Original Research Article

SEROLOGICAL MARKERS OF HEPATITIS B VIRUS INFECTIVITY AMONG HEPATITIS B SURFACE ANTIGEN NEGATIVE BLOOD DONORS AT THE UNIVERSITY COLLEGE HOSPITAL, IBADAN.

ABSTRACT

Background: Transmission of HBV infection has been documented from hepatitis B surface antigen negative blood donors.

Objective: To determine the prevalence of serological markers of hepatitis B virus infectivity among hepatitis B surface antigen negative blood donors at the University College Hospital Ibadan.

Materials and Methods: A cross-sectional study was carried out among 490 blood donors who were negative for HBsAg. anti-HBc and other viral markers such as anti-HBs, HBeAg and anti-HBe were tested using ELISA kits by DIAPRO Diagnostic Bioprobes Milano, Italy.

Results: The mean age of participants was 32.5 years (±9.5), majority were males, 462 (94.3%). Eighty-three (16.9%) were positive for anti-HBc, out of which 35 (7.1%) had anti-HBc alone, 30 (6.1%) had both anti-HBc and anti-HBs while 18 (3.7%) were positive for anti-HBc, anti-HBs and anti-HBe. Antibody to HBsAg (anti-HBs) was detected in 54 (11%) donor samples, of which 6(1.2%) were positive for anti-HBs alone. The number of donors positive for anti-HBeAg was 18 (3.7%). However, no subject was positive for HBeAg.

Conclusion: This study has showed that some blood units containing other markers of HBV are being transfused to recipients even after screening for HBsAg is negative. These blood units are potentially infectious and can cause post-transfusion hepatitis in the recipients. There is need to consider introduction of testing for other markers of HBV infection in our blood banks.

Keywords: Blood donors, anti-HBc, HBsAg negative, HBV infectivity, Ibadan, Nigeria

INTRODUCTION

The detection of hepatitis B surface antigen (HBsAg) in blood is the mainstay in the diagnosis and screening for HBV infection in most developing countries, including Nigeria.[1,2] However, it has been reported that transmission of HBV infection by blood transfusion still occurs in a proportion of cases even if the transfused blood tested negative for HBsAg using highly sensitive assays.[3,4] Therefore, Hepatitis B virus (HBV) remains a major risk of transfusion-transmitted infection. The other modes of HBV transmission are perinatally (mother to child), close interpersonal contact with blood and other body, unsafe injection practices and sexual contact.[5] Nucleic acid testing (NAT) of all collected units of blood would give near zero risk of transfusion-associated HBV[6]. However, NAT has not been adopted in most developing countries, including Nigeria due to cost.

It is estimated that worldwide more than two billion people have been infected by HBV and 257 million have chronic infection. The HBV carrier rate variation is 1-20% worldwide. HBV infection accounts for 500,000 to 1.2 million deaths each year.[7] Studies have shown that the prevalence of HBV infection is relatively higher in the tropics particularly African region, where it has been reported to be endemic for HBV infection, accounting for the high number of patients chronically infected with HBV. [8]

The production of antibodies against HBsAg confers protective immunity and can be detected in patients who have recovered from HBV infection or in those who have been vaccinated. Antibody to HBcAg is detected in almost every patient with previous exposure to HBV. The Immunoglobulin M (IgM) subtype is indicative of acute infection or reactivation, whereas the IgG subtype is indicative of chronic infection. Antibody to HBeAg is suggestive of a nonreplicative state and one in which the antigen has been cleared.[9]

Several studies have reported the prevalence of HBsAg positivity among blood donors from various regions of Nigeria[10,11]. However data are scarce on serological markers of Hepatitis B virus infectivity among hepatitis B surface antigen negative blood donors. This study determined

the prevalence of antibodies to hepatitis B core antigen (anti-HBc), anti-HBeAg, anti-HBs, and HBeAg with the aim to determine the presence of previous HBV infection in Nigerian blood donors that might have been missed by an isolated assay of HBsAg. This would help in reducing the risk of transfusion of HBV-infected blood units with its attendant complications like liver cirrhosis and hepatocellular carcinoma.

Material and Method

Study Population and area: This was a descriptive cross-sectional study. The study population consisted of 490 consecutive consenting HBsAg negative blood donors who were also negative for HIV, HCV, Syphilis seen at the blood bank of the University College Hospital, Ibadan over a 6-month period. Other inclusion criteria include the following age range 17 to 65 years; haemoglobin concentration (Hb) greater than 13.5 g/dL in males and greater than 12.5 g/dL in females and nil blood donation in the previous 3 months.

