Evaluation of hepatitis B viraemia and corresponding antibodies among infected patients attending Abuja Teaching Hospital, Nigeria

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Abstract

In most under-developed and developing countries, diagnosis and treatment of hepatitis B relied mainly on detection of hepatitis B virus (HBV) serological biomarkers. The reliability of these markers in comparison with HBV DNA viral load is required to review their diagnostic value. Thus, this study investigated the serological and HBV viral load profile of persons with hepatitis B attending the University of Abuja Teaching Hospital, Gwagwalada, Nigeria. Attributes of hepatitis B-infected participants (February-May, 2018) were assessed. They included hepatitis B antigens (HBsAg, HBeAg), antibodies (HBsAb, HBcAb, HBeAb) and HBV DNA, using rapid immunichromatigraphical and real-time polymerase chain reaction (qPCR), respectively. Structured questionnaires were used to collate participants biodata. Out of 53 participants, 30 were male and 23 were female. 90.6% (48/53) were positive for HBsAg, 28.3% (15/53) were positive for HBsAb, 60.4% (32/53) were positive for HBcAb, 17.0% (9/53) were positive for HBeAg, while HBeAb was detected in 58.5% (31/53). HBV DNA was significantly associated with HBcAb (χ^2=28.622, P=0.000), HBeAg (χ^2=11.820, P=0.008), and HBeAb (χ^2=16.440, P=0.001). The on-site point of care serological test has significant impact in diagnosis and monitoring Hepatitis B when compared to qPCR.

Introduction

Hepatitis B virus (HBV) is a DNA virus that infects the liver cells (hepatocytes) and leads to either acute infection or chronic infection. Worldwide, an estimate of 650,000 people die each year from the complications of chronic hepatitis B (CHB).[1] More than 350 million people in the world are chronic carriers, despite the existence and effective use of vaccines.[2] In Nigeria, HBV infection is hyperendemic with the seroprevalence of HBsAg ranging from 10-40%.[3-6]

Acute infections where the virus is cleared from the body by immune response and chronic infect where the virus persists and lead to liver disease such as cirrhosis and hepatocellular carcinoma.[7] Infection with HBV results in acute hepatitis infection followed by recovery in 85% to 95% of human adults.[7] Recovery is achieved when the human immune response mounts an adequate immune response by producing protective, neutralizing antibodies against HBV surface antigens (HBsAg),[8,9] activation of strong and diversified CD4 and CD8 T cells,[8,10] release of antiviral cytokines in the liver such as gamma interferon and tumor necrosis factor alpha,[11-13] and generation of cells that are protected from reinfection.[14] In contrast, progression to chronic HBV infection is predominantly observed in immune-compromised adults and unvaccinated infants,[15] thereby exhibiting weak and inefficient humoral and cellular immune responses, which result into continuous virus replication, and HBsAg circulation in blood.[10] The contributions of different arms of the immune response, especially the roles of neutralizing antibodies in the onset and outcome of the infection is yet to be fully understood. Free antibody to surface antigen is not detected until after the resolution of HBV infection.[16] In both acute and chronic infection, circulating immune complexes containing antibody and HBsAg are found, suggesting that antibodies are produced much sooner than detected, and that they might play a role in the pathology of the disease.[17,18] HBsAg-specific antibodies have neutralizing properties and can mediate protective immunity.[19]

For persons with CHB, it is recommended to monitor their HBV viral load over time, in order to detect failure of the immune system to prevent progression to cirrhosis and hepatocellular carcinoma. In most under-developed and developing countries, where majority of HBV infected persons leave below US$1, diagnosis and treatment of hepatitis B relied mainly on detection of HBV serological biomarkers. The reliability of these markers in comparison with HBV DNA viral load, are required to review their diagnostic value. Thus, this study investigated the serological and HBV viral load profile of persons with hepatitis B attending the University of Abuja Teaching Hospital, Gwagwalada, Nigeria.

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Materials and Methods

Study design

This was a hospital-based prospective study which involved HBV-seropositive adult patients attending the General Out Patient Clinic (GOPC) of the University of Abuja Teaching Hospital. Data for study were generated following the diagnostic investigation of these participants enrolled for this study between the first week of February and mid-May, 2018. There were two types of analysis performed on blood sampled from the study participants: i) in order to determine the HBV viral load of the hepatitis B infected participants, RT-PCR [COBAS® Ampliprep/ COBAS® Taqman® HBV Test, version 2 (Roche Diagnostics, USA)] was used for the quantification of HBV DNA at the mRNA level; ii) serological markers of hepatitis B antigens (HBsAg, HBeAg) and antibodies (HBsAb, HBeAb, HBcAb) were detected using lateral flow chromatographic immunoassay technique (Onsite HBV-5 Rapid, CTK Biotech, Inc, USA).

