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Hepatitis B virus serological profile and associated risk factors in surface antigen negative blood donors in Nigeria

*Ahaneku Iherue Osuji [*1], Nneka Regina Agbakoba [2], Martin Ositaodinma Ifeanyichukwu [2], Idris Nasir Abdullahi [3], Chinyere Charity Ezeanya-Bapka [4], Georgina Chinenye duru [5]*

1- *Department of Microbiology and Immunology, University of Abuja Teaching Hospital Gwagwalada, Abuja, pmb 228 Abuja.*
2- *Department of Medical Laboratory Science, Nnamdi Azikiwe University, Nnewi, Campus, Anambra State, Nigeria.*
3- *Department of Medical Laboratory Science, Faculty of Allied Health Sciences, College of Medical Sciences, Ahmadu Bello University, Zaria, Nigeria..*
4- *Department of Microbiology, Edo University Iyamho, Edo State, Nigeria.*
5- *Department of Hematology and Blood Transfusion, University of Abuja Teaching Hospital Gwagwalada, Abuja, Nigeria.*

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ABSTRACT

Background: This cross-sectional study investigated the serological profile, socio-demographic characters and risk factors of hepatitis B virus (HBV) infection among HBV surface antigen (HBsAg)-negative blood donors at the University of Abuja Teaching Hospital (UATH) Gwagwalada and Nnamdi Azikiwe University Teaching Hospital (NAUTH) Nnewi, Nigeria. **Methods:** Blood samples collected from 176 HBsAg-negative blood donors (96 from UATH and 80 from NAUTH) were screened using a commercially available HBV 5-Panel (CTK Biotech, USA) and anti-HBc IgM ELISA kits. Determination of HBV-DNA was done on 36 HBV positive and 100 negative samples using COBAS Roche Real-time qPCR. Structured questionnaires were used to collate subjects' socio-demographic variables and risk factors of HBV infection. **Results:** Out of 176 samples tested, 140 (79.5%) were negative for HBV serologic markers while 36 (20.5%) were positive. The pattern of seropositivity showed that 19 (10.8%) samples were positive for anti-HBs, 9 (5.1%) were positive for anti-HBc, 3 (1.7%) were positive for both anti-HBc & anti-HBs and 5 (2.8%) were positive for anti-HBc IgM. None was positive for HBeAg and anti-HBe markers. Of the 36 HBV positive and 100 negative samples, 15 (41.7%) and 3 (3%) were positive for HBV-DNA respectively p=0.006. Not heard of HBV, no vaccination with HBV vaccines, previous blood transfusion, history of sexually transmitted diseases and visiting commercial barbers were significantly associated with HBV infection. Socio-demographic data showed that male blood donors were more infected with HBV than the females (*p=0.284*) and age group 26-40 years old was more affected than other age groups (*p=0.015*). **Conclusion:** The study revealed the endemicity of HBV infection and recommends that blood donors with critical risk factors be deferred from blood donation to reduce HBV transmission risk in Nigeria. Anti-HBc and anti-HBs markers could be included as screening tests for blood donors since HBV-DNA testing is not readily available nor affordable.

Introduction

Hepatitis B virus (HBV) infection is a worldwide challenge of public health significance [1]. It has been reported that one-third of the world population representing over 2 billion people has been infected with the HBV. [2]. Of this figure, about 400 million people are chronically infected, approximating to about 5% of the world's population at risk of developing the complications of chronic HBV

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* *Corresponding author:* Ahaneku Iherue Osuji
E-mail address: ahanekuos@gmail.com.

infection such as liver cirrhosis and hepatocellular carcinoma [3]. The detection of HBV surface antigen (HBsAg) in the blood of an individual is the mainstay in the diagnosis of HBV infection in most developing countries, including Nigeria [4]. Studies have shown that the carrier rate of HbsAg among blood donors in Nigeria is between 5% and 17% depending on the geographical location [5,6]. The risk of transfusion-transmitted HBV infection has been reduced as most blood banks in resource-limited economies screen all blood donations for HBsAg [7]. Although this serologic method reduces transfusion transmissible HBV infections, some HBsAg-negative blood samples but positive for other HBV serologic markers (anti-HBc and anti-HBc IgM) can still induce post-transfusion hepatitis in recipients [8, 9]. HBV-DNA testing of all collected units of blood would give near zero risks of transfusion-associated HBV [10]. However, this technology has not been adopted in many resource constraint economy, including Nigeria because it is not readily available in most blood transfusion centres and even when available, it is not affordable.

