

New strains of hepatitis B virus genotype E circulating in Nigeria

Bankole H. Oladeinde¹,
Ifeoma M. Ekejindu², Richard
Omoriegie^{3,4}, Ikpomwonosa
Odia⁵, Obiora D. Aguh⁴,
Uchechukwu M. Okwu⁶

¹Department of Medical Laboratory Science, Edo University, Iyamho, Edo State, Nigeria,

²Department of Medical Laboratory Sciences, Faculty of Health Sciences, College of Health Science, Nnamdi Azikiwe University, Awka, Nigeria, ³School of Medical Laboratory Sciences, University of Benin Teaching Hospital, Benin City, Nigeria, ⁴Medical Microbiology Unit, Medical Laboratory Services, University of Benin Teaching Hospital, Benin City, Nigeria,

⁵Institute of Lassa Fever Research and Control, Irrua Specialist Hospital, Irrua, Edo State, Nigeria, ⁶Department of Biological Sciences (Microbiology), College of Natural and Applied Sciences, Igbinedion University, Okada, Edo State, Nigeria

Address for correspondence:

Bankole H. Oladeinde, Department of Medical Laboratory Science, Edo University Iyamho, Edo State, Nigeria. Tel.: +2348053096120.
E-mail: oladeinde.bankole@edouniversity.edu.ng

WEBSITE: ijhs.org.sa

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ABSTRACT

Objective: Hepatitis B virus (HBV) is not uncommon among persons infected with human immunodeficiency virus (HIV). Severity of HBV infection and treatment outcome are associated with specific HBV genotypes. No study has reported the types of HBV genotypes circulating among HIV-infected subjects in Nigeria. This study was designed to determine the prevalence of HBV, as well as its genotypic distribution among HIV-infected subjects in Benin City, Nigeria.

Methods: Whole blood was collected from a total of 564 HIV-infected and 250 apparently healthy HIV-negative subjects. Serodiagnosis of HBV infection was done using an immunochromatographic kit. Detection of HBV-DNA and sequencing of amplicons were done using standard molecular techniques.

Results: HIV status was not significantly associated with HBV seroinfection (HIV vs. non-HIV: 4.6% vs. 4.0%; odds ratio = 1.168, 95% confidence interval = 0.550, 2.444, and $P = 0.854$). HIV-infected subjects were observed to have an insignificantly ($P = 0.645$) higher prevalence of true HBV infection than their non-HIV-infected counterparts (HIV positive vs. HIV negative: 23.1% vs. 10.0%). All patients with true HBV infection were found to harbor HBV genotype E, which did not cluster around other HBV genotype E.

Conclusion: This study reports novel strains of HBV genotype E circulating in Nigeria.

Keywords: Hepatitis B virus genotypes, human immunodeficiency virus infected patients, Nigeria

Introduction

Hepatitis B virus (HBV) is a leading cause of liver diseases worldwide. It is reported to be responsible for over 360 million cases of chronic hepatitis and 620,000 deaths each year.^[1] HBV infection is endemic in Sub-Saharan Africa.^[2] So far, eight genotypes of HBV have been reported based on sequence diversity.^[3] Factors such as genotypes, viral load, presence of Hepatitis B E antigens, and escape mutations have been reported to affect the outcome of HBV infection.^[3] Seven drugs have been approved for the management of patients with chronic HBV infection, namely standard interferon (IFN)- α , pegylated IFN- α (PEG-IFN- α), lamivudine, telbivudine, entecavir, adefovir dipivoxil, and tenofovir disoproxil fumarate.^[4] Patients infected with HBV genotype D or E have been reported to have a lower response rate to PEG IFN than those infected with genotype A.^[5] Furthermore, some studies have documented that patients infected with HBV genotype B

responded much more favorably to treatment with PEG IFN than those infected with genotype C.^[6,7] HBV genotype B has been reported to be independently associated with the earlier emergence of lamivudine-resistant strains than genotype C.^[8]

HBV is common among human immunodeficiency virus (HIV)-infected patients. This is because HIV and HBV share similar mode of transmission.^[9] HIV/HBV coinfection is associated with a poorer disease outcome.^[10] Spontaneous clearance of HBV is poorer in HIV infected than non-HIV infected ones. This often results in higher HBV-DNA levels and HBV antigenemia among HIV infected patients, increasing the risk for development of liver disease, cirrhosis and hepatocellular carcinoma.^[11,12]

In Nigeria, the routine screening of HIV-infected patients for HBV is not done in most hospitals. At present, there is a gap in knowledge on the genotypic distribution of HBV among

HIV patients in Nigeria. In the light of the above, this study aimed at determining the prevalence of true HBV infection, as well as the genotypic and subtype distribution of HBV among HIV-infected subjects in Nigeria.

