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Advancing Hepatitis B Virus Testing in Prospective Blood Donors Beyond Current Single Marker Rapid Technique: Is it a Luxury or Necessity?

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Authors' contributions

This work was carried out in collaboration between all authors. Author GIA designed the study and proof-read the manuscript. Author MOI collaborated in designing the study and managed the statistical analysis. Author KAF wrote the first draft of the manuscript, managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Blood transfusion service comes with its various risks which include transfusion of transmissible infections especially hepatitis B. Though Nigeria is noted as a high endemic region for hepatitis B virus (HBV) infection, yet most techniques–focus on detection of only hepatitis B surface antigen marker in serum or plasma. This study aims at optimizing 'safe blood' practice by advancing hepatitis B virus testing in prospective blood donors (PBD) beyond single marker serologic screening and estimating HBV endemicity among blood donors based on HBV markers seroprevalence. Up to 4 ml of K3EDTA anticoagulated sample was obtained from PBD and aliquot into two separate plain containers following informed consent and ethical approval. A total of four

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hundred and seventy (470) of PBD were initially screened for HBV markers using NOVA HBV 5-in-1 rapid one-step enzyme immunoassay between August, 2014 and November, 2015. Results were analyzed using SPSS version 21. The results showed that the overall gender ratio and mean age of PBD screened for HBV are 1.45:1 and 26.87±7.51 respectively. Chi square revealed right knowledge of most of the routes of hepatitis B viral transmission by PBD (χ^2 range = 11.6 – 102.3, $p < 0.05$). Also, this study revealed HBsAg seroprevalence of 6.4% based on NOVA 5-in-1 rapid EIA. Cummulative HBV markers seroprevalence was 19.36% including 34 (7.23%) $HBsAb^+$ and 1(0.21%) $HBsAb^+HBeAb^+HBcAb^+$ showing evidence of vaccinated and naturally immunized donors respectively. In conclusion, the use of more stringent serologic techniques and workable algorithm to reduce risks associated with blood transfusion and enhance both blood donors' and recipients' safety is no longer a luxury but a necessity.

Keywords: Transfusion; hepatitis; hepatocellular carcinoma; HBV; seroprevalence; enzyme immunoassay; vaccination; immunization.

1. INTRODUCTION

Blood transfusion is still associated with the risks of transmission of hepatitis B virus [1-5]. Different authors both in Ekiti state and South-west have published several data on the prevalence of these blood-borne infections in prospective blood donors based on HBsAg detection techniques only [6-9]. Hepatitis B virus is 50-100 times more infectious than human immunodeficiency virus [10]. Studies based on HBsAg detection only have the tendency of underestimating HBV endemicity, increasing the risk of blood transfusion-associated infection, promotion of incomplete post-test counselling of blood donors and poor prognosis in those with the risks of liver cirrhosis and hepatocellular carcinoma due to late diagnosis. Previous studies by Adam and his co-researchers as well as Kwon et al. described the significance of detecting various HBV markers in hepatitis B virus infected subjects and observed varying prevalence based on geographical locations and type of research subjects tested [1,10]. However, to the best of our knowledge, there was no known published data in Ekiti state that screened for HBV markers in PBD as at the time of this study and investigated occult hepatitis B.

Hepatitis B virus has five viral markers. These include hepatitis B surface antigen (HBsAg), hepatitis B surface antibody (anti-HBs), hepatitis B envelope antigen (HBeAg), hepatitis B envelope antibody (anti-HBe) and hepatitis B core antibody (anti-HBc) [11]. HBsAg appears 4 weeks following exposure to the virus but can be detected any time after the first week. Individuals with positive HBsAg are considered to be infected and are potentially infectious. Presence of the antigen longer than 6 months after initial exposure indicates chronic infection. However, it can be cleared before the sixth month suggesting hepatitis B virus infection is a self-limited disease. Presence of hepatitis B surface antibody (anti-HBs) is an indication of active or passive immunization that usually persists for life and a sole evidence of vaccination. Individuals with hepatitis B core antibody (anti-HBc) in addition to anti-HBs have natural immunity against hepatitis B virus. Anti-HBc is the first detectable antibody in the course of HBV disease. IgM anti-HBc indicates acute infection and is the only serologic marker detectable during the "window period," when neither HBsAg nor anti-HBs is detectable. Once IgG anti-HBc appears in the serum, it persists for life [11]. Detection of IgG anti-HBc indicates previous or ongoing infection. Individuals with positive HbeAg results have been shown to have higher rates of viral transmission [12]. Therefore, the antigen is used as a marker of viral replication and infectivity [13]. However, HbeAg testing is indicated primarily during follow-up of chronic infection rather than acute infection because of its variable level during the acute phase [14]. Loss of HBeAg and appearance of anti-HBe in serum is called seroconversion. It is a common finding in sera of individuals with chronic inactive hepatitis B. Seroconversion is associated with a lower level of HBV DNA or a low replicating state of the virus [12]. It has been used in many clinical settings as an end-point of antiviral therapy and possible remission of the disease [13]. HBV DNA level (viral load) indicates viral burden and viral replication. It is used to assess recovery from infection and candidacy for antiviral therapy and to differentiate between inactive carrier state and chronic active hepatitis in chronic HBV infection.

Prospective blood donors are individuals who come to the laboratory or are recruited for the purpose of blood donation and have to be

certified fit based on screening tests. Three categories of prospective blood donors have been identified worldwide (and all the three categories of blood donors were represented in our research study): Voluntary non-remunerated blood donors (VNBD), replacement blood donors (RBD) and paid blood donors (PDBD) [15-16]. The advocacy of the World Health Organization is 100% voluntary blood donation. Voluntary non-remunerated blood donors are the safest and low risk group of donors [17]. Replacement blood donors are relatives or friends who donate units of blood to replace those loaned from the blood bank and constitute the vast majority of blood donors in many of Nigerian Health Institutions. Paid or commercial blood donors are still the major source of donated blood where there are difficulties in recruiting donors based on cultural, religious or personal reasons. They are the high risk group of blood donors considering their social and sexual lifestyles, uncontrolled frequency of donations and wrong perceptions about blood donation as a means of meeting personal needs [18-20].

The aim of this study is to optimize 'safe blood' practice by advancing hepatitis B virus testing in PBD beyond single marker serologic screening and estimating HBV endemicity among blood donors based on HBV markers seroprevalence.

2. MATERIALS AND METHODS

2.1 Sampling

Up to four millilitres (4ml) of K_3EDTA anticoagulated samples were collected from four hundred and seventy research volunteers between August, 2014 and November, 2015 following informed consent. Plasma samples were separated according to Clinical and Laboratory Standard Institute [21].