Serological markers of HBV are antigen and antibodies that serve as markers of HBV infection. These viral markers are used to evaluate the stages of HBV infection. Detection of HBV serologic markers such as HBsAg, HBeAg, Anti-HBc, Anti-HBs and Anti-HBe in individuals signifies infection, immunity or exposure to the virus [11]. Surface antigen to HBV is the first serological marker to appear during HBV infection. It is a protein on the surface of the HBV that can be detected in high levels in the serum of infected individuals during acute or chronic HBV infection [12]. The production of antibodies against HBsAg confers protective immunity and can be detected in patients who have recovered from HBV infection or in those who have been vaccinated. Antibody to HBcAg is detected in almost every patient with previous exposure to HBV. The Immunoglobulin M (IgM) subtype is indicative of acute or reactivated infection, whereas the IgG subtype is indicative of chronic infection. Antibody to HBeAg is suggestive of a nonreplicative state in which the antigen has been cleared whereas the detection of HBeAg is a mark of infectivity and active replication [13].

Most blood banks in Nigeria use only HBsAg biomarker, rapid test device to screen prospective blood donors for HBV infection before donation based on its seronegativity. Besides this, other HBV serological markers are not included in the screening tests of blood donors. Many studies have reported varying detection rates of HBsAg among blood donors in Nigeria [5,6,14]. However, there is a paucity of data on HBV serologic markers among blood donors in Nigeria, particularly in our study sites. It is on these facts that the study was conceived to determine the prevalence and significance of HBV serological markers among blood donors that tested negative for HBsAg. The study also investigated the socio-demographic variables and risk factors of HBV infection among blood donor participants. We anticipate that the findings from this study will necessitate the need for thorough screening of blood donors beyond HBsAg tests to reduce HBV transfusion risk among blood recipients in Nigeria.

Methods

Study population and design

The study population comprises blood donors attending the blood banks of the University of Abuja Teaching Hospital (UATH) and Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi, Nigeria. The research is a cross-sectional study.

Ethical permission

Approval for this study was obtained from the health research ethics committee (HREC) of both hospitals where the study was conducted and individuals that agreed to be part of the study signed the consent form before sample collection. The approval letters from Health Research Ethics Committees with reference numbers for the University of Abuja Teaching Hospital Abuja is FCT/UATH/ HREC/PR/514 and that of Nnamdi Azikiwe University Teaching Hospital, Nnewi is NAUTH/CS/66/VOL.9/40.

Sample size determination

A total of 204 healthy blood donors participated in this study. This was obtained by calculation using the Fisher formula as described by **Araoye** [15]. The formula is $n = z^2p\ (1\text{-}p)/d^2$ Where n= required sample size, z= confidence level 95% (Standard value of 1.96).

P= estimated prevalence of 9% among blood donors as reported in Nigeria by **Erhabor et al.** [16]. d= margin of error at 5% (standard value is 0.05). Sample size calculation performed obtained 126 blood donors. Finally, the sample size of 204 blood donors was recruited and enrolled in the study.

Subjects and selection criteria

These include 204 (100 participants from UATH and 104 participants from NAUTH) healthy blood donors who had been previously screened and found eligible by the respective blood banks for donation. The recruitment and enrollment of the subjects was done

within five months, from June to October 2016. All participants who gave informed consent were selected for the study. Subjects who declined to offer consent were excluded from the study. Also, blood donors that tested positive for HBsAg by ELISA were excluded from the study.

Specimen collection and processing

Venous blood (10mL) was collected from each blood donor and 5mL of the sample was dispensed into K+ EDTA containers (Medi-Scsn, UK) and plain bottles. The samples on plain tubes were allowed to clot and thereafter were centrifuged with the samples in EDTA tubes at room temperature at 3500 rpm for 10 minutes. The plasma samples and sera were then separated into cryovials appropriately labelled and stored at −70 °C until testing was performed.