Sample collection and processing

This study was contingent on blood sampling from the phlebotomy units at the

University of Abuja Teaching Hospital. Prior to sampling, the HBV Research Team liaised with the Medical Laboratory staff in blood collection and sampling, using the purposive sampling technique. Three milliliter of blood samples was collected from individual participants using standard venipuncture. Following sampling and arrival at the research laboratory, blood samples were assigned unique identification numbers, allowed to clot at room temperature and sera were harvested by centrifugation at 3, 000 rpm for 10 minutes and stored at -20˚C.

Laboratory setting

Laboratory analysis was done at the Immunology unit of Medical Laboratory Department, University of Abuja Teaching Hospital, Gwagwalada, FCT-Abuja.

Study participants

The participants were age-matched of both genders who were identified serologically as being positive for HBV and from whom informed consent were obtained. Individuals with challenges consenting and/or with prior vaccination for HBV were not enrolled into the study.

Recruitment

The identification of participants was done by Physicians and Nurses of the General Outpatient Clinic. Written informed consent was obtained after careful explanation of the concept of the study to every participant before enrolling them into the study. This was accompanied with the issuance of an information sheet describing the study and a short structured questionnaire.

Ethical issues

Ethical approval (ref. number: UATH/HREC/PR/2018/01/014) for this study was obtained from the Ethical and Human Research Committee of the University of Abuja Teaching Hospital, Gwagwalada, FCT Abuja. Informed consent was obtained from participants. Confidentiality was ensured as samples were analyzed anonymously (using number code). A database (password protected), linked to the code, containing some information (date of birth, gender, vaccination history, medications) were stored electronically. Demographic, serological, and virologic data were delinked from participants' identity to ensure confidentiality.

Laboratory investigations

All samples were investigated for hepatitis B antigens (HBsAg, HBeAg) and antibodies (HBsAb, HBeAb, HBcAb) using enzyme immunoassay technique (Onsite HBV-5 Rapid, CTK Biotech, Inc, USA) and HBV DNA using RT-PCR [COBAS® Ampliprep/ COBAS® Taqman® HBV Test, version 2 (Roche Diagnostics, USA)]. Investigations were conducted based on manufacturer's instruction.

Detection of HBV serological markers by enzyme immunoassay

The serological techniques for the detection of hepatitis B and antibodies were performed using sandwich and competitive enzyme immunoassays. While HBsAg and HBeAg strips are antibody-based sandwich immunoassays, the HBsAb strip is an antigen-based sandwich immunoassay. Both HBeAb and HBcAb strips are competitive immunoassays.

The conjugate pad for all strips contains polyclonal antibodies specific to the HBV immunoglobulins (anti-HBsAg, anti-HBsAb, anti-HBcAb, anti-HBeAg, anti-HBeAb) which are conjugated with colloidal gold and the nitrocellulose membrane strip pre-coated with respective monoclonal immunoglobulins (anti-HBsAg, anti-HBsAb, anti-HBcAb, anti-HBeAg, anti-HBeAb) (control and test lines), and absorbent pad. These investigations were based on antigen-antibody reactions in the presence of chromogen that makes the reaction visible after 15 minutes. Results were interpreted based on the kit manufacturer's instructions.

HBV DNA isolation, amplification and quantification

HBV viral load was performed using the COBAS® Ampliprep/ COBAS® Taqman® HBV Test, version 2 (Roche Diagnostics, USA) for automated amplification and quantification with a detection limit of between 20-170, 000, 000 IU/mL. Sample preparation and polymerase chain reaction (PCR) amplification of the target DNA was done followed by quantification of cleaved dual□labeled oligonucleotide detection probe specific to the target according to the manufacturer's instructions. Selective amplification of the target nucleic acid from the specimen for HBV S gene was achieved by using target virus-specific forward (5′-CACATCAGGATTCCTAGGACC-3′) and reverse (HBSR1 (nt 339 to 321), 5′-GGTGAGTGATTGGAGGGTTG-3′) primers, while that specific for HBV X gene was achieved by using target virus-specific forward [HBXF1 (nt 1414 to 1435), 5′-ACGTCCTTTGTTTACGTCCCGT-3′] and reverse [HBXR1 (nt 1744 to 1723), 5′-CCCAACTCCTCCCAGTCCTTAA-3′] primers. These primers were selected from highly conserved regions of HBV.

