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Hepatitis B virus genotypes in Nigerian patients with hepatitis B virus-associated hepatocellular carcinoma: A hospital-based cross-sectional study

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Abstract
Background: Hepatitis B Virus Infection is a common cause of hepatocellular carcinoma (HCC) and liver disease mortality worldwide. About 10 HBV genotypes (A-J) have been identified and their roles in clinical outcomes of HBV infection have been emphasized. There is paucity of data in Nigeria on the possible role of HBV genotypes in the development of HCC. This study determined the association, if any, between HBV genotypes and the development of HCC in Nigerian patients
Methods: A hospital based cross-sectional study of 60 patients with HBV associated HCC. A structured questionnaire was used to obtain relevant data. Subjects gave informed consent and ethical approval was obtained from the ethics committee of the hospital. HBV genotypes analysis was carried out using the multiplex-nested Polymerase Chase Reaction method. Data obtained were entered into SPSS version 20 and analyzed using simple and inferential statistics.
Results: Total number of participants was sixty (60), majority were male 48(80%) and their mean age (SD) was 43.6 (11.53) years. The predominant age groups were 21-40 years, 21(35%) and 41-50, 21(35%), while those greater than 60 years accounted for the least, 4(6.7%). The commonest HBV genotype was E, 38(63.3%). A high proportion of patients with genotype E, 28(73.7%) were HBeAg negative and also had advanced HCC BCLC D, 30 (78.9%).
Conclusion: The commonest HBV genotype found in subjects with HCC in this study was E. Higher proportions of the subjects irrespective of their HBV genotypes presented in advanced disease of BCLC Class C and D.

Keywords: Hepatitis B, virus genotypes, hepatocellular carcinoma

Introduction
Hepatitis B virus (HBV) infection is a global health problem, about 2 billion people have been affected with HBV [1], majority in sub-Saharan Africa including Nigeria, South East Asia and the Far East [2]. Approximately 250 million people have lifelong chronic infection world wide and 0.5% spontaneously seroconvert annually [1]. More than one million people with chronic Hepatitis B die annually. About 20-30% of patients with chronic HBV infection would develop cirrhosis and hepatocellular carcinoma [3]. HBV is the 2nd most important carcinogen worldwide after tobacco [4].
In Nigeria, about 7.3-24% of the population have serological evidence of HBV infection (average 13.7%) with 20 million Nigerians currently infected [5], and approximately 5 million will die of HBV-related complications [5]. Areas of high incidence include: South East Asia, (10-20%), Alaskan Eskimos (45%), pacific Islands (50%) and Australian Aborigines (85%). Modes of transmission include parental exposure to blood and blood products, percutaneous and mucous membrane (heterosexual, homosexual and needle pricks), maternofetal (vertical) and child to child (horizontal) [1]. Ten (10) different HBV genotypes have been recognized so far (named with capital letters A to J) on the basis of a divergence of 8% in the nucleotide sequence of the whole genome [6]. HBV genotypes have different geographic distributions with a predominance of genotype A in North-Western Europe, North America and South Africa, genotypes B and C are highly endemic in Asiatic areas and genotype D in the Mediterranean basin and Eastern Europe [7].

Genotype E is found predominantly in West and South Africa, genotypes F and H in Central and South America and genotype G has been detected in France and the USA [7]. Genotype I has been found in Vietnam and Laos and J in Ryukyu Island, Japan. The possibility of influence of HBV genotypes on determining the clinical outcome of the infection and particularly on the risk of development of HCC has grown in the last few years [6, 7]. HBV genotypes may also be further divided into sub-genotypes based on a nucleotide intra-genotypic difference between 4 and 7.5%, more than 40 sub-genotypes have been identified (are named by adding a number to the letter representing a particular genotype e.g. A1) [6].

Majority of studies in this field were conducted in Far East Asia, in Japan and in Taiwan [8, 9]. These studies support the theory that there is a higher risk of development of HCC with genotype C rather than genotype B whereas cohort studies in the same geographic areas failed to demonstrate differences in HCC prevalence with respect to these two genotypes [10].

