Hepatitis B Virus Infection in Low and Middle – Income Countries: Combined Serological Markers for Efficient Diagnosis

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Abstract: Hepatitis B virus (HBV) infection is a global problem with Asia and sub-Saharan Africa mostly affected. Unfortunately, residual risk of transfusion associated HBV (TAHBV) is greater in low- and middle-income countries where virus prevalence is higher and implementation of Nucleic Acid Testing (NAT) and/or anti-HBc testing remain high-priced due to cost and loss of donors/blood products. There is therefore the need for cheaper and practical alternatives to reducing TAHBV. For this study, blood samples were collected from 273 consenting blood donors, aged 18-60 years. Five HBV serological markers: HBV surface and envelope antigens (HBsAg, HBeAg), and HBV core, surface and envelope antibodies (anti-HBc,anti-HBs, HBeAb) were detected using Enzyme Linked Immunosorbent Assays. A high anti-HBs prevalence of 37.7% was detected among the donors while HBsAg prevalence was 5.1%, a rate lower than 8% value for high endemic regions to which Nigeria is classified. Among the donors HBcIgM prevalence was 4.8% (13/273), with twelve donors (4.4%; 12/13) having anti-HBc IgM as the only detectable marker of HBV infection. Anti-HBs presence of 200 mIU/mL or more has been reported safe as a transfusion component in anti-HBc-positive blood. A high anti-HBs observed among blood donors in this study could be explored in routine HBV screening of anti-HBc-positive blood donors. Including anti-HBs screening and anti-HBc IgM found as the only HBV infection marker in 12 (4.4%) donors could reduce TAHBV in Nigeria where HBV NAT screening is not affordable and discarding anti-HBc IgG-positive blood not feasible because blood transfusion is critical to treatment of diverse pathologies.

Keywords: Hepatitis B Virus, Diagnosis, blood transfusion, serological markers, Nigeria.

INTRODUCTION

In sub-Saharan Africa (SSA), blood transfusion is critical to the treatment of diverse pathologies including malaria-associated anaemia, obstetric haemorrhage, and trauma, hence the need for safe blood. Despite these need, access to a safe and adequate blood supply remains an enduring public health challenge in much of SSA (Weimer *et al.,* 2019). Hepatitis B Virus (HBV) infection is one of the most important Transfusion Transmissible Infections (TTI). It is a global health problem, with an estimated 257 million chronic hepatitis B surface antigen (HBsAg) carriers and about 500, 000 to 1.2 million deaths occurring annually with SSA accounting for 87, 890 HBV deaths annually (Stanaway *et al.,* 2016; WHO, 2017). Transmission of HBV is common through blood transfusion, aside other routes of transmission such as sexual contact, close interpersonal contact through blood and body fluids, mother-to-child (vertical), intravenous drug use (IVDU), and unsafe traditional practices including tattoos, ear piercing, circumcision among others (Ogunfemi *et al.,* 2017).

Despite the introduction of universal hepatitis B vaccination and effective antiviral therapy, the estimated overall seroprevalence of hepatitis B surface antigen (HBsAg) remains high in Africa (Spearman *et al*., 2017). Transmission of HBV through blood transfusion could account for one of the reasons for this, amidst others. The risk of transfusion transmitted HBV has been greatly reduced in developed countries by combining sensitive HBsAg assays, nucleic acid testing (NAT) and in some places, antibodies against the HBV core antigen (anti-HBc) screening (Candotti *et al*., 2019; Prati and Valenti 2019; Velati *et al.,* 2019) but the residual risk of transmission of HBV via transfusion has been reported

to be greater in low and middle income countries, where the prevalence of the virus is higher and the implementation of anti-HBc testing and/or NAT for HBV DNA is not affordable (Candotti *et al*., 2019; Prati and Valenti 2019).

