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Research Article

Molecular Detection of Hepatits B Virus Genotypes in Tertiary Hospitals in Bayelsa State, Nigeria

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Abstract

In Africa, the Hepatitis B Virus (HBV) is considered endemic or even hyper-endemic. However, there is lack adequate comprehensive data regarding the genotypes of HBV and their distribution within Nigeria. This study seeks to identify the prevailing HBV genotypes among patients who sought medical care at the Federal Medical Center, Yenagoa and Niger Delta University Teaching Hospital Okolobiri, Bayelsa State, Nigeria. Between January and June 2022, 656 patients' blood samples were collected from both hospitals for analysis, consisting of 475 females (72.4%) and 181 males (27.6%). The samples were tested for Hepatitis B surface antigen (HBsAg) and genotyping using immunochromatography and multiplex Polymerase Chain Reaction (PCR) techniques with type-specific primers. Among the 656 patients screened for HBsAg, 66 individuals (10%), comprising 36 females (5.4%) and 30 males (4.6%), tested positive using immunochromatography. These positive cases underwent molecular genotyping using specific primers for genotypes A, B, C, D, E, and F. Of these, 33 (50%) exhibited strong or active positivity. In comparison, the remaining 50% displayed passive positivity due to viral degradation, as HBV is a non-enveloped virus. The results further revealed that HBV genotypes E and B were the predominant types, with a prevalence of 82.4% and 11.8%, respectively, in the study area. Interestingly, the observation showed that co-infections involving HBV/B and HBV/E had a majority of 5.9% and were detected among two female patients aged 26 and 25. It is noteworthy that in the study area and its environs, healthcare providers commonly prescribe tenofovir, a nucleotide analog drug. Previous research by various authors has indicated that HBV genotype E is more responsive to nucleotide analogs, while HBV genotype B responds better to interferon-based therapies. In conclusion, this study highlights the prevalence of HBV genotypes B, E, and B+E coinfection in Yenagoa within the Niger Delta region of Nigeria. This knowledge underscores the importance of healthcare providers accurately diagnosing HBV genotypes before prescribing treatment regimens, particularly considering combination therapy, to optimize the care of infected patients. This approach is vital for effectively managing HBV infections and improving patient outcomes.

Keywords: Hepatitis B surface antigen; HBV genotypes A, B, C, D, E, F, tenofovir drug; Nucleotide analog, interferon-based therapies, HBV B+E coinfection

Introduction

Hepatitis is a term employed in the medical field to denote inflammation of the liver, which can be caused by infectious as well as non-infectious agents. Its symptoms can vary among individuals, with some remaining asymptomatic. Conversely, others may display symptoms such as jaundice, characterized by the yellowing of the skin and eyes, in addition to ailments like nausea, vomiting, fatigue, abdominal discomfort, and diarrhea. Cirrhosis, which also results in yellowing of the skin and eyes, can lead to jaundice [13]. Hepatitis is categorized as acute when it resolves within six months and as chronic when it persists be-

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yond this period. Acute hepatitis may either resolve, progress into chronic hepatitis, or, in rare cases, culminate in sudden liver failure. These are all potential outcomes. Significant complications of chronic hepatitis encompass liver scarring (cirrhosis), liver failure, and even liver cancer. Viral hepatitis pertains to hepatitis caused by hepatotropic viruses, which includes Hepatitis A, B, C, D, and E (RNA viruses) and with the Hepatitis B Virus (HBV) (a DNA virus). Other potential causes of hepatitis encompass excessive alcohol consumption, specific medications, chemical exposure, various infections, autoimmune diseases, and Non-Alcoholic Steatohepatitis (NASH) [15].

Healthcare challenges associated with hepatitis B are widespread, particularly in economically disadvantaged regions. It is estimated that approximately one-third of the global population carries the HBV [1], and the annual rate of spontaneous conversion from Hepatitis B Surface Antigen (HBsAg) to hepatitis B surface antibody is 0.5%. This means around 350–400 million people worldwide live with a lifelong chronic infection. While chronic hepatitis B infection can lead to Liver Cirrhosis (LC) and Hepatocellular Carcinoma (HCC), cirrhosis develops in only a tiny fraction of individuals. HCC and, on occasion, cirrhosis are complications of hepatitis B. Continual vaccination programs have proven effective in reducing the prevalence of HBV infection in various countries worldwide.

