International Research Journal of Public and Environmental Health
Vol.10 (1), pp. xx-xx March 2023
ISSN: 2360-8803(e) ISSN: 2814-3817(p)
Available online at https://www.journalissues.org/IRJPEH/
DOI: https://doi.org/10.15739/irjpeh.23.001

Original Research Article

Seroprevalence and molecular characterization of Hepatitis B Virus isolated from HIV patients attending referral center in Abuja

Accepted 5 March, 2023

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The co-infection of the hepatitis B virus HBV) with the human immunodeficiency *virus (HIV) is one of the major challenges in the management of HIV. Both viral* infections are among the clinical conditions of public health importance with high mortality and morbidity worldwide, especially in developing countries like Nigeria. They share common route of transmission such as body piercing, intravenous drug abuse, blood and blood products, unsafe injections and sexual contact which puts HIV positive individuals at risk of co-infection. It is therefore, necessary to document the molecular epidemiological dynamics of HBV among HIV patients in HIV referral center in Abuja, Nigeria. Using stratified random sampling, 200 HIV infected individuals on Antiretroviral Therapy ART were sampled in a referral center in Abuja, Nigeria and then evaluated for HBV using Rapid Test Device (RTD) strip for HBsAg, HBV Combo Rapid Test Cassette 5 Panel HBV for HBsAg, HBsAb, HbeAb, HBeAg and HBcAb. HBV and HIV viral DNA and RNA were extracted and characterized genotypically for HBV genotype A, B, C, D, E, and F specific genes by polymerase chain reaction (PCR) followed by sequencing. Obtained sequences were edited using the bioinformatics softwares. The overall prevalence of HBV among the study population was 10.0% (20/200), 15.0% (30/200), and 20.0% (40/200), for RTD strip, RTD HBV cassette and PCR, respectively. The genotypic characterization is HBV genotype E. Based on the study, the genotypic characterization is 100% of the study population. The HIV isolated is HIV-1 subtype G. Gender, occupation, marital status, level of education and place of residence were the risk determinants of HBV among study population. The relatively high prevalence of HBV/HIV coinfection and the presence of circulating HBV genotype E has provided important epidemiological information on the molecular characteristics of HBV in HIV-infected in Nigeria, and this has important clinical relevance in the management of HBV/HIV co-infection. Also, PCR has proven to be the most sensitive technique in the diagnosis of HBV, and so it should be used for routine HBV diagnosis for accuracy and precision. The findings of this research confirm that HBV is a major co-morbid infection and a threat to HIV patients. The PCR is the best method of diagnosis. The health sector, federal and state ministry of health should improve in providing PCR machines, creating awareness and vaccinating the populace against HBV to reduce the prevalence of these infections.

Keywords: Hepatitis B virus, Human Immunodeficiency Virus, Co-infection, referral centre, prevalence

INTRODUCTION

Human Immunodeficiency Virus (HIV) and Hepatitis B viral infection (HBV) are public health menace with high morbidity and mortality worldwide, especially in developing countries with deplorable healthcare systems (Sam, 2022); WHO, 2022). According to the WHO (2022)., the burden of hepatitis B infection is highest in the WHO Western Pacific Region and the WHO African Region (where Nigeria falls in) where about 116 million and 81 million people are chronically infected respectively. There are about 38.4 million people living with HIV/AIDS worldwide in 2021 and more than 95% of them live in developing countries, of which about 70% are in Sub-Saharan Africa (WHO, 2022).. Hepatitis B virus is an enveloped DNA virus which can infect Human and result in both acute and chronic infection, with symptoms ranging from asymptomatic infection or mild disease to severe or fulminant hepatitis B infection. In most cases, acute hepatitis B is self-limiting condition characterised by acute inflammation and hepatic necrosis, with a case fatality rate of 0.5–1%in the majority of cases. A broad spectrum of disease is encompassed by chronic hepatitis B (CHB), which is defined as persistent HBV infection (the presence of detectable HBsAg in the blood or serum for more than six months), with or without associated active viral replication and evidence of hepatocellular injury and inflammation. The patients' age plays a major role in the risk of developing a persistent infection. Chronicity develop commonly following acute infection in newborns (90% of neonates born to mothers who tested positive for hepatitis B e antigen [HBeAg]) and in 21 young children under the age of 5 years (20–60%), but it occurs less frequently (less than 5%) when infection is acquired in adulthood. The vast majority of people who have chronic hepatitis B were infected during their early childhood or at birth around the world (Maimuna et al., 2008; Zhang et al., 2014).