2.2 Assay Kit and Serologic Testing

Hepatitis B viral markers screening was carried out using NOVA HBV 5-in-1 multi-test kit (HBV 260) which is a rapid one-step enzyme immunoassay. Analysis was carried out immediately on separated plasma according to manufacturer instructions.

2.3 Quality Control Measures

Haemolysed or lipaemic samples were not used for analysis. Besides in-built internal controls of the test strips, known positive and negative plasma samples were run in parallel with research samples to validate test results. Individuals with recent HBV vaccination history were excluded from the study.

2.4 Informed Consent and Social Demographic Data

The informed consent and social demographic data of the research volunteers were obtained through administered questionnaires.

2.5 Ethical Clearance

Ethical clearance for this study was obtained from the Ethics Committee of the Federal Teaching Hospital, Ido Ekiti.

2.6 Study Location

This study was carried out at the Federal Teaching Hospital, Ido Ekiti.

3. RESULTS

3.1 Social Demographic Characteristics of Prospective Blood Donors

Tables 1 and 2 summarized the study findings on social demographic characteristics of blood donors. As observed in Table 1, study results showed that of the four hundred and seventy research volunteers screened for HBV during the study period, 363 (77.23%), 72 (15.32%) and 35 (7.45%) were VNBD, RBD and PDBD respectively. A total of 278 (59.1%) were male blood donors of whom 115 (24.4%) and 163 (34.7%) were first timers and previous donors respectively. Of the 192 (40.9%) female blood donors enrolled, 131 (27.9%) and 61 (13.0%) were first timers and previous donors respectively. The male-to-female ratios of VNBD, RBD and PDBD were 1:1, 11.0:1 and 10.7:1. Overall mean age was 26.87± 7.51 years. Mean age of VNBD, RBD and PDBD were 26.0 ± 7.4 30.7 ± 7.2 and 27.4 ± 6.6 year respectively. Overall, 241 (51.2%) of the prospective blood donors age grouped 16 – 25 years constituted the largest number of research volunteers while 2 (0.4%) of prospective blood donors age grouped 59 – 65years were the least.

From Table 3, irrespective of blood donor category, more than 75.0% of the prospective blood donors were Christians and a total of 389 (80.2%) had tertiary education. Both singles and

married individuals participated in the study with the overall number of the singles being slightly higher, 287 (61.1), compared to the married, 175 (37.2%). However, based on blood donor category, at least 63.0% of prospective blood donors were VNBD and PDBD except for the RBD which were composed of more married individuals (51.4%) than the singles (44.4%). Only 6 (1.3%) of the total population of blood donors screened had history of blood donation rejection due to hepatitis B virus infection.

3.2 Prospective Blood Donors' Knowledge on Mode of Hepatitis B Viral Transmission

Prospective blood donors' knowledge of the mode of transmission of hepatitis B virus before performance of procedure (pre-test) was studied. As shown in Table 3, statistically significant numbers of prospective blood donors' had a perfect knowledge of hepatitis B mode of transmission for most of the variables tested (χ^2 range: 11.6 – 102.3, $p < 0.05$) except for intravenous drug abuse ($\chi^2 = 2.0$, $p = 0.36$) and sharing of tooth-brush and eating together (($\chi^2 = 1.2$, $p < 0.54$).

3.3 The Prevalence of Each Pattern of HBV Markers Seropositivities Based on the Population of Each Prospective Blood Donor Category Screened

Table 4 presented the prevalence of each pattern of HBV markers seropositivities based on the population of each prospective blood donor category screened. HBV markers seropositivities occurred either singly or in combination with others. Overall, a total of 91 of the blood donors were seropositive for HBV viral markers thus yielding a cummulative HBV markers seroprevalence of 19.36% and these were distributed within the population of each blood donor category screened. Thirteen patterns of HBV markers seroprevalence were detected through this study which include: $HBsAb^+$,$HBsAg^+HBeAb^+HBcAb^+$, $HBsAg^+HBeAg^-HBcAb^+$, $HBsAg^+HBeAg^+HBcAb^+$, $HBcAb^+$, $HBcAb^+HBeAb^+$, $HBsAb^+HBeAb^+HBcAb^+$, $HBeAb^+$, $HBsAg^+HBeAg^+$, $HBsAg^+HbeAg^-$, $HBcAb^+HBeAg^+$, $HBsAb^+HBeAg^+$, and $HBeAg^+$. Also, 379 (80.74%) of the prospective blood donors were considered susceptible for HBV infection based on seronegativity of all HBV markers.

Moreover, the prevalence of HBV markers seropositivities demonstrated that age groups of prospective blood donors had statistically significant impact on the cummulative seroprevalence ($p < 0.002$). The mean ages (±SD) of the age groups of PBD were 21.1 ± 2.1, 29.8 ± 2.8, 39.9 ± 2.4, 48.3 ± 2.5 and 58.5 ± 0.7 for 16-25, 26-35, 36-45, 46-55 and 56-59 respectively. Overall, PBD of age groups 16-25, 26-35, 36-45, 46-55 and 56-59 years had cummulative HBV markers seroprevalence of 8.1%, 7.2%, 3.8%, 0.2% and 0% respectively. Table 5 presented the study findings.

4. DISCUSSION

The enrolment of more voluntary non-remunerated blood donors (77.23%) compared to other category of blood donors RBD (15.32%) and PDBD (7.45%) as observed in this study was a result of new policy formulation targeting 80.0% voluntary blood donation and strong collaboration between Federal Teaching Hospital and the National Blood Transfusion Service to enhance growth towards achievement of 100% voluntary blood donation as recommended by the World Health Organization [13]. Based on further screening for other transfusion transmissible infections not captured by this study, it was revealed that 75.5% of the VNBD eventually donated. This is quite high compared to the recent reports by the Federal Ministry of Health. Based on the recent facts on blood donation in Nigeria, Federal Ministry of Health [22] reported that voluntary, non-remunerated blood donation accounts for only 10% of the total blood collection in Nigeria (compared to less than 5% reported by Ahmed and Bashawri and their co-researchers [16,20]) while family replacement donations and commercial donations account for 30% and 60% respectively. This study result was lower than 90.0% voluntary blood donors who showed readiness to donate blood should the need arise as reported by Kulkani and Kulkani [24] and 86.0% voluntary blood donation reported by Lavanya et al. [25] among Indian populace [23-24]. However, this is several folds higher than 1% reported by Salaudeen and his co-researchers in Ilorin, Nigeria [25]. Nwogoh et al. reported 0% voluntary blood donation in Benin City, Nigeria. Moreover, the finding of male: Female ratio of nearly 1:1 among VNBD was quite unusual but male: female ratios of 11:1 and 10.7:1 for the RBD and PDBD follow the current trends that majority of blood donors in Africa are males [3,26]. Reason for nearly gender equality among prospective VNBD when

compared to the RBD and PDBD and overall PBD findings seems to be due to more female first timer volunteers recruited during blood drive in response to comprehensive hepatitis B viral screening motivation.

