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Detection of Hepatitis B Virus Serological markers among Adult HIV Positive Female Patients on HAART in Ogun State, Nigeria

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Abstract

Hepatitis B virus (HBV) infection is endemic in sub-Saharan Africa, Nigeria inclusive, and it is common among HIV/AIDS patients. The aim of this study is to determine the prevalence of hepatitis B virus serological markers and associated risk factors among HIV-positive female patients on HAART in Ogun State, Nigeria. After receiving ethical permission from the Babcock University Health Research Ethics Committee (BUHREC), 100 female HIV-infected patients from Babcock University Teaching Hospital (BUTH), Ilishan-Remo, Ogun State, and General Hospital, Ijebu-Ode, Ogun State, were recruited for the study. The patients' HIV status was confirmed using three rapid diagnostic kits, all of which were used according to the manufacturer's instructions: Determine (Abbott Laboratories, Tokyo, Japan), Unigold HIV (Trinity Biotech Plc Bray, Co. Wicklow, Ireland), and 1/2 Stat Pak (Abbott Laboratories, Tokyo, Japan) (Chembio Diagnostic Systems, New York, USA). While an HBV 5-in-1 Panel supplied by Innovation Biotechnology Co., Ltd, Beijing, China was used for the qualitative detection of HBV markers in serum specimens. Out of the 100 study participants examined, 4(3.6%) were positive for HBsAg, 2 (1.8%) were positive for HBsAb, 81 (73.6%) were positive for HBeAg, 3 (2.7%) were positive for HBeAb, while 65 (59.1%) were positive for HBcAb. There was no significant association between the occurrences of HBsAg and HBsAb and the socio-demographic characteristics of the study participants ($P>0.05$), except for HBeAg and HBeAb ($P<0.05$). Identified risk factors include: lack of knowledge of HBV, lack of a history of HBV vaccination, history of blood transfusion, organ transplant, and unprotected sex among others. The findings demonstrate that Hepatitis B Virus (HBV) infection exists among HIV-positive female patients on HAART in Ogun State, Nigeria, particularly among the age categories of 18-25 years and 26-30 years. This necessitates ongoing and persistent public health interventions among the study population.

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Introduction

Infections with the hepatitis B virus (HBV) cause a large number of liver illnesses across the world[1][2][3][4][5]. Globally, it is estimated that 5%–10% of people living with HIV are co-infected with hepatitis B virus (HBV), while HIV/HBV frequency in sub-Saharan Africa varied from 0.0% to 28.4%[6]. The global prevalence of hepatitis B virus (HBV) infection among people infected with human immunodeficiency virus (HIV) is 7.4%, according to the World Health Organization[7], and about 1% of people infected with HBV (2.7 million people) are also infected with HIV[8].

HBV and HIV infections are major global health problems in Sub-Saharan Africa, with over 2% of the population infected with HIV and at least 8% infected with hepatitis B[9]. Overall, between 40% and 60% of seropositive people are likely to be HBV infected, and the prevalence of chronic hepatitis B, defined as the persistence of hepatitis B surface antigen (HBsAg) for more than 6 months among HIV infected people, is higher, differing from 15% to 20% of all seropositive people, with higher rates in West African and Southern African cohorts[9].

HIV and HBV share common risk factors, and many cases of HIV occur in people with HBV, resulting in an increased risk for HIV/HBV co-infection[10]. Co-infection with HIV and HBV viruses causes complex interactions. HBV infection poses hazards to antiretroviral therapy (ART)-related liver damage, limits CD4 recovery, accelerates immunologic progression, and increases morbidity and mortality in HIV-infected patients[11]. HIV infection alters the natural course of HBV infection. So if applied to people who do not have HIV, chronic HBV has a more aggressive course, which can lead to higher rates of chronic HBV, lower HBsAg seroconversion, higher levels of HBV replication and reactivation (HBeAg, and HBV DNA detection), accelerated cirrhosis, an increased risk of developing hepatocellular carcinoma, and a lower treatment response. Although co-infection with HBV has been linked to higher HIV RNA turnover, increased liver morbidity, and complex HIV pathology, it is not typically considered in the therapy of HIV infection[11].

Hepatitis B virus (HBV) infection is endemic in Sub-Saharan Africa, Nigeria inclusive[12], and it is common among HIV/AIDS patients. Both illnesses are extremely dangerous to people's health and are spread in similar ways. Because of the patient's immunosuppression and higher risk of HBV exposure, several different HBV serological patterns may be found among HIV-infected individuals. In the presence of high levels of HBV replication, flares can occur during immune reconstitution in HBV/HIV co-infected patients after the initiation of HAART[13][14].

Flares may also occur as a result of hepatotoxicity associated with HAART therapy. It is critical to explore the distribution of HBV serological markers in HIV patients on HAART[15][16]. To the best of our knowledge, no research has been done on the prevalence of Hepatitis B Virus serological markers in HIV Positive Patients on HAART in Ogun State, Nigeria, using the HBV 5 in 1 Panel rapid test kit. Furthermore, risk factors related to the incidence of HBV infection in this scenario

must be identified. This research is required because of the scarcity of knowledge in this regard. The aim of this study is to determine the prevalence of hepatitis B virus serological markers and associated risk factors among HIV-positive female patients on HAART in Ogun State, South-west, Nigeria.

Methodology

Study Design

This research is a hospital-based cross-sectional study.

Study area

This research was conducted among HIV-positive patients at Babcock University Teaching Hospital (BUTH) in Ilishan-Remo, Ogun State, and General Hospital in Ijebu-Ode, Ogun State. BUTH is a 300-bed private hospital that serves as the community's only tertiary care facility. Ilishan-Remo community is one of the geopolitical wards of Ogun State's Ikenne Local Government Area, which is located in the tropical area of the country's south-western section at 70 29'00"N, 20 55'00"E. General Hospital, Ijebu-Ode, Ogun, on the other hand, is a government-owned hospital with a 1000-bed capacity and the only tertiary hospital in the Itoro Local Government Area (LGA), which is also located in the tropical area of Nigeria's south-western region, at coordinates 6.81310 N, 3.92490 E.

Study population

This hospital-based cross-sectional study was conducted among HIV-positive female patients at Babcock University Teaching Hospital (BUTH) in Ilishan-Remo, Ogun State, and General Hospital in Ijebu-Ode, Ogun State.