Ethical clearance: Ethical approval was obtained from the Joint Ethical committee of the University of Ibadan and University College Hospital Ibadan before the commencement of the study.

Sample collection: Semi-structured, self-administered questionnaire was used to obtain subjects' sociodemographic details. Five (5mls) millimeters of venous blood was collected in a plain vacutainer tubes from the participants after obtaining a written informed consent. The blood was allowed to clot and sera separated by centrifugation at room temperature at 3000 gyration, and stored at -20^{0}C in the deep freezer until analyzed.

Laboratory Investigation: All samples were screened for HBsAg, using Monolisa HBsAg ULTRA by BIORAD which is a sandwich third generation enzyme linked immunosorbent assay (ELISA) according to the manufacturer's instructions. All samples found to be negative for HBsAg were further tested for anti-HBs, anti-HBc, HBeAg and anti-HBeAg using HBsAb ELISA Kit (DIA.PRO Milano Italy), HBcAb ELISA kit (DIA.PRO Milano Italy), HBeAg & Ab ELISA Kit (DIA.PRO Milano Italy) respectively.

Statistical Analysis: Data collected were subjected to descriptive and inferential statistical analysis using the SPSS version 20 (SPSS Inc, Illinois, USA). Quantitative variables were summarized using mean and standard deviation while qualitative variables were summarized in frequencies and proportions. Level of significance was set at 5%.

RESULTS

A total number of 550 blood donors were screened for Hepatitis B surface antigen using ELISA, out of which 60 (10.9%) were positive while 490 (89.1%) tested negative.

Of the 490 that tested negative for HBsAg, 462 (94.3%) were males and 28 (5.7%) were females giving a male to female ratio of 17:1.Their ages ranged from 18-60 with a mean of 32.5 ± 9.5 years. More than half of them were married, 290 of 490 (59.2%) while the remaining 200 (40.8%) were single. Forty-two (8.6%) had primary education while 237 (48.4%) and 210 (43%) had secondary and tertiary education respectively. Two hundred and one (41%) were employed while 71 (14.5%) and 218 (44.5%) were unemployed and students/ housewives respectively.

Eighty-nine of the 490 (18.2%) prospective donors considered fit for blood donation based on Hepatitis B surface antigen negativity were found to be positive for at least one other serological marker (anti-HBc, anti-HBs and anti-HBe) of Hepatitis B virus infection. The sero-prevalence of anti-HBc was 83 (16.9%), out of which 35 (7.1%) were positive for anti-HBc alone, 30(6.1%) were positive for both anti-HBc and anti-HBs and 18 (3.7%) were positive for anti-HBc, anti-HBs and anti-HBe. Anti-HBs was detected in a total of 54 (11%) donor samples, however, only 6 (1.2%) were positive for anti-HBs alone. The prevalence of anti-HBe was 3.7% (18 of 490). No subject was positive for HBeAg (Table 1 and 2).

Table 1: Prevalence of hepatitis B virus markers in Hepatitis B surface antigen negative blood donors.

HBV marker	No. Examined	Prevalence (%) (n =490)
Anti-HBc	**83**	**16.9**
Anti-HBs	**54**	**11.0**
Anti-HBe	**18**	**3.7**
HBeAg	**0**	**0**
Total	**89[a]**	**18.2**

111

112 *a = presence of more than one marker is common*

113 *Anti-HBc= antibody to hepatitis B core antigen; anti-HBs = antibody to hepatitis B surface*
114 *antigen; anti-HBe = antibody to hepatitis B e antigen. HBV= Hepatitis B virus*

115 **Table 2: Serological characteristics of Hepatitis B surface antigen negative blood donors.**

Characteristics	No. Examined (%)
Anti-HBc only	35 (7.1)
Anti-HBc + Anti-HBs	30 (6.1)
Anti-HBc+ Anti-HBs + Anti-HBe	18 (3.7)

