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Case report

Molecular detection of Hepatits B virus genotypes in tertiary hospitals in Yenagoa, Nigeria

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Abstract

Despite HBV being endemic or hyper endemic in Africa, there is paucity of data on the genotypes and their distribution in this part of the country. This study is aimed at detecting the prevalent genotypes of HBV among patients attending two tertiary hospitals in Yenagoa, Nigeria. A total of 656 patients [females 475(72.4%) and males 181(27.6%)] were tested for Hepatitis B surface antigen (HBsAg) between the period of January to June 2022. Blood samples collected were analyzed using immunochromatography techniques for HBsAg detection and multiplex Polymerase chain reaction (PCR) using type-specific primers for genotyping. Of the 656 patients screened for HBsAg, 66 (10%) [36, 5.4% female; 30, 4.6% male] were positive using immunochromatography and were then subjected to molecular genotyping using specific primers to A, B, C, D, E and F. Likewise, 33(50%) were positive using PCR while the remaining showed passive positivity as a result of degradation of the virus being a non-enveloped virus. This finding showed that HBV/E and HBV/B were the major identified genotypes with prevalence of 82.4% and 11.8% respectively in Yenagoa, Bayelsa State. HBV/B+E mixed infections was seen with a prevalence of 5.9%, found amongst 2 female subjects within age group 26-25. In the clinical sector in Yenagoa, tenofovir a nucleotide analog is used. It has been shown from previous studies that HBV E is more sensitive to nucleotide analogs while HBV B is more sensitive to interferon-based therapies. In conclusion, HBV genotypes B, E and B+E were discovered in Yenagoa, Bayelsa State.

Background of the study

The inflamed tissue of the liver is what medical professionals refer to as hepatitis. Some people who have hepatitis do not show any symptoms, while others develop jaundice, which is characterized by a yellow discoloration of the skin and the whites of the eyes, as well as an inability to eat, vomiting, weariness, abdominal pain, and diarrhea. Jaundice can also be caused by cirrhosis, which is characterized by a yellow discoloration of the skin and the whites of the eyes (WHO, 2022). If one has hepatitis and it goes away in less than six months, then you had acute hepatitis; however, if it lasts longer than six months, then one had chronic hepatitis. It is possible for acute hepatitis to clear up on its own, progress into chronic hepatitis, or even (very rarely) result in sudden liver failure. All of these outcomes are possible. Chronic hepatitis can lead to a number of serious complications, including scarring of the liver (sometimes called cirrhosis), liver failure and even cancer of the liver. Viral hepatitis is a term that refers to hepatitis that is caused by infection with any one of a wide variety of hepatotropic viruses (Lamontagne et al., 2016). These viruses include hepatitis A, B, C, D, and E. Hepatitis caused by a virus is a systemic illness that mostly manifests itself in the liver and is distinguished by an inflammation of the liver's primary tissue. These viruses are all RNA viruses, with the exception of the hepatitis B virus (HBV), which is a DNA virus. Other probable causes of hepatitis include: excessive use of alcohol, the use of certain medicines, exposure to chemicals, a variety of infections, autoimmune diseases and non-alcoholic steatohepatitis, which is also referred to as NASH (Wang et

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al., 2012).