Quantitative HBV DNA viral load levels below the sensitivity level of 20 IU/mL was defined as undetectable, levels less than 2, 000 IU/L were regarded as low-level HBV replication, while levels greater than 2000 IU/mL were regarded as rapid viral replication in the liver.[20,21]

Statistical analysis of data

Data generated was analyzed using SPSS version 20 (California, USA). Results of categorical variables were proportions and association between these variables were assessed using the Chi-square test for likelihood ratio. P≤0.05 was considered statistically significant.

Results

Distribution of HBV serological markers into categories of gender and age groups

The evaluation of the preponderance of male gender on the basis of the ratio of several age groups, showed that the male to female ratio was mostly maximum between the ages of 30-39 years across the frequency of all HBV serological biomarkers. The ratio was mostly minimum at the age of under 19 years.

The rate of seropositivity was categorized according to age-groups. The prevalence of HBsAg seropositivity in individuals based on gender and age-groups were as follows: <19 years (2 males and 1 female), 19-29 years (13 males and 10 females), 30-39 years (7 males and 5 females), >39 years (7 males and 3 females). The highest HBV seroprevalence of HBsAg was observed amongst the male participants [13 (27.08%)] between the age range of 19 and 29 years which declined with advancing age (Table 1).

Subsequent subcategorization of HBsAb seropositivity by gender and age-groups were as follows <19 years (0 males and 0 females), 19-29 years (1 male and 7 females), 30-39 years (4 males and 0 females), >39 years (2 males and 1 female). The highest HBV seroprevalence of HBsAb was observed amongst the female participants [7 (46.67%)] between the age range of 19 and 29 years which declined with advancing age (Table 1).

Further subcategorization of HBcAb seropositivity by gender and age-groups were as follows <19 years (1 male and 1 female), 19-29 years (7 males and 8 females), 30-39 years (6 males and 3 females), >39 years (4 males and 2 females). The highest HBV seroprevalence of HBcAb was observed amongst the female participants [8 (25.0%)] between the

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age range of 19 and 29 years which declined with advancing age (Table 1).

The prevalence of HBeAg seropositivity was highest in individuals with the age range of 19-29 years and the male participants [3(33.33%)] within this age group had a higher prevalence compared to their female counterparts [1(11.11%)]. Younger participants were observed to be more seropositive based on HBeAg status when compared to their older counterparts. Subcategorization of HBeAg seropositivity on the basis of gender and age-groups were as follows <19 years (0 males and 0 females), 19-29 years (3 males and 1 female), 30-39 years (1 male and 1 females), >39 years (2 males and 1 female) (Table 1).

On the order hand, subcategorization of HBeAb seropositivity by gender and age-groups were as follows <19 years (1 male and 1 females), 19-29 years (7 males and 10 females), 30-39 years (5 males and 1 female), >39 years (4 males and 2 females). The highest HBV seroprevalence of HBeAb was observed amongst the female participants [10 (32.6%)] between the age range of 19 and 29 years which declined with advancing age (Table 1). In most cases of age-groups, the female counterparts dominated in terms of HBV serological markers seropositivity.

Relationship between socio-demographic features of participants and HBV serological markers

During the study period, 53 consenting HBV-seropositive adults were selected using purposive sampling method. The mean age of the fifty three participants enrolled was 31.25±10.27 years. The youngest was 12 years while the oldest was 58 years. Majority (50.9%) of the participants were aged between 19 and 29 years. There were 30 (56.6%) males and 23 (43.3%) females which was in a ratio of 1.3:1.

The highest HBV seroprevalence 23 (47.9%) of HBsAg was observed between in participants between the age range of 19 and 29 years which declined with advancing age. Based on gender, males had a higher HBV seroprevalence compared to their female counterparts with a male to female ratio of 1.5:1. There was no significant association between HBsAg status and age (χ^2=3.418, P=0.332) as well as gender

Table 1. The frequency of HBV serological markers in males and females and ratio of occurrence based on age.

