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RESEARCH ARTICLE

Profile of Hepatitis B Sero Markers Among Blood Donors Attending Usmanu Danfodiyo University Teaching Hospital, Sokoto-Nigeria

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ABSTRACT

Introduction: Hepatitis B Virus (HBV) is a double-stranded circular DNA virus, it is one of the major blood transmissible infections. The prevalence of HBV is highest in sub-Saharan Africa and East Asia, where 5-20% of the adult population are infected. In Africa, up to 15 to 60% of the population are positive for at least one of the serological markers of HBV. This study aimed to determine the prevalence markers of HBV among prospective blood donors in Sokoto.

Materials/methods: About 170 blood donors were randomly recruited into this research study and 5ml of blood was aseptically collected from each of the research participants, the sample was screened for Hepatitis B virus, using a rapid step-wise HBV-5 panel immunoassay of Combo Cassette Manufactured by Lusys Laboratories Inc U.S.A. The HBV-5 is capable of detecting HBsAg, HBsAb, HBeAg, HBeAb and HBcAb simultaneously. The test component was opened from the notch, and the device was removed, 2-3 drops (60-90µl) plasma sample was added into the sample wells in the device. The result was read within 15 minutes.

Results: The prevalence rates of the markers are HBsAg 8(4.7%), HBeAg 1(0.6%), HBsAb 7(4.1%), HBeAb 8(4.7%), and HBcAb 9(5.3%). Donors aged (20-30) years had the highest prevalence rate of HBV infection compared to other age groups. Male blood donors had higher seropositivity for the five markers than their female counterpart. The prevalence of HBV markers was high among the First time donors (non-vaccinated donors) who have a higher prevalence than other donors (vaccinated donors).

Conclusion: This study has shown that screening for HBsAg alone may not be sufficient for the diagnosis of hepatitis B virus infection, and thus other markers should be included in the routine screening.

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ABBREVIATIONS

Anti HBc IgG: Anti- Hepatitis B core Immunoglobulin G; Anti-HBc IgM : Anti- Hepatitis B core Immunoglobulin M; DNA: Deoxyribonucleic Acid; HBcAb: Hepatitis B Core Antibodies; HBc IgM: Hepatitis B core Immunoglobulin M; HBeAg: Hepatitis B enveloped Antibodies; HBeAg: Hepatitis B enveloped Antigen; HBIG: Hepatitis B Immune Globulins; HBsAb: Hepatitis B Surface Antibodies; HBsAg: Hepatitis B Surface Antigen; HBV: Hepatitis B virus; HBV DNA: Hepatitis B Virus Deoxyribonucleic Acid; IgG: Immunoglobulins G; IgM: Immunoglobulins M; UDUTH: Usmanu Danfodiyo University Teaching Hospital.

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INTRODUCTION

Hepatitis B Virus (HBV) is of the Hepadnaviridae family, which has a double-stranded circular DNA genome [1]. The virus has proteins that form significant markers for its diagnosis and treatment. They are five (5) different proteins markers that are found in HBV, they are:

- Hepatitis B surface antigen (HBsAg); this constituent the outer protein coat of the virus,
- Hepatitis B envelope antigen (HBeAg), this is normally shed during the viral replication
- Hepatitis B core antibodies (HBcAb): this found in the hepatocytes [2]. Others are Anti-Hbe-Ab and Anti-HBs Ab.

The replicative and life cycle of this virus can be divided into four stages in the human host, stage one and two are termed the replicative phase, and the two stages are described as the integrative phase [3]. HBsAg, HBeAg and HBV DNA are detected in blood in the first stage, while Anti HBe is found to be negative. This is defined as the active period in the HBV life cycle and is described by immune tolerance where there is the presence of active viral replication occasioned by upregulations of HBV DNA and infectiousness in the host. The second stage in the life cycle is akin to the first except that the level of HBV DNA is drastically reduced and the profile of liver enzymes is found to be elevated [3]. HBV DNA is found to be negative, during the third stage, while Anti-Hbe and is detectable in blood and HBeAg is, negative as well. This is the point where host immune control is achieved and active viral replication stops, this stage is characterised by a complete clearance of the virus in the fourth stage with HBsAg becoming negative or undetectable and infectiousness of the host is drastically reduced [3]. An occult infection could be described as the presence in the liver of replicative-competent HBV DNA, with the absence of HBsAg in the serum [4]. Hepatitis B virus infection is one of the deadly transmissible blood infections, it has chronically infected over 400 million people globally [4]. The prevalence of HBV is much higher in East Asia and sub-Saharan Africa, where 5.5–20.5% of the adult population is infected [5] and 17 to 65% of the general population are found to be positive for one or more of the serological markers of HBV infection. HBV causes a spectrum of infections which often ranges from inactive chronic carrier states to progressive chronic hepatitis which could lead to cirrhosis, followed by primary liver cancer [6]. Regions with the prevalence of HBsAg above 9% are described as HBV endemic region [2]. Despite the availability of vaccines in most countries, like Nigeria, the prevalence of this virus infection is still dangerously high in Nigeria and other countries of the world, and this viral infection is an important cause of morbidity and mortality [7]. HBV is a major cause of liver disease morbidity and mortality worldwide, accounting for over 360 million cases of chronic