Malagnino *et al*., [11] carried out a cross-sectional study in Italy on West African migrants and found out that genotype E was the predominant genotype and was associated with higher incidence of development of CLD.

There is paucity of data on the possible role of HBV genotypes in the development of HCC in Africa including Nigeria and our environment. Data obtained from this study will increase our knowledge of HBV biology and assist in public health planning to create more awareness on the predominant genotypes associated with HCC in our environment and the need to be more aggressive in both monitoring and treatment when they are detected.

This study determined the predominant HBV genotype in subjects with hepatocellular carcinoma, genotypes associated with HCC among young subjects (<40 years) and old subjects (≥40 years), the relationship between HBV genotypes and HBeAg status and the relationship between HBV genotypes and Barcelona Clinic Liver Cancer (BCLC) Classification of the subjects.

Methods

Study design and site: This was a hospital-based cross-sectional study conducted among adults' patients with HBV associated Hepatocellular carcinoma to determine the predominant HBV genotype found in the patients.

Setting: This study was conducted at the Gastroenterology unit of the Obafemi Awolowo University Teaching Hospitals Complex (OAUTHC), Ile-Ife, Osun State, Nigeria between October 2018 to October 2019. OAUTHC is a 600-bed Federal Tertiary Hospital that provides both Surgical and Medical Care to residents of Ile-Ife and other towns in Osun State and neighbouring states.

Study participants: Consenting adult patients aged 18 years or older of both sexes with hepatitis B virus-associated hepatocellular carcinoma attending the Gastroenterology clinics or on admission at the OAUTHC, Ile-Ife, who satisfied the inclusion criteria constituted the study population. The study excluded those unable to provide a written signed consent, below 18 years, with other malignancies and or HCV infections.

Variables: Variables were grouped into sociodemographic characteristics, genotypes distributions in the younger (< 40), the older subjects (≥ 40), mean ages and HBeAg status, the Barcelona Clinic Liver Cancer (BCLC) Classification Staging and genotypes distributions of the subjects and HBV genotypes distribution among all the subjects.

Data sources and measurement

Data collection tool: Structured proforma was used to capture relevant data.

Data collection: A structured proforma which was designed to capture socio-demographic information of subjects, history of present illness, alcohol, family history of liver disease including HCC and liver cirrhosis, findings on physical examination, Barcelona Clinic Liver Cancer (BCLC) Staging Classification**,** relevant laboratory results including HBV genotypes and final diagnosis.

Sample size was determined using the Fisher's Statistical formula for estimating minimum sample size in health studies. n= minimum sample size, P= proportion in the target population, Z= the standard normal deviation (using 95% confidence level = 1.96), D = degree of accuracy desired, set at 0.05, Q = 1.0 - P. Using a Prevalence of Liver cancer in Nigeria of 3.7%. [12] Where P = 0.037, Z = 1.96, q = 1-0.037, $(1.96)^2$ X 0.037(1.0 - 0.037)/ $(0.05)^{2.}$ N= 54, to account for 10% non-response (attrition) rate, the estimated minimal sample size was 60.

Sampling strategy: Patients with HBV associated Hepatocellular carcinoma attending gastroenterology clinic or who were admitted at the medical ward of OAUTHC, Ile-ife. Relevant data were extracted from their folders. Diagnosis of Hepatocellular was established using: Triple-phase helical computed tomography CT; with demonstration of hypervascularity in late arterial phase defined as arterial phase hyper hyperdensity and hypo density (washout) on portal venous and or delayed phases is diagnostic of HCC. [13]. Liver biopsy and histopathological diagnosis using International Consensus Group for Hepatocellular Neoplastic classification [14] was conducted for those whose Triphasic CT results were inconclusive for HCC, with no contraindications for liver biopsy. Indication, procedures, risk, benefits and alternatives were explained to all subjects and a dully signed written consent was obtained. Biopsies are done by the Consultants and Senior Registrars while the House officer monitors the patients until discharge. Participants for liver biopsy observe an overnight fast before the morning of the procedure. Ultrasound guided liver biopsy using Tru-cut needle was done via either the transthoracic approach. Aseptic measures were adopted in carrying out the biopsy.

