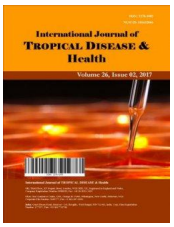

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Hepatitis B Serological Markers and Risk Factors among Pregnant Women and Prospective Blood Donors in Southwestern Nigeria

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Authors' contributions

This work was carried out in collaboration between both authors. Author OAA designed the study, performed the statistical analysis, wrote the protocol and wrote part of the first draft of the manuscript. Author MOJ wrote the part of the manuscript and made useful contributions in the analysis of the results. Both authors read and approved the final manuscript.

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ABSTRACT

Background: Hepatitis B control, especially among pregnant women and prospective blood donors, in the developing world is still a disease that must be given attention considering its mortality and morbidity rates.
Methods: This study was conducted among 153 subjects including pregnant women and prospective blood donors using appropriate hepatitis B serological marker ELISA kits (WANTAI, China) for each marker.
Results: Among the subjects screened, 38 had at least one of the markers. Among those that showed no detectable HBsAg were 2 prospective blood donors with HBeAg and another 6 prospective blood donors and 2 pregnant women with HBcAb-IgM detected in them all indicating an ongoing infection and replication of hepatitis B virus. Sexual activities were found to be of statistical significance in the study.
Conclusion: It is imperative to give more attention to control of HBV spread through more sex education and administration of vaccination.

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Keywords: Pregnant; blood donor; HBV; markers; sexual activities.

1. INTRODUCTION

Hepatitis B virus (HBV) infection is a serious public health problem causing most of the acute and chronic liver disease [1]. It is estimated globally that 2 billion people (approximately 30% of the world population) have HBV infection, of which 300-400 million have chronic infection [2,3]. About 5% of the world population are at risk of developing chronic infection leading to 5 million to 1.2 millon death yearly [4,5]. The causative agent is a member of the family Hepadnaviridae and belongs to Orthohepadnavirus genus. HBV invades the liver and causes acute hepatitis which may resolve without complication but in some cases may lead to chronic form of the disease [6].

HBV infection is highly infectious and can be transmitted covertly by percutaneous routes and overtly by blood transfusion [3]. Transfusion transmitted hepatitis B infection (TTHBVI) has always been a dreaded disease with an increased incidence in donated blood [7]. However, the introduction of HBV nucleic acid tests (NAT) in blood donor screening, has brought about a decrease in the residual risk of HBV infection by transfusion [8]. Yet the use of unscreened blood transfusion keeps the patient at a high risk of acquiring such transfusion-transmitted infections (TTI) [3] especially in developing countries. The essence of screening for individual or combinations of HBV markers are to ascertain different phases of the HBV infection and to determine whether a patient is at the acute or chronic infection stage, is immune to HBV as a result of prior infection or vaccination, or is susceptible to infection. The serological markers of hepatitis B virus infection are hepatitis B surface antigen (HBsAg), and its corresponding antibody, (antiHBs), hepatitis B core antigen (HBcAg), and antibodies to the core antigen (antiHBc- IgM and IgG), hepatitis B envelope antigen (HBeAg), and hepatitis B envelope antibody (antiHBe) and hepatitis B virus DNA (HBV DNA) [1]. HBV DNA can be detected very early after HBV infection and it generally indicates active viral replication. HBsAg is the first serological marker to appear during incubation period and can initially be detected in serum from 1-12 weeks after infection. During convalescence, it declines to undetectable levels and if it persists for more than 6 months then it indicates a carrier state and a risk for chronic hepatitis.

The discovery of HBsAg was a major breakthrough in decreasing the incidence of transfusion transmitted hepatitis B. However, in many developing countries, including Nigeria, screening of blood before transfusion for HBsAg alone is still in practice in the prevention of HBV transmission during blood transfusion [9]. The screening of donated blood by enzyme-linked immunosorbent assay (ELISA) is the common method used for the detection of the viral marker. HBsAg screening however does not totally rule out the risk of transmission of hepatitis B [10,11]. This is because during serological response of the host to infection, there is a phase called the 'window period' in which HBsAg cannot be detected in the blood. The window period represents a carrier state of the disease although the infection is present. During the window period, when the HBsAg has disappeared and HBsAb has not yet appeared, the detection of the antibody to the hepatitis B core antigen serves as a useful serological marker for HB infection [12]. The IgM class of the anti-HBc is the first to appear indicating recent infection while the IgG variety of anti-HBc appears later during infection and shows a previous HBV infection [10].