Determination of sample size

Araoye's formula[17] was used to calculate the sample size for this study.

$n = Z^2PQd^2$

Where:

n = sample size

Z = Z statistic indicates a level of confidence, which is commonly set at 1.96 at a 95% confidence interval.

P = Prevalence or proportion expected (proportion in the population having the particular trait).

Q =1- p

d = precision (in proportion of one; if 5%, d = 0.05).

For the calculation, a 95% confidence interval, a P value of 0.063,*i.e,* a prevalence rate of 6.3% for HIV-HBV co-infection from a previous study by Okocha *et al*.[18], and a margin of error (d) set at 0.05 was used to determine the minimum sample size required. To minimize errors arising from the likelihood of non-compliance, 10% of the sample size was added.

Therefore:

$$n = \frac{(1.96)^2 (0.063)(1-0.063)}{(0.05)^2}$$

$n = 90.7 \approx 91$

10% of 91: 10/100 X 91 = 9.1 ≈ 9

Sample size was therefore: 91 + 9 = 100

A total of 100 HIV-positive female patients were required for the study.

Sampling technique

Fifty (50) HIV-infected female patients attending the HIV clinic of each hospital were randomly enrolled until the desired sample size is achieved. Following their consent, each was interviewed individually and a blood sample was taken.

Eligibility of subjects

Inclusion Criteria

Consenting HIV positive Patients on HAART attending Babcock University Teaching Hospital (BUTH), Ilishan- Remo, Ogun State and General Hospital, Ijebu-Ode, Ogun State, who were eighteen years or more (≥18 years) were randomly selected for the study.

Exclusion Criteria

Patients who were HIV negative, HAART naive HIV positive, and under the age of eighteen (18 years) were excluded

from the study.

Consent

Each subject gave informed consent. Participants were required to freely complete the consent in their own handwriting and sign it as confirmation of their wish to submit samples for the test after receiving a detailed overview of the study's purpose and nature, as well as the sample collecting technique. They had their privacy protected.

Sample Collection

To get the sera from the patients, five (5) ml of venous blood samples were collected into plain bottles and allowed to clot. Following blood clotting, the serum was separated by aspiration using a Pasteur pipette.

Sample Transportation

The blood samples were transported to Babcock University's medical laboratory science department's laboratory unit and processed within two hours of collection. All samples were sent to the laboratory as quickly as possible and processed the same day they were collected. Each participant's specimens were collected and labeled on the specimen container with their identification number.

Specimen Storage

Specimens were analyzed as quickly as possible. The sera were held at 2-8^{0}C for up to 3 days when a delay was expected. Specimens were maintained below -20^{0}C for long-term storage. Prior to testing, frozen specimens were completely thawed and well mixed. Repeated freezing and thawing of sera were avoided.

Laboratory investigations

HIV detection

The current National HIV serodiagnosis algorithm was utilized to detect HIV. This entails utilizing three rapid diagnostic kits according to the manufacturer's instructions. Each patient's serum was tested for the presence of HIV antibodies using Determine (Abbott Laboratories, Tokyo, Japan) and Unigold HIV (Trinity Biotech Plc Bray, Co. Wicklow, Ireland). If both kits test positive, the patient is diagnosed with HIV and vice versa. When the results of the tests did not agree, the Tie Breaker 1/2 Stat Pak (Chembio Diagnostic Systems, New York, USA) was used. One of the first two kits that agreed

with the third kit's results was used to determine the patient's HIV serostatus[19][20].

HBV detection

An HBV 5-in-1 Panel supplied by Innovation Biotechnology Co., Ltd, Beijing, China, was used to assess the qualitative detection of HBV markers in serum specimens.

Principle of HBV 5 in 1 Panel

The kit is intended for the qualitative measurement of hepatitis B markers such as (HBsAb, HBsAg, HBeAg, HBeAb, HBcAb) in human serum or plasma in the assessment of hepatitis B infection. The HBsAg test is a double antibody sandwich immunoassay in which anti-HBsAg antibody complexes are colloidal gold conjugated and dry-immobilized in the test instrument. When the sample is added, it migrates through the strip via capillary diffusion, rehydrating the gold conjugate complexes. If HBsAg is present, it will react with gold conjugate complexes to create particles. These particles will move along the strip until they reach the test zone, where they will be caught by anti-HBsAg antibodies immobilized there, resulting in a visible red line. There will be no red line in the test zone if there is no HBsAg in the sample.
HBsAb is a sandwich immunoassay with two antigens. When the sample is added, it migrates by capillary diffusion through the strip rehydrating the gold conjugate complexes forming particles. These particles will continue to migrate along the strip until the test zone where they are captured by HBsAg immobilized there and a visible red line appears. If there is no HBsAb in the sample, no red line will appear in the test zone.
HBeAg; this test is a double antibody sandwich immunoassay. When the sample is added, it migrates through the strip via capillary diffusion, rehydrating the gold conjugate complexes and producing particles. HBeAg will react with gold conjugate complexes to create particles if it is present. These particles will move along the strip until they reach the test zone, where they will be caught by anti-HbeAg antibodies immobilized there, resulting in a visible red line. If there is no HBeAg in the sample, no red line will show in the test zone. The gold conjugate complexes will travel on their own until they are trapped in the control zone by immobilized goat anti-mouse IgG antibody, forming a red line that indicates the test's validity.
A competitive immunoassay is the HBeAb test. When the specimen is inserted, it migrates through the strip with the gold conjugate complexes via capillary diffusion. If HBeAb is present, gold conjugate complexes will compete for the restricted amount of HBeAg immobilized in the test zone. The gold conjugate complexes will not react with HBeAg, and no red line will form in the test zone. If the specimen lacks HBeAb, gold conjugate complexes react with HBeAg, resulting in a visible red line. A red line will always show in the control zone, indicating that the test is valid.
A competitive immunoassay is the HBcAb test. When the specimen is inserted, it migrates through the strip with the gold conjugate complexes via capillary diffusion. If HBcAb is present, gold conjugate complexes will compete for the restricted amount of HBcAg immobilized in the test zone. The gold conjugate complexes will not react with HBcAg, and no red line will form in the test zone. If the specimen lacks HBcAb, gold conjugate complexes react with HBcAg, resulting in a visible

red line. The legitimacy of the test to act as a procedural control is always indicated by a red line in the control zone.