Diagnosis of HBV was made using AASLD guideline [15]. The presence of HBsAg establishes the diagnosis of hepatitis B. Chronic infection was diagnosed by the presence of HBsAg for at least 6 months or presence of total Anti-HBcAb in the absence of IgM-anti-HBcAb or presence of IgG-Anti-HBcAb in the absence of IgM-Anti-HBcAb. The HBV profile was assessed using the Quick profile HBV-5 Panel Test, (LumiQuick Diagnostics Inc. USA) which is a rapid immunochromatography assay for the qualitative detection of the markers of Hepatitis B virus.

Blood sample collection: Blood was collected at the ante-cubical vein using aseptic procedures into an ethylenediaminetetraacetic acid-anticoagulated (EDTA) bottle, centrifuged at 3000 rpm for 5 minutes. All samples collected were first tested immunochromatographically for HCV for exclusion, then reconfirmed for HBsAg status using Immuno-comb kit (LabACON HBsAg/Anti-HCV Rapid Test Strip Hangzhou Biotest Biotech Co. Ltd,

China)). The separated plasma was then transferred into cryovials and stored at −20°C till analysis. All the plasma was separated at the Virology Laboratory of the OAUTHC, Ile-Ife and transported in cold chain to the Virology Gene Laboratory, Ibadan, Nigeria, where Hepatitis B viral genotyping was carried out.

Hepatitis B genotyping was done using the multiplex nested PCR method, it involved DNA extraction, first round and second round PCR, electrophoresis and HBV genotyping were conducted by an experienced virologist with a doctorate degree in the Virology. The researcher was also present with the virologist and watched the whole process in the laboratory.

HBV DNA extraction: HBV DNA was extracted from two hundred (200) µL of plasma of HBsAg-positive participants using the QIAGEN HBV DNA extraction mini kit according to the manufacturer's instructions. The extracted DNA was stored at 4 °C.

Statistical analysis: The data generated from the structured questionnaires were entered into and analyzed using Statistical Package for Social Sciences (SPSS) version 20 Software, they were entered using numerical codes. Categorical variables were summarized as frequencies and percentages while continuous variables were summarized as Mean ± standard deviation. Chi-Square was used to test for the association between two categorical variables. Student's t- test, Analysis of Variance (ANOVA) and Duncan multiple comparison were used to compare means of quantitative (continuous) normally distributed variables. A p value of ≤0.05 and confidence interval of 95% were considered as being statistically significant. Results were presented in tables and charts.

Ethical considerations: Participant gave informed consent and ethical approval was obtained from the hospital Ethics and Research committee (approval numbers IRB/IEC/0004553 and NHREC/27/02/2009a, protocol number ERC/2019/04/01).

Results

Participants: A total of seventy samples were sent but 85% (60/70) HBV DNA was detected and genotyped. Thus, total of 60 genotyped HBV subjects with HCC were studied.

Socio-demographic characteristics of the study participants: A total of 60 genotyped HBV subjects with HCC were studied**.** There was a higher proportion of males 48(80%) vs. females 12(20%) with a male to female ratio of 4:1. The mean age (SD) of the patients was 43.6 (11.5) years. Participants in the age groups 21-40 years and 41-50 years accounted for the highest proportion of the subjects 21(35%), while those greater than 60 years accounted for the least proportion of the subjects 4(6.7%). Majority of the subjects were Yoruba 54(90%), married 48(80%), Christians 41(68.3%), Artisan 23(38.3%) and had tertiary education 27(45%) (Table 1).

HBV genotypes distribution in the subjects: The commonest HBV genotype present in the subjects was genotype E occurring in 38(63.3%) of the subjects. Other genotypes present though in small proportion included mixed genotypes A&E in 6(10%), genotype A in 5(8.3%), genotypes B, C&E in 4(6.7%) and genotype C in 3(5%) in descending order (Fig 1).

Comparison of mean ages of subjects with various HBV genotype: The Duncan multiple comparison test shows that the significant difference exists between the mean age of patients with genotype C and the rest of the genotypes put together (C 62.20 ± 23.30 vs. E 43.92 ± 10.91; A 42.20 ± 5.01, B 35.25 ± 3.95, A&E 43.17 ± 7.55, C&E 37.75 ± 12.69, F = 2.440, p= 0.0046) (Table 2).