Individuals with IgG variety of anti-HBc may not be infectious as they may have sufficiently high titres of antibodies to HBsAg which are protective in nature and the affected individuals may actually be disease free [7,10]. It has been demonstrated that some patients with no detectable HBsAg in them but showing HBc IgM antibody in their sera continue to replicate HBV [13]. Thus, the absence of HBsAg in the blood sample may not be enough to prove absence of an ongoing HBV infection. Blood containing anti-HBc (IgM) with or without detectable presence of HBsAg might be infectious, therefore routine prospective blood donor screening for anti-HBc (IgM) may be necessary to decrease the risk of transfusion transmitted hepatitis B. This study was designed to determine the prevalence of HBV serological markers and the risk factors associated with HBV transmission in Southwestern Nigeria among blood donors and pregnant women.

2. MATERIALS AND METHODS

2.1 Study Population and Location

A total of 153 participants (Male/Female: 67/ 86) were enrolled in the study. All the females were

pregnant women while the males were prospective blood donors, recruited from private and government hospitals in Osun, Oyo and Ondo states, all in the Southwestern Nigeria. The samples were collected from September 2015 to January 2016.

2.2 Sample Collection and Preparation

Three milliliters (3 ml) of venous blood was collected into sterile plain bottles from each patient, centrifuged at 3000 rpm for 10 minutes and the serum transferred aseptically into labelled cryovials. The sera were stored at −20°C until analysed. The sociodemographic and risk factors data were collected using structured questionnaire.

2.3 Serological Testing

All samples were tested for HBsAg, anti-HBs, HBeAg, anti-HBe and anti-HBc (total and IgM), using ELISA (Wantai AiD^{TM}, Wantai Biopharmaceuticals, China) according to manufacturer's instruction. The subjects who tested positive to the markers were compared in terms of gender, age group and risk factors by use of chi-square and Fisher's exact test at 95% confidence interval, using SPSS version 20.0.1 for Windows.

3. RESULTS

3.1 Prevalence of Hepatitis B Virus Serological Markers in South-western Nigeria

The occurrence of all the serological markers, except HBeAg, detected in the study population were relatively high with at least one serological marker detected in 24.8% (n=38) of the study population. Specifically, the prevalence of the markers HBsAg, anti-HBs, HBeAg, anti-HBe, anti-HBc (total) and HBcIgM detected in the 153 samples are respectively 19(12.4%), 21(13.7%), 3(2.0%), 20(13.1%), 52(34.0%), 14(9.2%).

3.2 Socio-demographic Data and Study Location of Samples Positive for HBV Serological Marker

Table 1 shows the distribution of the detectable HBV markers according to the socio-demographic data, study location and months of sample collection and their levels of significance.

Out of the 69 prospective blood donor samples that showed no detectable HBsAg, 2(1.5%; 2/134) had HBeAg in them indicating active HBV replication. One of these two prospective blood donor had anti-HBcIgM, showing recent infection while the other is negative for all other markers tested. The distribution and occurrence of other HBV serological markers in HBsAg negative subjects is as shown below.

3.3 Serological Marker and Risk Factors of HBV Transmission

Table 4 shows the risks factors associated with HBV transmission and the serological markers. There was no correlation between history of blood transfusion, occupation, surgery, dental extraction, tattoo, sharing sharps and prevalence of HBsAg (P = 0.05). However, there were significant correlation between sexual activity, number of sex partners, use of protection during sexual activity and HBsAg prevalence (P=0.03, 0.04 and 0.02 respectively). There was no correlation between HBV vaccine record and the distribution of the markers as many of the subjects could not ascertain their vaccination history.

The Table 4 shows the significance values obtained for some of the risk factors considered.

4. DISCUSSION

High prevalence of serological markers of HBV is reported in the study population, except for HBeAg which shows a prevalence of 2%. The prevalence of HBsAg and its corresponding antibody in this study was 12.4% and 13.7% respectively. In Nigeria the prevalence of HBsAg ranges from 2.4% to 19.6% [14–17], depending on the study population while anti-HBs prevalence is between 2.5 to 35% [15,19,20]. Hepatitis B e antigen and its associated antibody (HBeAb) has been found among different population to be between 1.5% -16.2% and 23% -81% respectively [20,18,16,19] while antiHBc total and HBcIgM values are between 32%-67% and 4% to 18.4% respectively [14,15, 20,18,19]. The prevalence of the serological markers observed in this study falls within the range reported in previous studies above.