Procedure

1. The foil pouch was opened and the test panel was taken out.
2. 100µl or 2-3 drops of the sample was added into each of the sample wells on the panel
3. The outcome was read after 15 minutes. At 25 minutes, the HBsAg negative result was confirmed.

The results' interpretation

HBeAg Positive, HBsAg Positive, HBsAb Positive: In addition to the pink-colored control band, a separate pink-colored band will appear in the test region.
Negative: On the control region, just one colored band will show.
Invalid: A procedural error or degradation of the test reagent could explain why no band emerges in the control zone. A new test should be performed on the specimen.
Interpretation for HBeAb, HBcAb
Positive: In the control section, only one colored band will appear; no pink-colored band will appear in the test location.
Weak positive: In addition to a pink-tinted control band, a light pink band appeared in the test region.
Negative: In both the tests and the control zone, two colored bands emerge.
Invalid: No band appears in the control zone, possibly due to a methodological error or reagent degradation. Retest the specimen.

Data analysis

Microsoft Excel was used to enter the raw data. The SPSS Statistics software suite was used for statistical analysis (version 18.0). Tukey-Kramer Multiple Comparisons and One-Way Analysis of Variance test was used to determine if the prevalence of HBV serological markers among the subjects differed significantly. P values <0.05 were considered significant. Tables and charts were used to display the results of statistical analysis.

Results

This present study assessed the prevalence of hepatitis B virus serological markers among HIV-infected patients on HAART in Ogun state, South-west, Nigeria. The socio-demographic characteristics of the study participants including age range, religion, tribe, educational status, and marital status are presented in Table 1. The majority of the participants are within the age range of 18-25 years (36.4%), while the least were between 42-49 years and >50 years, 10.0% each. 50.9% of the participant are singles, 13.6% of the participants are divorced, 17.3 % of the participant are married, 10.0% of the participant are separated, while 8.2% of the participant are widows. More than half of the study subjects were

Christians (59.1%), followed by a higher minority- Muslim (31.8%), 1.8% practiced traditional religion, while 7.3% practiced other religions. As regards ethnicity, the tribe with the higher frequency was Igbo (30%), followed by Yoruba (26.4%), Hausa (24.5%), and then finally other tribes (19.1%).

As regards educational background, 14.5% of the study participants did not have any formal education, 4.5% had primary education, 31.8% had secondary education, while slightly less than half of the study subjects had tertiary education (49.1%). The majority of the study participants (52.7%) live in a rural area, while 47.3% live in an urban area. With regards to monthly income, the majority of the study participants (36.4%) earn above ₦100,000 monthly; 24.5% earn between ₦20-50000, 20.9% earn between ₦51,000 - ₦100,000 monthly, while 16.4% earn less than ₦20,000 monthly. Two (2) of the study participants (1.8%) do not have any source of monthly income (Table 1).

The clinical history of HIV infection and adherence to HAART medication of the study participants is presented in Table 2. All the study participants are aware of their HIV status (100%), with the majority of them having a diagnosis time of 1-5 years (38.2%), followed by > 6 months (20.0%), 5-10 years (18.2%), >10years (15.5%) and finally less than 6 months (8.2%). Still, the majority of them indicated that they always adhere to HAART medication (63.6%), followed by often (32.7%) and sometimes (3.6%).
Figure 1 shows the serological profile of the research participants for the hepatitis B virus. 4 (3.6%) were positive for HBsAg, 2 (1.8%) were positive for HBsAb, 81 (73.6%) were positive for HBeAg, 3 (2.7%) were positive for HBeAb, and 65 (59.1%) were positive for HBcAb out of the 100 study participants.
Table 3 shows the frequency of HBsAg incidence in relation to the socio-demographic features of the research subjects. Occurrence of HBsAg was recorded among ages 18-25 YRS (1.8%) and 26-33 YRS (1.8%). The highest occurrence was recorded among singles (2.7%), Christians (2.7%), Yoruba (1.8%), and equally among those with secondary and tertiary education (1.8%, each). There was no significant association between the occurrence of HBsA and all the socio-demographic characteristics of the study participants (P>0.05).

Table 4 shows the frequency of HBsAb incidence in connection to the socio-demographic parameters of the research subjects. HBsAb was found in 0.9 percent of 18-25 year-olds and 26-33 year-olds (0.9 percent). A non-married and a widow tested positive for HBsAb based on their marital status (0.9 percent each). Christians (1.8 percent), Yoruba (1.8 percent), and individuals with secondary and higher education all had the greatest rates of incidence (0.9 percent, each). There was no significant relationship between the presence of HBsAb and any of the research participants' socio-demographic variables (P>0.05).

Table 5 also shows the frequency of HBeAg incidence in connection to the socio-demographic features of the research subjects. The highest occurrence was recorded among age 18-25 YRS (30.0%), followed by 34-41 YRS (15.5%), 26-33 YRS (12.7%), 42-49 YRS (8.2%) and finally >50 YRS (7.3%).

On the basis of marital status, the highest occurrence was recorded among the singles (40.9%), followed by the divorced

(10.9%), married (9.1%), widow (7.3%), and finally the separated (5.5%). On the basis of religion, tribe, and educational status, the highest occurrence was recorded among Christians (46.4%), Igbo (24.5%), and those with tertiary education (38.2%). There was no significant association between the occurrence of HBeAg and all the socio-demographic characteristics of the study participants ($P>0.05$), except for educational status ($p<0.05$).

Table 6 also shows the frequency of HBeAb incidence in connection to the socio-demographic features of the research subjects. HBeAb was found in those aged 18-25 years (0.9%) and 34-41 years (1.8%). Singles (2.7%), according to marital, religious, tribal, and educational status, had the greatest prevalence. those who practice other forms of religion (besides Christianity, Islam, and Traditional) (1.8%), Igbo (1.8%), and those with secondary education (2.7%).

