

Prevalence Of Hepatitis B Surface Antigen And Hepatitis B Core Antibody Among Prospective Blood Donors In Abuja, Nigeria

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Abstract

Hepatitis B (HB) virus (HBV) remains a major risk factor for safe blood use. Compulsory pre-transfusion screening for HB core antibody (anti-HBc) to detect occult HBV infection in HB surface antigen (HBsAg)-negative persons and prevent post-transfusion HBV infection in the target area is an unmet need. This study aimed to evaluate the presence of HBsAg and anti-HBc in prospective blood donors in Abuja, Nigeria and determine their association with age, gender, marital status (MS) and education. The research adopted the principle of enzyme-linked immunoassay (ELISA) method for the evaluation of the presence of Total anti-HBc positivity and HBsAg-status among blood donors; and analysed their association with age, gender, MS and education using chi-squared (X^2) test. The results were presented in simple tables and figures. A total of 300 individuals participated in the study. The p-value was set at $P \leq 0.05$ as significant level. Data for 300 participants were analysed with male:female dominant ratio of 24:1, recording prevalence of 7.7% (HBsAg+) and 17.7% (anti-HBc). Age group 25-34 years had the highest prevalence: 3.7% for HBsAg+ and 8.3%

for anti-HBc; participants with formal education had higher prevalence for HBsAg+ (6.7%), anti-HBc (14.3%) than informal education (1.0% and 3.3%) respectively. Married participants recorded higher prevalence for HBsAg+(6.0%) and anti-HBc (14.0%) than unmarried participants (1.7% and 3.6%) respectively. Anti-HBc positive without HBsAg-positivity was recorded among 10% of the participants. There was no significant association between prevalence of the markers and the demographic variables studied. The high prevalence of anti-HBc (17.7%) among HBsAg+(7.7%) and (10%) among HBsAg-negative noticed in this study showed that the virus is actively replicating in chronic HBV carriers and persistently enhancing silent spread within the population in the target area.

Keywords: Abuja, anti-HBc, HBsAg, Nigeria, pre-transfusion screening, Prevalence

Introduction

Blood transfusion service (BTS) is an integral and indispensable part of the healthcare system. It ensures safe, adequate and accessible blood supply when the need arises.^[1] Blood borne pathogens including HBV may be transmitted through blood transfusion which can be potentially life threatening to those transfused. HBV infection constitutes a worldwide health threat with various levels of seriousness in different population. More than 350 million individuals have chronic, lifelong HBV infections and the World Health Organization (WHO) estimated that those who died globally in 2002 from acute and chronic liver diseases due to HBV infection were more than 600,000.^[2,3] HBV is classified into eight genotypes: A – H by phylogenetic analysis, using alignment of whole genome.

Sub-genotypes with distinctive sequence characteristics and divergence in complete genome have been elucidated within the genotype A – C, E and F with genotype E and subtype ayw4 endemic in Nigeria.^[4,5] The difference in biological properties and heterogeneity in their global distribution may account for the differences in prevalence of HBV mutant in different geographic distribution.^[2,3] However, protection against one subtype seems to confer immunity against the other, and no differences in clinical symptoms have been related to subtypes.^[6] Nigeria is hyper-endemic for HBsAg-positivity; prevalence: ($\geq 8\%$) of the population.^[3,7] A major route of spread is through blood. The infection can occur either as symptomatic disease or asymptomatic disease. Patients can clear the HBV and develop anti-HBs; but individuals who develop antibody to HBcAg are at risk of virus reactivation, showing persistence of virus, regardless of the time of infection or how high the titre of anti-HBs was.^[8] HBcAg is an HB viral protein which is an indicator of active viral reactivation, and makes transmission easily possible. The core is considered “particulate,” not secreted, and does not circulate in the blood.^[9]