HBV is composed of hepadnaviruses with a 42-nanometer diameter virion [18]. It consists of tiny spheres and rods with an average width of 22 nanometers and is structured in two layers, resembling a double shell. Its exceptional resilience allows it to withstand high and low humidity levels. HBV can endure temperatures as low as -20°C for 15 years, -80°C for 24 months, average room temperature for 6 months, and 44°C for only 7 days [17].

Recent studies have identified eleven HBV genotypes (A through J), each predominantly in specific regions worldwide [20]. The genomic sequence differences must be at least 8% to accurately classify a person's genotype. In Sub-Saharan Africa, genotype E is the most prevalent, followed by A and D, with genotype D being the least common [21]. The HBV genotype significantly influences the clinical outcome and the patient's response to therapy, such as interferon-based treatment (Mustapha, 2014). For instance, genotypes A and B progress to the chronic phase more slowly than genotypes D and C [17]. However, genotypes D and A have higher rates of spontaneous HBeAg seroconversion than genotypes C and D, although infections with genotypes A and D carry a higher risk of developing hepatocellular carcinoma [17]. Genotype E, prevalent in West Africa shows only tangential associations with pre-core and basal core promoter changes and exhibits a suboptimal response to interferon-based treatment (Anna, 2014). This genotype is responsible for the majority of HBV infections in the region. Recent research has also uncovered rare mixed-genotype HBV infections in various areas, potentially indicating similar clinical implications [1].

The objectives of this present study are to molecularly characterize the Hepatitis B virus genotypes among individuals attending two major tertiary health institutions in Bayelsa State for treatment and determine the current genotypes of HBV that are widespread in the area. HBV genotypes variations has been shown to be helpful in viral indicators for the prediction of disease progression and for assisting practicing doctors in identifying patients who are most likely to gain the most benefit from IFN-based therapy. The findings of this study will raise awareness about the appropriate treatment and control measures to take. The findings of the study will provide information about the the most common HBV genotypes and their patterns of infection in the study area

Materials and Methods

Study Area

This study was conducted in Yenagoa, a city located in Bayelsa State, situated within the Niger

Ethical Approval

The Ethics Committee of the Federal Medical Centre and Niger Delta University Teaching Hospital in Bayelsa state gave their approval for this study.

Sample Size

Sample size will be calculated using the formula derived by Daniel (1999):

$$N = \frac{Z^2P(1-P)}{d^2}$$

Sample size calculation will be done using the 95% confidence interval and 0.05 precision rate.

The prevalence rate of HBV infection in Nigeria is 12.2% [3].

N= Minimum sample size

Z= Confidence interval (95%) who's equivalent coefficient is 1.96

P= Prevalence rate 12.2% (0.122)

d=degree of precision (5%)

$$N = \frac{(1.96)^2 \times 0.122 \times 0.878}{0.05^2}$$

N =165

Sample Collection

For this analysis, 4mls of whole blood sample was collected from each patient into ethylene diaminetetra-acetic acid bottle and centrifuged at 3000rpm for 5 min to separate the plasma.

Sample Processing

Using a Surge-lab rapid immuno-chromatographic test strip, the HBsAg status of all samples was confirmed after initial immuno-chromatographic testing for HCV and HIV to rule out those conditions. After that, the separated plasma was put into plain bottles and refrigerated at -4°C until analysis. The whole blood was also stored at 4°C for extraction.

Laboratory Methods and Procedures

HBsAg, HCV and HIV Status Determination

The WHO standard of serial alogarithm using skytec and determine test kits was adopted [12].