Table 1. Socio-demographic characteristics of the study participants

Characteristics	Category	Frequency	Percent
Age range	>50 YRS	11	10.0
	18-25 YRS	40	36.4
	26-33 YRS	25	22.7
	34-41 YRS	23	20.9
	42-49 YRS	11	10.0
Marital status	Divorced	15	13.6
	Married	19	17.3
	Separated	11	10.0
	Single	56	50.9
	Widow	9	8.2
Religion	Christianity	65	59.1
	Islam	35	31.8
	Others	8	7.3
	Traditional	2	1.8
Tribe	Hausa	27	24.5
	Igbo	33	30.0
	Others	21	19.1
	Yoruba	29	26.4
Educational status	None	16	14.5
	Primary	5	4.5
	Secondary	35	31.8
	Tertiary	54	49.1
Location of Residence	Rural	58	52.7
	Urban	52	47.3
Household monthly income (₦)	<20,000	18	16.4
	>100,000	40	36.4
	20-50,000	27	24.5
	51-100,000	23	20.9
	Nil	2	1.8

There was no significant association between the occurrence of HBeAb and all the socio-demographic characteristics of the study participants (P>0.05), except for educational status (p<0.05).

Table 7 also shows the frequency of HBcAb incidence in connection to the socio-demographic features of the research subjects. The highest occurrence was recorded among age 18-25 YRS (25.5%) and the lowest among age 42-49 YRS (7.3%) Singles (34.5%) and separated (3.6%) had the most and lowest occurrences, respectively, based on marital status. In terms of religion, Christians (37.3%) and Traditional worshippers (1.8%) had the largest and lowest occurrences, respectively. According to tribe and educational background, the Igbo (24.5%) and those with secondary education

(23.6%) had the highest rates. There was a significant association ($p<0.05$) between the occurrence of HBcAb and the age, marital status, tribe, and educational level of the study participants.

Table 2. Clinical History of HIV infection and adherence to HAART medication

Characteristics	Category	Frequency	Percent
Do you know your HIV status?	Yes	110	100.0
	No	0	0
Time of HIV diagnosis	<6 months	9	8.2
	>10 YRS	17	15.5
	>6 months	22	20.0
	1-5 YRS	42	38.2
	5-10 YRS	20	18.2
How often do you adhere to HAART Medication	Always	70	63.6
	Often	36	32.7
	Sometimes	4	3.6

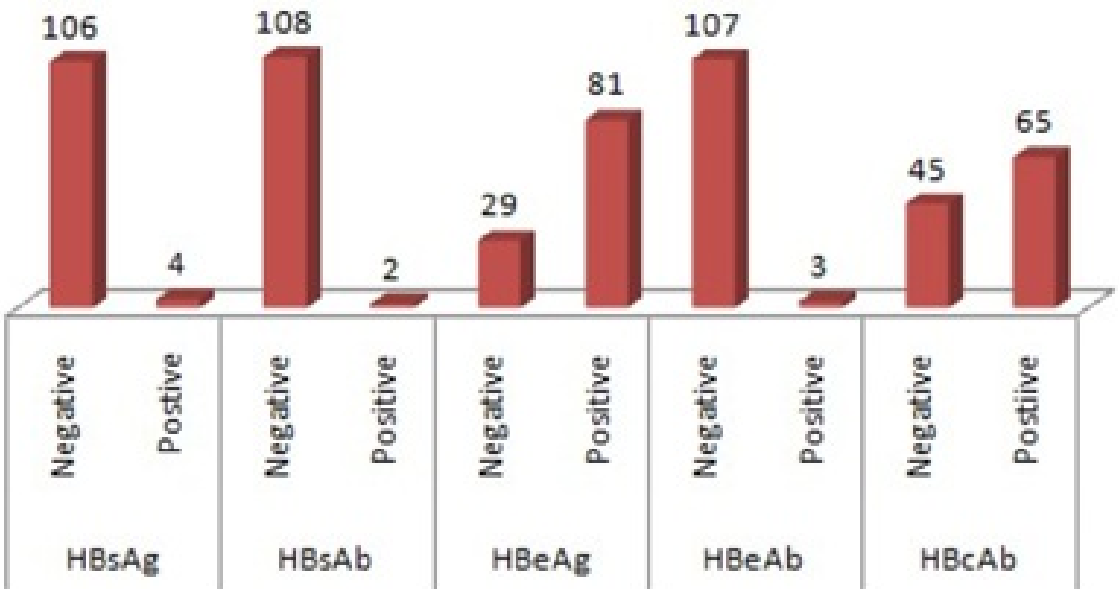

Fig. 1. Hepatitis B virus serological profile of the study participants

The risk factors associated with the occurrences of HBV serological markers among the study participants are presented in Table 8. Among the 15 (13.6%) respondents who indicated that they have no knowledge of Hepatitis B virus, 8 (7.3%) and 6 (5.5%) of them tested positive for HBeAg and HBcAb, respectively. Among 61 (55.5%) respondents who indicated they have no history of Hepatitis B Virus vaccination, 2 (1.8%), 1 (0.9%), 47 (42.7%) and 33 (30.0%) tested positive for HBsAg, HBsAb, HBeAg and HBcAb. Among the 35 (31.8%) respondents who indicated history of blood transfusion, 2 (1.8%), 1 (0.9%), 26 (23.6%) and 20 (18.2%) tested positive for HBsAg, HBsAb, HBeAg and HBcAb, respectively. Among

the 3 (2.7%), who indicated a history of organ transplant, 3 (2.7%) and 2 (1.8%) of the respondents tested positive for HBeAg and HBcAb.