Lower mean age of VNBD (26.06 ± 7.43) compared to PDBD (27.43 ± 6.63) and RBD (30.69 ± 7.22) revealed that much younger people (especially between 16 and 25 years old) participated as voluntary blood donors. Generally, age range for blood donation is 18 - 65 years. World Health Organization encouraged participation of younger people to give blood regularly as VBD while older people give blood when they are well past 65 years if they had been consistent regular blood donors [27]. Climbing the age ladder, from the overall PBD screened as well as VNBD, RBD and PDBD, it was observed that the percentage of PBD decreased as the age group increased except for the replacement blood donors. 57.3% of VNBD fell into 16-25 years age group while 31.7% belonged to 26-35 years age group. This is higher than the figures published by the World Health Organization which reported that 45% of donors were aged 25 or less [28]. Study findings showed that a lot of individuals of younger age group in Ekiti state especially in our institutions are well informed on the importance of giving blood to safe life. This differs from another finding which showed under-representation of younger people in blood donation [29]. 31.7% of VNBD of 26-35 years age group was reported in this study. However, a higher percentage of RBD (47.2%) fell within 26-35 years. This is slightly higher than 46.5% reported by other researchers in a study from Ethiopia which was made up 98.0% RBD [30]. Exactly 45.7% and 42.9% of PDBD fell within 16-25 years and 26-35 years age group and that revealed the financial inequalities in Ekiti state that prompted individuals of these age groups to engage in paid donations as life support. Anaemia has been reported to be the most common consequence of paid blood donations especially when there are uncontrolled donations for monetary gain [31-33].

Irrespective of blood donor category, enrolment for blood donation was at least 77% among Christians and less than 25.0% among Islamic faithfuls. This was higher than that 61.1% reported by a group of researchers in Ilorin [26]. Possible reasons might stem from more population of Christians in Ekiti state and their positive response towards blood donation. Although higher percentages of the prospective blood donors had tertiary education irrespective of category but it was more pronounced among the VBD population. The findings in this study underscored the place of targeted population in blood donor selection. Most of the VNBD (88.7%) compared to RBD (65.2%) and PDBD (57.1%) had tertiary education and were well-informed on the relevance of blood donation to safe life. The outcomes of this study were corroborated by similar studies among VNBD by Shenga et al. who found that as the level of education increased, percentage of voluntary blood donors increased [34-35].

In the overall PBD screened, the outcomes study revealed positive blood donation attitude was optimal among the volunteers with tertiary education and decreases progressively as we considered blood donors without formal education. Furthermore, study also revealed the marital status of PBD. This study in comparison with other studies showed singles and married individuals participated in all categories of blood donations with varying findings [23,36]. Only 6 (1.3%) of the overall population of PBD. This was made up of 5 or 1.4% of VNBD, and 1 (1.4% of RBD) of indicated that they had blood donation rejection history due to HBV infection. Interestingly, none of the paid blood donors declared ever being rejected due to HBV. That lent credence to the report by Nwogoh and his co-researchers that paid blood donors often conceal information in an attempt to engage in blood donation for monetary gain [18].

The results of the assessment of the prospective blood donors' baseline knowledge on the mode of transmission of hepatitis B virus prior testing (pre-test) showed that prospective blood donors had the right knowledge of most of the routes of transmission of hepatitis B virus (χ^2 range: 11.6 – 102.5, $p < 0.05$) except for intravenous drug abuse ($\chi^2 = 2.0$, $p = 0.36$) and sharing of tooth-brush and eating together ($\chi^2 = 1.2$, $p = 0.54$). This emphasizes the need for more public awareness on viral hepatitis including hepatitis B virus transmission routes with more emphasis on roles of intravenous drug abuse in HBV while de-emphasizing sharing of toothbrush and eating together as HBV transmission route.

Similarly, within each population of prospective blood donors HBV markers seroprevalence was studied. Overall, 34 (7.23%) of research subjects were seropositive for $HBsAb^+$ (category 1) showing evidence of successful immunization. $HBsAb^+$ seroprevalence pattern according to