Comparison between genotypes distributions in the younger (<40) and the older subjects (≥40): The frequencies of genotypes A, B, C&E were higher in subjects <40 years than in those ≥40. (10%, 15%, 10% vs. 7.5%, 2.5%, 5.0%). While genotypes E and C occurred in higher proportion in subjects aged ≥40 years than those less than 40 years (67.5% and 7.5% vs. 55% and 0%, p=0.532). There was no significant association between genotype distributions in the older and the younger subjects. (X^2 = 5.554, p = 0.352) (Table 3).

Associations between genotypes distributions and HBeAg status of the subjects: Very high proportion of subjects with HBeAg negative status was observed in those with genotype E 28(73.7%). Also, a high proportion of the subjects with genotypes A, C, E and A&E were negative for HBeAg (60%, 66.7%, 73.7%, and 66.7% respectively). There was no significant association between genotype distributions among subjects with HBeAg positive and those with HBeAg negative status. (X^2 = 1.917, p = 0.860) (Table 4).

Associations between the Barcelona Clinic Liver Cancer (BCLC) classification staging and genotypes distributions of the subjects: A significantly higher proportions of subjects with HBV genotypes E 30(78.9%), A&E 5(83.3%), C&E 4(100.0%) and C 2(66.7%) presented with more advanced disease BCLC-stage D (X^2 = 11.906, p = 0.036) while higher proportion of those with genotypes A 3(60.0%) and B 3(75.0%) presented at BCLC-stage C (Table 5).

Table 1: Socio-demographic characteristics of the study participants

Variable	Total N = 60 n (%)	Male N = 48 n (%)	Female N = 12 n (%)	Statistics	P-value
Age in years					
(Mean ± SD)	43.6±11.53	43.2 ± 11.9	45.2±10.2	t-test df=4 LR=2.512	0.588 0.643
21- 30	5(8.3)	5(100)	0(0)		
31- 40	21(35)	16(76.4)	5(23.8)		
41-50	21(35)	17(81)	4(19)		
51-60	9(15)	7(77.8)	2(22.2)		
>60	4(6.7)	3(75)	1(25)		
Gender	**60(100)**	**48(80)**	**12(20)**		
Level of Education					
No formal education	5(8.4)	3(60)	2(40)	df=4 LR=3.181	0.364
Primary	14(23.3)	10(71.4)	4(28.6)		
Secondary	14(23.3)	11(78.6)	3(21.4)		
Tertiary	27(45)	24(88.9)	3(11.1)		

Ethnicity					
Yoruba Ibo Hausa Fulani Efik	54(90) 2(3.3) 1(1.7) 2(3.3) 1(1.7)	42(77.8) 2(100) 1(100) 2(100) 1(100)	12(22.2) 0(0) 0(0) 0(0) 0(0)	df=4 LR=2.840	0.585
Religion					
Christianity Islam	41(68.3) 19(31.7)	33(80.5) 15(78.9)	8(19.5) 4(21.1)	X^2=0.019 df=1	0.890
Marital status					
Married Single Widowed Divorced	48(80) 7(11.7) 4(6.7) 1(1.7)	39(81.2) 7(100) 1(25) 1(100)	9(18.8) 0(0) 3(75) 0(0)	df=3 LR=9.222	0.026*
Occupation					
Artisan Civil servant Trading Unemployed/Retired	23(38.3) 19(31.7) 12(20) 6(10)	20(86.9) 17(89.5) 5(41.7) 6(100)	3(13.4) 2(10.5) 7(58.3) 0(0)	df=3 LR=13.297	0.004*

Keys: *=Statistically significant, LR=Likelihood ratio, X^2=Chi-square test

Table 2: Comparison of mean ages of subjects with various HBV genotype

HBV genotype (Number of cases)	Mean age Mean ± SD	F	p value
A (5)	42.20 ± 5.01	2.440	0.046
B (4)	35.25 ± 3.95		
C (3)	*62.20 ± 23.30		
E (38)	43.92 ± 10.91		
A & E (6)	43.17 ± 7.55		
C & E (4)	37.75 ± 12.69		