Reports have shown that ‘donors’ who are HBsAg-negative but positive for anti-HBc continue to replicate HBV even when such blood or human organs are transfused/given to recipients;^[10] putting them at increased risk of continuous HBV infection. Therefore, knowing the significance of establishing HBV infection through serological testing and the clinical significance of anti-HBc in chronic-HBV infection is essential. There is limited data about the process of active and sustained HBV infectivity and infection in the target area; hence the need for complete routine screening of blood for HBV markers, including anti-HBc to aid identification of persons with high risk of transmission of the virus due to occult HBV infection (OHBVI) represented by the presence of HBV-DNA in serum and/or liver tissue in the absence of HBsAg positivity;^[11] safe guide blood transfusion services and plan for management of patients. This study evaluated the prevalence of HBsAg and anti-HBc among blood donors in three healthcare centers in Abuja, Nigeria; and their association with age, gender, marital status and education.

Materials and Methods

Study Area

This was a prospective cross-sectional study; conducted in three hospitals in Abuja, the Capital City of Nigeria. The area has a population of about 2.153 million, a land mass of about 2,829 square miles (7,315 square yards) and lies on latitude 9.0667°N and longitude 7.4833°S.^[12] It is bounded on the North, South-east, South-west and West by states of Kaduna, Nasarawa, Kogi and Niger.^[13]

Sample Size Determination and sample selection

The sample size was determined using the formula: $n = \frac{t^2 \times p \times (1-p)}{d^2}$,^[14] where **n** is the required sample size, **t** represented confidence level at 95% (standard value of 1.96), **p** represented 25% average prevalence of HBsAg+ in Nigeria from previous studies, **m** equaled 5% (standard value of 0.05) margin of error. With 4.16% (approximating to 12 donors) provided for dropouts or spilled samples, a total of 300 donor-samples was taken. Therefore, 300 blood samples were collected from prospective blood donors in three representative hospitals in the ratio of 169:78:103.

Inclusion/Exclusion criteria

Included were prospective blood donors between the ages of 15-45 years of both sexes who were present at the selected hospitals at the time of study. They were asked to sign informed written consent. Excluded were blood donors below 15 years and above 45 years of both sexes and those who did not give their consent.

Ethical Clearance

Ethical approval was obtained from the Federal Capital Territory (FCT) Health Research Ethics Committee, Abuja.

Informed consent

Participation was voluntary and informed written consent was obtained from participants before the study.

Data collection Instrument-Pilot testing

The questionnaire was first tested among 20 randomly selected health workers and hospital visitors to ascertain the validity of the data collection instrument. Prior to the collection of the blood samples, the piloted structured questionnaire was administered in order to obtain demographic information of the blood donors.

Blood Sample collection

Blood samples were collected from prospective blood donors between June and September 2014. Two milliliter of blood sample was collected from each donor using standard venipuncture technique described by Ochei and Kolhatkar (2000).^[15] The blood samples collected were transferred into EDTA bottles and labeled accordingly for easy identification. The samples were transported to the serology laboratory at Fereprod Medical Centre, Abuja, for analysis.

Sample Preparation

The blood samples were centrifuged (Hackett Incorporation England, Model no:0029AB) at 1000000 revolution per minutes (rpm) for five minutes. The plasma from each sample was aspirated and transferred into a sterile sample bottle and stored at -20°C prior to screening for further analysis.^[15]

Sample Screening and interpretation

A commercially available ELISA kit (Advanced Quality Company China, Model; A1099. Batch B) designed for qualitative determination of HBsAg and Anti-HBc, was used to screen the blood samples. The assay was prepared according to the manufacture's instruction in order to determine HBV positive samples. The one step test strip has a test region and a control region. Presence of a red coloured band in the test region indicates a positive result while the absence of a coloured band indicates a negative result. To ensure quality control, a coloured band will always appear at the control region while absence of this renders the result invalid.

Statistical analysis

The data were tabulated and analysed using Statistical Package for Social Sciences (SPSS), version 20 software (SPSS Inc. Chicago, Illinois, USA). Categorical data were presented as numbers and percentages. Chi-squared (X^2) test was used for associations between prevalence and categorical variables of age, gender, MS and education. The level of 0.05 ($P \leq 0.05$) was considered significant.