hepatitis and 620,000 deaths per year [8]. It is hyperendemic (that is >8% of the population infected) in Sub-Saharan Africa and a major cause of chronic liver disease [9]. Estimated that 44% of cirrhotic liver disease and 47% of hepatocellular carcinoma cases in SSA are attributed to HBV [10]. Hepatitis B virus infection is arguably the most significant global public health problem [11]. Of the approximately 2 billion people infected worldwide, 300 million infected patients are chronic carriers of Hepatitis B Virus (HBV) and it is the 10th leading cause of death [12]. HBV is known as one of the first viral infection discovered to be found in the blood and transmitted mainly through blood and blood products [13]. In countries with high prevalence, HBV is seen as one of the major complications of transfusion of blood and its products [14], this is due to the facts that, there exist an extended window period between infection with HBV and the actual detection of HBsAg, within which the virus could be transmitted [14]. Researchers have shown that individual who are anti-HBc positive have the possibility of harbouring an occult HBV infection thus maintaining in their liver and blood, HBV-DNA [15] and this serves as a potential source of transmission. The mere absence or undetectable presence of HBsAg in the blood or other bodily fluids of healthy individuals may not be enough to guarantee the absence of circulating HBV, so blood and blood products containing anti-HBc should be treated as infectious, either with or without the detectable presence of HBsAg [16]. Studies from different parts of Nigeria has reported a varying prevalence rate of HBsAg among blood donors, however, information on other markers of HBV is scarce, because DNA testing of all collected units is not feasible because of the cost of running the test in Nigeria [15]. This study was therefore conducted primarily to determine the prevalence of other markers of hepatitis B virus and to evaluate the reliability of infection in screening blood donors in our transfusion centres in Nigeria using HBsAg marker alone in the diagnosis of HBV.

Since an individual with anti-HBc positive may harbour an occult HBV infection and maintain HBV-DNA sequence in their blood and liver. This presents a potential source of HBV transmission, the absence of HBsAg in the blood of apparently healthy individual may not be sufficient to ensure the lack of circulating HBV, this containing anti-HBc with or without the detectable presence of HBsAg might be an infection. This study will provide up-to-date data about the hepatitis B status of the screened donors in the UDUTH blood bank. The data obtained from this research will assist the hospital in deciding transfusion using screened donors and taking preventives measures. It creates awareness among the screened donors to go for regular hepatitis B screening. It also assists in the proper screening of donors to avoid HBV transmissions. This study aims to screen and assess the prevalence of Hepatitis B Virus (HBV) infection among screened donors attending Usmanu Danfodiyo University Teaching Hospital Sokoto using Hepatitis B sero markers cassettes.

MATERIALS AND METHODS

Study area

The study was carried out in the Faculty of medical laboratory sciences in collaboration with Usmanu Danfodiyo University Teaching Hospital (UDUTH), Sokoto. Sokoto has a total population of 4.2million people [17]. The state has a landed area of 26,64km² and is located between longitude 11.30 to 13.50 degrees east and latitude 4 to 6-degree north. It is bounded to the North by the Niger Republic, Kebbi state to the South-west and the East by Zamfara state. The state comprised of 23 local governments areas [17].

Study design

This is a descriptive, cross-sectional study design to determine the prevalence markers of HBV among prospective blood donors in Usmanu Danfodiyo University Teaching Hospital (UDUTH) Sokoto, between May 2018 to July 2019.

Determination of sample size: The sample size was calculated using the standard formula for calculating the minimum sample size (18). Sample size (n) is given by the formula below:

$$n = \frac{Z^2 Pq}{d^2}$$

where;

n = Minimum sample size

Z = Standard normal deviation at 95% level of confidence = 1.96

Since there was no published work on prevalence of the cement company workers, 50% is considered as the prevalence.

P = Prevalence to be estimated is 14% =

q = Compliment of p i.e. 1-p = 1-0.14 = 0.86

d = Tolerance margin of error = 90% i.e. (100-95%) = 5% = 0.05 therefore then plus 10% alteration ratio = 14.9

$$149 + 14.9 = 163.9164$$

Inclusion and exclusion criteria

HBV negative blood donors attending UDUTH were included in the study, donors who found to be positive for the HBV screening test were excluded from the research study.