Hepatitis is a liver disease implicated as a co-infection in people living with HIV/AIDS caused by hepatitis B and C viruses. Hepatitis is a leading cause of death in most developing countries including Nigeria – where the disease is misdiagnosed with malaria that share similar symptoms (Eze et al., 2014). Progression of chronic hepatitis B (CHB) disease to severe liver diseases, such as Liver Cirrhosis (LC) and Hepatocellular Carcinoma (HCC), LC and HCC, is determined by the genetic characteristics of the host, as well as by viral and environmental factors. The transmission route for both HIV and HBV infection can be through blood and blood products, body piercing, intravenous drug abuse, unsafe injections and sexual activity (Sam, 2022; WHO, 2022; Jafari et al., 2010; Chessbrough, 2010). This puts HIV positive individuals at risk of co-infection with hepatitis B. Infection with chronic hepatitis B virus (HBV) is estimated to affect about 296 million people worldwide and can lead to cirrhosis and hepatocellular carcinoma, most of them are thought to be in the developing countries (Sam, 2022; Chessbrough, 2022). Despite the effective decline of the mortality and morbidity rate from HIV/AIDS as the result of Highly Active Antiretroviral Therapy (HAART), liver diseases due to chronic HBV infection is still a leading cause of death globally Despite the effective decline of the mortality and morbidity rate from HIV/AIDS as the result of Highly Active Antiretroviral Therapy (HAART), liver diseases due to chronic HBV infection is still a leading cause of death for both HIV and non-HIV patients globally (Jafari et al., 2010).

The rates of co-infection of HIV with HBV is dependent on several factors including study population and associated-risk factors for HIV acquisition, and this usually vary from region to region (Wondimeneh et al., 2010). A systematic review in 18 sub-Saharan African countries showed that the prevalence of HBV in HIV-infected individuals ranged from 3.9-7.3% to 6.9%, respectively. Diwe at al. (2013) reported HBV co-infection prevalence of 2.2% in Nigeria. This perhaps may be related to the low incidence of intravenous drug abuse and needle sharing amongst the people compared to the developed countries where such practices still hold sway. With prolonged survival of HIV infected patients, co infection with either HBV correlates with reduced survival rate. The impact of HBV could not be limited in causing liver hepatotoxicity but also results in failure in immunological recovery in HIV positive patients. This is seen in a study in Tanzania in which slow rate of immunologic recovery after initiation of HAART treatment and higher risk of hepatotoxicity among HIV/HBV co-infected patients was reported (Christian et al., 2010 The epidemiology of hepatitis B virus can be described in terms of prevalence of the hepatitis B surface antigen (HBsAg) in a population, broadly classified as high (>8% HBsAg prevalence), intermediate (2-7%) and low prevalence (< 2%) (Jennifer et al, 2015). The endemicity of these infections in Nigeria necessitates the need to determine the seroprevalence and molecular characterization of HBV infections among HIV patients in order to guide therapy and ensure proper prognosis of HIV/AIDS patients undergoing retroviral therapy in Abuja, Nigeria.

IMPORTANCE

The endemicity of Hepatitis B virus infection in North-Central, Nigeria is high. For both HIV and non HIV patients, the prevalence of Hepatitis B virus infection is greater than 10% irrespective of the diagnostic method used. The circulating hepatitis B in this region is molecularly characterized as the hepatitis B, genotype E. There is no significant difference between the prevalence of hepatitis B virus in HIV and non-HIV patients which means anybody can contact the infection if precautionary measures are not strictly adhered to. This study reveals an important epidemiological information which will help both the federal and State Ministries of health and the healthcare providers in the management and control of the spread of this virus. It has been proved that hepatitis B viral infection can be transmitted through contacts with sharp objects, blood and blood products contact, tattooing, and sexual contacts. In this study, the greater prevalence of hepatitis was seen among the injectable drug users, uneducated, unemployed and civil servants. This calls for urgent attention for population-based screening, counselling and educating the populace on the impact of this disease to avoid further spread and the complications associated with these infections like liver cirrhosis and hepatocellular

carcinoma. This infection has been spreading silently and people are not given much attention to curtail the spread and device means to prevent and eradicate it. Though there is a vaccine to prevent this infection but majority of the inhabitants are ignorant of it and those who are informed about it feel reluctant to go for the vaccine. The most commonly used diagnostic method for this disease in this part of the world is the rapid test device which is cheap and affordable but from this study, we can see that the best diagnostic method is the PCR which is very expensive and most health facilities cannot afford it.

MATERIALS AND METHODS

Study design

Ethical Approval

Ethical approval was obtained from Federal Capital Territory Health research Ethics Committee with protocol approved number: FHREC/2014/01/77/16-12-14 and the Ethical Committee in the referral Centre.