HBsAg-positive Age (years)	Male 29 (60.42%)	Female 19 (39.58%)	Ratio Male : Female (1.1:1)	Total 48 (100.00%)
<19	2 (4.17%)	1 (2.08%)	02:01	3 (6.25%)
19-29	13 (27.08%)	10 (20.83%)	1.3:1	23 (47.92%)
30-39	7 (14.58%)	5 (10.41%)	1.4:1	12 (25.00%)
>39	7 (14.58%)	3 (6.25%)	2.3:1	10 (20.83%)
HBsAb-positive Age (years)	**Male 7 (46.67%)**	**Female 8 (53.33%)**	**Ratio Male : Female**	**Total 15 (100.00%)**
<19	0 (0.00%)	0 (0.00%)	0:0*	0 (0.00%)
19-29	1 (6.67%)	7 (46.67%)	0.1:1	8 (53.33%)
30-39	4 (26.67%)	0 (0.00%)	4:0°	4 (26.67%)
>39	2 (13.33%)	1 (6.67%)	02:01	3 (20.00%)
HBcAb-positive Age (years)	**Male 18 (56.25%)**	**Female 14 (43.75%)**	**Ratio Male : Female**	**Total 32 (100.00%)**
<19	1 (3.13%)	1 (3.13%)	01:01	2 (62.50%)
19-29	7 (21.88%)	8 (25.00%)	0.9:1	15 (46.88%)
30-39	6 (18.75%)	3 (9.38%)	02:01	9 (28.13%)
>39	4 (12.50%)	2 (6.25%)	02:01	6 (18.75%)
HBeAg-positive Age (years)	**Male 6 (66.67%)**	**Female 3 (33.33%)**	**Ratio Male : Female**	**Total 9 (100.00%)**
<19	0 (0.00%)	0 (0.00%)	0:0*	0 (0.00%)
19-29	3 (33.33%)	1 (11.11%)	03:01	4 (44.44%)
30-39	1 (11.11%)	1 (11.11%)	01:01	2 (22.22%)
>39	2 (22.22%)	1 (11.11%)	02:01	3 (33.33%)
HBeAb-positive Age (years)	**Male 17 (54.84%)**	**Female 14 (45.61%)**	**Ratio Male : Female**	**Total 31 (100.00%)**
<19	1 (3.23%)	1 (3.23%)	01:01	2 (6.45%)
19-29	7 (22.58%)	10 (32.26%)	0.7:1	17 (54.84%)
30-39	5 (16.13%)	1 (3.23%)	05:01	6 (19.35%)
>39	4 (12.9%)	2 (6.45%)	02:01	6 (19.35%)

*Absence of participants for comparison; °absence of female participants within age range for comparison with male counterparts.

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(χ^2=3.099, P=0.078), which means that HBsAg status is independent from age and gender (Table 2). As regards HBsAb, participants between the age range of 19 and 29 years recorded the highest seroprevalence. Based on gender, male had a lower seroprevalence compared to the female counterparts (male to female ratio=1:1.1). There was no significant association between HBsAb status and age (χ^2=2.072, P=0.558) as well as gender (χ^2=0.841, P=0.359). Similarly, the seroprevalence of HBcAb among participants between the age range of 19 and 29 years had the highest seroprevalence. Also, males had higher seroprevalence compared to their female counterparts (male to female ratio=1.3:1). There was no significant association between

Table 2. Association between socio-demographic factors and sero-prevalence of HBV serological markers among infected persons attending Abuja Teaching Hospital, FCT.

Variables	HBV markers		χ^2	P-value
	HBsAg-positive, n (%)	HBsAg-negative, n (%)		
Age (years)				
<19	3 (6.2%)	0 (0.0%)	3.418	0.332
19-29	23 (47.9%)	4 (80.0%)		
30-39	12 (25.0%)	1 (20.0%)		
>39	10 (20.8%)	0 (0.0%)		
Gender				
Male	29 (60.4%)	1 (20.0%)	3.099	0.078
Female	19 (39.6%)	4 (80.0%)		
Age (years)	**HBsAb-positive, n (%)**	**HBsAb-negative, n (%)**		
<19	0 (0.0%)	3 (7.9%)	2.072	0.558
19-29	8 (53.3%)	19 (50.0%)		
30-39	4 (26.7%)	9 (23.7%)		
>39	3 (20.0%)	7 (18.4%)		
Gender				
Male	7 (46.7%)	23 (60.5%)	0.841	0.359
Female	8 (53.3%)	15 (39.5%)		
Age (years)	**HBcAb-positive, n (%)**	**HBcAb-negative, n (%)**		
<19	2 (6.2%)	1 (4.8%)	0.750	0.861
19-29	15 (46.9%)	12 (57.1%)		
30-39	9 (28.1%)	4 (19.0%)		
>39	6 (18.8%)	4 (19.0%)		
Gender				
Male	18 (56.2%)	12 (57.1%)	0.004	0.949
Female	14 (43.8%)	9 (42.9%)		
Age (years)	**HBeAg-positive, n (%)**	**HBeAg-negative, n (%)**		
<19	0 (0.0%)	3 (6.8%)	2.260	0.520
19-29	4 (44.4%)	23 (52.3%)		
30-39	2 (22.2%)	11 (25.0%)		
>39	3 (33.3%)	7 (15.9%)		
Gender				
Male	6 (66.7%)	24 (54.5%)	0.456	0.499
Female	3 (33.3%)	20 (45.5%)		
Age (years)	**HBeAb-positive, n (%)**	**HBeAb-negative, n (%)**		
<19	2 (6.5%)	1 (4.5%)	1.119	0.772
19-29	17 (54.8%)	10 (45.5%)		
30-39	6 (19.4%)	7 (31.8%)		
>39	6 (19.4%)	4 (18.2)		
Gender				
Male	17 (54.8%)	13 (59.1%)	0.095	0.758
Female	14 (45.2%)	9 (40.9%)		