Anti-HBs only	6 (1.2)
Anti-HBe only	0
HBeAg	0

DISCUSSION

Hepatitis B virus transmission through blood transfusion is still a great source of concern despite screening for hepatitis B surface antigen (HBsAg) in blood; which is the mainstay of diagnosis for HBV infection in most blood banks in developing countries, including Nigeria. The first serological marker of HBV infection is HBV DNA, followed by HBsAg and HBeAg. Thereafter, anti-HBc, anti-HBe and anti-HBs appear. Antibody to hepatitis B core antigen is the first antibody to appear following acute hepatitis B infection and persists at high level following resolution of infection. [12] It is a marker of acute, chronic or resolved infection, although, the degree of protection depends on anti-HBs levels. Anti-HBc remains detectable for life [13] and its significance in screening of blood donors as a way of reducing the residual risk of post transfusion hepatitis B infection has been investigated.[14]

There have been concerns about risk of transfusion transmissible HBV infection from blood donors in whom anti-HBc is the only detectable hepatitis B virus marker with no evidence of HBsAg or anti-HBs, particularly in highly endemic regions. The prevalence of "anti-HBc only" in this study of 7.1% is similar to prevalence of 8% reported by Pourazar *et al* among Iranian blood donors [15]. El-Zaatari *et al* [16] and Salawu *et al* [17] reported a lower prevalence of 3.7% and 4.4% in Lebanon and Ife respectively among blood donors. However, higher prevalence rates of 18.9% and 30.1% respectively were reported by Asim et al. [18]and Panigrahi *et al* [19].

The variations in the seroprevalence of anti-HBc in blood donors may be due to differences in the prevalence of HBV infection in these regions. It may also be due to difference in the specificity, sensitivity and positive predictive value of the test method. Likewise, the difference in the socio-cultural practices such as tattooing, scarifications, may explain the variations

observed. Countries with intervention measures and health policies such as access to health care, immunization practices as found in developed countries are bound to have lower prevalence rate reported. Co-infection of HBV with Human immunodeficiency virus and Hepatitis C virus as suggested by some authors could down-regulate the synthesis of HBsAg.[20,,21] The importance of anti-HBc in screening for occult HBV infection has been argued extensively. Studies have demonstrated that some HBsAg-negative individuals but anti-HBc positive continue HBV replication.[22, 23] The infectivity of blood donations positive for anti-HBc only was reported by Allain *et al* [24] as 4% in immune competent recipients. However, Mosley *et al* [25] reported 17% infectivity of anti-HBc only blood products, although the immune status of the recipients was not indicated. In order to determine the rate of HBV transmission via anti-HBc- positive and HBsAg-negative blood donations in this environment, a retrospective studies on regular blood donors and their respective recipients will be necessary.

In this study, both anti-HBc and anti-HBs were found in 30 of 89 (33.7%) individuals constituting 6.1% of the total number (30/490) tested for HBV markers. These subjects were considered to be previously infected and to have become immune to HBV infections. It has been documented that blood components positive for anti-HBc and anti-HBs do not appear to transmit HBV and there is clearly an inverse correlation between anti-HBs level and infectivity.[26] However, on the contrary, the presence of anti-HBs is not a sign of total HBV eradication as being suggested by Thedja *et*al [27]. Reactivation of HBV infection despite high levels anti-HBs levels has been revealed by Gartner *et al* [28]and further reported by Levicnik-Stezinar *et al* [29]. Manzini *et al* [30] observed that some blood donors with high titres of anti-HBs, over 100IU/L still had detectable HBV DNA. In a more recent study, Ashim *et al* [31] reported HBV DNA positive cases were detected in donors with low titres of anti-HBc positive and anti-HBs positive antibodies.

No participant was found positive for hepatitis B e antigen in this study. This is similar to findings by Japhet *etal* [32] in Ife, Nigeria, but in contrast with finding by Salawu *etal* [17] who reported a prevalence of 0.22% (1 of 459) in Ile-Ife. In similar studies done in Africa, El-Ghitany *et al* [33] reported 0.4% among Egyptian blood donors, while Ashim *etal* (2010)[31], in India found none of the subjects positive for HBeAg. The presence of HBeAg is associated with relatively high infectivity and severity of HBV infection.

This study reveals under-diagnosis of HBV infection with the use of only HBsAg as its surrogate marker and suggests that anti-HBc antibody should be tested routinely in addition to surface antigen in our blood banks.

CONCLUSION

The result in this study highlights the high prevalence of Hepatitis B core antibody in Hepatitis B surface antigen negative blood donors in Ibadan, Southwestern Nigeria. There is need to further screening of our blood donors for other serological markers of HBV even if we cannot embark on Nucleic acid testing due to cost.