Hepatitis B viral infection is the most common cause of hepatitis and it leads to serious liver disease such as cirrhosis and hepatocellular carcinoma (Prabina *et al.,* 2019), hence, a country should do everything possible to limits its transmission. Implementation of both HBsAg and anti-HBc, and HBV NAT have been reported to provide optimal safety levels against post transfusion HBV infection (WHO 2009; Candotti *et al.,* 2019; Prati and Valenti, 2019; Velati *et al.,* 2019) as it permits detection of the window phase of acute infection, persistent occult infection, and HBV variant strains. One of the major challenges to eradicating post transfusion HBV in developing countries including Nigeria has been the unaffordability of NAT screening for blood donors and discouragement of anti-HBc screening because it would result in a huge loss of blood donors with subsequent blood inventory collapse in region where blood is mostly needed (Berkem and Karakoç 2019; Hoshi *et al.,* 2019).

Considering the importance of HBV infection and its associated sequel, there is then the need to look for alternative methods of reducing TAHBV in low and middle income countries where the prevalence is high. Studies have reported transfusion associated HBV (TAHBV) infection (Gerlich *et al., 2007;* Satake *et al.*, 2007; Yuen *et al.,* 2011; Candotti *et al*., 2019) and in Nigeria, the most populous nation in Africa, the possibility of transfusion associated HBV (TAHBV) infection have been documented (Japhet *et al.,*2011; Oluyinka *et al.,*2015; Olotu *et al.,* 2016; Ogunfemi *et al.,*2017); however, the possibility of reducing TAHBV in the country has not been fully explored, hence this study.

MATERIALS AND METHODS

Study population

Two hundred and seventy-three (Male=246, F=27; Age range:18-60 Mean:26.9years) apparently healthy consenting blood donors in Ibadan were enrolled in the study between October and December 2012 and their demographic data was obtained. Ibadan is the capital city of Oyo state, located in the South-western part of Nigeria, 128km inland northeast of Lagos and 530km southwest of Abuja, the Federal Capital of Nigeria. The number of female blood donors available for this study was smaller than the male population due to the fact that females are not encouraged to donate blood or exempted from blood donation due to anaemia, pregnancy, breastfeeding or childbirth.

Sample Collection and Preparation

About 5ml of blood was collected from each donor by venepuncture using sterile needle and syringe into appropriately labelled sterile container, free of anticoagulants or preservative. Each blood specimen was subsequently separated in the laboratory by centrifugation at 3000 rpm for 10 minutes. The serum was transferred aseptically into appropriately labelled cryovial. Thereafter, all sera were kept frozen at -20C until analysed.

Screening for Serological Marker

HBV Serological marker and kit used for detection

All sera were subjected to five different HBV serological markers detection procedures using commercial Enzyme Linked Immunosorbent Assays. The frequency of HBsAg was tested using Bio-Rad Monolisa HBsAg Ultra Kit®. The 4 other serological markers tested: antibody to the HBsAg (Anti-HBs), Hepatitis B 'e' antigen and its corresponding antibody (HBeAg and HBeAb) and IgM class of antibody to the HBcAg (Anti-HBcIgM) were detected using Diapro Diagnotic Bioprobes Milano- Italy. All assays were carried out according to manufacturer's instruction.

Assay procedure

For Diapro ELISA test kits, dilution of samples (1:101) were first carried out. For each sample, 1000 µL sample diluent was dispensed into labelled disposable Eppendoff tube, then 10 µL of sample was added and vortexed before use. Same dilution method was applied to all the samples using different Eppendorf tubes. The calibrators were not diluted as they were supplied ready to use. For each microplate, the first well (A1) was left empty for blanking purpose. Thereafter, 100µl of calibrator 1 (0 U/ml), calibrator 2 (5 U/ml), calibrator 3 (10 U/ml), calibrator 4 (20 U/ml), Calibrator 5 (50 U/ml) and calibrator 6 (100 U/ml) were dispensed into appropriate wells (B1, C1, D1, E1, F1, and G1 respectively) while the control serum was also added into well H1. Subsequently, 100µl of each diluted sample was dispensed into appropriately labelled well. The plate was sealed with adhesive seal after careful rocking and the microplate was incubated for 60 minutes at 37^{o}C.