population of each of the prospective blood donors category showed it was highest among paid blood donors with 4 (11.43%) and lowest among the replacement blood donor population with 2 (2.78%). It is a well-known fact that $HBsAb^+$ seropositivity is an indication of successful vaccination among immunized group [37] and it has been used to determine the need for vaccination if HBsAb is absent. It may also be detected in naturally resolved HBV infected subjects previously exposed with consequent immune protection. Such individuals will also have detectable anti-HBc antibody with or without anti-HBe antibody. Chronic inactive hepatitis B, chronic $HBeAg^+$ and $HBeAg^-$ cases were observed in the study as evidenced by the detection of $HBsAg^+HBeAb^+HBcAb^+$(category 2); $HBsAg^+HBeAg^+HBcAb^+$ (category 4) and $HBsAg^+HBeAg^-HBcAb^+$ (category 3) respectively [38,39]. 21 (4.47%) of the overall population of PBD screened were diagnosed as potential chronic inactive hepatitis B based on serologic findings until proved otherwise with the evidence of elevated alanine aminotransferase and HBV-DNA level greater than 2000 IU/ mL. Study also showed that 4 (0.85%) and 2 (0.43%) were $HBeAg^+$ and $HBeAg^-$ cases respectively. According to the PBD population categories, for the chronic inactive hepatitis B category, 14 (3.86%), 3 (4.17%) and 4 (11.43%) were detected among the VNBD, RBD and PDBD respectively. This is accordance with the conclusion from various researches that voluntary non-remunerated blood donation is the safest blood donation recruitment method [17,15, 40]. Majority of the PBD were unaware of their HBV serostatus until decision to give blood. $HBcAb^+$ (total) alone (category 5) was detected in 18 (3.83%) of the overall PBD and were found among the VNBD (16 or 4.40%) and PDBD (2 or 5.71%) categories only. Several published data have shown the association of anti-HBc alone ($HbcAb^+$) with occult hepatitis B infection [41-44]. Other patterns of HBV antibody markers were equally detected in this study. $HbcAb^+HbeAb^+$ (category 6) constituted 0.85% of the study population while $HbsAb^+HbeAb^+HbcAb^+$ (category 7) and $HbeAb^+$ (category 8) individually constituted 0.21% of the entire PBD population screened. While $HbcAb^+HbeAb^+$ might indicate clinically resolved past infection but no evident cure, $HbsAb^+HbeAb^+HbcAb^+$ is an evidence of natural immunity against hepatitis B virus. No vaccination is indicated for this category of donors. Detection of isolated $HbeAb^+$ (category 8) may imply HBV infection or immune blood donor. Hepatitis B viral infection in incubation phase (category 9) was also detected in 2 (0.43%) of our research subjects with evidenced $HbsAg^+HbeAg^+$. $HbsAg^+HbeAg^-$ (category 10) was detected in 1 (0.21%) of the VNBD [45]. HbeAg has been shown to be a marker of viral replication, high infectivity and is associated with the higher risk of developing of hepatocellular carcinoma. While published articles have showed that chronic carriers of HBV have 100-fold risk of developing hepatocellular carcinoma [46-47], chronic inactive carriers of hepatitis B have lower risk of developing liver cirrhosis or hepatocellular carcinoma compared to HbeAg-positive subjects. A study showed an 87% cumulative hepatocellular carcinoma risk from age 30 to 70 years for those that were persistently seropositive for HbsAg and HbeAg and 12.0% for those with seropositivity for HbsAg only [48]. These first two cases ($HbsAg^+HbeAg^+$ and $HbsAg^+HbeAg^-$) reported here have been found to be associated with chronic hepatitis B infection in affected subjects. Moreover, of note in Ekiti State, and this study were the unprecedented findings of $HbsAb^+HbeAg^+$ (category 11), $HbcAb^+HbeAg^+$ (category 12) and $HbeAg^+$ alone (category 13) in RBD, PDBD and VNBD respectively and require careful interpretations. Interestingly, the first two were unusual combinations of HBV markers detected in two blood donors which have not been previously reported in Ekiti state especially among blood donors. The first was a paid blood donor with $HbsAb^+HbeAg^+$ markers and the second a replacement blood donor with $HbcAb^+HbeAg^+$ markers. Each constituted 0.21% of the total number of prospective blood donors screened. The detection of $HbsAb^+HbeAg^+$ markers in the paid blood donor was an indication of chronic hepatitis B infection in HbsAg seroconverted state. Persistence of $HbeAg^+$ marker despite HbsAg seroconversion predicts viral replication in, and high infectivity of chronic HBV-infected blood donor with potential danger of transmitting the virus through blood transfusion. Detection of both serologic markers ($HbcAb^+HbeAg^+$) predicts chronic hepatitis B infection at higher risk of developing hepatocellular carcinoma [49]. Published research has shown that in patients who develop chronic hepatitis B, IgM anti-HBc can persist at low levels during viral replication and can result in positive tests for IgM anti-HBc [50]. On the other hand, HBc (IgG) antibody persists for a life time in chronic infected blood donors. In the absence of comprehensive serologic screening, non-detection of HbsAg in the two aforementioned blood donors would have resulted in three potential challenges in clinical

practice. Transfusion of such units of blood would have occurred thus constituting potential risks to recipients of blood transfusion. Besides, these individuals would have been ignorant of their status until liver-related symptoms ensued. Finally, due to high infectivity rate in such individuals, other persons might have been infected inadvertently following exposure to blood of such individuals. Moreover, detection of $HbeAg^+$ alone without $HbsAg^+$ in one of the VNBD is unusual. It could mean that hepatitis B core antigen is present in the hepatocyte of the donor but the anti-HBc is yet undetected in serum and presence HBV-DNA polymerase as in occult hepatitis B [51] or a false positive test result from contaminated sample.

HBV surface antigenaemia (both singly and in combination with other markers) summed up to 30 (6.4%) of the overall blood donor population. That is, 1 (0.21%) occurred singly while in combinations with $HbeAb^+$ and $HbcAb^+$, $HbcAb^+$ only, $HbeAg^+$ and $HbcAb^+$, and $HbeAg^+$ only, 21 (4.47%), 4 (0.85%), 2 (0.43%) and 2 (0.43%) respectively were positive for HbsAg and were certified unfit for donation. Approximately 6.4% HbsAg seroprevalence in this study suggests that Ekiti State can be classified as an intermediate endemic region [41,52] for HBV infection based on hepatitis B surface antigenaemia. This result is slightly higher than 6.2% HbsAg seroprevalence among patients routinely screened for HbsAg at the Blood Transfusion unit of Ekiti State University Teaching Hospital (EKSUTH) in 2015 as reported by Adekoya-Benson and her co-researchers and confirms the intermediate endemicity of Ekiti with reference to HBV infection [6]. This is lower than 10.9% reported by both Yusuf and Alemayehu, and Shittu research groups [30,53], 9.8% by Motayo et al. [7], 27.0% by Vem [54], and 7.5% by Salawu and his co-researchers [26]. However, based on HBV markers of significance in blood transfusion (i.e. detection of HBV markers other than $HbsAb^+$ detected as evidence of successful vaccination, and $HbsAb^+HbeAb^+HbcAb^+$ found in those with natural immunity following exposure to the virus), this study revealed HBV endemicity of 11.9% in Ekiti state, thus making it a high endemic region. That is, reports based on HbsAg detection alone under-estimated HBV endemicity in Ekiti state. Asymptomatic individuals with chronic hepatitis B may not necessarily have HbsAg but detection of other serologic markers of HBV, HBV-DNA level and serum alanine aminotransferase may be very diagnostic. This is particularly important in clinical situation involving relapse of the disease when individuals once immune to the virus without evident cure (based on previous exposure to hepatitis B infection) become immunosuppressed. Underlying mechanism of immunosuppression may be co-infection with human immunodeficiency virus (HIV) which progressively depleted the immune system, primary or secondary stem cell disorders or exposure to toxic chemicals. For blood transfusion purposes and surveillance, it is preferable based on clinical judgment not to underestimate HBV endemicity in high endemic region such as Nigeria due to non-detection of HbsAg. This is imperative to prevent risk of HBV transmission through vertical or horizontal means, identify eligible individuals for vaccination exercise, promote safe blood practices, and provide reliable national epidemiologic data. Moreover, 80.64% of the overall PBD population screened was susceptible to HBV infection if exposed. Observance of seronegative results for all HBV markers relatively confirmed absence of infection, susceptibility to HBV infection and eligibility of PBD for hepatitis B vaccines (and/ or hepatitis B immunoglobulins) if not contraindicated. Ignorance of HBV infection, route of transmission or effective HBV vaccination programmes can predispose once seronegative blood donor to HBV infection.