Study population

A total of 200 participants (15-75years) with CD4 count of 200 ± 490 drawn from HIV patients attending a referral clinic in a local hospital in Abuja participated in this study. Additional 200 non-HIV infected individuals within the same age limit in the same area served as control. Stratified Random Sampling (SRS) technique was used to select the study participants of HBsAg and anti-HBc from the proposition of the seropositive individuals among the HIV positive population and the control population studied and expressed as a percentage. Measurement of frequency of socio-demographic variables and other characteristics of sampled population were computed. A 95% confidence interval (CI) was used to describe the prevalence of hepatitis in the population. Odd ratio (OR) and 95% CI were calculated for each association. A p-value ≤ 0.05 was considered to be statistically significant.

Sample collection and processing

Confirmation of HIV Status of Patients

3ml veinous whole blood from the median cubital was aseptically collected from each participant, and was transferred to sterile test tube for separation into serum by centrifuging sample at 3000 rpm for 5 min (Chessbrough 2010; Ochei and kolhatker, 2009). The Alere Determine HIV ½ (Alere Medical, 2012) and chembio HIV 1/2 STAT-PAK (Chembio Diagnostic system, Inc. 2011). Kits were respectively used to screen the blood samples for the detection of antibodies to HIV types 1 and 2 (HIV 1/2) in serum specimens of participants. Their CD4 count was determined using the flow Cytometry method.

Variables of the study

The Two hundred subjects under study were divided into age groups ranging between 15 to 75 in the following order 15-25, 25-35, 35-45, 45-55, 55-65 and 65-75 respectively.

Screening for hepatitis B surface antigen (HBsAg)

The HBsAg One Step Hepatitis B Surface Antigen Test Strip (Blumerg, 2012) was used according to the manufacturer's instruction for screening the blood samples for the presence of HBV antigen. The membrane of the test strip for detection of HBsAg is pre-coated with anti-HBsAg antibodies on the test line region of the strip.

PCR assay for detection of HBV-DNA

DNA extraction was performed using Zymo extraction kit (Larry, 2014) according to manufacturer's instruction. HBsAg negative/positive samples of both HIV positive and HIV negative subjects was used for DNA extraction. To extract DNA, 400µl of genomic lysis buffer was added to 100µlof serum (4:1), mixed completely by vortexing for 6s, and allowed to stand for 10 min at room temperature. The mixture was transferred to a zymo-spin IIC column in a collection tube and centrifuged at 10,000×g for 1 min. The collection tube with the flow through was discarded and the zymo-spin IIC column was transferred to a new collection tube in which 200µl of DNA pre-wash buffer was added and centrifuged at 10,000×g for 1 min. 500µl of DNA wash buffer was added to the spin column and centrifuged at 10,000×g for 1 min, and the spin column was transferred to a clean microcentrifuge tube. An aliquot of 50µl of DNA elution buffer was added to the spin column, incubated for 5 min at room temperature and centrifuged at top speed for 30s to elute the DNA. Nanodrop 1000 was used for DNA quantification. PCR assay for amplification of HBV-DNA was performed in a final reaction volume of 20µl comprising 2µl template DNA, 4.2µl nuclease-free water, 10ul primers (Forward Primer: → 5^{I}-TCACCATATTCT TGG GAACAACA-3^{I} and Reverse Primer: 5^{I}-ACC ACT GAA CAA ATG GC-3^{I}←), and 1X concentration of 10µl Taq pol, DNATPs, and MgCl. Gene amplification was carried out in a 9700 Applied Biosystem Thermo cycler (9700 Applied Biosystem USA. 2015). with the PCR cycling profile consisting an initial step at 94°C for 5 min, 35 cycles of 94°C for 20 s, 60°C for 30 s, 72°C for 1 min and a 2-min final extension at 72°C. Finally, 8 µL of the PCR products were electrophoresed in a 1.5% agarose gel containing 0.5 mg/mL ethidium bromide and photographed on an Ultraviolet Transilluminator (NRI Technologies UV Transilluminator 2000).

Extraction of RNA from plasma

RNA extraction was performed using Zymo extraction kit according to manufacturer's instruction (Larry, 2014). RNA was extracted from HIV positive samples of HIV positive

Table 1. Prevalence of HBV among HIV patients attending a Referral Centre Using RTD, ELISA and PCR Methods

Test	No of subjects	+ve HbsAg	+ve HBsAb	+ve HBeAg	+ve HbeAb	+ve HbcAb
RTD	200	20(10%)	-	-	-	-
ELISA	200	30(15%)	5(2.5%)	7(3.5%)	70(35%)	68(34%)
PCR	200	44(22%)	-	-	-	-
Non HIV Volunteers						
RTD	200	22(11%)	-	-	-	-
ELISA	200	30(15%)	20(10%)	9(4.5%)	60(30%)	55(27.5%)
PCR	200	20(20%)	-	-	-	-

p>0.05

subjects and then PCR was used for detection of HIV RNA. Briefly, 300µl of viral RNA buffer was added to 100µl of plasma sample and mixed briefly by vortexing. The samples were transferred to the Zymo-spin IC Column in a collection tube and centrifuged at 10000g for 2 min. The flow through was discarded. 500µl of viral wash buffer was added to the column and then centrifuged for 2 min at 10000g, and the column was carefully transferred into DNase/RNase-free tube. Later, 15ul of DNase/RNase free water was added to the column matrix and centrifuge for 30s to elute the RNA. Eluted RNA was stored at -70^{o}C for HIV detection.