*Duncan multiple comparison test indicating means not significantly different

Table 3: Comparison between genotypes distributions in the younger (< 40) and the older subjects (≥40)

HBV genotypes	< 40 years N (%)	≥ 40 years N (%)	X^2	P value
A	2 (10.0)	3 (7.5)	5.554	0.352
B	3 (15.0)	1 (2.5)		
C	0 (0.0)	3 (7.5)		
E	11 (55.0)	27 (67.5)		
A & E	2 (10.0)	4 (10.0)		
C & E	2 (10.0)	2 (5.0)		

Keys: x^2=Chi-square test, HBV=Hepatitis B virus

Table 4: Associations between genotypes distributions and HBeAg status of the subjects

HBV genotypes	HBeAg negative n (%)	HBeAg positive n (%)	X^2	P value
A	3 (60.0)	2 (40.0)	1.917	0.860
B	2 (50.0)	2 (50.0)		
C	2 (66.7)	1 (33.3)		
E	28 (73.7)	10 (26.3)		
A & E	4 (66.7)	2 (33.3)		
C & E	2 (50)	2 (50)		

Keys: ^{2} =Chi-square test, HBeAg=Hepatitis e antigen

Table 5: Associations between the Barcelona Clinic Liver Cancer (BCLC) classification staging and genotypes distributions of the subjects

Variables	BCLC-Stage C n (%)	BCLC-Stage D n (%)	X^2	P value
A	3 (60.0)	2 (40.0)	11.906	0.036
B	3 (75.0)	1 (25.0)		
C	1 (33.3)	2 (66.7)		
E	8 (21.1)	30 (78.9)		
A & E	1 (16.7)	5 (83.3)		
C & E	0 (0.0)	4 (100.0)		

Keys: ^{2} =Chi-square test

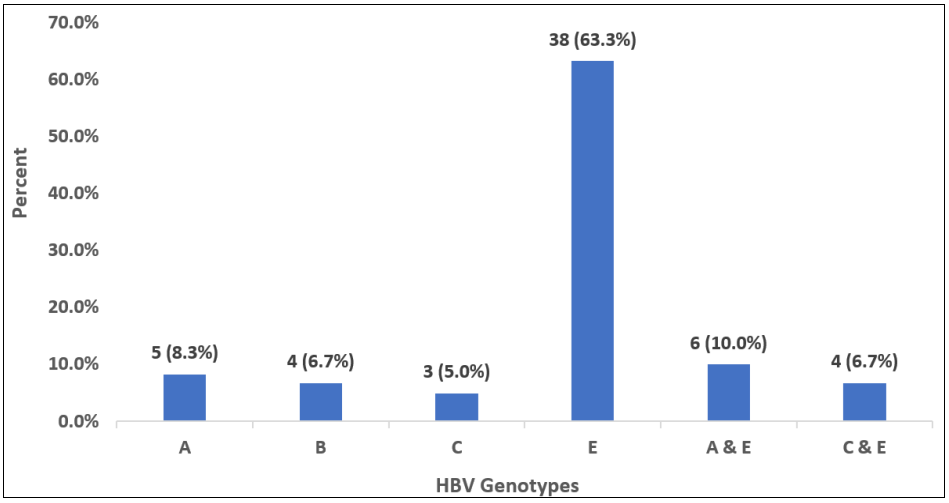

Fig 1: HBV genotypes distribution in the subjects

Discussion

This study was able to detect and genotype the infecting HBV in 85% of the total samples sent. This finding is comparable to the detection rate of 83.3% reported by Mustapha *et al*., [16]. In Zaria, North West Nigeria using the same method. The result is also comparable to studies in

Egypt using innogenetics line probe assay [17] and in India [18] that used nucleic acid amplification technique. Both reported detection rates of 71.4 and 69.7% respectively. Among the various methods of HBV genotyping that were used in the studies above [16, 17, 18] multiplex PCR was the most sensitive. And it also reflected on the high HBV genotype detection rate of 85% obtained from this study. The different methods used may not be fully responsible for the variability in HBV-DNA isolation rate in the HBsAg positive samples. The fact that HBV-DNA is a non-encapsulated virus and tends to degrade rapidly, HBsAg may remain in circulation for a prolonged period of time even in the absence of or very low levels of HBV DNA {due to treatment or natural clearance) may explain the different detection rates [19, 20].