Study population

The population comprised of 170 blood donors attending UDUTH Sokoto, Sokoto state. The target population are selected at random to include HIV negative blood donors

attending UDUTH, Sokoto. Blood donor with positive HBV screening test were all excluded from the research study, this is because those that are confirmed to be positive for HBV cannot serve as Blood donors and thus cannot give blood

Data collection and analysis

The socio-demographic data and other relevant information of each participant were obtained using a self-administered questionnaire, data obtained were entered into SPSS version 22 for analysis. The results obtained were presented using Tables.

Scope and limitation of the study

The study is limited to a randomized screening of 170 blood donors attending UDUTH Sokoto. The information obtained from this study will be treated with the utmost confidentiality and will only be used for this research. This work is only limited to a screening of blood donors for hepatitis B sero-markers.

Ethical consideration

Ethical clearance for the study was sought and obtained from the ethical committee of Usmanu Danfodiyo University Teaching Hospital (UDUTH), Sokoto, with the reference number, UDUTH/HREC/2017/No.630.

Sample collection

The blood sample was taken from each participant for the serological test. Informed consents were obtained from participants before the collection of the sample. A sample of blood was collected from each participant with a sterile disposable syringe and needle, after disinfection of the selected venipuncture site with 70% alcohol in an expanding circular scrub from the centre to the periphery of the needle insertion. About 5ml of blood was collected by venipuncture and was dropped into an anticoagulated bottle, the plasma was separated and used for serology.

Serological test procedure

Clinically, HBsAg, HBsAb, HBeAg, HBeAb and HBcAb are the important markers in the diagnosis of HBV infections. The onsite HBV-5 Rapid Test can detect HBsAg, HBsAb, HBeAg, HBeAb and HBcAb simultaneously. The plasma specimen and test component were brought from room temperature, thawed and mixed properly. The test component was opened from the notch, and the device was removed. The device was labelled with the specimen Identification number and 2-3drops (60-90l) plasma specimen was added into each of the sample wells in the device. The result was read within 15minute and recorded.

RESULTS

Out of 170 screened blood donors recruited for the study, the following tested positive: 8(4.7%) for HBsAg, 7(4.1%) for

HBsAb, 1(0.6%) for HBeAg, 8(4.7%) for HBeAb and 9(5.3%) for HBeAb. This is as shown in figure 1.

DISCUSSION

Hepatitis B virus infection is a major blood transmissible infections known to man with about 400 million people chronically infected globally [3]. In 170 blood samples tested, HBsAg was detected in 8(4.7%), HBeAg 1(0.6%), HBsAb 7(4.1%), HBeAb 8(4.7%) and HBeAb 9 (5.3%) respectively (Figure 1). The result of this study showed that 9(5.3%) blood donors had anti-HBc as the serological markers. This finding is in agreement with Geraldine (2006), who reported that testing blood donors for HBsAg alone is not sufficient to eliminate transfusion mediated hepatitis B viral infection. It was observed that the presence of HBV-DNA in one-fifth of anti-HBc only positive donors studied and stated that, reactivity to anti-HBc only can predict HBV infection [19]. It may be possible that some of the blood donors are in their window period and may have HBc IgM antigen at that stage when HBsAg is not detectable in the blood. The only serologic evidence of hepatitis B infection is the presence of IgM antibody to the core antigen [19]. The screening test for detection of HBsAg does not rule out the transmission of Hepatitis B as the donors might be in the window period and detection of the antibody to hepatitis B core antigen (HBc IgM), serves as a useful serological marker during the window period. A prevalence of 5.3% observed for anti-HBc in this study is high compared with 0.56% in the United Kingdom [20], 0.84% in the United State [21], 1.13% in Canadian blood donors [22], 4.85% in Italy [23]. This may due to proper awareness for HBV infection in that area than where this research was conducted. The result from this study showed lower anti-HBc prevalence compared to those obtained in Indian 18.9%, Pakistan 17.28%, Turkey 20.0% [24], 18.1% in Maiduguri, Borno State, Nigeria [15]. IgM class of the anti-HBc is a marker that indicates recent infection, IgG variety of anti-HBc appears later during the infection and points to a past HBV infection. Anti HBc IgG may remain positive for life in an infected individual, although the

Figure 1 The prevalence of HBV Makers among blood donors. 4.7% for HBsAg, 4.1% for HBsAb, 0.6% for HBeAg, 4.7% for HBeAb and 5.3% for HBeAb.

Figure 2 Prevalence of HBV markers among blood donors by tribes, 67.3% for Hausa, 17.1% for Yoruba, 10% for Igbo and 5.3% for others.

individual has protective levels of anti-HBs, the blood of such a donor might be free from transmitting HBV [25].

In figure 2, it was indicated that the prevalence of HBV markers among blood donors is tribe dependent, in that Hausa has 67.3% of positive markers, Yoruba has 17.1% of positive markers, Igbo has 10% of positive markers and 5.3% of the positive markers are from the other tribes, but this result could also be attributed to the fact that the research was conducted in a predominantly Hausa dominated state.