Table 3. The frequency of occurrence of HBsAg in relation to the socio-demographic characteristics of the study participants

Characteristics	Category	Number examined N (%)	Number negative N (%)	Number positive N (%)	Pearson Chi-Square (χ^2)	P-Value	Likelihood Ratio
Age range	>50 YRS	11(10.0)	11(10.0)	0(0.0)	3.269	0.514	4.547
	18-25 YRS	4(36.4)	38(34.5)	2(1.8)			
	26-33 YRS	25(22.7)	23(20.9)	2(1.8)			
	34-41 YRS	23(20.9)	23(20.9)	0(0.0)			
	42-49 YRS	11(10.0)	11(10.0)	0(0.0)			
Marital status	Divorced	15(13.6)	15(13.6)	0(0.0)	3.606	0.462	4.690
	Married	19(17.3)	19(17.3)	0(0.0)			
	Separated	11(10.0)	11(10.0)	0(0.0)			
	Single	56(50.9)	53(48.2)	3(2.7)			
	Widow	9(8.2)	8(7.3)	1(0.9)			
Religion	Christianity	65(59.1)	62(56.4)	3(2.7)	0.616	0.893	0.970
	Islam	35(31.8)	34(30.9)	1(0.9)			
	Others	8(7.3)	8(7.3)	0(0.0)			
	Traditional	2(1.8)	2(1.8)	0(0.0)			
Tribe	Hausa	27(24.5)	26(23.6)	1(0.9)	2.201	0.532	3.216
	Igbo	33(30.0)	33(30.0)	0(0.0)			
	Others	21(19.1)	20(18.2)	1(0.9)			
	Yoruba	29(26.4)	27(24.5)	2(1.8)			
Educational status	None	16(14.5)	16(14.5)	0(0.0)	1.224	0.747	1.926
	Primary	5(4.5)	5(4.5)	0(0.0)			
	Secondary	35(31.8)	33(30.0)	2(1.8)			
	Tertiary	54(49.1)	52(47.3)	2(1.8)			

NB: *There was no significant association between the occurrence of HBsA and all the socio-demographic characteristics of the study participants (P>0.05).*

Furthermore, 2 (1.8%), 1 (0.9%), 17 (15.5%), 2 (1.8%) and 14 (12.7%) among the 26 (23.6%) who indicated that they had tattoos/ear piercing, tested positive to HBsAg, HBsAb, HBeAg, HBeAb and HBcAb, respectively. Still, among the 8 (7.3%) who indicated that they share sharp objects, 7 (6.4%) and 5 (4.5%) tested positive for HBeAg and HBcAb, respectively. Also, among the 36 (32.7%), who indicated that they engaged in unprotected sex, 3 (2.7%), 1 (0.9%) 19 (17.3%), 3 (2.7%) and 19 (17.3%), tested positive to HBsAg, HBsAb, HBeAg, HBeAb and HBcAb, respectively.

Table 4. The frequency of occurrence of HBsAb in relation to the study participants' socio-demographic parameters

Characteristics	Category	Number examined N (%)	Number negative N (%)	Number positive N (%)	Pearson Chi-Square (χ^2)	P-Value	Likelihood Ratio
Age range	>50 YRS	11(10.0)	11(10.0)	0(0.0)	1.604	0.808	2.243
	18-25 YRS	40(36.4)	39(35.5)	1(0.9)			
	26-33 YRS	25(22.7)	24(21.8)	1(0.9)			
	34-41 YRS	23(20.9)	23(20.9)	0(0.0)			
	42-49 YRS	11(10.0)	11(10.0)	0(0.0)			
Marital status	Divorced	15(13.6)	15(13.6)	0(0.0)	5.188	0.269	3.681
	Married	19(17.3)	19(17.3)	0(0.0)			
	Separated	11(10.0)	11(10.0)	0(0.0)			
	Single	56(50.9)	55(50.0)	1(0.9)			
	Widow	9(8.2)	8(7.3)	1(0.9)			
Religion	Christianity	65(59.1)	63(57.3)	2(1.8)	1.410	0.703	2.130
	Islam	35(31.8)	35(31.8)	0(0.0)			
	Others	8(7.3)	8(7.3)	0(0.0)			
	Traditional	2(1.8)	2(1.8)	0(0.0)			
Tribe	Hausa	27(24.5)	27(24.5)	0(0.0)	5.690	0.128	5.437
	Igbo	33(30.0)	33(30.0)	0(0.0)			
	Others	21(19.1)	21(19.1)	0(0.0)			
	Yoruba	29(26.4)	27(24.5)	2(1.8)			
Educational status	None	16(14.5)	16(14.5)	0(0.0)	0.601	0.896	0.952
	Primary	5(4.5)	5(4.5)	0(0.0)			
	Secondary	35(31.8)	34(30.9)	1(0.9)			
	Tertiary	54(49.1)	53(48.2)	1(0.9)			

NB: *There was no significant association between the occurrence of HBsAb and all the socio-demographic characteristics of the study participants (P>0.05).*

Table 5. *The frequency of occurrence of HBeAg in relation to the socio-demographic characteristics of the study participants*

Characteristics	Category	Number examined N (%)	Number negative N (%)	Number positive N (%)	Pearson Chi-Square (x^2)	P-Value	Likelihood Ratio
Age range	18-25 YRS	40(36.4)	7(6.4)	33(30.0)	6.009	0.198	5.783
	26-33 YRS	25(22.7)	11(10.0)	14(12.7)			
	34-41 YRS	23(20.9)	6(5.5)	17(15.5)			
	42-49 YRS	11(10.0)	2(1.8)	9(8.2)			
	>50 YRS	11(10.0)	3(2.7)	8(7.3)			
Marital status	Divorced	15(13.6)	3(2.7)	12(10.9)	9.078	0.059	8.679
	Married	19(17.3)	9(8.2)	10(9.1)			
	Separated	11(10.0)	5(4.5)	6(5.5)			
	Single	56(50.9)	11(10.0)	45(40.9)			
	Widow	9(8.2)	1(0.9)	8(7.3)			
Religion	Christianity	65(59.1)	14(12.7)	51(46.4)	5.640	0.130	6.032
	Islam	35(31.8)	14(12.7)	21(19.1)			
	Others	8(7.3)	1(0.9)	7(6.4)			
	Traditional	2(1.8)	0(0.0)	2(1.8)			
Tribe	Hausa	27(24.5)	7(6.4)	20(18.2)	1.992	0.574	2.048
	Igbo	33(30.0)	6(5.5)	27(24.5)			
	Others	21(19.1)	7(6.4)	14(12.7)			
	Yoruba	29(26.4)	9(8.2)	20(18.2)			
Educational status	None	16(14.5)	10(9.1)	6(5.5)	13.760	0.003	13.495
	Primary	5(4.5)	0(0.0)	5(4.5)			
	Secondary	35(31.8)	7(6.4)	28(25.5)			
	Tertiary	54(49.1)	12(10.9)	42(38.2)			

NB: *There was no significant association between the occurrence of HBeAg and all the socio-demographic characteristics of the study participants (P>0.05), except for educational status (p<0.05).*