Test Procedure

50ul of plasma was applied on the sample pad and allowed to flow after the protective foil cover on the test card was removed. The outcome was read after 15 minutes. The control window of the strip, labelled "control," and the patient window

of the strip, labelled "patient," both contain red bars to indicate a positive result. In the patient window, any red bar that is plainly visible is regarded as positive. In contrast, a single red bar in the "control" window of the strip and no red bars at all in the "patient" window denote a negative result. Even if a red bar appears in the patient window but not in the control window of the strip, the result was redone if there isn't a red bar there.

HBV 5 panel test

Test Procedure

The test device was removed from its pouch and place on a flat surface.5µl of plasma was dispensed into the sample pad for each of the 5 viral markers. Results were read after 10 minutes.

Interpretation of Results

For HBsAg, HBsAb, and HBeAg, the presence of just one band in the control zone denotes a negative result, but the presence of two bands in both the test and control zones denotes a positive outcome. When there is just one band present at the control zone, an invalid result is indicated.

One band in the control zone indicates a positive result for HBeAb and HBcAb, whereas the presence of two bands in both the test and control zones indicates a negative result. When there is just one band present at the control zone, an invalid result is indicated.

The Process of Extracting DNA from a Blood Sample

To process 100 uL of blood, 400 uL of Genomic Lysis Buffer were utilized. After mixing thoroughly on a vortex for four to six seconds, the mixture was left to stand at room temperature for five to ten minutes. Following this, the mixture was transferred to a collection tube containing a Zymo-Spin IIC Column. The centrifuge was operated at 10,000xg for one minute, and the collection tube with the flow-through was discarded. The Zymo-Spin IIC Column was then moved to a new Collection Tube. After adding 200uL of DNA Pre-Wash Buffer, the spin column underwent centrifugation at 10,000 x g for one minute. Subsequently, 500uL of g-DNA Wash Buffer was added to the spin column, which was again subjected to a one-minute centrifugation at 10,000xg.

The spin column was then transferred to a freshly cleaned microcentrifuge tube. To elute the DNA, an additional 50 uL of DNA Elution Buffer were applied to the spin column. After letting it stand at room temperature for two to five minutes, the centrifuge was operated for 30 seconds at maximum speed to extract the DNA. The eluted DNA was stored frozen at -20 degrees Celsius for future use.

DNA Quantification

The collected genomic DNA underwent quantification and analysis using the Nanodrop 1000 spectrophotometer. The Nanodrop icon on the desktop was double-clicked to initiate the device's software. The instrument was first calibrated using 2µl of sterile, deionized water, then blanked with ordinary saline solution. Following this calibration, 2 microliters of the extracted DNA were carefully placed onto the lower pedestal, after which the upper pedestal was lowered to ensure contact between the DNA on both pedestals. The "measure" button on the toolbar was clicked to calculate the DNA content of the sample. The measured DNA concentration was reported in ng/ul, with a range typically falling between 5 to 100 ng/ul and yielding values within the range of 1.5 to 2.0. Additionally, the purity of the DNA was determined based on the 260/280 absorbance ratio.

'Multiplex-Nested Polymerase Chain Reaction' (PCR)

This done using type-specific primers used to assign genotypes A through F based on pre-S1 through S genes of the HBV genome.

➢ **First-round polymerase chain reaction for detecting DNA from the hepatitis B virus.**

The first round of nested PCR utilized a total volume of 20µL for the reaction. Each premix tube was labeled with the respective sample ID. Following DNA extraction, it was combined with the Master Mix, consisting of 16µL of deionized water [D.H2O], 250 M of each dNTP, 1X PCR buffer, 15 mM of $MgCl_2$, 1U of heat-resistant Taq polymerase, and 1 L of both outer primers P1 (forward) and S1 2 (reverse) in equal proportions.

The PCR procedure was carried out in a thermal cycler with the following reaction conditions:

- Initial activation at 95ºC for five minutes.
- Denaturation at 94ºC for twenty seconds.
- Annealing at 60ºC for thirty seconds.
- Extension at 72ºC for one minute.