Table 1. Socio-demographic parameter and prevalence of hepatitis B markers in screened individuals

Sociodemographic parameter		Samples positive for HBV Serological Markers (%)						P value (95% CI)
		HBsAg (%)	HBsAb (%)	HBeAg (%)	HBeAb (%)	HBcAb (Total) (%)	HBcAb-IgM (%)	
Sex	Male	8 (12)	9 (13.4)	2(3)	11(16.4)	29 (43.3)	8(12)	
	Female	11(12.8)	12 (14)	1(1.1)	9 10.5)	23(26.7)	6(7)	
	Total	19	21	3	20	52	14	
Marital status	Single	4 (7.4)	6(11.1)	1(1.9)	7(13)	20(37)	5 (9.3)	
	Married	15 (15.2)	15 (15.2)	2 (2.0)	13(13.1)	32 (32.3)	9 (9.1)	
	Total	19	21	3	20	52	14	
Age (Years)	11-20	1(25)	2(50)	0(0)	0 (0)	1(25)	0 (0)	
	21-30	8 (9.3)	13 (15.1)	1 (1.2)	9 (10.5)	30 (34.9)	8 (9.3)	
	31-40	9 (18)	3 (6)	1 (2)	9 (18)	14 (28)	4 (8)	
	41-50	1 (9.1)	2(18.2)	0 (0)	2 (18.2)	7 (63.7)	1 (9.1)	0.529
	51-60	0 (0)	1 (100)	1 (100)	0 (0)	0 (0)	1 (100)	
	Total	19	21	3	20	52	14	
Occupation	Civil Servant	2 (7.1)	3 10.7)	1 (3.6)	2 (7.1)	13 (46.4)	3 (10.7)	
	Trading	7 (21.9)	4 (12.5)	0 (0)	7 (21.9)	11	2 (6.3)	
	Artisan	6 (12.8)	5 (10.6)	1 (2.1)	6 (12.8)	14 (29.8)	5 (10.6)	
	Housewife	0 (0)	1 (16.7)	0 (0)	0 (0)	1 (16.7)	0 (0)	0.754
	Bank Staff	0 (0)	1 (25)	0 (0)	1 (25)	3 (75)	0 (0)	
	Students	4 (14.3)	6 (21.4)	1 (3.6)	4 (14.3)	8 (28.6)	4 (14.3)	
	Others	0 (0)	1 (12.5)	0 (0)	0 (0)	2 (25)	0 (0)	
	Total	19	21	3	20	52	14	
State	Ondo	10 (20.8)	7 (14.6)	0 (0)	11 (22.9)	26 (54.2)	4 (8.3)	
	Osun	7 (10.1)	9 (13.0)	3 (4.3)	8 (11.6)	26 (37.7)	8 (11.6)	
	Oyo	2 (5.6)	5 (13.9)	0 (0)	1 (2.8)	-(0)	2 (5.6)	0.082
	Total	19	21	3	20	52	14	
Months of collection	September	1 (2.6)	2 (5.3)	1 (2.6)	4 (10.5)	17 (44.7)	5 (13.2)	
	October	3 (8.8)	6 (17.6)	0 (0)	4 (11.8)	16 (47.1)	2 (5.9)	
	November	11(21.2)	8 (15.4)	1 (1.9)	10 (19.2)	17 (32.7)	5 (9.6)	
	December	1 (25)	1 (25)	0 (0)	0 (0)	-(0)	0 (0)	
	January	3 (20)	4 (26.7)	1 (6.7)	2 (13.3)	2 (13.3)	2 (13.3)	
	Total	19	21	3				

Table 2. Other hepatitis B virus serological markers in HBsAg negative individuals

	HBsAg +ve	HBsAg -ve	HBsAg –ve & HBeAg +ve	HBsAg –ve & HBcAb +ve	HBsAg –ve & HBcAb-IgM +ve
Blood Donors	9	69	2	27	6
Pregnant Women	10	65	0	13	2
Total	19	134	2	40	8

Table 3. Detailed serological profile of HBsAg negative but HBcIgM positive samples

S/N	Lab ID	Age group	Sex	State	Marital status	Group	Occupation	HBsAb	HBcAb Status	HBeAg Status	HBeAb
1	A003	21-30	Male	Osun	Single	Blood Donor	Student	Negative	Positive	Negative	Negative
2	A006	>60	Male	Osun	Married	Blood Donor	Civil Servant	Positive	Negative	Positive	Negative
3	A017	41-50	Male	Osun	Married	Blood Donor	Civil Servant	Negative	Positive	Negative	Positive
4	A036	21-30	Male	Osun	Single	Blood Donor	Student	Negative	Positive	Negative	Negative
5	A037	21-30	Male	Osun	Single	Blood Donor	Artisan	Negative	Positive	Negative	Negative
6	IB013	21-30	Female	Oyo	Married	Pregnant	Artisan	Positive	Negative	Negative	Negative
7	MC006	21-30	Female	Osun	Single	Blood Donor	Student	Negative	Positive	Negative	Negative
8	S008	21-30	Female	Oyo	Married	Pregnant	Artisan	Negative	Negative	Negative	Negative

Table 4. The risk factors associated with HBsAg distribution and the *P* values at 95% CI