Nested PCR for amplification of HIV V3 region

This was performed in two phases: **in primary amplification,** a portion of the extracted RNA was amplified by PCR using primers that flank the HIV sequence (F: →5^{I}-GGCATCAAACAGCTCCAGGCAAG-3^{I} and R: 5^{I}-AGCAAAGCCCTTTCTAAGCCCTGTCT-3^{I}←). PCR conditions included initial denaturation at 95^{o}C for 5 min, denaturation at 95^{o}C for 30s; annealing at 55^{o}C for 30s; extension at 72^{o}C for 30s, and final extension at 72^{o}C for 2 min. **In secondary amplification,** a portion of the HIV primary amplicon was amplified by PCR using primers that flank the HIV sequence (F: → 5^{I}-TCCTGGCTGTGGAAAGATACCTA-3^{I} and R: 5^{I}-GTCCCCTCGGGGCTGGGAGG-3^{I}←). The PCR conditions included initial denaturation at 95^{o}C for 5 min, denaturation at 95^{o}C for 30s; annealing at 55^{o}C for 30s; extension at 72^{o}C for 30s, and final extension at 72^{o}C for 2 min.

Amplification of HCV gene and sequencing

A portion of the extracted RNA was amplified by PCR using primers that flank the HCV sequence (F:→5^{I}-ACTGTCTTCACGCAGAAAGCGTCTAGCCAT-3^{I} and R:→5^{I}-CGAGACCTCCCGG GGCACTCGCAAGCACCC-3^{I}←). PCR components included a master mix (final volume was 20ul) supplied by Inqaba Biotech, (2017). This comprised Taq polymerase, DNTPs, MgCl at 1X concentration and volume of 10ul; forward and reverse primers at a concentration of 0.2uM and 0.16ul volume respectively; template at 1ul volume and nuclease-free water at 8.68ul volume. PCR conditions included initial denaturation at 95°C for 5 min, denaturation at 95°C for 30s; annealing at 55°C for 30s; extension at 72°C for 30s, and final extension at 72°C for 2 min. The PCR conditions included initial denaturation at 95°C for 5 min, denaturation at 95°C for 30s; annealing at 55°C for 30s; extension at 72°C for 30s, and final extension at 72°C for 2 min. Sequencing was done for HBVand HIV templates using the BigDye Terminator kit on a 3510 ABI sequencer by Inqaba Biotechnological, South Africa.

Phylogenetic analysis

Obtained sequences were edited using the bioinformatics algorithm Trace edit, and similar sequences were downloaded from the National Center for Biotechnology Information (NCBI) database using BLASTN. These sequences were aligned using Clustal X. The evolutionary history was inferred using the Neighbor-Joining method in MEGA 6.0 (Saitou and Nei, 1987). The bootstrap consensus tree inferred from 500 replicates (Saitou and Nei, 1987). was taken to represent the evolutionary history of the taxa analyzed. The evolutionary distances were computed using the Jukes-Cantor method (Jukes and Cantor, 1969).

Data analysis

All data were statistically analyzed using Graphpad Prism version 7. The P values were calculated and the significant difference was also obtained.

RESULT

Table 1 shows the prevalence of hepatitis B virus (HBV) among HIV/non-HIV patients attending a referral center in Abuja. Among the 200 HIV positive patients from the referral center, 20(8%), 30(15%) and 44(22%) were recorded positive using the RTD, ELISA and PCR methods respectively. Determination of the prevalence of hepatitis B virus among HIV patients attending the referral Centre with respect to age is represented on Table 2. Result shows the demographic study of the population. Within the age range studied, 36(18%)/200, 30(15%)/200 and 12(6%)/200 of the individuals in age brackets 25-35, 35-45 and 45-55

Table 2: Prevalence of hepatitis B Virus among HIV patients attending the Referral Center with respect to Demograpghical Study