Both acute and chronic hepatitis may be brought on by the hepatotropic, enveloped, non-cytopathic HBV virus. It exclusively infects humans and a small number of other primate species, exhibiting a limited host range. Hepatitis B virus (HBV) is a serious public health concern that can be fatal, even though there is a safe vaccination for it today. This is especially true in Asia, Africa, and South America. The clinical manifestations of HBV infection can range from the asymptomatic carrier state through acute hepatitis, fulminant hepatitis, chronic hepatitis, liver cirrhosis (LC), and hepatocellular cancer (HCC). Genetic traits of the host, viral characteristics, and environmental variables all have a role in the progression of chronic hepatitis B (CHB) illness to severe liver disorders including LC and HCC (Mustafa, 2014). All hepdnaviruses, like HBV, have the same distinct three morphologic forms, counterparts to the HBV envelope and nucleocapsid virus antigens, replicate in the liver but emerge in extrahepatic locations, encompass their own endogenous DNA polymerase, possess partially double-stranded and partly single-stranded genomes, and rely on a replicative strategy that is unique among DNA viruses but typical of opportunistic bacteria. Hepadnaviruses rely on reverse transcription (performed by the DNA polymerase) of minus-strand DNA from a "pregenomic" RNA intermediary rather than DNA replication straight from a DNA template. After that, the DNA-dependent DNA polymerase converts the plus-strand DNA from the minus-strand DNA template into a covalently closed circular DNA in the hepatocyte nucleus, which is used as a blueprint for messenger RNA and pregenomic RNA. The messenger RNA translates viral proteins, which are then packed into virions and released from the hepatocyte together with the viral DNA. Although HBV is challenging to grow in vitro in the traditional sense from clinical material, HBV DNA has been transfected into a number of cell lines. These transfected cells enable the in vitro replication of the whole virus and its constituent proteins (Jules and Kurt, 2012).

Healthcare issues related to hepatitis B are widespread, particularly in areas with a lower level of economic development. It is estimated that one third of all persons on the earth have the hepatitis B virus in their bodies. (Ahmad et al., 2019) and only 0.5 percent can spontaneously seroconvert from having the hepatitis B surface antigen (HBsAg) to having the hepatitis B surface antibody each year. This means that roughly 350–400 million people are living with a lifetime chronic infection. Chronic hepatitis B infection can lead to liver cirrhosis (LC) and hepatocellular carcinoma (HCC), however cirrhosis only develops in a tiny fraction of individuals. Hepatocellular carcinoma (HCC) development and on occasion cirrhosis, are side effects of hepatitis B. Numerous dermatologic, cardiac, joint, neurologic, hematologic, and gastrointestinal (GI) tract symptoms are observed, along with glomerulonephritis and polyarthritis nodes. Since the 1970s, significant progress has been achieved in the areas of knowledge relevant to the epidemiology, virology, natural history, and treatment of the hepatitis B virion, which is a hepatotropic viral particle. These areas of study concern the infectious agent that causes hepatitis B. In addition, it has been demonstrated that continuing vaccination programs are effective in reducing the prevalence of HBV disease in a number of different nations around the world (Anna, 2014).

The virus can be transmitted by exposure to infectious blood or body fluids (through child birth or from contact with other people's blood). Intravenous drug use, tattooing, acupuncture, blood transfusion, dialysis, sexual intercourse are the most frequent routes of infection. The virus cannot be spread by holding hands, sharing eating utensils, kissing, hugging, coughing, sneezing or breastfeeding (CDC, 2015)

Recent studies have led to the discovery of eleven different HBV genotypes (A through J), each of which is found in a specific region of the world. To correctly classify a person's genotype, the differences in their genome sequence must be at least 8%. E is the genotype that is found in the most people in Sub-Saharan Africa, followed by A and D. Genotype D is the one that occurs the least frequently. Both the clinical outcome and the patient's response to therapy based on interferon are significantly influenced by the HBV genotype. Interferon-based therapy is one such example. In spite of the fact that genotypes A and B have higher rates of spontaneous HBeAg seroconversion in comparison to genotypes C and D, infections with HBV genotypes A and D typically progress further into the chronic phase than infections with HBV genotypes B and C do. This is due to the fact that infections with HBV genotypes A and D have a higher risk of developing hepatocellular carcinoma. The HBV genotype E that is most prevalent in West Africa is only marginally related to pre-core and basal core promoter alterations, and it also has a poor response to interferon-based therapy. This genotype is responsible for the majority of HBV infections in the region. Additionally, recent investigations conducted elsewhere have revealed rare HBV mixed genotype infections, which may indicate similar clinical results Additionally, recent investigations conducted elsewhere have revealed rare HBV mixed genotype infections, which may indicate similar clinical results (Ahmad et al., 2019).

Aim

This study was aimed to pinpoint the HBV genotypes that are most common in Yenagoa, Bayelsa State, Nigeria.