Table 6. The frequency of HBeAb incidence in relation to the study participants' socio-demographic parameters

Characteristics	Category	Number examined N (%)	Number negative N (%)	Number positive N (%)	Pearson Chi-Square (χ^2)	P-Value	Likelihood Ratio
Age range	>50 YRS	11(10.0)	11(10.0)	0(0.0)	4.414	0.353	4.586
	18-25 YRS	40(36.4)	39(35.5)	1(0.9)			
	26-33 YRS	25(22.7)	25(22.7)	0(0.0)			
	34-41 YRS	23(20.9)	21(19.1)	2(1.8)			
	42-49 YRS	11(10.0)	11(10.0)	0(0.0)			
Marital status	Divorced	15(13.6)	15(13.6)	0(0.0)	2.974	0.562	4.132
	Married	19(17.3)	19(17.3)	0(0.0)			
	Separated	11(10.0)	11(10.0)	0(0.0)			
	Single	56(50.9)	53(48.2)	3(2.7)			
	Widow	9(8.2)	9(8.2)	0(0.0)			
Religion	Christianity	65(59.1)	65(59.1)	0(0.0)	16.840	0.461	9.449
	Islam	35(31.8)	34(30.9)	1(0.9)			
	Others	8(7.3)	6(5.5)	2(1.8)			
	Traditional	2(1.8)	2(1.8)	0(0.0)			
Tribe	Hausa	27(24.5)	26(23.6)	1(0.9)	2.881	0.410	3.885
	Igbo	33(30.0)	31(28.2)	2(1.8)			
	Others	21(19.1)	21(19.1)	0(0.0)			
	Yoruba	29(26.4)	29(26.4)	0(0.0)			
Educational status	None	16(14.5)	16(14.5)	0(0.0)	6.609	0.085	7.053
	Primary	5(4.5)	5(4.5)	0(0.0)			
	Secondary	35(31.8)	32(29.1)	3(2.7)			
	Tertiary	54(49.1)	54(49.1)	0(0.0)			

NB: *There was no significant association between the occurrence of HBeAb and all the socio-demographic characteristics of the study participants (P>0.05).*

Table 7: The frequency of occurrence of HBcAb in relation to the study participants' socio-demographic parameters

Characteristics	Category	Number examined N (%)	Number negative N (%)	Number positive N (%)	Pearson Chi-Square (χ^2)	P-Value	Likelihood Ratio
Age range	>50 YRS	11(10.0)	2(1.8)	9(8.2)	11.254	0.024	11.502
	18-25 YRS	40(36.4)	12(10.9)	28(25.5)			
	26-33 YRS	25(22.7)	15(13.6)	10(9.1)			
	34-41 YRS	23(20.9)	13(11.8)	10(9.1)			
	42-49 YRS	11(10.0)	3(2.7)	8(7.3)			
Marital status	Divorced	15(13.6)	5(4.5)	10(9.1)	11.736	0.019	11.757
	Married	19(17.3)	13(11.8)	6(5.5)			
	Separated	11(10.0)	7(6.4)	4(3.6)			
	Single	56(50.9)	18(16.4)	38(34.5)			
	Widow	9(8.2)	2(1.8)	7(6.4)			
Religion	Christianity	65(59.1)	24(21.8)	41(37.3)	3.452	0.327	4.148
	Islam	35(31.8)	18(16.4)	17(15.5)			
	Others	8(7.3)	3(2.7)	5(4.5)			
	Traditional	2(1.8)	0(0.0)	2(1.8)			
Tribe	Hausa	27(24.5)	9(8.2)	18(16.4)	19.157	0.000	19.950
	Igbo	33(30.0)	6(5.5)	27(24.5)			
	Others	21(19.1)	16(14.5)	5(4.5)			
	Yoruba	29(26.4)	14(12.7)	15(13.6)			
Educational status	None	16(14.5)	16(14.5)	0(0.0)	29.193	0.000	34.929
	Primary	5(4.5)	3(2.7)	2(1.8)			
	Secondary	35(31.8)	9(8.2)	26(23.6)			
	Tertiary	54(49.1)	17(15.5)	37(33.6)			

NB: *There was a significant association ($p<0.05$) between the occurrence of HBcAb and the age, marital status, tribe, and educational level of the study participants.*

Table 8. Risk factors associated with the occurrences of HBV serological markers among the study participants.