Socio-demographic data and HBV risk factors

Relevant socio-demographic information and HBV-associated risk factors were obtained from the blood donors using a validated structured questionnaire. The questionnaire was self-administered. The demographic data included age, sex, marital status, academic status and occupation. The risk factors obtained from the questionnaire include: Not heard of HBV, number of sexual partners and history of sexually transmitted diseases (STDs). Other risk factors captured include the presence of tattoos/scarification marks, history of alcohol/drug abuse, sharing of sharps/occupational or domestic accidents with sharp, hepatitis B vaccination status, history of blood transfusion and previous surgery/dialysis.

Serological analysis

- ***Detection of HBsAg by 4th generation ELISA***

Surface antigen for hepatitis B was tested by ELISA technique on the 204 specimens (100-UATH and 104-NAUTH) that were negative for HBsAg with rapid test device using the method of **Burtis et al.** [17]. The 4th generation ELISA kit (Fortress Diagnostics, UK) was used. Manufacturer's instructions and test procedures were strictly followed.

- ***Detection of HBV serologic markers using HBV 5-panel assay***

One hundred and seventy six (176) samples that tested negative for HBsAg 4th generation ELISA were assayed for HBV serologic markers using HBV 5 panel assay (CTK Biotech, USA). Hepatitis B virus 5-panel assays used was a rapid diagnostic test kit devised to detect five serological markers (HBsAg, HBeAg, anti-HBs, anti-HBc & anti-HBe) associated with hepatitis B virus infection. Two to three drops of serum were placed in each of the samples well using the disposable pipette accompanying the test kit. The reading of the result was taken after 15 minutes according to the manufacturer's instruction. In-house positive and negative controls were performed before testing the blood donors' samples to validate the reagent kit.

- ***Detection of hepatitis B core antibody IgM by ELISA***

Hepatitis B core antibody IgM was determined on 176 sample negative for HBsAg using 4th generation ELISA kit (Fortress Diagnostics, UK) as described by **Burtis et al.** [17]. The assay procedure as outlined by the manufacturer was strictly followed.

HBV DNA testing

Thirty six samples positive for HBV serologic markers and 100 seronegative samples were examined for HBV DNA using COBAS Roche Real-time PCR. HBV DNA quantification was done as described by **Osuji et al.** [18]. All procedures were done according to manufacturer's instructions. High positive control (HPC) and low positive control (LPC) controls were included in each run.

Statistical analysis

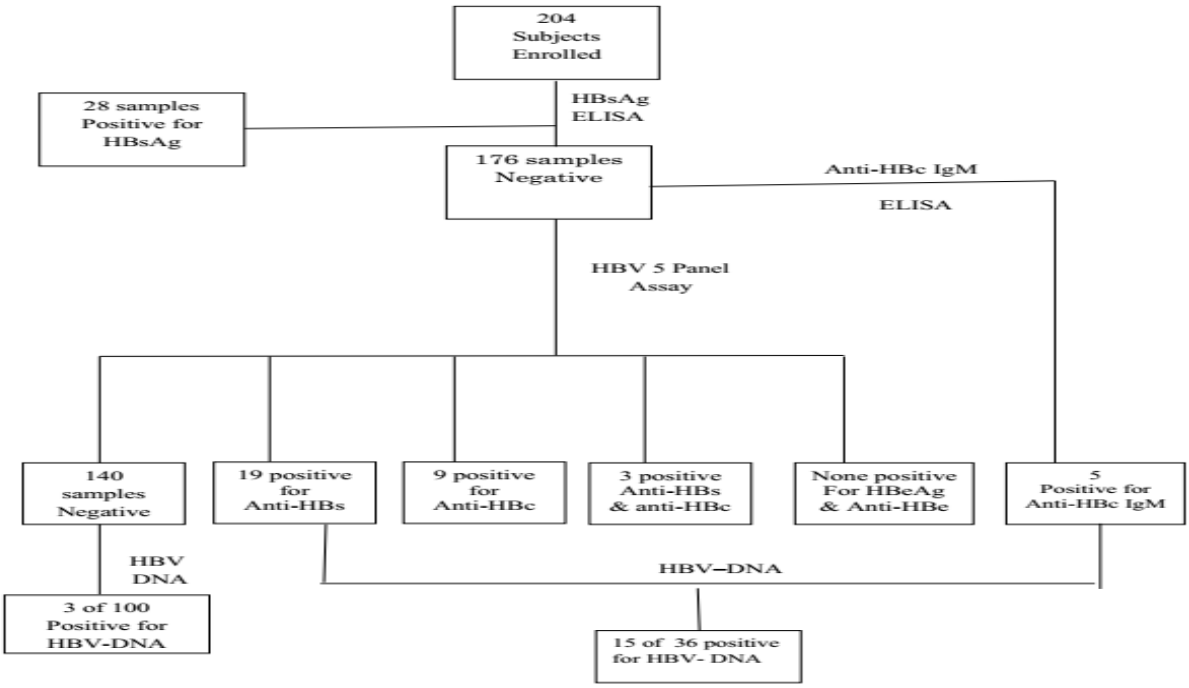
Data obtained from this study were analyzed by Statistical Package for Social Science (IBM, New York, USA) version 26. Descriptive statistics which include percentages were used to describe the frequency of categorical variables. A two-tailed chi-square test was used to compare categorical variables. A *p-value <.05* was considered to indicate statistical significance.