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Table 3. Association of HBV DNA with gender and age.

Variable	HBV DNA (IU/mL)				χ^2	P-value
	<20	20-1,000	1,001-1,000,000	>1,000,000		
Age (years)						
<19	1 (8.3%)	1 (5.6%)	1 (5.9%)	0 (0.0%)		
19-29	5 (41.7%)	9 (50.0%)	9 (52.9%)	4 (66.7%)		
30-39	3 (25.0%)	5 (27.8%)	4 (23.5%)	1 (16.7%)		
>39	3 (25.0%)	3 (16.7%)	3 (17.6%)	1 (16.7%)		
Total	12 (100.0%)	18 (100.0%)	17 (100.0)	6 (100.0)	1.858	0.997
Gender						
Male	7 (58.3%)	9 (50.0%)	10 (58.8%)	4 (66.7%)		
Female	5 (41.7%)	9 (50.0%)	7 (41.2%)	2 (33.3%)		
Total	12 (100.0%)	18 (100.0%)	17 (100.0%)	6 (100.0%)	0.619	0.892

HBcAb status and age (χ^2=0.750, P=0.861) as well as gender (χ^2=0.004, P=0.949). On the other hand, the seroprevalence of HBeAg among participants between the age range of 19 and 29 years were observed with the highest seroprevalence. Males had a higher seroprevalence compared to their female counterparts with a male to female ratio of 2:1. There was no significant association between HBeAg status and age (χ^2=2.260, P=0.520) as well as gender (χ^2=0.456, P=0.499), which means that HBeAg status is independent from age and gender.

Participants between the age range of 19 and 29 years were observed with the highest seroprevalence. Males had a higher seroprevalence compared to their female counterparts with a male to female ratio of 1.2:1. There was no significant association between HBeAb status and age (χ^2=1.119, P=0.772) as well as gender (χ^2=0.095, P=0.758), which means that HBeAb status is independent from age and gender.

In contrast to the subcategorization of HBV serological markers based on gender and age groups, the male participants in most cases had higher seroprevalence for these markers compared to their female counterparts which was in accordance to what was observed in the study conducted by Forbi *et al.*[22] who suggested the poorer outcome of HBV infection among males instead of their male counterparts. Younger participants (those between 19-29 years) was also observed to have the highest seroprevalence for all HBV serological markers investigated during this study.

Table 4. HBV prevalence among infected persons attending Abuja Teaching Hospital, FCT.

Variable	Frequency	Percentage (%)
HBV DNA (IU/mL)	53	100.00
<20	12	22.64
20-1, 000	18	33.96
1, 001-1, 000, 000	17	32.08
>1, 000, 000	6	11.32
HbSAg	53	100.00
Positive	48	90.57
Negative	5	9.43
HbSAb	53	100
Positive	15	28.30
Negative	38	71.70
HbcAb	53	100
Positive	32	60.38
Negative	21	39.62
HbeAg	53	100
Positive	9	16.98
Negative	44	83.02
HbeAb	53	100
Positive	31	58.49
Negative	22	41.51

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Association between hepatitis B viral load and socio-demographic data

There was no significant association between HBV DNA status and age (χ^2=1.858, P=0.997) as well as gender (χ^2=0.619, P=0.892), which means that HBV DNA status is independent from age and gender (Table 3).

Prevalence of serological markers of hepatitis B

Out of the 53 HBV-infected participants, 48 (90.6%) were positive for HBsAg, 15 (28.3) were positive for HBsAb, 32 (60.4%) were positive for HBcAb, 9 (17.0%) were positive for HBeAg, and 31 (58.5%) were positive for HBeAb (Table 4).

