 2(10): 1104-1108.
11. Marusawa H, Imoto S, Ueda y, Chiba T. Reactivation of latently infected hepatitis B virus in a leukemia patient with antibodies to hepatitis B core antigen. Gastroenterol. 2001 Sep; 36(9): 633-636.

12. Nordbo SA, Skaug K, Holter E, Waage A, Brinch L. Reactivation of hepatitis B virus infection in an anti-HBc and anti-HBs positive patient after allogeneic bone marrow transplantation. Eur J Haematol. 2000 Jul; 65(1): 86-87.
13. Hennig H, Puchta L Luhm J, Schlenke P, Gocrg S. Frequency and load of hepatitis B virus DNA in first-time blood donors with antibodies to hepatitis B core antigen. Blood 2002 Oct 1; 100(7): 2637-2641.
14. Allain JP. Occult hepatitis B virus infection. Transfus Clin Biol. 2004 Feb; 11(1):18-25.
15. Norder H, Hammas B, Larsen J, Skaug K, Magnius LO. Detection of HBV DNA by PCR in serum from an HBsAg negative blood donor implicated in cases of posttransfusion hepatitis B. Arch Virol Suppl. 1992;4: 116-118.
16. Gomes SA, Yoshida CF, Niel C. Detection of hepatitis B virus DNA in hepatitis B surface antigen-negative serum by polymerase chain reaction: evaluation of different primer pairs and conditions. Acta Viro1. 1996 Jun; 40(3): 133-138.
17. Mosley JW, Stevens CE, Aach RD, Hollinger FB, Mimms LT, Solomon LR, Barbosa LH, Nemo GJ. Donor screening for antibody to hepatitis B core antigen and hepatitis B virus infection in transfusion recipients. Transfusion 1995 Jan; 35(1):5-12.
18. Roth WK, Weber M, Seifried E. Feasibility and efficacy of routine PCR screening of blood donations for hepatitis C virus, hepatitis B virus, and HIV-1 in a blood-bank setting. Lancet. 1999 Jan 30; 353(9150):359-363.
19. Amini S, Mahmoodi MF, Andalibi S, Solati AA. Seroepidemiology of hepatitis B, delta and human immunodeficiency virus infections in Hamadan province, Iran: a population based study. J Trop Med Hyg 1993 Oct;96(5): 277-87.
20. Pourazar A, Salehi M, Jafarzadeh A, Kazemi-Arababadi M, Oreizi F, Shariatinezhad K. Detection of HBV DNA in HBsAg negative normal blood donors. Iran J of Immunol 2005; 2: 172-176.
21. Kwok S, Higushi R. Avoiding false positives with PCR. Nature 1989 May 18; 339(6221): 237-8.
22. Behbahani BA, Mafi-Nejad A, Tabei SZ, Lankarani KB, Rashidi M. Indication of anti-HBc antibody screening and HBV -DNA detection in diagnosing latent hepatitis B virus infection. Iran J Med Sci. 2005; 30:28-33.
23. Kleinman SI-I, and M.P. Busch MP. HBV: amplified and back in the blood safety spotlight. Transfusion. 2001 Sep; 41(9):1081-1085.
24. Hennig H, Puchta I, Luhm .1, Schlenke P, Goerg S, Kirchner H. Frequency and load of hepatitis B virus DNA in first-time blood donors with antibodies to hepatitis B core Antigen. Blood. 2002 Oct 1; 100(7): 2637-2641.
25. Allain JP, Hewitt PE, Tedder RS, Williamson LM. Evidence that anti-HBe but not HBV DNA testing may prevent some HBV transmission by transfusion. Br J Haematol. 1999 Oct; 107(1): 186-195.
26. Comanor L, Holland P. Hepatitis B virus blood screening: unfinished agendas. Yox Sang 2006 Jul;91(1):1-12.
27. Kleinman SH, Kuhns MC, Todd DS, Glynn SA, McNamara A. Frequency of HBV DNA detection in US blood donors testing positive for anti-HBc: implications for transfusion transmission and donor screening. Transfusion 2003 Jun; 43(6): 696-704.
28. Almeida-Neto C, Strauss E, Sabino EC, Sucupira MC, Chamone DA. Significance of isolated hepatitis B core antibody in blood donors from Sao Paulo. Rev Inst Med Trop Sao Paulo. 2001 Jul-Aug;43(4):203-208.
29. Ren F, Li H, Zhao H. Studies on hepatitis B virus infection in blood donors with positive anti-HBc and negative HBsAg. Zhonghua Yu Fang Yi Xue Za Zhi. 1998 Jan; 32(1):7-9.
30. Sato S, Ohashi W, Ihara H, Sakaya S, Kato T, Ikeda H. Comparison of the sensitivity of NAT using pooled donor samples for HBV and that of a serologic HBsAg assay. Transfusion. 2001 Sep; 41(9): 1107-1113.
31. Bernvil SS, Andrews V, Kuhns MC, McNamara AL. Hepatitis B core antigen antibody as an indicator of a low grade carrier state for hepatitis B virus in a Saudi Arabian blood donor population. Transfus Sci. 1997 Mar; 18(1):49-53.
32. Zahn A, Li C, Danso K, Candotti D, Owusu-Ofori S, Temple J, Allain JP. Molecular characterization of occult hepatitis B virus in genotype E-infected subjects. J Gen Virol. 2008 Feb; 89(Pt 2):409-418.
33. Grob P, Jilg W, Bornhak H, Gerlich W. Serological pattern "anti-HBc alone": report on a workshop. J Med Virol. 2000 Dec; 62(4): 450-455.
34. Nandi J, Benerjee K. Detection of hepatitis B virus DNA in donor blood by polymerase chain reaction. Natl Med J India. 1992 Jan-Feb; 5(1):5-7.