After the first incubation, the microplate was washed with wash buffer solution for 5 cycles with a soaking time of 20-30 seconds between cycles. A second incubation at the same temperature and time was carried out after addition of enzyme conjugate to all the wells except the blank and the plate was washed as previously described following the incubation. Addition of substrate (a chromogen) followed, with a final incubation in the dark at room temperature for 20 minutes. Stop solution was immediately added and the plate read at 450/630nm.

For Bio-Rad Monolisa kit, used for detection of HBsAg, the assay procedure and washing steps was similar to that of Diapro except that there was no blanking, no calibrators and no control serum but positive and negative controls were used. Also, the first incubation was carried out at 37^{o}C for 60 minutes, the second at room temperature for 30 minutes while the final incubation was done in the dark for 30 minutes at room temperature

RESULTS

Prevalence of HBV serological markers among the donors

From the 273 sera tested for the five different HBV serological markers, HBsAg was detected in 14 (5.1 %) of the donors while HBsAb prevalence was 37% (103/273). Of the 14 HBsAg positive donors, HBeAg was detected in 2 (14.3%) donors while HBeAb was detected in 3 (21.4%) donors. The prevalence of anti-HBc IgM was 4.8% (13/273). Twelve (4.4%) donors (M=11; F=1) had anti-HBc IgM as the only detected marker of HBV (Table 1)

HBV Serological Markers by Gender

From the 273 blood donors enrolled for this study, 246 were males while 27 were females. The prevalence rates of HBsAg, HBsAb, HBcIgM, HBeAg and HBeAb among the males were 5.3% (13/246), 37.8% (93/246), 4.9% (12/246), 14.3% (2/13) and 21.4% (3/13), respectively. It is noteworthy that only 14 subjects with detectable HBsAg were screened for HBeAg and HBeAb markers. They are markers of high HBV replication/infectivity and its corresponding antibody. Among the females, HBsAg, HBsAb and HBcIgM prevalence rates were 3.7% (1/27), 38.5 (10/27) and 3.8% (1/27). However, there was no statistical correlation between HBV serological marker and sex. Among the females tested in this study, HBeAg and HBeAb were not found (Table 2)..

Age Distribution of HBV Serological Markers

For all the serological markers, highest prevalence was detected among blood donors aged 18-40 years with more than 80% prevalence in this age group. For blood donors older than 50 years, no serological marker was detected, except HBsAb with a prevalence of 2.2% (Table 3).

DISCUSSION

This study reports HBsAg prevalence of 5.1% among blood donors.

Transfusion-transmitted infections (TTIs), among blood donors especially HBV (among others) remain a major threat to blood safety (WHO, 2017). Hepatitis B Virus endemicity is classified as high, intermediate or low according HBsAg prevalence, with high areas having HBV prevalence of ≥ 8% (Asia Pacific and sub-Saharan African re-gions of the world), intermediate areas with 2-7% and low areas having < 2% (MacLachlan and Cowie, 2015). Nigeria among other sub-Saharan African countries is considered high endemicity region since HBV chronic infection can be present in more than 8% of the population (Ott *et al.,* 2012). Results of this study agrees with other HBsAg prevalence studies in Nigeria, where HBV prevalence is lower than 8%. The prevalence of HBsAg in these studies were 4.7%, 6.7%, 3.9%, 1.5%, 4.1% and 2.1% respectively (Nwogoh et al., 2011; Onyekwere and Hameed 2015; Aba and Aminu 2016; Abiola *et al.,* 2016; Okoroiwu *et al.,* 2018; Meka *et al.,* 2019). A lower prevalence recorded in this study may reveal a shift in HBV endemicity in Nigeria, from high to intermediate classification, however, further studies among different populations and in different locations in the country is needed to corroborate this assertion.