Status	**HBV +ve**	**HBV-ve**	**Total**
15-25	9(4.5%)	12(6%)	21(10.5%)
25-35	36(18%)	48(24%)	84(42%)
35-45	30(15%)	30(15%)	60(30%)
45-55	12(6%)	15(7.5%)	27(13.5%)
55-65	0(0%)	5(2.5%)	5(2.5%)
65-75	3(1.5%)	0(0%)	3(1.5%)
Total	90(45%)	110(55%)	200(100%)
Status	HBV +ve	HBV-ve	Total
15-25	9(4.5%)	12(6%)	21(10.5%)
25-35	36(18%)	48(24%)	84(42%)
35-45	30(15%)	30(15%)	60(30%)
45-55	12(6%)	15(7.5%)	27(13.5%)
55-65	0(0%)	5(2.5%)	5(2.5%)
65-75	3(1.5%)	0(0%)	3(1.5%)
Total	90(45%)	110(55%)	200(100%)
Status	HBV +ve	HBV-ve	
Nationality			
Nigerian	90(100%)	110(100%)	
Non-Nigerian	0(0%)	0(0%)	
Total	90(100%)	110(100%)	
Tribe			
Igbo	21(23.33%)	25(22.72%)	
Hausa	24(26.70%)	18(16.36%)	
Yoruba	3(3.33%)	15(13.64%)	
Others	42(46.67%)	52(47.27%)	
Total	90(100%)	110(100%)	
Tribal Mark			
Yes	32(35.56%)	31(28.18%)	
No	58(64.44%)	79(71.82%)	
	90(100%)	110(100%)	
Medical history			
Status	HBV +ve	HBV-ve	
History of Blood transfusion			
Yes	24(26.66%)	26(23.64%)	
No	66(73.33%)	84(76.36%)	
Total	90(100%)	110(100%)	
History of Surgery			
Yes	30(15%)	47(23.5%)	
No	60(30%)	63(31.5%)	
Total	90(45%)	110(55%)	
History of Injecting drug use			
Yes	60(66.7%)	29(26.36%)	
No	30(33.3%)	81(73.64	
Total	90(100%)	110(100%)	
Knowledge of STD			
Fully informed	24(26.67%)	34(30.91%)	
Little knowledge	20(22.22%)	34(30.91%)	
No idea	46(51.11%)	42(38.18%)	
Total	90(100%)	110(100%)	
Family History of HBV			
Yes	30(33.33%)	24(21.81%)	
No	60(66.66%)	86(78.18%)	
Total	90(100%)	110(100%)	

respectively were positive to HBV. A total of 9(4.5%)/200 individuals were recorded HBV positive in age bracket 15-25. Within the age group 55-65, five (5(2.5%))/200 patients were screened and none of them was found with HBV. Age range of 25-35 had the highest prevalence of 18% and 65-75 was recorded to have the least prevalence of

Occupational exposure		
Status	HBV +ve	HBV-ve
Civil servant	20(22.22%)	6(5.45%)
Public Servant	6(6.66%)	26(23.63%)
Unemployed	33(36.66%)	18(16.36%)
Petty trader	24(26.66%)	56(50.91%)
International businessman	3(3.33%)	3(2.73%)
Apprentice	4(4.44%)	10(9.09%)
Total	90(100%)	110(100%)
Status	HBV +ve	HBV-ve
Nationality		
Nigerian	90(100%)	110(100%)
Non-Nigerian	0(0%)	0(0%)
Total	90(100%)	110(100%)
Tribe		
Igbo	21(23.33%)	25(22.72%)
Hausa	24(26.70%)	18(16.36%)
Yoruba	3(3.33%)	15(13.64%)
Others	42(46.67%)	52(47.27%)
Total	90(100%)	110(100%)
Tribal Mark		
Yes	32(35.56%)	31(28.18%)
No	58(64.44%)	79(71.82%)
Total	90(100%)	110(100%)
Medical history		
Status	HBV +ve	HBV-ve
History of Blood transfusion		
Yes	24(26.66%)	26(23.64%)
No	66(73.33%)	84(76.36%)
Total	90(100%)	110(100%)
History of Surgery		
Yes	30(15%)	47(23.5%)
No	60(30%)	63(31.5%)
Total	90(45%)	110(55%)
History of Injecting drug use		
Yes	60(66.7%)	29(26.36%)
No	30(33.3%)	81(73.64%)
Total	90(100%)	110(100%)
Knowledge of STD		
Fully informed	24(26.67%)	34(30.91%)
Little knowledge	20(22.22%)	34(30.91%)
No idea	46(51.11%)	42(38.18%)
Total	90(100%)	110(100%)
Family History of HBV		
Yes	30(33.33%)	24(21.81%)
No	60(66.66%)	86(78.18%)
Total	90(100%)	110(100%)
Occupational exposure		
Status	HBV +ve	HBV-ve
Civil servant	20(22.22%)	
Public Servant	6(6.66%)	
Unemployed	33(36.66%)	
Petty trader	24(26.66%)	
International businessman	3(3.33%)	3(2.73%)
Apprentice	4(4.44%)	10(9.09%)
Total	90(100%)	110(100%)