The pattern of HBV markers seropositivities in overall prospective blood donors screened according to age groups was also studied. Age wise, this study showed a characteristic finding among the PBD enrolled. The percentage of the PBD enrolled decreased as the age groups increased. This differed slightly from the findings by Lavanya and his co-researchers who found that age group of PBD peaked at 26-30 years and then decreased steadily till age group 41-45 years [24]. Younger PBD, age grouped 16-25 (mean age: 21.1 ± 2.1years) constituted a largest percentage (51.3%). Study showed that age groups of PBD had statistically significant impact on HBV markers seropositivity ($p < 0.002$). Another study also published similar findings [55]. Optimal $HbsAb^+$ seroprevalence was observed among PBD age grouped 16-25 (3.0%) and 26-35 (3.0%) suggesting low level of vaccination among PBD and the need for more enlightenment programme on the benefits of HBV vaccination among PBD of different age groups [56]. In similar pattern as percentage of PBD enrolled in this study, overall HBV markers seropositivity decreased as the age groups of PBD increased. Overall hepatitis B viral markers

seropositivity was significantly higher in age group 16-25 years than any other age group. This differed from the outcomes of research by Kamel et al. who observed significantly higher HBV markers seropositivities among blood donors age grouped 30-39 and ≥40 years [57]. Percentage of chronic $HbeAg^{+}$ carriers was optimal among PBD age grouped 26-35 years. Overall, the risk of HBV transmission was significantly low in PBD age grouped 46-55 years (0.2%) and 0% in PBD age grouped 56-49 years. Study findings might probably be attributed to minimal number of prospective blood donors belonging to those age groups. Overall, the age groups of PBD had statistically significant impact on the seroprevalence of the HBV viral markers ($p = 0.002$).

Table 1. Social demographic characteristics of prospective blood donors

Social Demog. Variables	Overall PBD Demo. Data	VNBD N (%)	RBD N (%)	PDBD N (%)
PBD screened: N (%)	470 (100.0)	363 (77.23)	72 (15.32)	35 (7.45)
Sex				
Male	278 (59.1)	180 (49.6)	66 (91.7)	32 (91.4)
Female	192 (40.9)	183 (51.40)	6 (8.3)	3 (8.6)
Male: Female ratio	1.5:1	1:1	11.0:1	10.7:1
Donors status				
Male First timers	115 (24.4)	77 (21.2)	28 (38.9)	10 (28.6)
Male Previous Donors	163 (34.7)	103 (28.4)	38 (52.8)	22 (62.8)
Female First timers	131 (27.9)	126 (34.7)	4 (5.5)	1(2.9)
Female Previous Donors	61 (13.0)	57 (16.7)	2 (2.8)	2 (5.7)
Mean age (years)	26.87 ± 7.51	26.06 ± 7.43	30.69 ± 7.22	27.43 ± 6.63
Age group				
16-25	241 (51.2)	208 (57.3)	18 (25.0)	16 (45.7)
26-35	166 (35.3)	115 (31.7)	34 (47.2)	15 (42.9)
36-45	55 (11.7)	35 (9.6)	18 (25.0)	3 (8.6)
46-55	6 (1.3)	3 (0.8)	2 (2.8)	1 (2.9)
56-59	2 (0.4)	2 (0.6)	0 (0.0)	0 (0.0)

PBD = Prospective blood donors; VNBD = Voluntary non-remunerated blood donors
RBD = Replacement blood donors; PDBD = Paid blood donors
SD = Standard deviation

Table 2. Social demographic characteristics of prospective blood donors

Social Demog. Variables	Overall PBD Dem. Data	VNBD N (%)	RBD N (%)	PDBD N (%)
Religion				
Christianity	380 (80.9)	290 (79.9)	64 (88.9)	27 (77.1)
Islam	90 (19.1)	73 (20.1)	8 (11.1)	8 (22.9)
Educational status				
None	3 (0.6)	3 (0.8)	0 (0.0)	0 (0.0)
Primary	5 (1.1)	1 (0.3)	3 (4.2)	1 (2.9)
Secondary	73 (15.5)	37 (10.2)	22 (30.6)	14 (40.0)
Tertiary	389 (82.8)	322 (88.7)	47 (65.2)	20 (57.1)
Marital status				
Single	287 (61.1)	232 (63.9)	32 (44.4)	23(65.7)
Married	175 (37.2)	126 (34.7)	37(51.4)	12(34.3)
Widowed	7 (1.5)	4 (1.1)	3 (4.2)	0 (0.0)
Divorced/Separated	1 (0.2)	1 (0.3)	0 (0.0)	0 (0.0)
PBD rejection history				
Due to HBV Infection	6 (1.3)	5 (1.4)	1(1.4)	0 (0.0)

PBD = Prospective blood donors; VNBD = Voluntary non-remunerated blood donors
RBD = Replacement blood donors; PDBD = Paid blood donors
SD = Standard deviation

Table 3. Prospective blood donors' knowledge on mode of hepatitis B virus transmission

HBV MoT	*Yes* N (%)	*No* N (%)	*I don't Know* N (%)	*Total*	χ^2	*P Value
SISO	248 (52.8)	105 (22.3)	117 (24.9)	470 (100.0)	80.3	0.00
TIBBP	260 (55.3)	102 (21.7)	108 (23.0)	470 (100.0)	102.3	0.00
SSB	113 (24.0)	215 (45.7)	142 (30.2)	470 (100.0)	35.3	0.00
MTMS	194 (41.3)	116 (24.7)	160 (34.0)	470 (100.0)	19.5	0.00
S & C	122 (26.0)	177 (37.7)	171 (37.7)	470 (100.0)	11.6	0.003
IVDA	159 (33.8)	143 (30.4)	168 (35.7)	470 (100.0)	2.0	0.36
HBCIP	239 (50.9)	99 (21.1)	132 (28.1)	470 (100.0)	68.3	0.00
MTCT	232 (49.4)	102 (21.7)	136 (28.9)	470 (100.0)	58.0	0.00
OE	232 (49.4)	92 (19.6)	146 (31.1)	470 (100.0)	63.6	0.00
SUNS	243 (51.7)	96 (20.4)	131 (27.9)	470 (100.0)	75.2	0.00
STET	168 (35.7)	152 (32.3)	150 (31.9)	470 (100.0)	1.2	0.54
TSM	195 (41.5)	115 (24.5)	160 (34.0)	470 (100.0)	20.5	0.00

