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ORIGINAL ARTICLE

Hepatitis B Viral Markers in Surface Antigen Negative Blood Donors: The Need to Look Beyond Antibody Negativity

Contre l'hépatite B marqueurs viraux chez les donneurs de sang Antigène de surface négative: la nécessité de regarder au-delà d'anticorps Négativité

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ABSTRACT

BACKGROUND: **The presence of Hepatitis B Virus (HBV) in blood that is Hepatitis B Surface Antigen (HBsAg) negative is considered a potential risk for transmission of hepatitis B virus infection.**

OBJECTIVE: **To determine prevalence of antibodies to markers of hepatitis B virus infection in HBsAg negative prospective blood donors.**

METHODS: **A structured questionnaire to assess prospective donor's demographic data and past medical history was administered to 457 consenting HBsAg negative subjects. All the subjects were also negative for antibodies to hepatitis C virus (HCV), human immunodeficiency virus (HIV) and syphilis. Their serum samples were tested for the presence of anti-HBc, anti-HBe, anti-HBs and HBeAg.**

RESULTS: **Of the 457 samples tested, 20 (4.37%), 58 (12.69%), 1 (0.22%), and 1 (0.22%) were positive to anti-HBc, anti-HBs, anti-HBe, and HBeAg antibodies, respectively. Ten (50%) of those who were positive for HBc antibody were also positive to anti-HBe and anti-HBs. Similarly, two (3.4%) donors who were positive for anti-HBs were also positive for HBeAg and anti-HBe. Of the 20 who were anti-HBc positive, seven had tattoo/traditional marks on their body and one had previous history of blood transfusion.**

CONCLUSION: **This study has shown that some potential blood units containing HBV are being transfused to patients unknowingly by screening for HBsAg only. Screening for other markers of hepatitis B virus may increase the rejection rate, but will reduce HBV transmission. WAJM 2011; 30(4): 292–295.**

Keywords: **HBsAg negativity, anti-HBc, anti-HBs, anti-HBe, HBe antigen, Blood donors, Teaching Hospital, Nigeria.**

RÉSUMÉ

CONTEXTE: La présence de l'hépatite B (VHB) dans le sang qui est l'antigène de surface (HBsAg) négatif est considéré comme un risque potentiel de transmission du virus de l'hépatite B.

OBJECTIF: Déterminer la prévalence des anticorps marqueurs des virus de l'hépatite B dans AgHBs négatif donneurs de sang potentiels.

MÉTHODES: Un questionnaire structuré pour évaluer les données démographiques des donneurs potentiels et les antécédents médicaux a été administré à 457 sujets consentants AgHBs négatif. Tous les sujets ont également été négatifs pour les anticorps de l'hépatite C (VHC), virus de l'immunodéficience humaine (VIH) et la syphilis. Leurs échantillons de sérum ont été testés pour la présence d'anticorps anti-HBc, anti-HBe, anti-HBs et HBe.

RÉSULTATS: Sur les 457 échantillons testés, 20 (4,37%), 58 (12,69%), 1 (0,22%) et 1 (0,22%) étaient positifs aux anticorps anti-HBc anti-HBs, anticorps anti-HBe, et HBAg anticorps, respectivement. Dix (50%) de ceux qui étaient positifs pour les anticorps HBc étaient également positifs pour l'anti-HBe et anti-HBs. De même, deux (3,4%) des donateurs qui se sont avérés positifs pour l'anti-HBs étaient également positifs pour l'AgHBe et anti-HBe. Sur les 20 qui étaient anti-HBc positif, sept avaient tatouages / traditionnels sur leur corps et un seul avait des antécédents de transfusion sanguine.

CONCLUSION: Cette étude a montré que certaines unités de sang potentiels contenant du VHB sont transfusées à des patients sans le savoir par le dépistage de l'HBsAg seulement. Dépistage d'autres marqueurs de l'hépatite B pourrait augmenter le taux de rejet, mais permettra de réduire la transmission du VHB. **WAJM 2011; 30(4): 292–295.**

Mots-clés: la négativité AgHBs, anticorps anti-HBc, anti-HBs, les donneurs de sang anti-HBe, l'antigène HBe, au Nigeria.

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Abbreviations: HAART, Highly Active Anti-retroviral Therapy; **HBV,** Hepatitis B Virus; **HBsAg,** Hepatitis B Surgice Antigen; **HCV,** Hepatitis C Virus; **HIV,** Human Immunodeficiency Virus **TTIs,** Transmission Transmissible Infections.

INTRODUCTION

Screening for transfusion transmissible infectious (TTIs) agents is an established procedure in Blood Banks globally before accepting an individual to donate blood. Most important of these TTIs screened for include hepatitis viruses (including Hepatitis B and C), HIV, and syphilis. Hepatitis B virus (HBV) infection screening is particularly important because it is a major public health problem affecting about 350 million people worldwide.[1] Hepatitis B virus induce wide spectrum of clinical forms, ranging from a healthy carrier state, acute hepatitis B infection, and chronic hepatitis infection, which could lead to cirrhosis and Hepatocellular carcinoma.