The temperature was gradually reduced by 0.5ºC for the first six cycles. Subsequently, it remained constant at 57ºC for the subsequent 29 cycles. A total of 35 complete cycle sets were completed, encompassing denaturation through extension. The third and final extension step occurred for five minutes at 72 degrees Celsius.

Second-Round Polymerase Chain Reaction: Hepatitis B virus genotyping Each sample underwent two separate runs for the second round of PCR, utilizing distinct tubes. In the first tube, we combined the common sense primer (B2) with type-specific primers for genotypes A, B, and C. Meanwhile, the second tube contained the standard universal antisense primer (B2R) and primers unique to genotypes D, E, and FTo facilitate this second round of PCR, we introduced an additional 17µL of distilled water to each premix 'A' and 'B' tube. Furthermore, we increased the total amount of primers to 2µL by adding 0.5µL of each primer to both mixtures. Each premix tube was to contain 1µL of the product from the first PCR round. After gentle mixing and subsequent centrifugation, we retained the same PCR settings previously employed. The negative control, samples, and ladder underwent electrophoresis on a 2% agarose gel containing 1x TAE buffer to analyze the results. This process was conducted for 45 minutes at 100V.

Agarose Gel Electrophoresis

On a 1% agarose gel prepared in 1X Tri-Boris EDTA containing 5ls of 'Safe View,' the amplicons were measured against a 100bp molecular weight marker. The gel was then run in a horizontal tank filled with 1X Tri-Boris EDTA for 30 minutes at 350V. Under a blue light transillumination, each gel was examined with the PrepOneTM Sapphire EC135-90. Electrophoresis was performed at a voltage of 100 mV for 30 minutes. We were able to determine the size of the bands and the fragment size by comparing the bands to a DNA ladder that was 100 kb or longer.

Results and Discussions

Tables 2 and 3 show the demographic presentation of participants. Of 656 subjects screened for HBsAg, 475(72.4%) were females, while their male counterparts had 181(27.6%) participants (Table 2). The age distribution revealed that 301 (45.9%) were aged 26-35, 120 (18.3%) were aged 36-45,118 (18.0%) were within 16-25 and 49 (7.5%) were >56. 38 (5.8%) and 30 (4.6%) were within 46-55 and <15, respectively (Table 3).

Table 1: Primer Sequence (5'-3') Specificity Position Polarity.

Primer	Sequence (5'-3')	Specificity	Position	Polarity
1st round PCR				
P1	TCACCATATTCTTGGGAA-CAAGA	Universal	2823-2845	Sense
S1-2	S1-2 CGAACCACTGAA-CAAATGGC	Universal	685-704	Antisense
2nd round PCR: Mix A				
B2	GGCTCCAGTTCCGGAACAGT	Type A-E	67-86	Sense
BA1R	CTCGCGGAGATTGACGA-GATGT	Type A	113-134	Antisense
BB1R	GGTCCTAGGAATCCTGAT-GTTG	Type B	165-186	Antisense
BC1R	CAGGTTGGTGAGCTGGAGA	Type C	2979-2996	Antisense
2nd round PCR: Mix B				
B2R	GGAGGCGGATTTGCTGGCAA	Type D-F	3078-3097	Antisense
BD1	GCCAACAAGGTAGGAGCT	Type D	2979-2996	Sense
BE1	CACCAGAAATCCAGATT-GGGACCA	Type E	2955-2978	Sense
BF1	GTTACGGTCCAGGGTTACCA	Type F	3032-3051	Sense

Table 2: Demographic presentation of participants.

AGE GROUPS	MALE (%)	FEMALE (%)	TOTAL (%)
<15	11(1.7)	19(2.9)	30(4.6)
16-25	28(4.3)	90(13.7)	118(18.0)
26-35	50(7.6)	251(38.3)	301(45.9)
36-45	40(6.1)	80(12.2)	120(18.3)
46-55	25(3.8)	13(2.0)	38(5.8)
≥56	27(4.1)	22(3.4)	49(7.5)
TOTAL	181(27.6)	475(72.4)	656

Table 3: Distribution of HBsAg among participants.