Result

The descriptive prevalence distributions of HBsAg and Anti-HBc and demographic variable and their subsets are shown in tables 1 and 2; while the X^2 -squared statistical output of prevalence, P-value and correlation coefficient to ascertain the relationship between prevalence of HBV infection and demographic variables is shown in table 3.

Table 1: Description of HBsAg-status and Anti-HBc-status according to Demographic variables and their subsets

Covariates	Participants (screened)	HBsAg-status (Prevalence)		Anti-HBc-status	
		Positive	Negative	Positive	Negative
Gender					
Male	288	23(7.7%)		51(17.0%)	
Female	12	0(0.0%)		2(0.6%)	
Total	300	23(7.7%)		53(17.7%)	
Age-groups (Years)					
15-24	10	1(0.3%)		1(0.3%)	
25-34	115	11(3.7%)		25(8.3%)	
35-44	147	10(3.3%)		23(7.7%)	
45-54	28	1(0.3%)		4(1.3%)	
Total	300	23(7.7%)		53(17.6%)	
Marital status					
Unmarried	59	5(1.7%)		11(3.6%)	
Married	241	18(6.0%)		42(14%)	
Total	300	23(7.7%)		53(17.6%)	
Education					
Formal	221	20(6.7%)		43(14.3%)	
Informal	79	3(1.0%)		10(3.3%)	
Total	300	23(7.7%)		53(17.6%)	

Table 2: Composite distribution of prevalence according to demography

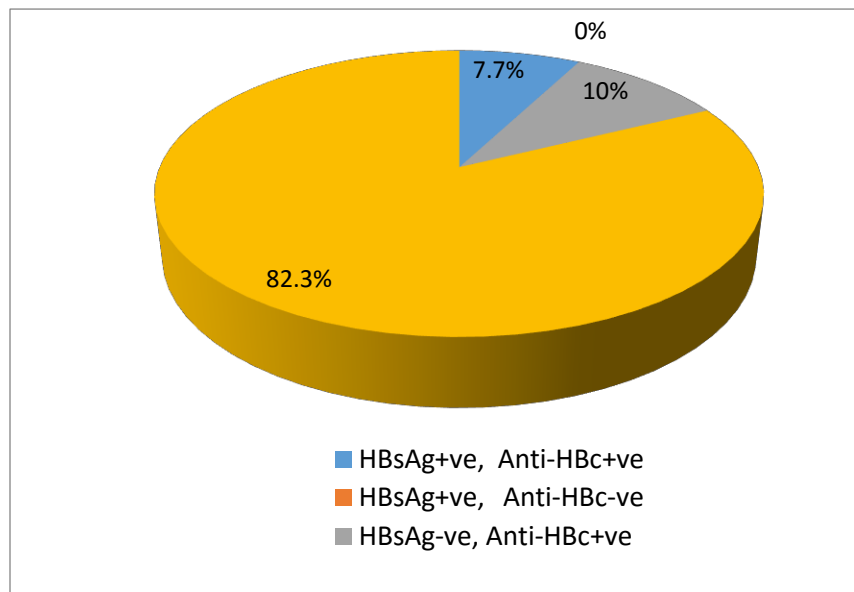
Demography	Sample Screened	Surface antigen		Core antibody	
		Positive (%)	Negative (%)	Positive (%)	Negative (%)
Gender					
Female	12	0 (0.0%)	12 (100.0%)	2 (16.7%)	10 (83.3%)
Male	288	23 (8.0%)	265 (92.0%)	51 (17.7%)	237 (82.3%)
Total	300	23 (7.7%)	53 (17.7%)	53 (17.7%)	247 (82.3%)
X²= 0.8,P>0.05, Coefficient of correlation (r) =0.993, Coefficient of regression (b) =2.26					
Age (Years)					
15-24	10	1(10.0%)	9 (90.0%)	1(10.0%)	9 (90.0%)
25-34	115	11(9.6%)	104 (90.4%)	25 (8.3%)	90 (78.3%)
35-44	147	10 (6.8%)	137 (93.2%)	23 (15.6%)	124 (84.7%)
45-54	28	1(3.6%)	27 (96.4%)	4 (14.3%)	24 (85.3%)
Total	300	23(7.7%)	277 (92.3%)	53 (17.7%)	247 (82.3%)
X² = 0.26 P>0.05, Coefficient of correlation (r) =0.998					
Marital status					
Unmarried	59	5 (8.5%)	54 (91.5%)	11 (18.6%)	48 (81.4%)
Married	241	18 (7.5%)	223 (92.5%)	42 (17.4%)	199 (82.6%)
Total	300	23 (7.7%)	277 (92.3%)	53 (17.7%)	247 (82.3%)
X²=0.1 P>0.05, Coefficient of correlation (r) =0.996, Coefficient of regression (b) = 2.5					
Educational Status					
Informal	79	3 (3.8%)	76 (96.2%)	10 (12.7%)	69 (87.3%)
Formal	221	20 (9.0%)	201 (91.0%)	43 (19.5%)	178 (80.5%)
Total	300	23 (7.7%)	277 (92.3%)	53 (17.6%)	247 (82.3%)