** $p < 0.05$ is statistically significant*

Key: SISO = Sharing of infected sharp objects; TIBBP = Transfusion of infected blood and blood products SSB = Sharing of same bed; MTMS = Male-to-male sexual transmission; S & C = Sneezing and coughing IVDA = Intravenous drug abuse; HBCIP = Hepatitis B and C infected partner; MTCT = Mother-to child transmission

OE = Occupational exposure; SUNS = Sharing of unsterilized needles and syringes; TSM = Tattoo and scarification marks

Table 4. The prevalence of each pattern of HBV markers seropositivities based on the population of each prospective blood donor category screened

HBVSPM	Categories of prospective blood donors			
	HBVMOP (N/%)	**VBD N (%)**	**RBD N (%)**	**PDBD N (%)**
$HBsAb^+$	34 (7.23)	28 (7.71)	2 (2.78)	4 (11.43)
$HBsAg^+ HBeAb^+ HBcAb^+$	21 (4.47)	14 (3.86)	3 (4.17)	4 (11.43)
$HBsAg^+ HBeAg^- HBcAb^+$	4 (0.85)	3 (0.83)	0 (0.00)	1 (2.86)
$HBsAg^+ HBeAg^+ HBcAb^+$	2(0.43)	2 (0.55)	0 (0.00)	0 (0.00)
$HBcAb^+$	18 (3.83)	16 (4.40)	0 (0.00)	2 (5.71)
$HBcAb^+ HBeAb^+$	4 (0.85)	3 (0.83)	1 (1.39)	0 (0.00)
$HBsAb^+ HBeAb^+ HBcAb^+$	1 (0.21)	1 (0.28)	0 (0.00)	0 (0.00)
$HBeAb^+$	1 (0.21)	0 (0.00)	0 (0.00)	1 (2.86)
$HBsAg^+ HBeAg^+$	2 (0.43)	2 (0.55)	0 (0.00)	0 (0.00)
$HBsAg^+ HbeAg^{--}$	1 (0.21)	1 (0.28)	0 (0.00)	0 (0.00)
$HBcAb^+ HBeAg^+$	1 (0.21)	0 (0.00)	1 (1.39)	0 (0.00)
$HBsAb^+ HBeAg^+$	1 (0.21)	0 (0.00)	0 (0.00)	1 (2.86)
$HBeAg^+$	1 (0.21)	1 (0.28)	0 (0.00)	0 (0.00)
C.HBVSPM: N (%)	91 (19.36)	71 (19.57)	7 (9.23)	13 (37.15)
C.HBVSNM: N (%)	379 (80.64)	292 (80.43)	65 (90.27)	22 (62.85)

Key: Category 1: $HBsAb^+$ Category 2: $HBsAg^+HBeAb^+HBcAb^+$
Category 3: $HBsAg^+HBeAg^- HBcAb^+$ Category 4: $HBsAg^+HBeAg^+HBcAb^+$
Category 5: $HBcAb^+$; Category 6: $HBcAb^+HBeAb^+$; Category7: $HBsAb^+HBeAb^+HBcAb^+$
Category 8: $HBeAb^+$; Category 9: $HBsAg^+HBeAg^+$; Category 10: $HBsAg^+HBeAg^-$
Category 12: $HBsAb^+HBeAg^+$; Category 11: $HBcAb^+HBeAg^+$; Category 13: $HBeAg^+$
HBVSPM = Hepatitis B virus seropositive markers % = Percentage
HBVMOP = Hepatitis B Markers overall prevalence N = Absolute number + = Positive,
C.HBVSPM = Cummulative Hepatitis B Virus Seropositive markers
C.HBVSNM = Cummulative Hepatitis B Virus Seronegative markers

Table 5. The patterns of HBV markers seropositivities in overall prospective blood donors screened according to age groups

Analytical variables	Different age groups (Years)					*P value*
	16-25	**26-35**	**36-45**	**46-55**	**56-65**	
Mean age (Years)	21.1 ± 2.1	29.8 ± 2.8	39.9 ± 2.4	48.3 ± 2.5	58.5 ± 0.7	
PBD Screened N (%)	241 (51.3)	166 (35.3)	55 (11.7)	6 (1.3)	2 (0.4)	
HBVSPM: N (%)						
$HBsAb^+$	14 (3.0)	14 (3.0)	6 (1.3)	-	-	
$HBsAg^+$ $HBeAb^+$ $HBcAb^+$	4 (0.9)	11 (2.3)	5 (1.0)	1 (0.2)	-	
$HBcAb^+$	13 (2.8)	3 (0.6)	2 (0.4)	-	-	
$HBsAg^+$ $HBcAb^+$	1 (0.2)	2 (0.4)	1 (0.2)	-	-	
$HBcAb^+$ $HBeAb^+$	1 (0.2)	2 (0.4)	1 (0.2)	-	-	
$HBsAb^+HBeAb^+$ $HBcAb^+$	1 (0.2)	-	-	-	-	
$HBsAg^+$ $HBeAg^+$ $HBcAb^+$	-	1 (0.2)	1 (0.2)	-	-	
$HBsAg^+$ $HBeAg^+$	2 (0.4)	-	-	-	-	
$HBsAg^+$	-	1 (0.2)	-	-	-	
$HBeAg^+$	-	-	1 (0.2)	-	-	
$HBsAb^+$ $HBeAg^+$	-	-	1 (0.2)	-	-	
$HBeAb^+$	1 (0.2)	-	-	-	-	
$HBcAb^+$ $HBeAg^+$	1 (0.2)	-	-	-	-	
C.HBVMSP: N (%)	38 (8.1)	34 (7.2)	18 (3.8)	1 (0.2)	0 (0.0)	*p = 0.002

**p< 0.03 is statistically significant*

Keys: Category 1: $HBsAb^+$; Category 2: $HBsAg^+HBeAb^+HBcAb$ Category 3: $HBsAg^+HBeAg^-$ $HBcAb^+$
Category 4: $HBsAg^+HBeAg^+HBcAb^+$; Category 5: $HBcAb^+$; Category 6: $HBcAb^+HBeAb^+$
Category 7: $HBsAb^+HBeAb^+HBcAb^+$; Category 8: $HBeAb^+$; Category 9: $HBsAg^+HBeAg^+$
Category 10: $HBsAg^+HBeAg^-$; Category 11: $HBcAb^+HBeAg^+$
Category 12: $HBsAb^+HBeAg^+$; Category 13: $HBeAg^+$
C.HBVSPM= Cummulative Hepatitis B Virus Seropositive Markers

5. CONCLUSION

Advancement of hepatitis B screening beyond routine HBsAg testing is no longer a luxury but a necessity. As evidence of this study showed, testing for other HBV serologic markers promotes safe blood practice, establishment of reliable epidemiologic data on HBV, successful evaluation of HBV vaccination programmes, HBV disease definitions and identification of individuals with potential risks of developing cirrhosis or hepatocellular carcinoma.