	MALE		FEMALE		
Age groups	N.E(%)	N.I(%)	N. E (%)	N.I(%)	TOTAL (%)
<15	11(1.7)	1(0.2)	19(2.9)	1(0.2)	30(4.6)
16-25	28(4.3)	4(0.6)	90(13.7)	5(0.8)	118(18.0)
26-35	50(7.6)	12(1.8)	251(38.3)	25(3.8)	301(45.9)
36-45	40(6.1)	10(1.5)	80(12.2)	4(0.6)	120(18.3)
46-55	25(3.8)	2(0.3)	13(2.0)	1(0.2)	38(5.8)
≥56	27(4.1)	1(0.2)	22(3.4)	0(0)	49(7.5)
TOTAL	181(27.6)	30(4.6)	475(72.4)	36(5.5)	656

- N.E: Number Examined
- N.I: Number Infected

Of the 656 participants, 66 (10%) were positive for HBsAg, with females having 36 (5.4%) positive subjects and 439 (66.9%) negative. Male participants had 30 (4.6%) positive subjects negative and 151 (23.0%) negative subjects.

Amongst the 34 HBV-DNA positive samples, it was observed tha 28 (82.4%) subjects had HBV E genotype while HBV B and HBV B + E mixed infections had 4 (11.8%) and 2 (5.9%) respectively (Table 4). There were more male participants, with 19 (55.9%) to females with 15 (44.1%) (Table 4).

Discussion

Nigeria has made significant progress in addressing viral hepatitis, following the WHO's four-pronged strategy adopted in 2010 to recognize it as a global health concern. This strategy encompasses monitoring cancer cases associated with hepatitis, establishing national infection control guidelines for healthcare workers, enforcing vaccination requirements, and screening all donated blood.

However, some key aspects remain unaddressed. Notably, no national policies are in place to prevent mother-to-child transmission of infections or eradicate Hepatitis B (HBV). This gap is evident in the findings of a study conducted by Musa et al. (2015), which revealed that between 2000 and 2013, 14% of Nigerians were exposed to HBV.

Hepatitis B is a preventable condition through vaccination. Nigeria updated its national vaccination schedule in 1995 to include the HBV vaccine, which became accessible to the general public in 2004. In this research project, participants ranged in age from 1 year to 31.0 years, with the median age been 41years, as depicted in Table 2.

Interestingly, our research findings indicated a higher prevalence of HBV among female participants than male participants, contrary to previous studies that suggested a higher risk for men due to associated factors. According to Vilibic et al. (2014), this result suggests that sexual activity and HBV infection are not necessarily correlated.

While PCR is considered the second gold standard after sequencing, it's noteworthy that in this investigation, only 51.5% (34 out of 66) of the samples could confirm the presence of HBV infection and determine its genotype. Several factors may contribute to this outcome, including potential sample deterioration before analysis, primer sensitivity, or factors unrelated to the technique.

Irrespective of the detection method used, as highlighted by Ahmad et al. (2019), various genotyping methodologies maintain variability in HBV-DNA isolation. Notably, not all samples that test positive for HBsAg (Hepatitis B surface antigen) will yield positive results for HBV-DNA detection. This phenomenon could be attributed to HBV being an unencapsulated virus, being prone to rapid DNA degradation. Additionally, the stage of

Table 4: Distribution of HBV genotypes by age and gender.

	Male			Female			
GENOTYPES/ AGE GROUPS	HBV B (%)	HBV E (%)	HBV B+E (%)	HBV B (%)	HBV E (%)	HBV B+E (%)	TOTAL
<15	-	-	-	-	-	-	-
16-25	1(2.9)	6(17.6)	-	1(2.9)	-	-	8(23.5)
26-35	-	4(11.8)	-	1(2.9)	8(23.5)	2(5.9)	15(44.1)
36-45	1(2.9)	5(14.7)	-	-	2(5.9)	-	8(23.5)
46-55	-	1(2.9)	-	-	-	-	1(2.9)
≥56	-	1(2.9)	-	-	1(2.9)	-	2(5.9)
TOTAL	2(5.9)	17(50.0)	-	2(5.9)	11(32.4)	2(5.9)	34

the illness, especially in individuals with long-term inactive infections, can impact the results. Other factors like sporadic viremia or relatively low and undetectable HBV-DNA levels due to prior therapy or natural clearance might also play a role.