KEY

-ve= Negative, +ve= Positive

NA= Not applicable

ART= Antiretroviral Therapy

RTD = Rapid Test Device

No of pts = Number of patients

NA= Not applicable

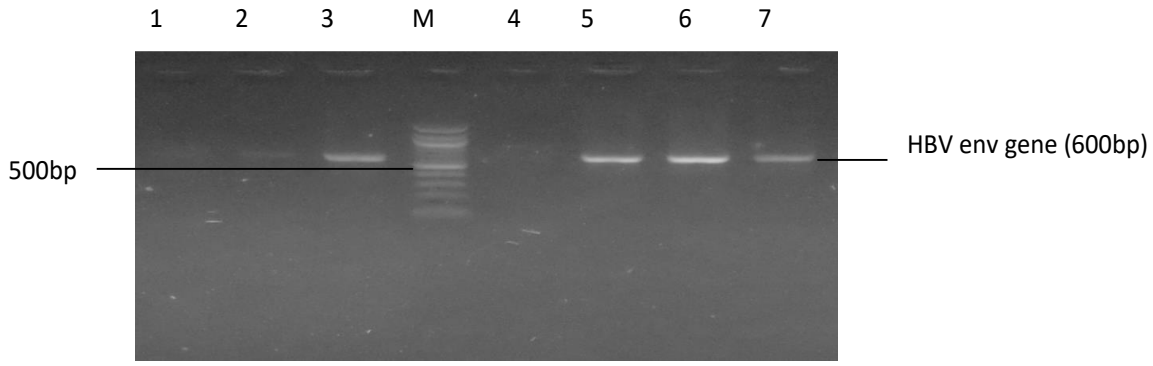

Figure 1: Agarose gel Electrophoresis of the HBV env gene. Lanes 3, 5, 6, 7 showing the amplified HBV env gene. Lane M represents the 100 bp ladder.

3(1.5%) for HBV in the referral centre.

The resultant effect of nationality/ethnicity/culture as a prevalence factor of hepatitis B virus among HIV patients attending the centre (Table 2). All the subjects were Nigerians by nationality, belonging to Igbo, Hausa or Yoruba tribes and presence of tribal incision was also considered. All subjects positive for HBV were Nigerians, 90(45%)/200 of them positive to HBV respectively. The ratio of Igbo: Hausa: Yoruba positive to HBV in the centre was 21(23.33%):24(26.70%):3(3.33%)/200, respectively. Thirty-two 32(35.56%) of the patients had tribal mark. The Hausas and others (i.e. other tribes) were observed to have the highest prevalence and the percentage of those without tribal marks were more than those with tribal mark.

In respect to medical history, the prevalence of hepatitis B virus among HIV patients was determined as denoted on Table 2. Parameters considered include; history of blood transfusion, surgery, drug abuse, knowledge of STD and family history of HBV. Most of the patient positive to HBV had no medical history of blood transfusion and surgery. Sixty of the patients 60(66.70%) positive to HBV had used injectable drugs. Individuals positive to HBV with no idea of sexually transmitted diseases (STDs) were 46(51.11%). Family history for HBV was seen to be low. Use of injectable drugs generally had the highest prevalence of all the parameters considered.

Occupational exposure as a cause of prevalence of hepatitis B virus among HIV patients as seen in the table 3, putting into consideration the nature of their jobs in terms of being a civil servant, public servant, unemployed, traders, international businessmen and apprentice. The highest prevalence of HBV was recorded for the unemployed, petty traders, civil servants in the centre Least prevalence was recorded for public servants, apprentice and international business men with 6(6.66%), 4(4.44%) and 3(3.33%) respectively individuals. The centre accounted for 33(36.66%) unemployed persons positive to HBV and 24(26.66%), 20(22.22%) for petty trader and civil servants while the least prevalence for HBV was recorded for business man 3(3.33%).

A total of 100 HIV samples and 100 non HIV samples positive to Hepatitis B infection, were randomly selected and were subjected to PCR-based detection for viral RNA, out of which 20(20%) samples from HIV positive patients and 20(20%) non HIV volunteers were proved as HBV positive, with a band size of approx. 600 bp in PCR, as shown in Figure 1.

The obtained sequence from the isolate produced an exact match during the megablast search for highly similar sequences from the NCBI non-redundant nucleotide (nr/nt) database. The sequence of HB2 and HB6 showed a percentage similarity to other species at 99%. The evolutionary distances computed using the Jukes-Cantor method were in agreement with the phylogenetic placement of the isolates within the Hepatitis viruses and revealed a closely relatedness to Hepatitis B virus strain AYNF241 (gb: KU984100.1) than other Hepatitis viruses (Figure 2). Figure 3 showed a closely relatedness to Hepatitis B strain partial preS/S (gb: AJ604932.1) than other Hepatitis viruses. The genotype is hepatitis genotype E.