6. RECOMMENDATIONS

The following recommendations become inevitable based on the outcomes of this study: A policy targeting 100% voluntary blood donation should be formulated in each blood transfusion laboratory facility and strategies to achieving this put in place. Secondly, serologic screening of HBsAg alone irrespective of method used should be phased out and replaced with a more comprehensive diagnostic tool that screen for the five markers of hepatitis B virus to promote safe blood practices, identify vaccinated subjects and give correct picture of HBV endemicity. Thirdly, identified blood donors susceptible to HBV infection should be vaccinated against HBV and those with evident failed vaccination based on HBsAb seronegativity should be re-vaccinated.

CONSENT

As per international standard or university standard, patient's written consent has been collected and preserved by the authors.

ETHICAL APPROVAL

As per international standard or university standard, written approval of Ethics committee has been

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Adam I, Gassim GI, Murad IA. Hepatitis B

and C virus infections among pregnant women in Arab and African countries. Journal of Infectious Disease in Developing Countries. 2013;8:566-578.
2. Harkisoen S, Arends JE, van Erpecum KJ, van den Hoek A, Hoepelman AI. Hepatitis viral load and risk of HBV-related liver disease: From East to West? Annals of Hepatology. 2012;11:164-171.
3. World Health Organization. Sixty-third world health assembly on viral hepatitis infection. Geneva; 2011.
4. Liang TJ. Hepatitis B: The virus and disease. Hepatology. 2009;49(5):S13-S21.
5. Kao JH, Chen DS. Global control of hepatitis B virus infection. Lancet Journal of Infectious Disease. 2002;4:395-403.
6. Adekoya-Benson T, Famoni PO, Ajala O, Akinbolaji TJ, Adeosun OC, Agbaje A, Olatimehin O, Ibraheem HO. Prevalence of HBV, HCV and syphilis among the people of Ekiti in South-western Nigeria. International STD Research & Reviews. 2015;3(2):40-44.
7. Motayo BO, Akpa OM, Ezeani I, Faneye AO, Udo UA, Onoja B. Seroprevalence rates of hepatitis c virus (HCV) antibody and hepatitis B virus surface antigen (HbsAg) in blood donors in a Southwestern Nigerian City. Journal of Immunoassay and Immunochemistry. 2014;36(1):91-99.
8. Seroprevalence rates of hepatitis C virus (HCV) Antibody and Hepatitis B Virus Surface Antigen (HbsAg) in Blood Donors in a Southwestern Nigerian City. Journal of Immunoassay and Immunochemistry. 2014;36(1):91-99.
9. Salawu L, Bolarinwa RA, Adegunloye AB, Muraina HA. HBsAg, anti-HCV, anti-HIV and VDRL in blood donors: Prevalence and trends in the last three and a half years in a tertiary health care facility in Ile-Ife, Nigeria. International Journal of Medicine and Medical Sciences. 2010; 2(11):335-341.
10. Omobolaji T, Campbell-Yesufu, Rajesh TG. Update on human immunodeficiency virus (HIV)-2 infection. Clinical Infectious Disease. 2011;52(6):780–787.
11. Centre for Disease Control and Prevention. Viral Hepatitis. CDC Recommendations for specific populations and settings. division of viral hepatitis, National Centre for HIV/AIDS, Viral Hepatitis, STD and TB Prevention. Atlanta; 2015.
12. Kwon SY, Lee CHC. Epidemiology and prevention of hepatitis B virus infection. Korean Journal of Hepatology. 2011; 17:87-95.
13. Cindy MW, Ian W, Eric EM, Susan AW, Lyn FAW, Stephanie MN, John WW. Recommendations for identification and public health management of persons with chronic hepatitis B virus infection; 2008. Available:http://www.cdc.gov/mmwr/pdf/rr/rr5708.pdf
14. Liaw YF. HBeAg seroconversion as an important end point in the treatment of chronic hepatitis B. Hepatology International. 2009;3:425-433.
15. Dienstag JL. Hepatitis viruses. Harrison's manual of Internal Medicine 17th Eds. 2009;1932-1939.
16. Van der Poel CL, Seifried E, Schaasberg WP. Paying for blood donations: Still a risk? Vox Sang. 2002;83:285-293.
17. Ahmed SG, Umar BA, Ibrahim UA, Saidu AT, Jolayemi B. Dynamics of blood donation and donor categories in Birnin Kudu, Nigeria. Nigerian Journal of Basic Clinical Science. 2004;1:66-70.
18. World Health Organization. Updated Factsheet on HIV/AIDS epidemiology; WHO, Geneva; 2012.
19. Nwogoh B, Ikpomwen OD, Isoa EM. Donor blood procurement and the risk of transfusion transmissible viral infections in a tertiary health facility in South-South Nigeria. Nigerian Medical Journal. 2011; 52(4):227–229.
20. World Health Organization The Melbourne declaration on 100% voluntary non-remunerated donation of blood and blood components; 2009. Available:http://www.who.int/entity/worldblooddonorday/MelbourneDeclaration2009.doc
21. Bashawri LA. Pattern of blood procurement, ordering and utilization in a University Hospital in Eastern Saudi Arabia. Saudi Medical Journal. 2002; 23:555-561.
22. Clinical and Laboratory Standard Institute. Tubes and additives for venous blood specimen collection. Approved guideline-fifth edition. HI-A5. 2003;16(13):45-46.
23. Federal Ministry of Health. National Blood Transfusion Service. 2009;21-26.
24. Kulkani PY, Kulkani AD. Mass counselling: Effective tool to improve knowledge, attitude and behaviour regarding blood donation. Annals of Medical & Health