$X^2=0.8$, $P>0.05$, Coefficient of correlation (r) =0.996, Coefficient of regression (b) = 2.5

Table 3: Output of X²-square statistical analysis on relationship between the prevalence of HBV infection and demographic variables

Factors	Number positive	Prevalence	P-value	Correlation coefficient
Age				
15-24	1	0.3		
25-34	11	3.7	0.26	0.998
35-44	10	3.3		
45-54	1	0.3		
Gender				
Female	0	0.0		
Male	23	7.6	2.8	0.993
Marital status				
Unmarried	5	1.7		
Married	18	6.0	0.10	0.996
Education				
Informal	3	1.0		
Formal	20	6.7	0.80	0.996

Figure 1: Mono-Positivity and Co-positivity Pattern of HBsAg and Anti-HBc Markers



Data for 300 participants were analysed with male:female dominant ratio of 24:1, recording prevalence of 7.7% (HBsAg+) and 17.7% (anti-HBc). Twelve females were HBsAg-negative of which two were positive for anti-HBc (0.7%) table 1. Thirty participants (10%) tested positive for anti-HBc without testing positive for HBsAg; figure 1. The distribution of blood donors according to age showed that the age group, 25-34 years had the highest prevalence of 3.7% for HBsAg and 8.3% for Anti-HBc, followed by the age group, 35-44 years which had a prevalence of 3.3%

for HBsAg and 7.7% for Anti-HBc respectively. The age group with the lowest positivity was observed among 15-24 year olds, with a prevalence of 0.3% for HBsAg and Anti-HBc respectively. Unmarried blood donors recorded 1.7% for HBsAg and 3.6% for Anti-HBc, while 6.0% for HBsAg and 14.0% for anti-HBc were recorded among married blood donors, table 1. Blood donors without formal education had 1.0% for HBsAg and 3.3% for Anti-HBc, but blood donors with formal education recorded 6.7% prevalence for HBsAg and 14.3% for Anti-HBc, table 1. The mono-positivity and co-positivity pattern of HBsAg and anti-HBc markers showed that 30 (10%) participants were positive for anti-HBc without positivity for HBsAg, figure 1. Despite the differences in prevalence, chi-squared statistical analysis revealed no significant relationship between prevalence of HBV infection and the studied demographic variables of the blood donors.