DISCUSSION

In this study, we investigated the seroprevalence and molecular characterization of hepatitis B virus among HIV infected individuals and various predisposing risk factors were studied. The prevalence of HBV among HIV patients attending a referral centre in Abuja surprisingly showed larger percentage of the non-HIV volunteers when the RTD is used. There was no significant difference in the prevalence of hepatitis B for the HIV infected and the non-HIV volunteers with ELISA and PCR methods. This implies that anybody can contact HBV irrespective of your HIV status, once exposed to the virus. There is urgent need to vaccinate the HBV negative to curb the spread of the disease. This correlates with previous studies which revealed that there is no statistical significance in the prevalence of hepatitis B infection among the HIV infected.

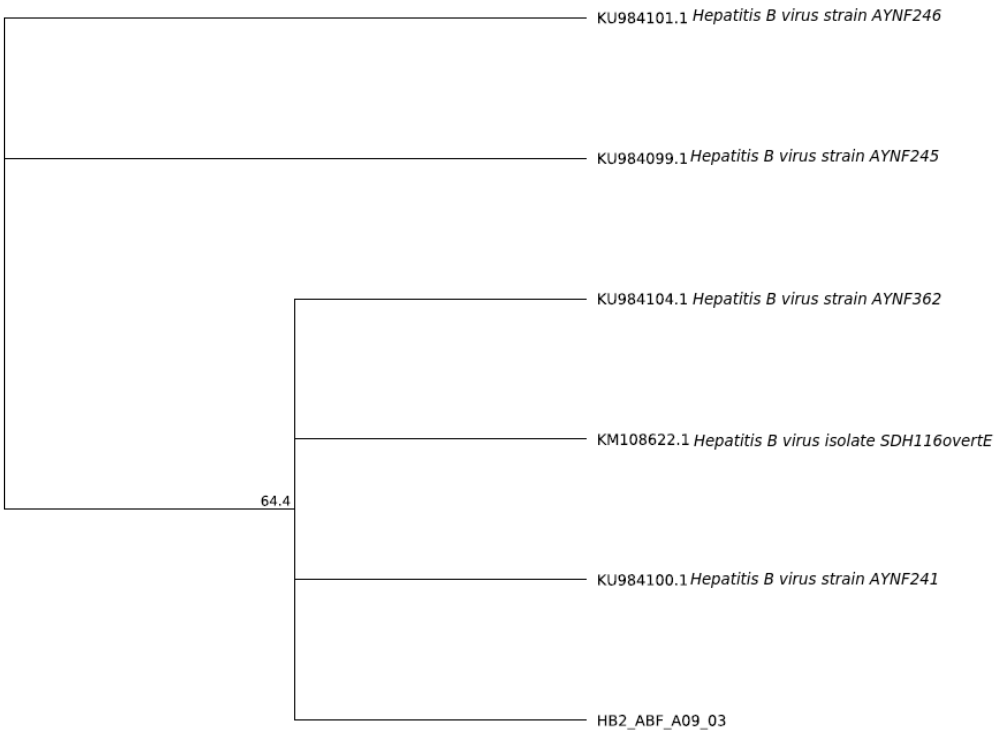

Figure 2: Phylogenetic tree showing relationship between HB2 and other Hepatitis viruses.