Most Blood Banks in resource-limited economies screen for hepatitis B virus infection mainly by screening for the hepatitis B surface antigen (HBsAg) and bleed donors based on its negativity.[2] Advances in the genomic amplifica-tion of viral DNA have, however, shown that it is possible to carry hepatitis B virus and still be negative on screening for HBsAg. This is known as occult hepatitis B infection.[3] Screening for occult hepatitis B infection is important to reduce the risk of HBV transmission through blood transfusion. Occult hepatitis B infection has been shown to increase hepatocellular carcinogenesis by eight times in patients with non-B, non-C liver cirrhosis.[4] Similarly, HBV may be associated with low CD4+ lymphocyte count and could lead to a flare of hepatitis in HIV patients when started on highly active anti-retroviral therapy (HAART).[5] Occult hepatitis B infections may also cause cryptogenic liver disease and fulminant hepatitis as well as affect disease progression and treatment response of chronic hepatitis C.[6]

Since hepatitis B virus infection depends on the infectious dose of the virus received by the individual, immuno-competence of the host[7] and transfusion of anti-HBc positive donor to immuno-compromised subjects.[8] The main objective of this study, therefore, was to evaluate the burden of occult hepatitis infection in the Nigerian setting where majority of the donors are paid commercial donors.

SUBJECTS, MATERIALS, AND METHODS

Subjects

Four hundred and ninety-five consecutive donors were investigated from the Blood Bank unit of the department of Haematology and Blood Transfusion, Obafemi Awolowo University Teaching Hospitals Complex (OAUTHC), Ile-Ife; after obtaining approval from the Ethics and Research committee of the hospital.

Materials and Methods

A structured questionnaire was administered to participants. Information obtained included donor's demographic data, past medical history, history of blood transfusion, and risk behaviour to hepatitis B infection and hepatitis B virus vaccination. Five ml of venous blood was obtained aseptically from each partici-pant. The serum was separated and stored at –20°C until analysed.

The specimens of donors that were negative for HBsAg (and also for antibody to HIV, HCV, syphilis) were tested for antibodies to hepatitis B virus core antigen (anti-HBc), surface antigen (anti-HBs), envelope antigen (anti-HBe), hepatitis B envelope antigen (HBeAg), using commercially available rapid ELISA kit (LumiQuick Diagnostic Inc., USA).

Statistical analysis was carried out using descriptive and inferential statistics (SPSS Inc., Chicago, IL, USA, Windows, version 16).

RESULTS

Table 1 shows the demographic and serological characteristics of the donor population. Of the 495 donors screened, 443 were male and 52 female, giving a male to female ratio of 9:1. Three hundred and thirteen (63.2%) had donated blood in the past, while the rest (36.8%) were first-time donors. Their ages ranged from 18 to 59 years with a mean of 29.4 $\pm$ 8.4 years. The majority of the donors (55.8%) were in the age bracket 21–30 years. Most of the donors (55.9%) were unmarried. The majority had not received blood transfusion ((98.6%) nor received hepatitis B virus vaccine (95.2%) in the past.

Of the 495 screened blood donors, 457 were negative to HBsAg, HIV, HCV and syphilis). Eighty (17.5%) of these prospective donors considered fit for donation based on HBsAg negativity were found to be positive for various other markers (anti-HBc, anti-HBs, anti-HBe, and HBeAg) of hepatitis B virus infection. The majority [56(70%)] of them were first-time donors. Of these, only one had history of blood transfusion in the past, while 10 had traditional and/or tattoo marks on their body. Ten had received hepatitis B vaccination. Twenty (4.37%), 58 (12.69%), 1 (0.22%), and 1 (0.22%) were anti-HBc, anti-HBs, anti-HBe, and HBeAg positive, respectively. Ten of those who were positive for HBc antibody were also positive to anti-HBe and anti-HBs antibodies. Similarly, two donors who were positive for anti-HBs were also HBeAg and anti-HBe positive. Of the 20 who were anti-HBc positive, 15(75%) were first-time donors; seven had tattoo/traditional marks on their body and one had previous history of blood transfusion. Two had received hepatitis B vaccine. Of those who were anti-HBs positive, the majority (68.96%) were first-