Results

Summary of laboratory tests and results

Figure 1 summarizes the tests done and the results obtained in the form of a flow chart. There were 204 subjects negative for HBsAg by rapid test kit recruited for the study. Their sera were tested for HBsAg using ELISA and 28 samples were positive. The remaining 176 HBsAg negative samples were then screened for HBV serologic markers using HBV 5 panel assay and anti-HBc IgM ELISA kits. Thirty six HBV positive and 100 negative samples were screened for HBV-DNA using real-time PCR. HBV-DNA was found in 15 of 36 HBV positive samples and 3 of 100 negative samples.

Figure 1. Flow chart of the study.



HBsAg rapid test kit versus HBsAg ELISA

Out of 204 specimens that tested negative by rapid test kit, 28 (13.7%) were positive by HBsAg ELISA while 176 (86.3%) were negative. This is presented in **table (1)**. The study observed a statistically significant difference between the two sites concerning the positivity of HBsAg ELISA over the rapid test kit.

Sociodemographic characteristics of blood donors

Table 2 presented HBV status and sociodemographic variables of blood donors that tested negative for HBsAg in the study population. The finding showed that 140 blood donors tested negative to HBV markers and were susceptible to HBV infection while 36 subjects were positive. It was observed that male blood donors are more infected with HBV than the females but there was no statistically significant association of HBV infection with the gender (*p=0.284*). The age bracket of 26-40 years had more HBV seropositivity than other age groups (*p=0.015*). Also, the occupation and academic status of blood donors were associated with HBV seropositivity *(p<0.0001*). Although married donors are more infected with HBV than the single, there was no statistically significant difference. The study observed a significant association of HBV infection with occupation and academic status of the participants and students and subjects with secondary academic attainment were more infected with HBV.

Hepatitis B virus risk factors assessment

The relationship between the risk factors of transmission and HBV status of blood donors negative for HBsAg is presented in **table (3)**. The finding showed that there is a significant association of HBV infection with subjects that had not heard of HBV (*p=0.04*), No HBV vaccination (p=0.*006*), previous blood transfusion (*p=0.02*), multiple sexual partners (*p=0.02*) and visiting commercial barbers, manicures and pedicures (*p=0.001*). There was no significant association of HBV infection with blood donors with occupational/domestic accident, drug/alcohol abuse and previous surgeries/dialysis.

Prevalence and pattern of HBV serologic markers

Out of HBsAg negative 176 samples tested for HBV serological markers, 36 (20.5%) were positive while 140 (79.5%) were negative for the viral markers. The pattern of seropositivity showed that out of 36 samples, 19 (10.8%), 9 (5.1%) and 3 (1.7%) tested positive for Anti-HBs, Anti-HBc and both anti-HBs & anti-HBc respectively. Five (2.8%) of 176 samples tested were positive for anti-HBc IgM. None of the blood donor participants was positive for HBeAg and anti-HBe markers. None was also positive for more than two HBV serologic markers **(Table 4)**.