Blood donors within the age group 20-30 years had the highest prevalence of HBV markers than 31-40 years, 41-50 years and 51-60 years as shown in figure 3. This study contradicts the earlier report with a high prevalence of HBV in older subjects 40 years and above than in younger people [26] and Luka [27], in their study, reported higher HBV prevalence among the older age group 30-34 years. The finding in this study supports the work of Buseri (2009), who noted that HBV infection is more prevalent in younger subjects within ages 20-29 years [28], the possible reason for this high prevalence rate in younger people than older people may be attributed to their active sexual activities and drug abuse, however, we observed that HBV is not limited to any particular age group.

The gender-specific difference shown that male blood donors had higher seropositivity for the five markers than the Female counterpart. This contradicts the report of Uneke, 2005, who stated that females are more infected with HBsAg than the male [29]. The reason for this difference might be due to a large number of male blood donors in this study than the females. However, his observation contradicts what had been previously reported by some authors, Mehmet [30] in his study reported a higher prevalence rate in male than females in both rural and urban, with the observation that male sex is an important factor for HBV positivity (Figure 4). The same high prevalence rate of HBsAg was reported among males than the females in Lagos, Nigeria [31]. A similar study also reported a higher HBsAg prevalence in males than

Figure 3 Prevalence of HBV Markers among blood donors by Age. 20-30 (1.9 for HBsAg, 2.4 for HBsAb, 2.4 for HBeAb, 2.6 for HBcAb) 31-40 (0.6 for HBsAg, 0.6 for HBsAb, 1.3 for HBeAb, 0.6 for HBcAb) 41-50 (1.2 for HBsAg, 0.6 for HBsAb, 1.2 for HBeAb, 0.0 for HBcAb) 51-60 (1.2 for HBsAg, 0.6 for HBsAb, 0.0 for HBeAg, 0.0 for HBeAb, 1.8 for HBcAb).

females among patients attending dental Clinic, University College Hospital, Ibadan, Nigeria, this was due to a shorter carrier HBsAg rate in female than the males [24]. The lack of statistical difference in HBV markers suggests that they were equally exposed to HBV in corroboration, with earlier findings [32].

Out of the 170 blood donors, 151(88.8%) samples were first time donors, (not vaccinated) 7(4.12%), were second-time donors received one dose of vaccine, 8(4.71%) were the third time donors received two doses of vaccine and 4(2.35%) were fourth-time donors received three or more dose of vaccine respectively. The prevalence of HBV markers was high among first-time donors (non-vaccinated donors) than the remaining donors (vaccinated donors) and the vaccinated donors are safest than the non-vaccinated donors (Figure 5). This result is in agreement with a previous study conducted among blood donors at Gondar University Teaching Hospital, Northwest Ethiopia [33]. This shows that HBV infection decreases as the frequency of vaccination

Figure 4 The prevalence of HBV markers among donors by gender Male (4.0 HBsAg, 4.0 HBsAb, 0.6 HBeAg, 2.9 HBeAb, 4.8 HBcAb) Female (0.4 HBsAg, 0 HBsAb, 0 HBeAg, 1.8 HBeAb, 0.6 HBcAb).

Figure 5 Frequency of vaccinate HBV positive marker. None (2.9 for HBsAg, 3.5 for HBsAb, 0.6 for HBeAb, 1.3 for HBeAb, 3.5 for HBcAb) Once (1.3 for HBsAg, 0.6 for HBsAb) Multiple (0.6 for HBsAg, 0 for HBsAb, 0 for HBeAg, 0.6 for HBeAb, 0 for HBcAb).

increases. The possible reason is that the more an individual get vaccinated the more the donor becomes immunized.

CONCLUSION

This study showed an overall prevalence rate of HBsAg to be 4.7%, HBsAb 4.1%, HBeAg 0.6%, anti-HBc 9(5.3%), among prospective blood donors in Usmanu Danfodiyo University Teaching Hospital Sokoto, Nigeria. This study, however, confirmed the presence of HBV markers among apparently healthy blood donors. The majority of those might have acquired the infection through unsafe sexual practices and blood transfusion during window period or late phase chronic HBV when HBsAg is at an undetectable level. Thus the need for the inclusion of HBc in routine screening of blood donors in Nigeria to reduce the risk of transfusion transmissible HBV infection.

RECOMMENDATIONS

In line with this result fine, it is recommended that all blood donors be routinely screened for HBV profile as part of the routine blood donation process, particularly during their first visit. There should be public awareness campaign for Hepatitis B infection and public vaccination/immunization. We equally recommended that HBV DNA testing should be included in blood donation screening test. This research should be replicated on a national scale with wider participation of major tertiary health institutions nationwide.

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