Positive predictive value of HBV structural proteins and their corresponding antibodies in diagnosis of HBV infection

Based on HBsAg status, 87.8% (positive predictive value) of the 41 participants who were seropositive for HBsAg were infected with HBV. 75.0% (sensitivity) of the 48 participants infected with HBV (36 individuals) were seropositive for HBsAg, while 0% (specificity) of the 5 participants not infected with HBV (no individual) were seronegative for HBsAg.

In terms of HBsAb status, 24.4% (positive predictive value) of the 41 participants who were seropositive for HBsAb were infected with HBV. 66.7% (sensitivity) of the 15 participants infected with HBV (10 individuals) were seropositive for HBsAb, while 18.4% (specificity) of the 38 participants not infected with HBV (7 individuals) were seronegative for HBsAb.

Regarding HBcAb status, 78.0% (positive predictive value) of the 41 participants who were seropositive for HBcAb were infected with HBV. 100.0% (sensitivity) of the 32 participants infected with HBV (32 individuals) were seropositive for HBcAb,

while 57.1% (specificity) of the 21 participants not infected with HBV (12 individuals) were seronegative for HBcAb.

Concerning the HBeAg status, 19.5% (positive predictive value) of the 41 participants who were seropositive for HBeAg were infected with HBV. 88.9% (sensitivity) of the 9 participants infected with HBV (8 individuals) were seropositive for HBeAg, while 25.0% (specificity) of the 44 participants not infected with HBV (11 individuals) were seronegative for HBeAg.

As regards the status for HBeAb, 70.7% (positive predictive value) of the 41 participants who were seropositive for HBeAb were infected with HBV. 93.5% (sensitivity) of the 31 participants infected with HBV (29 individuals) were seropositive for HBeAb, while 45.5% (specificity) of the 22 participants not infected with HBV (10 individuals) were seronegative for HBeAb (Table 5).

Relationship between hepatitis viral load and serological markers of HBV

There was no significant association between HBV DNA and HBsAg (χ^2=2.841, P=0.417) as well as HBsAb (χ^2=1.625, P=0.654), which means that HBV DNA status is independent from HBsAg and its corresponding antibody (HBsAb).

On the other hand, there was significant association between HBcAb (χ^2=28.622, P=0.000), HBeAg (χ^2=11.820, P=0.008), HBeAb (χ^2=16.440, P=0.001), and HBV DNA, which implies that HBV DNA status is dependent on HBcAb, HBeAg and HBeAb.

The odds of participants developing acute hepatitis infection by being HBcAb IgM-positive was five times significantly greater for those who were HBV DNA-positive than those who are HBV DNA-negative (OR=4.556, 95% CI=2.558-8.113). The odds of participants developing hepatitis infection by being HBeAg-positive was three times significantly greater for those who were HBV DNA-positive than those who are HBV DNA-negative (OR=2.667, 95% CI=0.299-23.779). The odds of participants developing hepatitis infection by being HBeAb-positive was twelve times significantly greater for those who were HBV DNA-positive than those who are HBV DNA-negative (OR=12.083, 95% CI=2.296-63.586) (Table 6).

Discussion

In several regions of sub-Saharan Africa, documentation on the serological features of hepatitis B infection have sufficiently consolidated. There are few studies on the determination of HBV DNA among the population which could be due the scare availability and accessibility in terms of molecular technology and the cost of molecular testing as well as maintenance. Our study confirms the high prevalence of hepatitis B infection in both the young and the middle-aged adults. This is in accordance with a generalized hepatitis virus epi-

Table 5. Relationship between the positive predictive values and prevalence of HBV structural proteins and their corresponding antibodies among HBV-infected persons attending Abuja Teaching Hospital, FCT.

Variable	Prevalence (%)	Positive predictive value (%)	Sensitivity (%)	Specificity (%)
HBsAg	90.6	87.8	75.0	0.0*
HBsAb	28.3	24.4	66.7	18.4
HBcAb	60.4	78.0	100.0	57.1
HBeAg	17.0	19.5	88.9	25.0
HBeAb	58.5	70.7	93.5	45.5

*Absence of true negative value.

Table 6. Association between HBV DNA and HBV serological markers among infected persons attending Abuja Teaching Hospital, FCT.