The frequency of hepatitis B viral serological markers among blood donors that tested negative to HBsAg at the study population is presented in **table (5)**. The data showed that 22 (12.5%) of 176 blood donors were

positive for Anti-HBs marker while 12 participants representing 6.8 % were positive for Anti-HBc marker. It was observed that blood donors that tested positive for HBV serologic markers were more at UATH Abuja than at NAUTH, Nnewi. This is statistically significant (*p= 0.001*). Also, there is a statistically significant difference observed between the two study sites concerning anti-HBs marker (*p= 0.006*).

HBV DNA testing

Table 6, depicts the HBV serological profile of blood donors non-reactive to HBsAg in relationship to HBV DNA load. Out of 36 samples positive for HBV biomarkers, 15 (41.7%) were positive for HBV DNA. Three (3%) of 100 samples seronegative for HBV serologic markers were positive for HBV DNA. There is a statistically significant difference observed between blood donors positive for HBV markers and seronegative to HBV DNA load (*p=0.006*). The mean viral load of seronegative and seropositive samples was <100 IU/ml.

Table 1. Prevalence of HBsAg by ELISA among blood donors that tested negative by rapid test device at UATH and NAUTH.

Study Sites	**No. of Samples Tested**	**No. (%) Positive**	**No. (%) Negative**	**Chi-Square (p-value)**
UATH	100	4 (4)	96 (96)	15.6 *(<0.0001)*
NAUTH	104	24 (23.1)	80 (76.9)	
Total	**204**	**28 (13.7)**	**176 (86.3)**	

Table 2. HBV status of HBsAg negative blood donors and sociodemographic variables.

Blood donors' demographics	**No. (%) Susceptible n= 140**	**No. (%) Immune/Exposed n=19**	**No. (%) Chronic n=12**	**No. (%) Acute n=5**	**No. (%) HBV Infection n=36**	**Chi-square *(p-value)***
Gender						
Male	127 (90.7)	18 (94.7)	11 (91.7)	5 (100)	34 (94.4)	1.15 *(0.284)*
Female	13 (9.3)	1 (5.3)	1 (8.3)	0 (0)	2 (5.6)	
Age (in years)	50 (35.7)	2 (9.1)	2 (16.7)	3 (60)	7 (19.4)	
18-25	71 (50.7)	16 (72.7)	6 (50)	1 (20)	21 (58.3)	8.43 *(0.015)* *
26-40	19 (13.6)	4 (18.2)	4 (33.3)	1 (20)	8 (22.2)	
41-60						
Marital Status	60 (42.9)	9 (47.4)	7 (58.3)	3 (60)	19 (52.8)	
Married	79 (56.2)	10 (52.6.5)	5 (41.7)	2 (40)	17 (47.2)	3.74 *(0.154)*
Single	01 0.9)	0 (0)	0 (0)	0 (0)	0 (0)	
Separated						
Occupation/Profession	6 (4.3)	5 (22.7)	3 (25)	0 (0)	8 (22.2)	
Applicants	45 (32.1)	7 (31.8)	2 (16.7)	1(20)	12 (33.3)	37.26 *(<0.0001)**
Students	46 (32.9)	4 (18.2)	2 (16.7)	2 (40)	8 (22.2)	
Business/Trading	17 (12.1)	4 (18.2)	4 (33.3)	2 (40)	6 (16.7)	
Civil Servants	26 (18.6)	2 (9.1)	1(8.3)	0 (0)	2 (5.6)	
Artisans						
Academic Status	10 (7.1)	2 (9.1)	2 (16.7)	1 (20)	5 (13.9)	16.98 *(0.0002)* *
Primary	65 (46.4)	15 (68.2)	9 (75)	3 (60)	24 (66.7)	
Secondary	65 (46.4)	5 (22.7)	1(8.3)	1 (20)	7 (19.4)	
Tertiary						

**Statistical significant p<0.05.*

Table 3. HBV status of blood donors negative for HBsAg and ssociated risk factors.