Characteristics	Category	No. examined N (%)	No. Positive for HBsAg	No. Positive for HBsAb	No. Positive for HBeAg	No. Positive for HBeAb	No. Positive for HBcAb
Have you heard of Hepatitis B Virus?	No	15(13.6)	0(0.0)	0(0.0)	8(7.3)	0(0.0)	6(5.5)
	Yes	95(86.4)	4(3.6)	2(1.8)	73(66.4)	3(2.7)	59(53.6)
Have you received Hepatitis B vaccine?	No	61(55.5)	2(1.8)	1(0.9)	47(42.7)	0(0.0)	33(30.0)
	Yes	49(44.5)	2(1.8)	1(0.9)	34(30.9)	3(2.7)	32(29.1)
History of Blood Transfusion	No	75(68.2)	2(1.8)	1(0.9)	55(50.0)	3(2.7)	45(40.9)
	Yes	35(31.8)	2(1.8)	1(0.9)	26(23.6)	0(0.0)	20(18.2)
Do you have a history of organ transplants?	No	107(97.3)	4(3.6)	2(1.8)	78(70.9)	3(2.7)	63(57.3)
	Yes	3(2.7)	0(0.0)	0(0.0)	3(2.7)	0(0.0)	2(1.8)
Do you have a history of dialysis?	No	101(91.8)	4(3.6)	2(1.8)	74(67.3)	3(2.7)	60(54.5)
	Yes	9(8.2)	0(0.0)	0(0.0)	7(6.4)	0(0.0)	5(4.5)
Do you have tattoos/ear piercings?	No	84(76.4)	2(1.8)	1(0.9)	64(58.2)	1(0.9)	51(46.4)
	Yes	26(23.6)	2(1.8)	1(0.9)	17(15.5)	2(1.8)	14(12.7)
Do you share sharp objects?	No	102(92.7)	4(3.6)	2(1.8)	74(67.3)	3(2.7)	60(54.5)
	Yes	8(7.3)	0(0.0)	0(0.0)	7(6.4)	0(0.0)	5(4.5)
Do you share toothbrush?	No	110(100.0)	4(3.6)	2(1.8)	81(73.6)	3(2.7)	65(59.1)
Smoke	No	89(80.9)	2(1.8)	2(1.8)	66(60.0)	0(0.0)	53(48.2)
	Yes	21(19.1)	2(1.8)	0(0.0)	15(13.6)	3(2.7)	12(10.9)
Alcohol Intake	No	81(73.6)	1(0.9)	1(0.9)	56(50.9)	0(0.0)	41(37.3)
	Yes	29(26.4)	3(2.7)	1(0.9)	25(22.7)	3(2.7)	24(21.8)
Engage in unprotected sex	No	74(67.3)	1(0.9)	1(0.9)	62(56.4)	0(0.0)	46(41.8)
	Yes	36(32.7)	3(2.7)	1(0.9)	19(17.3)	3(2.7)	19(17.3)
Number of sex partners	1-2 partners	71(64.5)	3(2.7)	2(1.8)	51(46.4)	0(0.0)	37(33.6)
	3-5 partners	4(3.6)	1(0.9)	0(0.9)	4(3.6)	3(2.7)	4(3.6)
	None	35(31.8)	0(0.0)	0(0.0)	26(23.6)	0(0.0)	24(21.8)
Recent change of sex partners	No	89(80.9)	2(1.8)	1(0.9)	68(61.8)	0(0.0)	55(50.0)
	Yes	21(19.1)	2(1.8)	1(0.9)	13(11.8)	3(2.7)	10(9.1)
Frequency of sexual intercourse per week	1-2 week	61(55.5)	1(0.9)	1(0.9)	49(44.5)	0(0.0)	34(30.9)
	3-5 week	11(10.0)	3(2.7)	1(0.9)	7(6.4)	3(2.7)	7(6.4)
	Nil	38(34.5)	0(0.0)	0(0.0)	25(22.7)	0(0.0)	24(21.8)
Frequency of Medical check-ups	Less often	35(31.8)	2(1.8)	0(0.0)	26(23.6)	3(2.7)	27(24.5)
	Often	56(50.9)	0(0.0)	0(0.0)	46(41.8)	0(0.0)	33(30.0)
	Very often	19(17.3)	2(1.8)	2(1.8)	9(8.2)	0(0.0)	5(4.5)

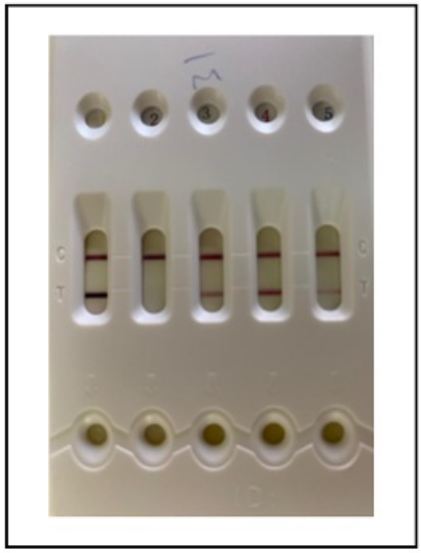

Fig. 2. *Hepatitis B Virus 5 in 1 Cassette showing a test positive for HBsAg and HBeAg only.*

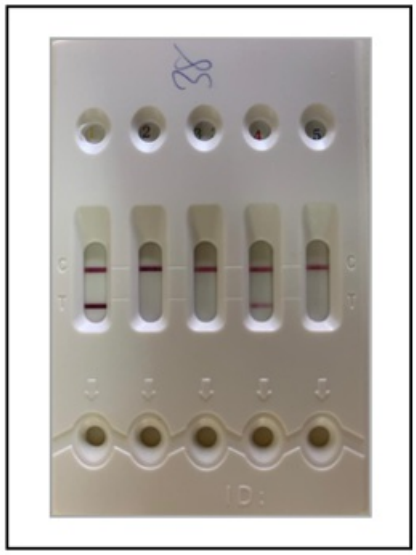

Fig. 3. *Hepatitis B Virus 5 in 1 Cassette showing a test positive for HBsAg and HBcAb only.*

Fig. 4. *Hepatitis B Virus 5 in 1 Cassette showing a test negative for all the serological markers.*

Discussion

This present study assessed the prevalence of HBV serological markers among HIV-positive female patients on HAART in Ogun State, Nigeria. The outcome of this study shows that Hepatitis B Virus (HBV) infection exists among HIV-infected female patients on HAART in Ogun state, South-west, Nigeria. Their HBV serological profile is as follow: HBsAg (3.6%), HBsAb (1.8%), HBeAg (73.6%), HBeAb (2.7%) and HBcAb (59.1%). These serological indicators are crucial in the clinical diagnosis of HBV infection. The first sign to show in the blood is HBsAg, which indicates the existence of a present infection, which might be acute or chronic. Antibody against HBsAg (HBsAb) is discovered in the bloodstream simultaneously with or shortly after the elimination of HBsAg. Its emergence predicts full recovery. Meanwhile, it gives lifetime protection after inoculation with the HBV vaccine[3].

Furthermore, because HBeAg is a marker of active intrahepatic viral replication and heightened infectivity, its presence in the blood indicates that the individual is highly infectious. The absence of HBeAg indicates that the positive participants are not more infectious. Meanwhile, HBeAb is commonly seen in the serum during the convalescent period, particularly in chronic hepatitis, and its carrier status indicates reduced infectivity. It emerges quickly after the antigen has vanished and can be detected for up to 2 years after the hepatitis has resolved[3].

In addition, HBcAg does not circulate freely in the serum of infected people; rather, antibodies to HBcAg (HBcAb) appear shortly after HBsAg, around the same time that serum ALT starts to rise, and remain elevated for the rest of their lives. Its presence implies an infection, which might be new or old, as well as a persistent HBV infection. The presence of both HBsAb and HBcAb shows innate infection immunity. The presence of HBsAg, HBcAb and HBeAb or HBeAg has been associated with Hepatitis B carrier state. Meanwhile; absence of the 5 HBV markers (HBsAg, HBsAb, HBcAb, HBeAg, and HBeAb) denotes absence of HBV infection and that the individual is susceptible to infection[3][21][22].