Methods

Study population

This study was conducted among patients registered at the University of Benin Teaching Hospital (UBTH), Benin City, a public tertiary hospital with referral status located in the Southern part of Nigeria.

A total of 814 subjects comprising of 564 HIV-infected and 250 apparently healthy non-HIV-infected subjects were recruited for this study. Whole blood was collected from all 814 participants, after receiving written informed consent from them. Minor consent was obtained from their parents or guardians. Exclusion criterion in the study was age <18 months. The study protocol was approved by the Ethics and Research Committee of the UBTH, Benin City, Nigeria.

Specimen processing

Specimen (serum) obtained from each patient was examined for the presence of hepatitis B surface antigen (HBsAg) using Determine™ HBsAg immunochromatographic test kit (Abbott Laboratories, USA). Test kit manufacturer instruction was strictly followed. 2 ml of patients' sera that tested positive to HBsAg were put in cryovials and kept at -80°C for further analysis.

Detection of HBV-DNA

HBV-DNA was harvested from 140 ul of patients' serum, with the aid of Zymo ZR Viral DNA/RNA kit, following the manufacturer's instruction. Detection of HBV was achieved by amplification of the S gene of HBV using OneTaq Quick-Load 2X Master Mix Kit (New England BioLabs). The primer set (forward primer 5'- TCACCATATTCTTGGGAACAAGA-3' and reverse primer 5'-CGAACCACTGAACAAATGGC-3') used for the amplification of target gene has been described in a previous study (Rashid *et al.*, 2014). The total volume of reaction mixture was 25 ul and consisted of the following components: 12.5 ul of OneTag Quick-Load 2X Master Mix, 0.5 ul each of forward and reverse primers, 2.5 ul of DNA extract, and 9 ul of nuclease-free water. The thermal profile for amplification used had been previously adopted by an earlier study (Rashid *et al.*, 2014) and is as follows: An initial cycle of 94°C for 5 min, followed by 40 cycles consisting of 94°C for 30 s, 55°C for 1 min, and 72°C for 1.5 min. The final elongation was 72°C for 5 min. Amplified polymerase chain reaction products were subsequently detected on 1.5% agarose gel following staining with ethidium bromide.

Characterization of HBV isolates

All HBV-DNA-positive amplicons were sequenced at Inqaba Biotec, South Africa. Resulting sequence output was opened

with the software FinchTV. After trimming, editing, and alignment of forward and reverse sequences, the identity of the sequence was confirmed with the use of Basic Local Alignment Search Tool. Confirmed HBV genotypes (A-H) retrieved from GeneBank database were used to determine the genotype of study isolates. Phylogenetic tree was created by the neighbor-joining method using study isolates and 29 HBV isolates from different countries as previously described.^[13,14]

Statistical analysis

Chi-square or Fischer's exact tests (INSTAT®) as appropriate were used to analyze data from this study. $P < 0.05$ was considered as statistically significant.

Results

The overall seroprevalence of HBV infection in this study was 4.4%. HIV-infected patients were observed to have a higher seroprevalence of HBV infection than non-HIV-infected subjects (HIV vs. non-HIV: 4.6% vs. 4.0%). HIV status was not significantly associated with HBV seroinfection in this study (HIV vs. non-HIV: odds ratio [OR] = 1,168, 95% confidence interval = 0.550, 2.444, and $P = 0.854$) [Table 1].

Of the 26 HIV patients' seropositive for HBV, 6 (23.3%) were observed to have detectable HBV-DNA, while 1 (10%) of 10 non-HIV subjects' seropositive for HBV infection had detectable HBV DNA. HIV-infected patients were observed to have about 3 times higher risk (OR = 2.700) of acquiring true HBV infection than their non-HIV-infected counterparts. However, HIV positivity was not identified as a risk factor for true HBV infection ($P = 0.645$) [Table 2].