Several genotyping techniques for HBV include sequencing, INNO-LiPA, multiplex PCR, oligonucleotide microarray chips, restriction fragmentation polymorphism, reverse dot blot, serotyping, invasion assay, and real-time PCR. Each method possesses unique characteristics such as sensitivity, specificity, cost, and time requirements, setting them apart.

Out of the 34 samples that tested positive for HBsAg, 32 of them (94.1%) showed a single-genotype infection, while the remaining 2 samples (5.9%) exhibited a mixed infection with both genotypes E and B. Genotype E was the predominant infection, accounting for 27 cases (79.4%), whereas genotype B mono infections were less common, totaling 4 cases (11.7%), as illustrated in Table 4.

In this study, we employed the multiple-nested PCR technology, known for its high accuracy rate of 93%, increased sensitivity in detecting mixed genotypes, cost-effectiveness for large populations, and user-friendly simplicity [6]. Our focus was on the six primary genotypes (A-F) among the patients involved in this investigation.

The prevalence of single-genotype infections (94.1% vs. 5.9% mixed infections) aligns with findings in Eritrea, where genotype D was predominantly single-genotype, and with a study in Egypt, where 87% of patients exhibited single-genotype infections [8]. In contrast to a study in Zaria, Nigeria by Ahmad et al., in 2019 which reported a higher percentage of multiple mixed infections with genotype E combinations at 82.6% of the population. This study underscores the prevalence of single-genotype infections among the population.

Furthermore, chronic hepatitis B patients with multiple genotype infections exhibited higher viral levels than those with a single genotype, as noted by Odemuyiwa et al. (2001). They also demonstrated greater in vitro HBV replication rates. Furthermore, this study has confirmed that in this region of Nigeria, genotype E, genotype B, and the combination of HBV/B + E infection are the most prevalent genotypes. These findings align with the research conducted by Mustafa in 2014, which also identified genotype E as the predominant genotype in Nigeria.

It is worth noting that patients infected with HBV genotype B, especially those who are young and develop fulminant hepatitis, are at a higher risk of HCC recurrence. Additionally, the HBV B genotype tends to exhibit slow seroclearance, although seroconversion occurs more frequently and is associated with a better response to interferon-based therapy in chronic hepatitis, as highlighted in the study by Odemuyiwa et al. (2001).

On the other hand, the therapeutic implications of genotype E are not well understood. Some suggest that this genotype is the most challenging to treat and may require a longer duration of medication, as indicated by Coa (2009).

Conclusion

This study indicates that HBV is quite prevalent in Nigeria. It highlights the importance of raising awareness, developing policies to avoid mother-to-child transmission, and providing universal immunization for all children and adults who have not yet contracted the disease. This study also illustrates the prevalence of HBsAg overall and the genotype that predominates in Bayelsa State. The study area has a 10% prevalence of HBV, with genotype E being the most common with 82.4% of cases, HBV/B coming in second with 11.8%, and combined HBV B+E infections coming in next with 5.9%. Hence, it is a reasonable assumption that the effective implementation of interventions, such as early immunization and screening of high-risk individuals, could significantly reduce the burden of HBV, concurrently leading to improvements in Nigeria's socioeconomic indicators.