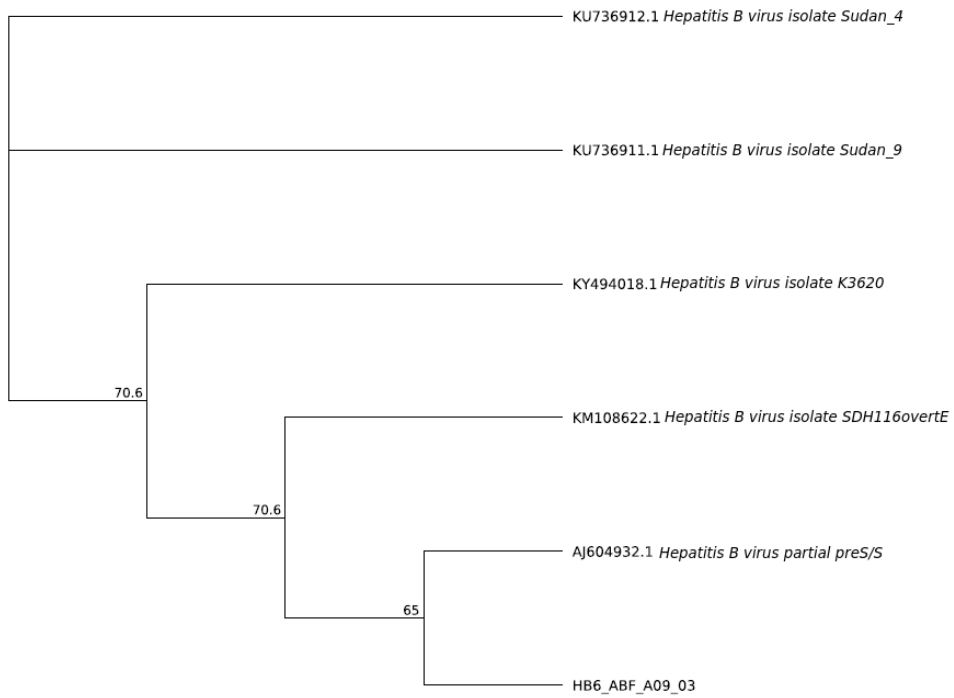

Figure 3: Phylogenetic tree showing relationship between HB6 and other Hepatitis viruses.

There is no significant difference in the prevalence of hepatitis B infection among the HIV un-infected. Finally, there is no evidence of an important effect of HBV carriage on HIV disease progression This correlates with previous studies which revealed that there is no statistical significance in the prevalence of hepatitis B infection

among the HIV infected and the HIV non-infected. Similarly, there is no evidence of an important effect of HBV carriage on HIV disease progression (Richard et al., 1997; Okocha et al., 2012). A report by Atefeh et al., (2015) indicated a wide range of HBV infection prevalence rate of 1.2 and 9.7% while that obtained in this research was 10%, 15% and 22% using RTD, ELISA and PCR respectively for HIV patients and 11%, 15% and 20% using RTD, ELISA and PCR respectively for non-HIV patients. The overall prevalence of HBsAg in this study population with ELISA increased to 15% and with PCR it increased to 22%. This result shows that ELISA and PCR methods gives higher prevalence and more reliable than other methods tested in this study. The prevalence rate of hepatitis B infection in Maiduguri and Ilorin was 11.6% and 5.7% (Thompson et al., 2015) are relatively low when compared with the values obtained with ELISA and PCR in this study. A study also carried out in an urban centre in Nigeria showed the prevalence of hepatitis B to be 11.5%. In New York, 25% of HIV patients were co-infected with 4.4% for HBV (Diwe et al., 2013), this is lower than the prevalence obtained in this study. The prevalence of HBV in the study area is considerably high and requires that the patients be adequately attended by healthcare providers so as to reduce the spread of the viral infection to the non-infected individuals and equally reduce the endemicity of the infection in this part of the world. Based on the results obtained in this study, Abuja can be classified as high endemic area for HBV since the prevalence is more than 8%. This agrees with the work reported earlier by other researchers that Nigeria has been classified as a hyper endemic nation for HBV with a prevalence of 12% (Lago et al., 2014).

In 2009, WHO reported a moderate endemicity for the presence of hepatitis B surface antigen (HBsAg) for HBV infection as 3.1%. The interesting factor is that hepatitis B virus infection is preventable. This can be achieved by population-based screening and educating the inhabitants of Abuja to get vaccinated against the disease that is for those who tested negative. Similar measures taken to prevent the transmission of HIV infection such as safe sex practice, safe handling of sharps, and avoidance of sharing of intravenous drug paraphernalia, among others can equally be emphasized to prevent HBV infection. The molecular sequencing of the hepatitis B virus DNA characterized the hepatitis B to be Genotype E, this is more predominant in Africa. Genotype F is more common in South America and Polynesia, genotype G is more predominant in the United States and France, none of the hepatitis B virus genotype sampled revealed the presence of genotypes F and G which is in line with previous studies made (Hubschen et al., 2011; Oluyinka et al., 2015; Abdurrahman et al., 2015). Studies made by Andernach et al. (2013) documented that one of the three genotypes that are predominates in Africa depending on the region includes; E, A and D. While genotype D is the most prevalent variant in Northern Africa, genotype A prevails in East and South Africa. Except for Cameroon, where genotype A is dominant, genotype E is highly endemic in most of sub-Saharan Africa. HBV genotype E has a high prevalence and wide geographic spread throughout large parts of Africa. This is in line with findings made in this research, all the HBV isolated from the three district hospitals are of genotype E. Conclusively, this study reports a no significant difference of non-HIV patients with hepatitis infection when compared to the HIV positive patients in this study and this necessitates the need for constant monitoring to evaluate control measures. The results classified Abuja as high endemic area for HBV infection in Nigeria. To offer an opportunity to identify individuals with diagnosed and undiagnosed infection, population-based screening should be conducted in the urban and rural areas of the country for HIV, HBV and other viral infection known to be endemic in the country. This will help reduce the national burden of complications resulting from these infections among HIV positive and non-HIV positive individuals alike.

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