Sciences Research. 2014;4(1):90-94.
25. Lavanya V, Viswanathan TS, Arul Sheeba MS, Malarvizhi A, Moorthy K. Prevalence of hepatitis B virus infection among blood donors with antibodies to hepatitis B core antigen. International Journal of Medicine and Medical Sciences. 2012;4(6):128–137.
26. Salaudeen AG, Odeh E. Knowledge and behaviour toward voluntary blood donation among students of a tertiary institution in Nigeria. Nigerian Journal Clinical Practice. 2011;14:303-307.
27. 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 African Journal of Medicine. 2011;30(4):292-295.
28. World Health Organization. Towards 100% voluntary blood donation; A Global Framework for Action. WHO. Geneva; 2010.
29. Teo KSK, Saparudin MS, Zaini Z, Morshidi MA, Metassan N, Jaberudin R, Teo BC, Yakup S, Wendy N, Lim A. Transfusion transmissible infections in Brunei Darussalam: A blood donor study. Brunei International Medical Journal. 2011;7(6): 321-327.
30. Shaz BH, James AB, Hillyer KL, Schreiber GB, Hillyer CD. Demographic patterns of blood donors and donations in a large metropolitan area. Journal of National Medical Association. 2011;103(4):351-357.
31. Mohammed Y, Bekele A. Seroprevalence of transfusion transmitted infection among blood donorsat Jijiga blood bank, Eastern Ethiopia: Retrospective 4 years study. Biomed Central Research Notes. 2016; 9:129.
32. Nwogoh B, Awodu OA, Bazuaye GN. Blood donation in Nigeria: Standard of the donated blood. Journal of Laboratory Physicians. 2012;4(2):94-97.
33. Tayou TC, Monny LM, Mbanya D. Evaluation of two methods for haemoglobin measurement in Cameroonian blood donors. Transfusion Clinical Biology. 2006; 13:331-334.
34. Adediran IA, Fesogun RB, Oyekunle AA. Haematological parameters in prospective Nigerian blood donors rejected on account of anaemia and/or microfilaria infestation. Nigerian Journal of Medicine. 2005;14:45-50.
35. Shenga N, Pal R, Sengupta S. Behaviour disparities towards blood donation in Shikkim, India. Asian Journal of transfusion Science. 2008;2:56-60.
36. Shenga N, Thankappan K, Kartha C, Pal R. Analysing social demographic factors amongst blood donors. Journal of Emerging Trauma Shock. 2010;3:21-25.
37. Maghsudlu M, Nasizadeh S. Iranian blood donors' motivations and their influencing factors. Transfusion Medicine. 2011;21(4): 247-252.
38. Singh RP, Harimoorthy V, Maheswari K, Vaidya K. Hepatitis B virus vaccination of voluntary blood donors and immunization status assessment by Anti-Hepatitis B Surface (HBs) antibody titre. Asian Journal of Transfusion Sciences. 2013;7(2):160.
39. McMahon BJ. The natural history of chronic hepatitis B virus infection. Hepatology. 2009;49(5):S45-55.
40. Hsu YS, Chien RN, Yeh CT. Long-term outcome after spontaneous HBeAg seroconversion in patients with chronic hepatitis B. Hepatology. 2002;35:1522-1527.
41. Glynn SA, Smith JW, Schreiber GB, Kleinman SH, Nass CC, Bethel J. Repeat whole-blood and platelet-pheresis donors: Unreported deferrable risks, reactive screening tests, and response to incentive programs. Transfusion. 2001;41:736–43.
42. Ray MM, Bradley DH. Seroprevalence of markers for hepatitis B viral infection. International Journal of Infectious Diseases. 2011;15:e78-e121.
43. Comanor L, Holland P. Hepatitis B virus blood screening: Unfinished agendas. Vox Sang. 2006;91:1-12.
44. Allain JP. Review article: Occult hepatitis B virus infection. Hepatitis B Annual. 2004; 2009:14-30.
45. Chaudhuri V, Nanu A, Panda SK, Chand P. Evaluation of serologic screening of blood donors in India reveals a lack of correlation between anti-HBc titre and PCR-amplified HBV DNA. Transfusion. 2003;43:1442-1448.
46. Chisari FV, Ferrari C. Viral hepatitis. In: Nathanson N. Viral pathogenesis, Philadelphia, Lippincott – Raven. 1997;745 -778.
47. Arun JS, Seung KY, Riccardo L. The aetiology of hepatocellular carcinoma and consequences for treatment. The Oncologist. 2010;15(4):14-22.

48. Szabo E, Paska C, Kaposi NP.-Similarities and differences in hepatitis B and C virus induced hepatocarcinogenesis. Pathology Oncology Research. 2004;10:5-11.
49. You SL, Yang HI, Chen CJ. Seropositivity of hepatitis B e antigen and hepatocellular carcinoma. Annals of Medicine. 2004; 36:215–224.
50. Yang HI, Lu SN, Liaw YF. Hepatitis B e antigen and the risk of hepatocellular carcinoma. New England Journal of Medicine. 2002;347:168-174.
51. Kao JH, Chen PJ, Lai MY, Chen DS. Acute exacerbations of chronic hepatitis B are rarely associated with superinfection of hepatitis B virus. Hepatology. 2001; 34(4):817–823.
52. Mayo Medical Laboratories. Chronic hepatitis B profile (Type B) Interpretive handbook. Mayo Foundation for Medical Education, Florida, USA; 2016.
53. Centers for Disease Control and Prevention. A comprehensive immunization strategy to eliminate transmission of hepatitis B virus infection in the United States: Recommendations of the advisory committee on immunization practices (ACIP); Part 1: Immunization of Infants, Children, and Adolescents. Morbidity and Mortality Weekly Report. 2005;54(16):1-32.
54. Shittu AO, Olawumi HO, Issa BA, Nwabuisi C, Durotoye IA, Yusuf AD, Ajiboye PO, Adegunloye AO, Sulyman D, Salami AK. Risk factors and seroprevalence of hepatitis B surface antigen among blood donors in University of Ilorin Teaching Hospital, Ilorin, Nigeria. East African Medical Journal. 2014;91(3):1-5.
55. Vem TS, Pondei J, Nimzing L, Bigwan E, Abdullahi S. A prevalence of hepatitis B surface antigen amongst blood donors in Jos, North Central Nigeria. International Journal of Current Research. 2012; 4(11):59-62.
56. El-Ghitany EM, Farghaly AG. Serological pattern of hepatitis B virus among HBsAg negative blood donors in Alexandria, Egypt. Eastern Mediterranean Health Journal. 2013;19(7):600-607.
57. Kamel E, El-Beltagy, Ibrahem A, Al-Balawi, Ziad AM. Prevalence of hepatitis B virus markers among blood donors in a tertiary hospital in Tabuk, North-western Saudi Arabia. International Journal of Infectious Diseases. 2008;12(5):495-499.

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