The pattern of distribution of HBV genotypes in the HCC patients in the current study showed that Genotype E was the most predominant type followed by mixed genotypes A&E, and then A. These findings are comparable to what was reported for West African migrants by Malignino *et al*., [11] in Italy, Mustapha *et al*., [16] in Zaria, North West, Nigeria and Shimakawa *et al*., [21] in Gambia. The predominance of and almost exclusive circulation of genotype E in West Africa may be partly due to its origin [22]. It has been postulated that HBV genotype E originated 200 years ago in West Africa through cross-species transmissions [23].

The mixed genotypes A&E was the most prevalent mixed pattern observed from this study, this finding is contrary to what was reported by Mustapha *et al*., [16] in Zaria, in their study, E&B was observed as the predominant mixed pattern (their study population were patients with chronic HBV infection different from HBV associated HCC in this study). Migration of people infected with different genotypes from other parts of the continent to West African or vice versa, may be a possible explanation for the mixed genotypes. Although there is paucity of information on the clinical and virologic characteristics of HBV genotype E. Some epidemiological studies have suggested carcinogenic potential of genotype E as higher incidence of HCC is found in West Africa regions endemic for genotype E [21-23] and recently an incidence of HCC of 58.5/100,000 in HBV genotype E patients has been reported in a study in Gambia, by Shimakawa *et al*., [21]. Both mutation in precore and basal core promoter mutations in the HBV genome which were found to favour the development of HCC have been observed to occur more frequently in persons infected with genotype E. [24, 25]. Reverse transcriptase sequencing analysis of HBV genotypes by Echevarria *et al*., [26] showed high prevalence of immune escape mutations in HCC subjects with HBV genotype E which is characterized with formation of stop codon resulting in production of truncated HBsAg which can accumulate in the endoplasmic reticulum and induce oxidative stress and enhance cell proliferations [26]. HBV genotype E have been found to harbour an abnormal amino acid with serine at position 140 which is known to affect HBsAg recognition by antibody this feature aids its ability to evade humoral immunity and may explain virologic breakthrough in HBV E genotype infection in vaccinated individuals from West Africa [27]. Contrary to our findings, HBV genotype A was the predominant genotype in South African isolates and was shown to be 4.5 times more likely than other genotypes to cause HCC [28].

Genotypes C and mixed genotype C&E were the least genotypes observed in the current study, this is not unexpected as genotype C is known to be more prevalent in Asia and North American countries like Alaska, Northern Canada & Greenland. The cases of genotypes C reported in the current study may have been imported into the country by travellers. This result is also similar to reports from other studies in West Africa [16, 21].

McMahon *et al*., [29] in their study in the USA observed that genotype C and sub genotypes B2-5, and F1, appear to be associated with a higher risk of developing HCC, while others, including genotypes B1, B6, and A2, appear to be associated with a lower risk of complications of HBV. In Thailand, Tangkijvanich *et al*., [30] in their study observed HBV genotypes C and B as the predominant genotypes and more prevalent genotypes in HCC patients compared to the others. These variations in geographical distribution of HBV genotypes have been postulated to be related to genetic characteristics of the populace, place of origin of the HBV genotype, climate and other environmental factors that may favour some genotypes to thrive in a particular environment over the others [31].

Majority of the patients studied were HBeAg negative, which was observed most commonly in those with genotype E and mixed genotypes A & E. These findings are similar to reports by Shimakawa *et al*., [21] in Gambia, West Africa and Malagnino *et al*., [11] in their study of West African immigrants in Italy. The absence of HBeAg in the subjects could be related to the presence of precore and or core ORF mutations which have been reported to be very common with genotype E and has been associated with persistence of viral infections, resistance to anti-viral therapy and promotes hepatic carcinogenesis [11, 32, 33]. The ages of the subjects studied may have also contributed to very high prevalence of HBeAg negative status in the subjects, majority of the subjects studied were adults. It has been reported that the prevalence of HBeAg positive status reduces with age (12% and 85% in subjects >50 years and <10 years respectively) [34].