HBV risk factors assessed	Susceptible (%) n= 140	Immune/Exposed (%) n=19	Chronic (%) n=12	Acute (%) n=5	HBV Infected (%) n=36	Chi-square *(p-value)*
Not heard about HBV	88 (62.9)	9 (47.4)	6 (50)	3 (60)	18 (50)	4.69 *(0.045)* *
No HBV vaccination	127 (90.7)	13 (68.4.7)	11 (91.7)	3 (60)	27 (75)	11.9 *(0.006)* *
Occupational/Domestic accident	75 (53.6)	10 (52.6)	6 (50)	2 (40)	18 (50)	1.05 *(0.305)*
Previous blood transfusion	6 (4.3)	1 (5.3)	3 (25)	0 (0)	4 (11.1)	5.14 *(0.02)* *
Multiple sexual partner	6 (4.3)	3 (13.6)	1 (8.3)	0 (0)	4 (11.1)	5.14 *(0.02)* *
History of sexually transmitted diseases	5 (3.6)	1 (4.5)	1(8.3)	0 (0)	2 (5.6)	0.43 *(0.509)*
Alcohol/drug abuse	20 (14.)	0 (0)	1(8.3)	1(20)	2 (5.6)	3.37 *(0.06)*
Previous Surgeries/Dialysis	7 (5)	1(4.5)	1(8.3)	1(20)	3 (7.7)	0.68 *(0.409)*
Tribal marks/Tattoo	13 (9.3)	0 (0)	0 (0)	1 (20)	1 (2.8)	2.57*(0.109)*
Visiting commercial Barber /Manicurer / Pedicurer	118 (84.3)	13 (59.1)	7 (58.3)	2 (40)	22 (56.4)	28.5*(<0.001)* *

**Statistical significant p<0.05.*

Table 4. HBV serologic markers pattern among blood donors and its relationship with HBV DNA assay.

HBV serological pattern	Frequency (%) of occurrence N=176	No. of samples tested for HBV DNA	No. (%) positive for HBV DNA	HBV Status
HBsAg-ve Anti-HBs-ve HBeAg-ve Anti-HBc-ve Anti-HBe-ve (Seronegative)	140 (79.5)	100	3 (3)	Susceptible
HBsAg-ve Anti-HBs-ve HBeAg-ve Anti-HB IgM+ve Anti-HBe-ve	5 (2.8)	5	2 (40)	Acute/Window period
HBsAg-ve Anti-HBs+ve HBeAg-ve Anti-HBc-ve Anti-HBe-ve	19 (10.8)	19	7 (36.8)	Immunity/Exposure
HBsAg-ve Anti-HBs-ve HBeAg-ve Anti--HBc+ve Anti-HBe-ve	9 (5.1)	09	4 (44.4)	Chronic infection
HBsAg-ve Anti-HBs+ve HBeAg-ve Anti-HBc+ve Anti-HBe-ve	03 (1.7)	03	2 (66.7)	Chronic infection

+ve: Positive, **-ve:** Negative, **%:** Percentage, **HBV DNA:** Hepatitis B Virus Deoxyibonucleic Acid

Table 5. Frequency of HBV markers among blood donors that tested negative to HBsAg at UATH and NAUTH.

HBV serologic markers	UATH, n=96 No. (%) of positive samples	NAUTH, n=80 No. (%) of positive samples	Total n=176 (%)	Chi-Square *(p-value)*
Anti-HBs	18 (18.8%)	4 (5%)	22 (12.5%)	7.6 *(0.006)* *
HBeAg	0 (0%)	0 (0%)	0 (0%)	NA
Anti-HBe	0 (0%)	0 (0%)	0 (0%)	NA
Anti-HBc	9 (9.4%)	3 (3.8%)	12 (6.8%)	2.16 *(0.141)*
Anti-HBc IgM	3 (3.1%)	2 (2.5%)	5 (2.8%)	1.5 *(0.21)*
Total	30 (31.3%)	9 (11.3%)	39 (22.2%)	10.45 *(0.001)* *

Statistically Significant (p< 0.05), **NA: Not Applicable.*

Table 6. Comparison of HBV biomarker seropositive and seronegative blood donors to HBV DNA levels.