An overall 3.6% HBsAg seropositivity was observed in this study which is lower than the 6.7% reported by Okonko *et al.*[23] among HIV-positive blood donors in Port Harcourt; thus suggesting a moderate prevalence of HBV infection among HIV-positive patients in this region. Nevertheless, HBsAg seropositivity rate of 3.6% among the HIV-positive study population in this region calls for great concern as HBV/HIV co-morbidity has been linked with a high risk of mother-to-child transmission and accelerated progression of HIV infection. The finding in this study is consistent with the 3.6% reported in Biu, Borno State, Nigeria by Bello *et al.*[24]. It is also in agreement with the 3.5% reported by Omatola *et al.*[25] in Anyigba, Kogi state, Nigeria. It is also similar to the 3.6% reported by Smit *et al.*[26], 3.2% by Rai *et al.*[8], 3% by Lodenyo *et al.*[27], and 3.7% by de Almeida *et al.*[28] previously reported among HIV patients in Netherlands, Japan, Johannesburg, and Brazil respectively. Lower prevalence of 2.7%, 1.2%, 1.13%, 1.8%, and 2.6% was earlier reported among HIV-positive patients in Nigeria, Tanzania, Mali, and Iran, respectively[29][30][31][32].

Okonko *et al.*[33] also reported 2.5% HBV/HIV coinfection among apparently healthy blood donors in Ibadan, Nigeria. The seroprevalence rate in this study is however lower than the prevalence of HBV/HIV co-infection range of 10-70 % earlier reported for Nigeria[34]. This lower prevalence rate is likely due to the efforts of the public health agencies on HIV/AIDS prevention in the Country since measures aimed at preventing HIV infection also protect against HBV which shares similar transmission routes with HIV.

It is noteworthy that only 1.8% of total study subjects were positive for HBsAb. This is an indication of lowered immunity with respect to the prevalence of HBsAg among HIV patients. It also suggests that the antibody against HBV is independent of HARRT compliance. The HBsAb positivity rate is higher than that reported by Aliyu *et al.*[35]. The presence of HBsAg without any antibodies is an indicator of an active hepatitis B infection. This antigen may be present before symptoms of an HBV infection are present. If this antigen level remains high for more than 6 months, the patient probably becomes a carrier of HBV, meaning that the patient can transmit it to others throughout life. Furthermore, 73.7% of the study participants had HBeAg, while a very small fraction (2.7%) had HBeAb. The development of anti-HBe indicates clearance of the infection as anti-HBe replaces HBeAg as the chronic HBV infection is resolving[36]. Anti HBe generally persists for a lifetime in over 80% of patients and indicates immunity[37]. However, the case is in retrograde with more HBV-infected subjects without immunity in form of an antibody to protect them.
Also, Anti HBc was found in 59.2% of the study participants. The detection of this antibody in blood is a clear indication that the body mounts an immune response to the virus leading to severity of symptoms and complications, especially in co-morbidity with HIV. However, 40.8% of total subjects do not have the HBc antibody indicating active infection. This result is similar to the report of Aliyu *et al.*[35].

The outcome of this study further revealed that Hepatitis B Virus prevalence was higher among young adults age group (18-25 years and 26-30 years). This supported previous studies in Uyo, Akwa-Ibom State, Nigeria[38], in Abuja, Nigeria[39], in North-West Ethiopia[40] , in Cameroon[41]. This may be associated with higher sexual activities within this age group, especially those within adolescent age. Ymele *et al.*[41] reported that HBV infection prevalence decreased with the age. Ogundeji[39] reported that the age group of 21-40 years had the predominant HIV, HBV, and HCV prevalence in

their study.

In relation to marital status, HBV seropositivity was higher among HIV-infected female individuals who were singles (2.7%) as compared to the married (0%) but this was not statistically significant. This is in agreement with a similar study by Okonko *et al.* [38]. This may imply that marital status is not really a risk factor for HBV infection, but an indicator to consider the sexual partner as a risk for infection, since unmarried people may tend to have many sexual partners or unprotected sex. Also, positivity of HBsAg among the infected married subjects in this study could be an indication that the infection might be through unprotected heterosexual intercourse or close contact with their infected partners as the virus can spread through body fluids[38].

As regards religion, though not significant, Christians had the highest seroprevalence of HBV with a prevalence of 2.7%. Subjects of the Yoruba tribe had the highest occurrence of HBV with a prevalence of 2.8% this might be due to the area of study where the majority is from the Yoruba tribe. As there was no association between tribe and HBV status among HIV-infected patients.

In this study, HBV infection was independent of the educational level of the study subject with secondary and tertiary education having the highest occurrence (1.8%). This is in disagreement with a similar study by Okonko *et al.*[38] who reported education-specific association with HIV-HBV co-infections and higher prevalence among those with primary educational status was observed (8.3%) compared to another educational status: tertiary (6.9%) and secondary (4.9%); but in agreement with a study by Katamba *et al.*[42] who reported that correlates of HIV and hepatitis B co-infections were primary education (especially low level of education).

In this study, as much as 86.4% of study subjects have knowledge of HBV. This is much higher than the 57.4% reported in a similar study by Omatola *et al.*[25]. The difference can be attributed to the difference in sample size between the two studies. The good standing rate of HBV awareness can be attributed to the level of education as about 70% of the subjects either had secondary or tertiary education and also lead to adherence to HIV clinical care evidenced by the higher rate of HAART adherence where patients have access to important information related to their health and risk factors at the HIV clinics.

Conclusion

The outcome of this study provides evidence that HBV infection is moderately endemic in Ogun state, South-west, Nigeria among HIV positive female population, particularly among young adults, age group (18-25 years and 26-30 years). Lack of vaccine against the disease, and social lifestyle that predisposes to acquiring infection, the transmission of the virus will probably increase and pose more potential public health concerns in the absence of concerted efforts to stem the disease.

Ethical Approval

The Babcock University Health Research Ethics Committee (BUHREC) granted ethical permission for the project, with the registration number BUHREC 559/21.

Competing Interests

The authors have stated that they have no competing interests.

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