RECOMMENDATION

1. Anti-HBc screening of blood donations should be advocated as part of the National policy on screening in blood banks with the view of curtailing transmission of HBV through this route.
2. There is need for a large multi-centre study to determine prevalence of occult hepatitis B infection among blood donors in Nigeria and its implications for blood transfusion.
3. Retrospective studies should be carried out on regular blood donors and their respective recipients to determine the rate of HBV transmission via anti-HBc-positive and HBsAg-negative blood donations.

LIMITATIONS OF THE STUDY

1. The sample size may not be fully representative of the entire donor population of the blood donors of the hospital.
2. Hepatitis B virus DNA was not done due to limited resources.

REFERENCES

1 Ola SO, Otegbayo JA, Odaibo GN, Olaleye OD, Olubuyide OI. Serum hepatitis C and hepatitis B surface antigenaemia in Nigerian patients with acute icteric hepatitis. West Afr J Med 2002;21:215-217

2 Ajayi AO, Komolafe AO Ajumobi K. Seroprevalence of hepatitis B surface antigenaemia among health care workers in a Nigerian tertiary health institution. Niger J Clin Pract 2007;10:287-289

3 Zervou EK, Dalekos GN, Boumba DS, Tsianos EV. Value of anti-HBc screening of blood donor for prevention of HBV infection: results of 3-year prospective study in Northwestern Greece. Transfusion 2001;41:652-658.

4 Kaviani MJ, Behbahani B, Mosallaii MJ, Sari-Aslani F, Taghavi SA. Occult hepatitis B virus infection and cryptogenic chronic hepatitis in an area with intermediate prevalence of HBV infection. World J Gastroenterol 2006;12:5048-5050.

5 Custer B, Sullivan SD, Hazlet TK, Iloeje U, Veenstra DL, Kowdley KV. Global epidemiology of hepatitis B virus. J Clin Gastroenterol 2004;38:158-168

6 Susan L. Stramer, Ulrike Wend, Daniel Candotti,, Gregory A. Foster, F. Blaine Hollinger, Roger Y. Dodd, et al. Nucleic Acid Testing to Detect HBV Infection in Blood Donors.N Engl J Med 2011; 364:236-247

7 World Health Organization (WHO) : GLOBAL HEPATITIS REPORT,2017 (http://www.who.int/hepatitis/publications/global-hepatitis-report2017/en/).

8 N Shahab and S Saqib. Epidemiology of Hepatitis A, B, C. IJIRB 2017;1(1):42-45

9 Hannachi N, Hidas S, Harrabi I, Mhala S, Marzouk M, Ghzel H et al. Seroprevalence and risk factors of HBV among pregnant women in central Tunisia. Pathol. Biol. 2009;42: 115-120.

10 A.M. Adekeye, A.A. Chukwuedo, P.N. Zhakom and R.S. Yakubu. Prevalence of Hepatitis B and C among Blood Donors in Jos South LGA, Plateau State, Nigeria. Asian J. Med. Sci.,5(5): 101-104, 2013

11 Okwesile A, Usman I, Abubakar W, Onuigue F, Erhabor O, Buhari H, et al. Prevalence of transfusion-transmissible hepatitis B infection among blood donors in Sokoto, North Western, Nigeria. Health Sciences Research. 2014:1(4);113-118

12 Allain JP. Occult Hepatitis B infection. Transfus. Clin Microbiol. 2004;11:18-25

13 Al-Mekhaizeem KA, Miriello M, Sherker AH. The frequency and significance of isolated hepatitis B core antibody and the suggested management of patients. CMAJ 2001;165:1063-1064

14 Behzad-Behbahani A, Mafi-Nejad A, Tabei SZ, Lankarani KB, Torab A, Moaddeb A. Anti-HBc and HBV DNA detection in blood donors negative for hepatitis B virus surface antigen in reducing the risk of transfusion associated HBV infection. Indian J Med Res 2006;123:37-42