Author Statements

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References

1. Ahmad AE, Bakari AG, Musa BOP, Mustapha SK, Jamoh BY, Abdullahi IN et al. Pattern of prevalent hepatitis B virus genotypes in Zaria, Nigeria. Niger Postgrad Med J. 2019; 26: 80-6.
2. Musa BM, Bussell S, Borodo MM, Samaila AA, Femi OL. Prevalence of hepatitis B virus infection in Nigeria, 2000-2013: A systematic review and meta-analysis. Niger J Clin Pract. 2015; 18: 163-72.
3. Olayinka AT, Oyemakinde A, Balogun MS, Ajudua A, Nguku P, Aderinola M et al. Seroprevalence of hepatitis B infection in Nigeria: A national survey. Am J Trop Med Hyg. 2016; 95: 902-7.
4. Vilibić-Cavlek T, Kucinar J, Ljubin-Sternak S, Kaić B, Lazarić-Stefanović L, Kolarić B. Prevalence of viral hepatitis in Croatian adult population undergoing routine check-up, 2010-2011. Cent Eur J Public Health. 2014; 22: 29-33.
5. Frank-Peterside N, Ayodele MBO. Sero-prevalence of hepatitis B virus Infection among HIV Co-infected patients in Port Harcourt, Nigeria. NY Sci J. 2016; 9: 4-8.
6. Hamida ME, Raja SM, Petros Y, Wahab M, Seyoum Y, Elkhidir IM, et al. Prevalence of hepatitis B virus genotypes among patients with liver diseases in Eritrea. Sci Rep. 2021; 11: 11323.
7. Kahila Bar-Gal G, Kim MJ, Klein A, Shin DH, Oh CS, Kim JW, et al. Tracing hepatitis B virus to the 16th century in a Korean mummy. Hepatology. 2012; 56: 1671-80.
8. Khaled IAEA, Mahmoud OM, Saleh AF, Bioumie EE. Prevalence of HBV genotypes among Egyptian hepatitis patients. Mol Biol Rep. 2011; 38: 4353-7.
9. Cao GW. Clinical relevance and public health significance of hepatitis B virus genomic variations. World J Gastroenterol. 2009; 15: 5761-9.
10. Odemuyiwa SO, Oyedele OI, Forbi JC, Elemuwa CO, Ibeh MA, Kfutwah AK et al. Hepatitis B surface antigen (HBsAg) in the sera of medical, nursing and microbiology students in Ibadan, Nigeria. Afr J Med Med Sci. 2001; 30: 333-5.
11. Sunbul M. Hepatitis B virus genotypes: global distribution and clinical importance. World J Gastroenterol. 2014; 20: 5427-34.
12. World Health Organization. Consolidated guidelines on HIV testing services 2015; 2015 [cited Jan 22 2023]. Available from: https://www.who.int/publications/i/item/9789241508926-.
13. World Health Organization. Hepatitis B; 2022 [cited Jun 22 2022]. Available from: https://www.who.int/news-room/fact-sheets/detail/hepatitis-.
14. Kramvis A. Genotypes and genetic variability of hepatitis B virus. Intervirology. 2014; 57: 141-50.

15. Wang YX, Luo C, Zhao D, Beck J, Nassal M. Extensive mutagenesis of the conserved box E motif in duck hepatitis B virus P protein reveals multiple; 2012.

16. Sunbul M. Hepatitis B virus genotypes: global distribution and clinical importance. World J Gastroenterol. 2014; 20: 5427-34.

17. Lamontagne RJ, Sumedha B, Michael JB. Hepatitis B virus molecular biology and pathogenesis. Hepatoma Resour. 2016; 2: 163-86.

18. Liang TJ. Hepatitis B: the virus and disease. Hepatology. 2009; 49: S13-21.

19. Toyé RM, Loureiro CL, Jaspe RC, Zoulim F, Pujol FH, Chemin I. The hepatitis B virus genotypes E to J: the overlooked genotypes. Microorganisms. 2023; 11: 1908.

20. Mahtab MA, Rahman S, Khan M, Karim F. Hepatitis B virus genotypes: an overview. Hepatobiliary Pancreat Dis Int. 2008; 7: 457-64.

21. Pourkarim MR, Amini-Bavil-Olyaee S, Kurbanov F, Van Ranst M, Tacke F. Molecular identification of hepatitis B virus genotypes/subgenotypes: revised classification hurdles and updated resolutions. World J Gastroenterol. 2014; 20: 7152-68.