HBV biomarker status	Frequency of Occurrence (%)	No. of samples tested for HBV DNA	No. (%) positive for HBV DNA	HBV DNA X±SD in IU/ml	Chi-square *(p-value)*
Seronegative	140 (79.5)	100	3 (3)	58±23	7.6 *(0.006)* *
Seropositive	36 (20.5)	36	15 (41.7)	86±32	

**Statistical significant p<0.05.*

Discussion

This study investigated the HBV serological profile, risk factors and socio-demographic characteristics of HBsAg negative blood donors at two Teaching Hospitals in Nigeria. Socio-demographic data among the subjects showed that male subjects had a higher prevalence of HBV serologic markers than the females, with anti-HBs being most prevalent but there was no statistically significant difference observed. This disagrees with the work of **Agbesor et al.** [19] that reported more prevalence in female than the male donors. There was a significant association of HBV markers with age as subjects of 26-40 years were more infected with HBV than other age groups (*p=0.015*). This collaborates with the study of **Agbesor et al.** [19] but contradicts with the work of **Buseri et al.** [20] that noted that HBV infection is more prevalent in young subjects within the age group of 21-29 years.

The risk factors assessment of participants showed that there is an association of HBV infection with blood donors who had not heard of HBV (*p=0.04*), no HBV vaccination (p=0.*006*), previous blood transfusion (*p=0.02*), multiple sexual partners (p=0.*02*) and visiting commercial barbers, manicures and pedicures (*p=0.001*). The finding from this study shows that most of the blood donors in the study population were at a higher risk and therefore susceptible to HBV infection. Also, these risk factors are critical and can be included as deferral criteria for blood donation. There is a need for vaccination of the populace with HBV vaccines, as there is low level of hepatitis B vaccination among health workers at University of Nigeria Teaching Hospital, Enugu, Nigeria as reported **by Ibekwe and Ibeziako** [21] and **Dayyab et al.** [22] in a study at Northeastern Nigeria. The low level of hepatitis B vaccination is due to non availability of the vaccines [22]. This finding correlates with the report of **Omatola et al**. [23] that observed lack of knowledge of HBV and sharing sharp objects were the most significant risk factors associated with HBsAg seropositivity at Ankpa, Kogi State, Nigeria. In another study by **Lavanya et al**. [24], they observed a high rate of HBV serological markers among blood donors in India and most of them have risk factors like alcoholism, smoking, tattooing, ear-piercing, visiting barber's shop and family history of jaundice. Preventive measures should be directed toward increasing knowledge of HBV infection to the populace, vaccination of people and precautions in handling sharps and sterility of equipment used in tribal markers/tattoo to reduce the spread of HBV infection in Nigeria.

The ELISA results of blood donors showed that out of 204 samples that tested negative for HBsAg by rapid test device, 28 (13.7%) samples were positive. This value is quite significant indicating that the use of HBsAg rapid test in screening blood donor could lead to transfusion of HBV to blood recipients. This finding agrees with the study of **Erhabor et**