Variable	HBV DNA (IU/mL) <20	20-1,000	1,001-1,000,000	>1,000,000	χ^2	P-value
HbSAg	12 (100. 00%)	18 (100.00%)	17 (100.00%)	6 (100.00%)	2.841	0.417
Positive	12 (100.00%)	16 (88.89%)	15 (88.24%)	5 (83.33%)		
Negative	0 (0.00%)	2 (11.11%)	2 (11.76%)	1 (16.67%)		
HbSAb	12 (100. 00%)	18 (100.00%)	17 (100.00%)	6 (100.00%)	1.625	0.654
Positive	5 (41.67%)	5 (27.78%)	4 (23.53%)	1 (16.67%)		
Negative	7 (58.33%)	13 (72.22%)	13 (76.47%)	5 (83.33%)		
HbcAb	12 (100. 00%)	18 (100.00%)	17 (100.00%)	6 (100.00%)	28.622	0.000*
Positive	0 (0.00%)	14 (77.78%)	14 (82.35%)	4 (66.67%)		
Negative	12 (100.00%)	4 (22.22%)	3 (17.65%)	2 (33.33%)		
HbeAg	12 (100.00%)	18 (100.00%)	17 (100.00%)	6 (100.00%)	11.820	0.008*
Positive	1 (8.33%)	5 (27.78%)	0 (0.00%)	3 (50.00%)		
Negative	11 (91.67%)	13 (72.22%)	17 (100.00%)	3 (50.00%)		
HbeAb	12 (100. 00%)	18 (100.00%)	17 (100.00%)	6 (100.00%)	16.440	0.001*
Positive	2 (16.67%)	10 (55.56%)	15 (88.24%)	4 (66.67%)		
Negative	10 (83.33%)	8 (44.44%)	2 (11.76%)	2 (33.33%)		

*Significant at P≤0.05.

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demic as documented by other studies conducted in Africa.[23-25] The categorization of HBV prevalence in our study was based on WHO classification of HBV severity in endemic countries. WHO recognizes low, moderate and high prevalence to be <2%, 2-8%, and >8% HBsAg positivity respectively.[26] The younger participants in our study were potentially infectious.[27]

In terms of gender, there was an observed preponderance or dominance of male participants among HBV-infected individuals in our study. In addition, the ratio of male to female generally increased during reproductive years when participants were divided based on age groups which was in accordance with previous studies.[28-30] Both Baig and Okwuraiwe *et al*. had similar finding.[23,31] While Braig proposed that the female reproductive hormone (estrogen) may have the ability to mount protection or re-enforce the immunity in the female population against the destruction of hepatocytes by the hepatitis B virus,[23] Okwuraiwe *et al*. which explained that the female population have relatively lower financial resources to test for the viral infection which may seem higher in the male population.[31]

In terms of age, the younger participants recruited for the study leaned towards positive detection for all the HBV serological markers than the older participants. These findings were supported by studies conducted in the northern region of Nigeria,[22] from Togo by Kolou *et al*.[24] and Japan by Tsukuma *et al*.[32] which indicated that the prevalence of these serological biomarkers decreased with age.

Recent studies have shown that hepatitis B infection is a dynamic infection with clinical presentations which include acute infection, chronic progressive liver disease, chronic inactive carrier state, liver cirrhosis and carcinoma.[23,33] The patterns for HBV panel serve as guide for determining the disease phase and requirement for antiviral treatment. The array of HBV serological markers has a definitive role in the detection of acute hepatitis B infections. Patients with the presence of HBV antigens (HBsAg, HBeAg) in their serum were highly viremic due to the presence of active viral replication in hepatocytes and seldom have HBV DNA level >200, 000 IU/mL as observed in this study.[34] In addition, investigating for HBeAg can be used to identify patients with high risk of developing liver cancer.[34,35] HBeAg is known to be a core antigen released by the HBV DNA and is considered as a surrogate marker of active replication of wild-type HBV.[36] Our study revealed a high seroprevalence for HBeAg and HBeAb which are in line with previous studies by Forbi *et al*. and Ola *et al*.[22,37] These group of patients with high prevalence of HBeAg reflect those with high risk of viral transmission and the likelihood of increased burden of liver carcinoma due to HBV in the future as posited by Forbi *et al*.[22] Based on this finding, it is imperative to re-enforce injection safety and vaccination protocols, and for healthcare centers to adopt HBeAg investigation of HBV-infected individuals so as to achieve efficient management of patients as well as the eradication of hepatitis B at all levels of development.