Table 1: Demographic and Serological Characteristics of all Donors

Characteristics	Number(%)
All Donors (Male : Female)	495(9:1)
Sex	
Males	443(89.5)
Females	52(10.5)
Age Groups (years)	
18 – 20	46(9.2)
21 – 30	279(55.8)
31 – 40	110(22.0)
41 – 50	48(9.6)
51 – 59	12(2.4)
First-time Donors	182(36.8)
Received Blood Transfusion in the past	7(1.4)
Received HBV Vaccine	24(4.8)
HBsAg Positive	38(7.6)
HBsAg Negative	457(92.3)
Anti-HBc (+) only	20(4.4)
*anti-HBc (+) +anti-HBe (+) + anti-HBs (+)	10(50)
Anti-HBs (+) only	58(12.7)
*anti-HBs (+) +HBeAg (+) + anti-HBe (+)	2(3.4)
Anti-HBe (+) only	1(0.22)
HBeAg (+) only	1(0.22)

** Presence of multiple antibodies in anti-HBc- and HBs-positive blood donors*

time donors, none had received blood transfusion in the past and only three had tattoo/traditional marks on their body. Seven of them had received hepatitis B vaccine (Table 2).

Table 2: Characteristics of Antibody Positive Blood Donors

	Number (%)	Positive
	Anti-HBc	Anti-HBs
First-time donor (%)	15(75)	40(69)
Received blood transfusion	1	0
Had tattoo/traditional marks	7	3
Received HBV vaccine	2	7

DISCUSSION

The detection of HBV DNA in the liver and/or serum, in individuals without detectable HBsAg in circulation has been termed occult hepatitis B virus infection (OBI).[1] The use of nucleic acid testing (NAT) has not been adopted in most developing countries, including Nigeria, obviously due to the cost and technology required. However, several studies have shown varying proportions of those positive for other markers of HBV infection aside from HBsAg with detectable HBV DNA to be capable of transmitting the virus.[9–15]

Of the markers detected as evidence of previous HBV infection, 20 (25%) were anti-HBc, with five of them also associated with anti-HBs; while the majority (58 or 72.5%) were anti-HBs. Antibodies to hepatitis B core antigen (anti-HBc) are markers of acute, chronic, or resolved HBV infection.[15] Its use in screening blood donors as a way of reducing the residual risk of post transfusion HBV infection has been variously investigated with varying prevalence. Hennig *et al*[9] reported a prevalence of 1.59% in their first-time blood donors, while Manzini *et al*[16] reported a prevalence of 4.86% from their own blood donors with positive HBV DNA. Behzad-Behbahani *et al*[15] and Panigrahi *et al*[17], reported a higher prevalence rates of 12.2 % and 30.1%, respectively, with positive HBV DNA. However, Ramezani *et al*[18], Sofian *et al*,[19] and Perez-Rodriguez *et al*,[20] reported prevalence rates of 2.07%, 2.1% and 29.4%, respectively in their blood donors, without the detection of HBV DNA in the serum. However despite non-detection of HBV DNA in serum, there have been reports of post transfusion HBV infection in recipients of blood positive for anti-HBc alone.[21]

Antibody to HBsAg (anti-HBs), though an evidence of recovery from HBV infection and protective immunity, anti-HBs samples have been shown to contain HBV DNA.[16] In a study of lymphoma and HIV positive patients, Koo *et al*[22] found 3.2% of lymphoma patients with positive anti-HBs to have detectable HBV DNA in their serum. Awerkiew *et al*[12] and Wu *et al*[14] have also reported the case of anti-HBs positive but negative for anti-HBc lymphoma patient who had reactivation of occult HBV infection on initiative anti-cancer chemotherapy. These reports suggest that despite negativity of HBV DNA, serum containing anti-HBs are capable of transmitting HBV infection. It is also important to note that immune complex formation between HBsAg and its antibody (anti-HBs) could result in false negative HBsAg. About 26.8% of the anti-HBc positive donor initial negative for HBsAg have been found to be positive for the surface antigen following immune complex dissociation,[11] thereby emphasising the importance of anti-HBc as an important marker of occult HBV infection that may reduce post transfusion HBV infection.

It is worthy of note that evidence of HBV infection was also found in ten of the donors who received hepatitis B vaccination. This finding has also been reported in a prospective study by Xu *et al*[23] who followed a neonatal HBV vaccination cohort until the age of 19 – 21 years. They found 4.2% of the vaccinees to be HBsAg negative, but positive for anti-HBc and anti-HBs, with 81 of 106 positive for HBV DNA.

It can be concluded from this study that there might have been under-diagnosis of the prevalence hepatitis B virus infection in our blood donors by the use of negativity for HBsAg alone. Eighty of those considered fit for donation based on HBsAg negativity were found to be positive for various other markers of hepatitis B infection suggesting previous exposure to the virus. It is particularly worrisome as 70% of those under-diagnosed were first-time donors and that 10 of them claimed to have received HBV vaccination. This has shown that HBsAg negativity alone may not be sufficient even in low economy setting as ours. There is need to further screen our blood donors for other markers of HBV infection even if we cannot embark on NAT due to cost. The high prevalence of HBV infection in our low economic environment could probably be due to transfusion of units with occult HBV, hence the need to consider introduction of testing for markers of HBV infection in our Blood Banks.

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