Table 1: Seroprevalence of hepatitis B virus infection among study subjects

Variable	N	No HBV Positive (%)	OR	95% CI	P value
HIV positive	564	26 (4.6)	1.168	0.550, 2.444	0.854
HIV negative	250	10 (4.0)			
Total	814	36 (4.4)			

N: Number of patients examined, HBV: Hepatitis B virus, OR: Odds ratio, CI: Confidence interval

Table 2: Prevalence of true HBV infection among HIV-infected and non-HIV-infected subjects

Variable	N	No HBV-DNA positive (%)	OR	95% CI	P value
HBV seropositive			2.700	0.282, 25.843	0.645
HIV positive	26	6 (23.1)			
HIV negative	10	1 (10.0)			

N: Number of patients examined, HBV-DNA: Hepatitis B virus-deoxyribonucleic acid, OR: Odds ratio, CI: Confidence interval

All HBV isolates were found to be genotype E [Figure 1]. Phylogenetic analysis revealed that none of the HBV isolates clustered around already known genotype E recovered from the GenBank, indicating new strains or variants [Figure 2].

Discussion

Reports indicate that HIV facilitates HBV replication leading to an increased risk for the development of liver diseases.^[11,12] Treatment outcome of HBV infections is associated with specific genotypes.^[15] Data on the genotypic prevalence of HBV in the Nigeria are sparse. Indeed, there is no report on the genotypic prevalence of HBV among HIV patients in Nigeria. This informed our study.

A total of 26 (4.6%) of the 564 HIV-infected patients studied were found to be seropositive to HBV. This is lower than the values reported in some African studies^[16-19] but higher than others.^[20,21] One Greek study^[9] and another Brazilian one^[22]

have, however, documented higher seroprevalence of HBV, while two others from Brazil^[23,24] reported lower values than that recorded in this study. The prevalence of HBV is reported to have geographical variation.^[25] This may explain the pattern of the result obtained in this study. Again, differences in serological diagnostic technique used may also account for the observed variation in these studies.

Among non-HIV-infected subjects, an HBV seroprevalence of 4.0% was recorded. This is higher than the value reported by a previous Nigerian study.^[26] Other Nigerian studies have, however, reported lower prevalence rate.^[27,28] The observed variation may be due to differences in nature of study population, as the studies conducted by Oladeinde *et al.*, 2013^[26,27] were carried out on pregnant women in contrast to our study population in which pregnant women accounted for <3%. Although a higher seroprevalence of HBV was recorded among HIV-infected patients, HIV was not identified as a risk factor for HBV seroinfection in this study. Similar findings have been reported by some Nigerian studies^[28,29] and a Ugandan one.^[30]

Molecular analysis of the sera of all 26 HIV-infected patients that were seropositive for HBV showed that 6 (23.1%) of them had a true HBV infection. Much higher prevalence of true HBV infection has been reported by other African studies.^[31-34] Much higher prevalence of true HBV infection has been reported by other African studies,^[31-34] with a lower value documented in one study conducted outside Africa.^[35] The role of CD4⁺ and CD8⁺ T-cell in the control of HBV infection is critical.^[36] The outcome of HBV infection is associated with specific HBV genotype.^[3] Thus, the observed variation in prevalence of true HBV infection may be related to differences in the immune status of the study population and circulating HBV genotypes. The disparity could also be attributed to differences in severity of HBV infection among study populations, variation in molecular diagnostic technique employed, and specific HBV gene targeted in these studies.

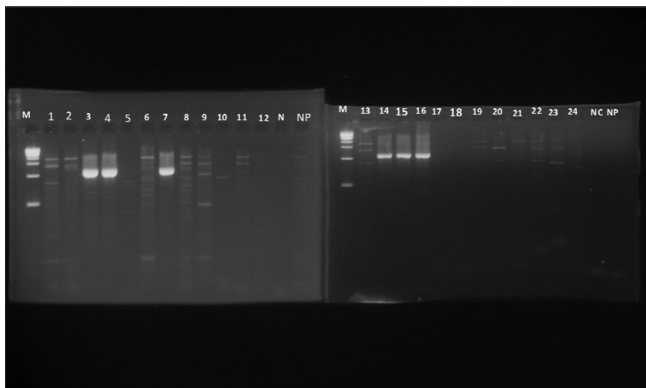


Figure 1: Hepatitis B virus-positive samples (1063 bp) detected by polymerase chain reaction (PCR) among HIV-infected patients after staining with ethidium bromide. Lane M - 10 kb DNA ladder, Lanes 3, 4, 7, 14-16: HBV-positive samples (1063 bp), Lane 1, 2, 5, 6, 8-13, 17-25: HBV-negative samples, Lane NC: Negative control, NP: Negative PCR