al. [16] that reported 9% of samples that initially tested negative with HBsAg rapid kits, were positive with ELISA technique among blood donors in University Teaching Hospital Sokoto, Nigeria. This indicates that ELISA is more sensitive and superior than the rapid test for screening of blood donors for HBsAg. The result of this study showed that of 176 samples tested for HBV serologic markers, 36 (20.5%) were positive. This figure collaborates with 18.4% prevalence reported by **Ebenezer et al.** [25] from University College Hospital, Ibadan, Southwest, Nigeria. This value is quite high indicating that subjects from these study sites had a higher risk of HBV infection and possibly some potential blood units containing HBV are being transfused to patients unknowingly. The pattern of seropositivity showed that of 36 seropositive samples, 19 (10.8%), 9 (5.1%) and 3 (1.7%) tested positive for Anti-HBs, Anti-HBc and both Anti-HBs & Anti-HBc respectively. Five (2.8%) of HBsAg negative 176 samples tested were positive for anti-HBc IgM. None of the blood donor participants was positive for HBeAg and anti-HBe markers. None was also positive for more than two HBV serologic markers. This value disagrees with a study by **Olotu et al.** [26] that found an anti-HBc frequency of 70.5 % in blood donors that tested negative to HBsAg in South-Western Nigeria. The variation and discrepancies in the detection of anti-HBc depend on many factors among which include the biomarker testing method used; patients' conditions as well as geographical areas as different prevalence were obtained from different regions [27]. However, this finding tally with the study of **Salawu et al.** [28] who reported a 4.4 % of hepatitis core antibody among blood donors that tested negative to HBsAg. They also recorded a frequency of 12.7% of anti-HBs in Ile-Ife among 457 blood donors negative for hepatitis B surface antigen (HBsAg). Whereas finding from this study recorded a 12.5% frequency of anti-HBs, 6.8% of anti-HBc and 2.8% of anti-HBc IgM. This study also correlates with that of **Japhet et al.** [29] that found a frequency of 15.2 % of anti-HBs among 92 donors also studied in Ile-Ife, Nigeria. In other studies by **Osuji et al.** [18] and **Oluyinka et al.** [30], they found that most of the blood donors with occult HBV infection (negative for a surface antigen) were HBV serologic markers positive indicating a risk of transfusion of hepatitis B surface antigen-negative blood units to recipients without screening for other HBV serological markers and HBV DNA. No participant was found positive for hepatitis B envelope antigen in this study. This is similar to findings by **Japhet et al.** [29] in Ife, Nigeria, **Ebenezer et al.** [25] in Ibadan, Nigeria but in contrast with finding by **Salawu et al.** [28] who reported a prevalence of 0.22% (1 of 459) in Ile-Ife, Nigeria.

The result from this study showed that 3 (3%) out of 100 seronegative blood donors and 15 (41.7%) of 36 HBV positive blood donors were positive for HBV DNA. This finding is discordant with the result of **Minuk et al.** [31] that recorded 8% frequency of HBV DNA among blood donors negative for HBV markers in a North American community-based population. The blood donors that were negative for all HBV markers have a high probability of not been infected with HBV. The individuals' positive only for HBV-DNA without any detectable HBV antibodies and antigens might be as a result of long-lasting persistence of HBV cccDNA or the possibility of integration of the HBV-DNA into the host genome [32]. The blood donors positive for anti-HBc, anti-HBs and HBV-DNA but negative for HBsAg represent the viral persistence after recovery with a low viral load as observed in previous reports of **Oluyinka et al** [30] and **Brojer et al.** [33]. A plausible explanation for this observation is that anti-HBs marker is poorly neutralized due to loss of recognition, allowing these mutant viruses to escape neutralization even when the antibody is present at protective levels [34, 35].

Conclusion

The prevalence of HBV serologic markers among blood donors negative for HBsAg was 20.5%. This signified that some potential blood units containing HBV are being transfused to patients unknowingly. This relatively high number of occult HBV infection observed in this study could pose a risk to blood transfusion services where only HBsAg is screened before blood donors are accepted for donation. It is hereby recommended that other HBV serologic markers (anti-HBc and anti-HBs) be included as screening tests for blood donors in Nigeria to reduce HBV transmission risk since DNA testing is not readily available and affordable. The study recommends that the critical risk factors be included as deferral criteria for blood donation. Also, the general populace should be vaccinated with HBV vaccine for their safety and protection and also have adequate knowledge of HBV and its mode of transmission. Future studies on HBV infection in Nigeria could focus on determination of performance characteristics of HBV rapid test kits and use of quantitative ELISA to detect the presence and level of anti-HBs.

Limitations of the study

The small number of samples of blood donors used in this study could place a limit to the outcome of this study. This is largely attributed to limited funds as there is no grant and sponsorship from donor agencies.

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Data availability

The data used to support the findings of this study are included in the article. The raw data of this study will be made available on request. All requests should be made to the corresponding author of this article.

Authors' contributions

The conception of the study was by AIO. AIO and MOI were involved in the recruitment of blood donors and collection of samples. AIO, INA, CCE and GCD performed laboratory testing; AIO and NRA analyzed the data. AIO wrote the manuscript; NRA, MOI and GCD provided ideas and useful comments during manuscript preparation. All authors read and approved the final manuscript.

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