We report overall HBc IgM prevalence of 4.8% (13/273) in this study with twelve (4.4%) of the donors having HBc IgM as the only evidence of HBV infection. The most frequently used marker to screen for the presence of HBV infection is HBsAg. However, in early infection, this antigen may not be present, and it may be undetectable later in the infection as it is being cleared by the host. During this 'window' in which the host remains infected, IgM antibodies to the hepatitis B core antigen (HBc IgM) may be the only serological evidence of disease (Brooks *et al.,* 2010; Willey *et al.,* 2011; Japhet *et al.,* 2011). Of note is the fact that detection of HBc IgM has also been reported by other studies in Nigeria and recommended its inclusion in the country's blood donation screening. (Japhet *et al.,* 2011; *Bakarey et al., 2017;* Ogunfemi *et al.,* 2017).

Hepatitis B surface antibody (HBsAb) is known as a marker of recovery or protection from HBV infection and vaccination (WHO, 2009; Brooks *et al.,* 2010; Willey *et al.,* 2011; Prabina *et al.,* 2019). In this study, a high HBsAb prevalence of 37.8% was found among the blood donor (though the values were not quantified). Studies have reported that anti-HBc-positive blood containing 200 mIU/mL or more of anti-HBs appears safe as a transfusion component since discarding anti-HBc-positive blood in HBV high or intermediate regions is discouraged as it may result in a huge loss of blood donors and subsequent blood inventory (Berkem and Karakoç 2019; Hoshi *et al.,* 2019). Including screening/quantification of HBsAb marker in routine screening of blood donors in Nigeria, in addition to HBc IgM could help to identify anti-HBc-positive blood donors whose blood is safe for transfusion, and disqualify infectious donor, even in the absence of HBsAg.

The major limitation of this study is the inability to carry out HBV DNA testing of the anti-HBc (Total and IgM) positive blood and quantification of anti-HBs among the blood donor. This is unconnected with limited or no funding for research in the country.

Table 1: Prevalence of Serological Markers among Blood Donors

Serological Markers	Number of Samples Tested	Positive (%)	Negatives (%)
HBsAg	273	14(5.1 %)	259 (94.9)
HBsAb	273	103 (37.7%)	170 (62.3%)
HBc IgM	273	13* (4.8%)	260 (95.2%)
HBeAg	14	2(14.3%)	12 (85.7)
HBeAb	14	3(21.4%)	12 (78.6%)

*12 donors had HBc IgM as the only serological marker of HBV infection

Table 2: Gender distribution of HBV Serological Markers

Serological Marker	Male Number tested (%)	Number positive (%)	Female Number tested (%)	Number positive (%)
HBsAg	246	13 (5.3%)	27	1 (3.7%)
HBsAb	246	93 (37.8%)	27	10 (38.5)
HBc IgM	246	12(4.9%)	27	1 (3.8%)
HBeAg,	13	2(14.3%)	1	0
HBeAb	13	3(21.4%)	1	0

Table 3: Age Distribution of HBV Serological Markers

Age Range	Number Tested	HBsAg	HBsAb	HBcIgM	Tested	HBeAg Positive	HBeAb Positive
18-20	25	1(0.4%)	25(9.6%)	1(0.4%)	0	0 (%)	0(%)
21-30	114	2(0.73%)	37(13.6)	7(2.6%)	2	0(%)	2(14.3%)
31-40	89	11(4.0%)	22(8.1)	4(1.5%)	9	2(14.3%)	0(%)
41-50	35	0 (%)	13(4.8%)	1(0.4%)	1	0(%)	1(7.1%)
51-60	10	0 (%)	6 (2.2%)	0(%)	0	0(%)	0(%)
Total	273	14 (5.1%)	103 (37.7%)	13(4.8%)	14	2(14.3%)	3(21.4%)

CONCLUSION

This study reports a lower than 8% HBsAg prevalence among blood donors, suggesting a decline in HBV prevalence in Nigeria as found in other HBV studies suggesting a need to possibly consider re-classifiying HBV endemicity in Nigeria. We also report high prevalence of HBsAb among the blood donors. This may help in reducing post transfusion HBV if this marker is screened/quantified in addition to HBcAb (Total and IgM) in the nation's routine blood donation screening.

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