Discussion

When infection of HBV occurs, there follows the development of host immunity; the nature determines the course and type of disease and its consequences. Some will develop persistent HBsAg+ while others will clear the virus and become HBsAg-negative.^[16] However, studies have shown that some HBsAg-negative individuals with anti-HBc or circulating HBV-DNA or both could remain infectious^[17,18] and continue to spread the infection through HBsAg-negative-anti-HBc positive donors.^[19] Anti-HBc is the first antibody produced after HBV infection and the only detectable marker in the window period. The isolation of anti-HBc in serum in the absence of HBsAg may be due to resolved HBV infection which HBsAb becomes undetectable. The prevalence of HBV infection in this study is 7.7% and 17.7% for HBsAg and anti-HBc respectively, aligning with the placement of Nigeria as hyper-endemic country for CHB disease.^[20] Studies conducted in different countries have shown in-patients undergoing haemodialysis and blood donation to have prevalence of 11.5%, 16.6%, 17%, 42%, 48% and 49.1% for anti-HBc.^[21-27] Mono-positivity and co-positivity pattern of HBsAg and anti-HBc observed in this study revealed that positivity to anti-HBc alone were observed in 10% of the blood donors. There could be false positive among anti-HBc-positives in HBsAg-negative individuals which can occur in low risk of past HBV infection or distantly resolved HBV infection in an area of endemicity such as the target area. However, using polymerase chain reaction (PCR) method to detect HBV-DNA in serum could help to resolve this doubt. Also, when protein mutation occurs, it may be undetectable during the window period or chronic infection in the absence of HBsAg if poor sensitive diagnostic assay was used.^[28] Donors who were HBsAg-negative but have occult HB infection detectable only by anti-HBc are potential sources of HBV infection.^[29] Almeida et al (2001) in a study in Sao Paulo about post-immunization screening concluded that testing for isolated hepatitis B core antibody was significant in the screening of blood donors.^[30] In line with this study, results of studies of organ transplantation have documented reactivation of HBV in HBsAg-negative-anti-HBc-positive patients.^[8,29,31] Therefore, it is suggested that this category of individuals who should be regarded as being at risk of reactivation of HBV could benefit from counseling. The no-correlation ($P>0.05$) observed in this study between prevalence and demographic variables: age, gender, marital status and education was similar to the findings of Japhet et al. (2011), and Adekeye et al. (2013).^[32,27] In summary, following the methods currently in use in the target area for pre-transfusion blood screening, this study has been able to evaluate the prevalence of anti-HBc+ along with HBsAg-status among blood donors and noted the necessity for both to be used as discriminatory markers for the risk of post-transfusion transmission of HBV.

Limitations and Strengths of the study

The protocol for this study did not include counseling of donors who tested positive for anti-HBc and are at risk of transmitting HBV to their contacts. Also, the study was not designed to include determination of the phase of infection or other sources of contracting the HBV to impart treatment and prevention. Being a cross-sectional study, participants who were anti-HBc were not followed up to check those who may have developed liver complications. Therefore, longitudinal follow-up study is needed to examine the clinical implications of being anti-HBc positive. The screening method (ELISA) used may not detect HBsAg-positive individuals during the Window period. Nevertheless, the high sample size employed and the provision made for dropouts and blood sample spills were reasonable enough to give strength to the outcome of this study.

Conclusion

Positive HBsAg individuals are at increased risk of chronic hepatitis B and liver diseases: cirrhosis, hepatocellular carcinoma (HCC) including death; and timely and rapid accurate diagnosis of HBV infection is seminal in the prevention of the disease and its sequelae. In the target area, the presence of HBsAg is the major diagnostic screening employed to determine HBV infection among prospective blood donors; anti-HBc is not used as a marker to determine previous exposure to HBV. This study reveals that HBV continues to be a major health burden even after adopting HBsAg as a marker for the screening of blood donors and notes that HBcAntigen (HBcAg) plays a role in the persistent propagation of the virus. We, therefore, suggest the mandatory inclusion of anti-HBc marker for the screening of blood donors to rule out the risk of post-transfusion HBV infectivity even in the presence of HBsAg-negativity. Awareness should be enhanced by public and private healthcare stakeholders about the danger posed by poorly screened blood.

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