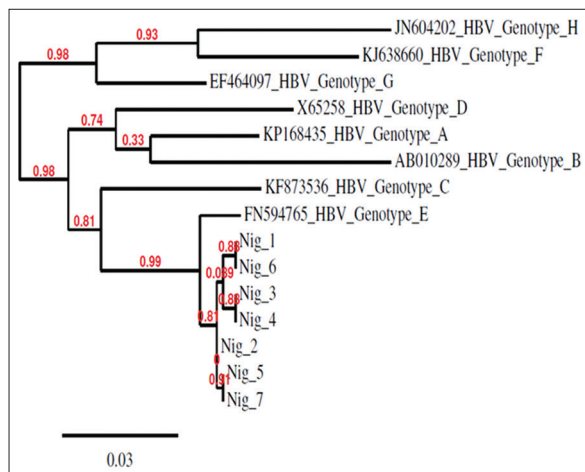


Figure 2: Genotypic characterization of hepatitis B virus isolates from the study

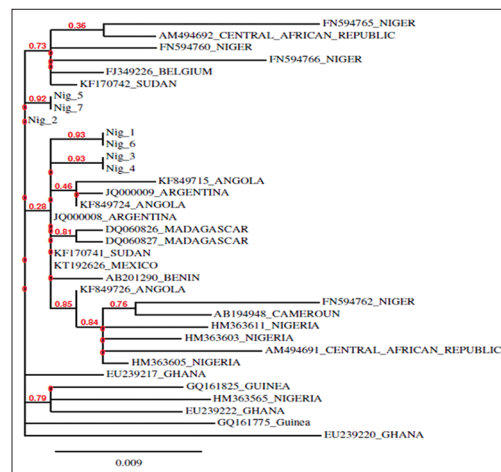


Figure 3: Phylogenetic analysis of study hepatitis B virus (HBV) isolates and other HBV genotype E isolates from GenBank database

Of the 10 non-HIV-infected subjects positive for HBsAg, only 1 (10.0%) was found to have a true HBV infection. This is lower than 83.3% recorded among blood donors in one Iranian study.^[37] The risk of acquiring true HBV infection was observed to be about 3 times (OR = 2.700) higher among HIV-infected than non-infected subjects. HBV disease progression is affected by the degree of immunodeficiency of a patient.^[38] HIV infection results in an immunodeficient state.^[39] Studies have shown that HIV-infected persons have a lower rate of clearance of Hepatitis B E antigen and a much more prolonged acute illness after infection with HBV than those without HIV.^[40] This may explain the pattern of result observed in this study. In general, the prevalence of true HBV infection did not differ significantly with respect to HIV status of subjects in this study.

All HIV-infected patients with true HBV infection in this study were found to harbor HBV genotype E. A preponderance of HBV genotype E has been reported among HIV-infected patients in several African studies.^[31,33,41] However, in one South African study, no HBV genotype E was detected among HIV-infected patients.^[34] Other studies conducted outside Africa also failed to detect HBV genotype E among HIV-infected patients.^[35,42,43] HBV genotype E has been reported to account for a majority of HBV infection in West Africa.^[44,45] Reports show that HBV genotype E is essentially not found outside of Africa, even among African-American populations, many of whom are of West African origin, which suggests that it is probably a recently evolved genotype.^[44]

Studies have reported an association between the spread of specific HBV genotypes and HIV. In one French study, an association was reported to exist between HBV genotype B and HIV seropositivity,^[46] while another conducted in the United States of America^[47] reported a preponderance of HBV genotype C among non-HIV-infected patients. Furthermore, marked differences in the genotypic distribution of HBV have been reported among HIV infected and non-infected patients in one Venezuelan study.^[48] In this study, no such association was found as all HBV isolates from HIV- and non-HIV-infected subjects were genotype E. The observed variation in report may be due to differences in geographical location. A genetic factor may also play a role in the pattern of result observed. This definitely will require further investigations to verify.

Phylogenetic analysis of all HBV isolates obtained in this study with 29 other reference HBV genotype E strains Figure 3 showed that they did not cluster around other HBV genotype E isolates. This indicates that these HBV isolates are new strains of HBV genotype E. To the best of our knowledge, this is the first Nigerian study to determine the HBV genotypic prevalence among HIV-infected patients. The sequences of HBV isolate from all six HIV-infected patients in this study have been deposited in the GenBank database with the accession numbers - KY886211, KY886212, KY886213, KY886214, KY886216, and KY886217. The HBV isolate from

non-HIV-infected subject has also been assigned the accession number - KY886215 in GenBank database.

Conclusion

The sero- and true-prevalence of HBV infection did not differ significantly between HIV-infected and non-infected patients. All HIV-infected and non-infected patients with true HBV infection were found to harbor HBV genotype E, which did not cluster around previous HBV genotype E isolates. The study highlights the presence of new variants of the HBV genotype E in Nigeria.

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