Risk factors	*P* values
Occupation	0.75
Marital status	0.15
Blood transfusion	0.51
Surgery	0.90
Hospital admission	0.95
Dental surgery/Extraction	0.93
Sexual activity	0.02**
Number of sex partners	0.04**
How often you have sex	0.02**
Protective measures in sex	0.02**
Use/share of sharps	0.07
Tattoo	0.37
Family member diagnosed of HB	0.18

*Note: ** show significant values at 95% CI*

Laboratory detection of hepatitis B virus infection is crucial for global control and prevention of HBV disease. Previous studies have suggested that performing HBsAg tests alone does not completely eliminate the risk of HBV transmission to blood recipients [7,10,11,21]. This study reports the presence of HBeAg (1.5%; 2/134) and HBcIgM (6.0%; 8/134) in the absence of HBsAg. These markers correspond to viral replication and recent HBV infection. The progression of liver disease in hepatitis B virus (HBV) infection is being fostered by active virus replication [22] and the presence of hepatitis B e antigen (HBeAg) in the serum generally indicates ongoing viral replication, disease progression and infectivity [23–26] hence HBeAg positive patients have high levels of serum HBV-DNA which indicates extremely intensive viral replication [23]. Transfusion of blood from the two HBeAg positive donors on the basis of HBsAg negativity as done in many developing countries including Nigeria is unsafe and will most likely result in post transfusion HBV infection since the donors are actively replicating the virus.

Anti-HBc (total) indicates history of infection whether present or past [21,27] while HBcIgM is a marker of recent HBV infection. Although the detection of HBV DNA was not carried out in this study to detect Occult HBV infection (OBI), the detection of anti-HBc in the serum of HBsAg-negative individuals is recognized as a marker of exposure or as being suggestive of occult infection [28-30,21]. In this study, we found the prevalence of HBsAg negative but anti-HBc (total) positive patients to be 29.9% (40/134) while 6.0% (8/134) are recent infection (HBcIgM positive). Interestingly, one of these samples shows HBcIgM as the only marker of HBV infection. Previous studies have suggested screening blood donors for HBcIgM in addition to HBsAg [14,19] as a way of preventing transmission of HBV through improperly screened blood.

In developed countries, the risk of transfusion-transmitted viral infections have been almost eliminated by the use of questionnaires to exclude donors at higher risk for HIV/HBV infection (donor selection) and the use of highly sensitive laboratory screening tests to identify infected blood donations. On the other hand, effective blood screening and banking system have remained elusive in most developing countries. From this study, there was a correlation between HBsAg and sexual activity as well as the number of sex partners. Blood donors who are sexually active, who have more than one sex partner and who had sex without protection were found to be more infected with HBsAg than their counterparts; use of donor selection could have excluded these prospective blood donors.

In patients with acquired immunity to HBV infection through vaccination, anti-HBs is the only serological marker detected in serum. We found 8 (5.2%) patients who have acquired immunity to HBV probably by vaccination while another five have developed immunity through exposure to the wild virus since they have anti-HBc in addition to the anti-HBs. This result shows that the rate of HB vaccination among the blood donor is low, hence the need for more enlightenment programs of HB vaccine and its benefits. It should be noted that proper HBV vaccination records of many of the subjects could not be confirmed thereby preventing a true picture of the source(s) of the immunity that some of them had acquired. The rate of acquired immunity through vaccination could be said to be low in the study population and we found HBeAg and HBcIgM separately as the only evidence of HBV infection in the population.

Although this study was not designed to identify occult HB infection and HBV genotypes in circulation in the study areas, further studies with larger sample size would attend to this and hence produce fuller information not only on selected markers but on the virus as a

whole. However, the study has provided useful information on HBV serological markers, HBV transmission and risk factors in the study area and population. It further buttressed the possibility of being transfused with HBV infected blood if the prospective blood donors are screened for HBsAg only. This should be extended to pregnant women as could be seen in this study of a woman who was HBsAg negative yet carrying the HBcIgM antibody and presenting with signs of ongoing fresh HBV infection.

To conclude, sexual activities and the number of sex partners were implicated by this study as routes of transmission, it is therefore important to educate the people in the areas on the importance of abstinence and /or faithfulness to one partner. Public enlightenment program on benefits of HBV vaccine and the need to properly keep records of vaccination is also important. Making HBV vaccination compulsory for women before pregnancy will help prevent vertical HBV and protect the neonates from HBV infection. This study advocates the use of donor selection through the use of questionnaire. The use of HBsAg as the only marker of HBV infection among blood donors should be discouraged while the government should make personnel and facilities available for the screening for other HBV serological markers and not only HBsAg.

5. CONCLUSION

It is imperative to give more attention to control of HBV spread through more sex education and administration of vaccination.

CONSENT

Additional informed consent was obtained from all patients for which identifying information is included in this article.

ETHICAL APPROVAL

The study received favourable ethical approval from Obafemi Awolowo University Health Services Research Ethics Committee and has therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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