15 Pourazar A, Salehi M, Jafarzadeh A, Arababadi MK, Oreizi F, Shariatinezhad K. Detection of HBV DNA in HBsAg negative normal blood donors. Iran J. Immunol, 2005;2(3):172-176
16 El-Zaatari M, Kazma H, Naboulsi-Majzoub M, Haidar M, Ramlawi F, Mahfoud Z, et al. Hepatitis B virus DNA in serum of anti-HBc only' positive healthy Lebanese blood donors: significance and possible implications. J Hosp Inf. 2007;66(3): 278-282
17 Salawu L, Adegoke AO, Aboderin AO, Huraina HA. Hepatitis B viral markers in surface antigen negative blood donors: The need to look beyond antibody Negativity. West Afr J Med 2011;30(4): 292-295
18 Ashim M, Ali R, Khan LA, Hussain SA, Singla R, Kar P. Significance of anti-HBc screening of blood donors and its association with occult hepatitis B virus infection: Implications for blood transfusion. Indian J Med 2010;132:312-317
19 Panigrahi R, Biswas A, Datta S, Banerjee A, Chandra PK, Mahapatra PK et al. Anti-hepatitis B core antigen testing with detection and characterization of occult hepatitis B virus by an in-house nucleic acid testing among blood donors in Behrampur, Ganjam, Orissa in Southern India: Implications for transfusion. Virol J. 2010;7: 204
20 Berger A, Presier W, Doerr HW. The role of viral load determination for the management of human immunodeficiency virus, hepatitis B virus and hepatitis C virus infection. J Clin Virol 2001;20:23-30
21 Carman WF, Van Deursen FJ, Mimms LT, Hardie D, Coppola R, Decker R, et al. The prevalence of surface antigen variants of hepatitis B virus in Papua Guinea, South Africa and Sardinia. Hepatology 1997;26:1658-1666
22 Kaminski G, Alnaqdy A, Al-Belushi I, Noqrales J, Al-Dhahry SH. Evidence of occult hepatitis B virus infection among Omani blood donors: a preliminary study. Med Princ Pract 2006;15:368-372
23 Zahn A, Li C, Danso K, Candotti D, Owusu-Ofori S, Temple J et al. Molecular characterization of occult hepatitis B virus in genotype E-infected subjects. J Gen Virol. 2008; 89(2): 409-418.
24 Allain JP, Hewitt PE, Tedder RS, Williamson LM. Evidence that anti-HBc but not HBV DNA testing may prevent some HBV transmission by transfusion. Br J Haematol 1999;107:186-95
25 Mosley JW, Stevens CE, Aach RD, Hollinger FB, Mimms LT, Solomon LR, et al. Donor screening for antibody to hepatitis B core antigen and hepatitis B virus infection in transfusion recipients. Transfusion 1995;35 (1): 5-12
26 Allain JP, Hewitt PE, Tedder RS, Williamson LM. Evidence that anti-HBc but not HBV DNA testing may prevent some HBV transmission by transfusion. Br J Haematol 1999;**107**:186-95

27 Thedja MD, Roni M, Harahap AR, Sirega NC, Ie SI, Muljono DH. Occult hepatitis B in blood donors in Indonesia: Altered antigenicity of the hepatitis B virus surface protein. Hepatol. Int. 2010;4: 608-614

28 Gartner BC, Jung W, Welsch C, Fischinger J, Schuber J, Zeuzem S, et al. Permanent loss of anti-HBc after reactivation of hepatitis B virus infection in an anti-HBs and anti-HBc-positive patient after allogeneic stem cell transplantation. J ClinVirol 2007;38:146-148

29 Levicni-Stezinar S, Rahne-Potokar U, Candotti D, Lelie N, Allain JP. Anti-HBs positive occult hepatitis B virus infections in two transfusion recipients. J. Hepatol 2008;48: 1022-1025

30 Manzini P, Girroto M, Borsotti R, Giachino O, Guaschino R, Lanteri M, et al. Italian blood donors with anti-HBc and occult hepatitis B virus infection. Haematologica. 2007; 92: 1664-1670

31 Ashim M, Ali R, Khan LA, Hussain SA, Singla R, Kar P. Significance of anti-HBc screening of blood donors and its association with occult hepatitis B virus infection: Implications for blood transfusion. Indian J Med 2010;132:312-317

32 Japhet MO, Adesina OA, Donbraye E, Adewumi MO. Hepatitis B Core IgM antibody (anti-HBcIgM) among hepatitis B surface antigen (HBsAg) negative blood donors in Nigeria.Virol J. 2011;8:513.

33 El-Ghitany EM, Farghaly AG. Serological pattern of hepatitis B virus among HBsAg negative blood donors in Alexandria, Egypt. EMHJ 2013;19(7):600-607