About 5.7% of study participants were categorized in a low replicative phase of chronic HBV characterized by HBeAg-negative status, HBeAb-positive status and low-level hepatitis viral replication (*i.e.* HBV DNA level below 2000 IU/mL). Without corresponding liver trauma and normal liver enzyme levels, these pools of individuals are regarded as chronic inactive carriers. Due to low risk of disease progression in this category of individuals, antiviral treatment not recommended.[27] On the other hand, it was observed that slightly over one-third (20/53) of participants who were HBeAg-negative had high-level hepatitis viral replication (HBV DNA >2000 IU/mL) in the presence of detectable HBeAb. This finding as observed in our study is called HBeAg-negative hepatitis and is regarded as the predominant form of chronic hepatitis B-associated hepatic disorder in Asia and sub-Saharan Africa.[29,38] In order to ensure efficient healthcare services to persons with hepatitis B infection, it is crucial to differentiate between these two groups of patients (*i.e.* the chronic inactive carriers and those with HBeAg-negative hepatitis). The active viral replication (HBV DNA >2000 IU/mL) in the case of HBeAg-negative hepatitis differentiates it from the chronic inactive carriers (HBV DNA <2000 IU/mL) and this serves as a predictive biomarker for monitoring disease progression and replication of viral mutants in the absence of HBeAg.[39] Unlike the chronic inactive carriers, those with HBeAg-negative hepatitis require antiviral therapy due to the high risk of disease progression.[25] The prevalence of hepatitis B disease in our study was 38% which was in consonance with reports from a Nigerian study by Lesi *et al*.[25] but not from a Gambian study by Lemoine *et al*.[40] Like the Nigerian study, our study involved hospital-based participants who were symptomatic with higher viral loads compared to those recruited for the Gambian study. The method for viral load detection using the Roche amplicon molecular assay with high level of sensitivity and specificity was a common technique used in our study as well as the work conducted by Lesi *et al*.,[25] but not for that conducted by Lemoine *et al*.[40]

Based on reliability of the HBV serological assays, our study sort to examine the sensitivity and specificity for all biomarkers by comparing the results generated by the chromatographic immunoassay (qualitative detection) to the results of the Roche amplicon molecular assay (quantitative detection) which is globally regulated and associated with high level of sensitivity and specificity. Apart from the sensitivities and specificities of all HBV serological biomarkers observed in our study, the sensitivity and specificity values for HBsAg were 75% and 0% respectively which was not in agreement with the manufacturer's specifications for the relative sensitivity (100%) and specificity (100%). This disparity could be due the absence of a true negative and negative predictive values. The negative predictive value of a test is the proportion of individuals who do not have detectable hepatic B viral load, are negative for HBsAg and do not have hepatitis disease.[41] This was not the case in our study as all of the enrolled participants were HBV-infected.

The association between hepatitis viral load and other HBV serological biomarkers besides HBsAg and its corresponding antibody were observed to be significant as observed in our study which was in line with a previous study by Lesi *et al*.[25] However, while 5 (9.4%) of those who were sero-negative for HBsAg had HBV DNA in their serum as in the case of occult hepatitis B, 12 (22.6%) of those who were sero-positive for HBsAg had HBV DNA absent from their serum as observed in our study. Occult hepatitis B infection is termed as the presence of low-level hepatitis viral load (<200 IU/mL) with the presence serological markers of past infection (HBcAb and/or HBsAb) and the absence of HBsAg in serum, cells of lymphatic system and/or hepatic tissue.[42] The proportion (22.6%) of participants with hepatitis viral load below 20 IU/mL and HBsAg-positive as observed in our study could either be due to rapid degradation of HBV DNA, anti-retroviral therapy/natural clearance by the immune system or sub-viral particles released by HBV-infected hepatocytes due to apoptosis to inhibit the propagation of HBV in the liver.[43]

The major limitation of our study is the sample size which was below the expected computed value which was dependent on the response rate from respondents. However, despite this setback, our study is unique as it represents the first among a few studies that assessed for a completed HBV panel of serological biomarkers in addition

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to the HBV viral load estimation using PCR molecular technology in this African region. There is need for a large-scale study of these variables so as to assess as well as monitor the progression and phases of hepatitis viral infection in HBV-infected Nigerian participants.

Conclusions

We have demonstrated occult hepatitis B infection, the high prevalence of HBsAg and the presence of HBeAg-negative hepatitis with significant viral loads in over one-third of infected hospital-based HBV-infected participants. We have also shown that slightly over one-sixth of HBsAg-positive participants are also HBeAg-positive, which is a sole marker of active viral replication and propagation. The positive status for all HBV serological biomarkers occurred more commonly in younger adults. This highlights the need to enhance injection safety and vaccination protocols, and for healthcare centers to adopt viral load testing or/and assessment for the HBV biomarkers in order to guard against unnecessary anti-retroviral therapies, begin appropriate treatment and avoid financial wastage in a bid to ensure quality healthcare and eliminate HBV infection.

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