These findings are contrary to reports from other studies were HBeAg positive status were reported more commonly with genotype C than B [30, 10]. Sarma *et al*., in India reported high prevalence of HBeAg positive status in HBV genotypes C, A and D [35]. Chan *et al*., [36] in China and Tangkijvanich *et al*., [30] in Thailand, observed higher proportion of positive HBeAg in subjects with genotype C than those with B but was not associated with higher risk of development of HCC. This could be partly explained by the fact that majority of subjects studied were adults and precore and basal core mutations that may result in loss of HBeAg have also been reported in subjects with genotype C but commoner in adults and elderly than in children [36].

There was no significant difference between the distributions of the various genotypes that were identified in this study in the younger and older subjects. Genotype E was the predominant type observed in both the young < 40 years and old subjects ≥ 40 years with HCC. This pattern was also reported by Shimakawa *et al*., [21] in Gambia West Africa. However, Yin *et al*., [37] in Maryland in China, found that HBV Genotype C was significantly more common in younger people (<40 years) with HCC than genotype B. He also found that HBV induced HCC genotype C harbours more frequent mutations in the young than genotype B. Also, Kew *et al*., [28] in Cape Town, South Africa reported HBV genotype A as the predominant genotype in African

isolates in patients with HCC at a younger age. On the other hand, a study in Asia, Japan, by Yoshikawa *et al*., [38] reported genotype B to be more prevalent in older age group. Some studies in Asia reported that males older than 40 years with chronic HBV infections is a risk factor for development of HCC and this has formed the basis for aggressive surveillance in such patients [39]. The relationship between advancing age and development of HCC has been postulated and partly attributed to increase in frequency of non-virological response to antiviral therapy with age, accelerated rate of progression of hepatic fibrosis [40] and increased prevalence of metabolic syndrome and NASH with age [41].

Majority of the patients irrespective of their genotypes presented with advanced HCC using the BCLC classifications, but genotypes E, mixed genotypes A & E, C and mixed genotypes C &E presented with more advanced HCC (BCLC-D). The high prevalence of advanced HCC in genotypes E, AE could be partly explained by the high prevalence of genotype E, low rate of HCC surveillance in patients with HBV in our environment (largely related to poverty), the inability of patients to afford regular surveillance tests as well as poor health seeking behaviour, cultural beliefs in herbal remedies, delay in presentation to hospital due to visitations to prayer houses contribute to high prevalence of advanced HBV associated HCC at presentation. These findings are similar to the report from Gambia, West Africa [21]. Though they are paucity of data on the link between advanced HCC and HBV genotype E, epidemiological studies have suggested a high association considering the facts that HCC is highly prevalent in west Africa where HBV genotype E is prevalent and patients usually present very late with advanced disease [42, 43]. But these findings differ from multiple studies in South Africa. [28], North America [28], Europe and Asia; China [37], Japan [44] and Taiwan [45] where genotypes A, B&C, C and D respectively and not E and AE are associated with high prevalence and advanced HCC.

Limitations to the study: The multiplex nested PCR method applied for genotyping HBV can identify only six genotypes; A-F, limitation to this study therefore mean that other genotypes G-J might have been missed by this method.

What is already known in this topic: The HBV genotypes C and D have been identified in many studies in Europe and Asia as the predominant types associated higher tendency to the development of HCC and chronic liver disease.

What this study adds: This study determined the predominant HBV genotype associated with development of HCC in Nigerian/African patients.

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Competing interests: Authors declare no competing interests

Authors contributions: Dr Obasi Emmanuel conceptualized, designed, entered data, wrote the manuscript, participated in sample collection and HBV genotyping. Prof Ndububa, Prof Adekanle and Dr. Ajayi collected some data, performed liver biopsies, supervised and reviewed the manuscript. Dr Fanaye conducted the HBV geotyping. All authors read and approved the final version of this manuscript.

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