MATERIALS AND METHODS

Study area

Between Delta state and Rivers state, in the central Niger Delta region of Southern Nigeria, sits the state of Bayelsa. On October 1, 1996, the military regime of General Sani Abacha separated the state from Rivers state. Eight Local Government Areas make up the state of Bayelsa. The mainstay of the economy in the state the government. The state is heterogeneous in nature with cultural varieties among the inhabitants in Sagbama and Ekeremor; Ogbia, Nembe, Brass and Yenagoa, Kolokuma-Opokuma and Southern Ijaw respectively. There are eight local government in Bayelsa state which include: Yenagoa, Ogbia, Brass, Nembe, Kolokuma/Opokuma, Southern Ijaw, Sagbama and Ekeremor local government. While Yenagoa stood as the capital of Bayelsa state. The population of Bayelsa state is put at about 1.7million people as at the 2006 census. Geographically, Bayelsa state is on latitude 04o151North, 05o231South and longitude 05o221West and 06o451East. The state is bounded with Delta State on the North, Rivers State on the East and the Atlantic Ocean on the West and South as shown in. This work was carried out in two selected health facilities in Yenagoa, Bayelsa State between the period of January and June, 2021. Most people residing in Bayelsa state are government workers. Other occupation of the people in the state includes: farming, trading and as well fishing.

Study Population

All patients that are sent to the laboratory to test for HBsAg make up the study population.

Ethical Approval

A letter of approval was issued by the H.O.D of the department of Medical Laboratory Science NDU, which was presented to the Ethics and research Committee of the Federal Medical Centre and Niger Delta University Teaching Hospital in Bayelsa state after which their approval for this study was given.

Inclusion criteria

All patients who present themselves to the laboratory department for HBsAg test and those attending the gastroenterology clinics of both hospitals were recruited for this research.

Exclusion criteria

All patients who are not running HBsAg test at the laboratory department and those not attending the gastroenterology clinics of both hospitals were excluded from this study. Also those who rejected verbal consent were excluded.

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. Plasma was used for screening of HBsAg, HIV and HCV while the red cells were taken to the molecular laboratory in NDU in ice packs for DNA extraction and genotyping.

Sample size

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

$$N = \frac{Z2P(1-P)}{d2}$$

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% (Olayinka et al., 2016).

N= Minimum sample size

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

P= Prevalence

N =656

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 40°C for extraction.

Laboratory Methods and Procedures

HBsAg Status Determination

The HBsAg status was determined using skytec test kits according to manufacturer's instructions following WHO standard alogarithm (WHO, 2015).

Test Procedure

The test card's protective foil cover was taken off, and 50ul of plasma was put to the sample pad before being allowed to flow. After 15 minutes, the result was read.

HCV Status Determination

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The Hcv status was determined using skytec test kits according to manufacturer's instructions following WHO standard alogarithm (WHO, 2015).

Test Procedure

The test card's protective foil cover was taken off, and 50ul of plasma was put to the sample pad before being allowed to flow. After 15 minutes, the result was read.

HIV Status Determination

The HIV status was determined using Determine test kits according to manufacturer's instructions following WHO testing allogarithm (WH0, 2015)

Test Procedure

The test card's protective foil cover was taken off, 50ul of whole blood was applied to the sample pad along with one drop of chase buffer. Results were read 15 to 60 minutes later.

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. Result were read after 10 minutes.

The process of extracting DNA from a blood sample

A total volume of 100 uL of blood was treated with 400 uL of the Genomic Lysis Buffer. After that, it was thoroughly combined by vortexing for four to six seconds, and then it was permitted to sit at room temperature for five to ten minutes. After that, the mixture was moved to a Zymo-Spin IIC Column that was contained inside of a collecting Tube. The centrifuge was set to 10,000 x g, and it ran for one whole minute. It was decided to throw away the Collection Tube that had the flow through. The Zymo-Spin IIC Column was subsequently moved to a different Collection Tube after the move. After adding 200 ul of DNA Pre-Wash Buffer to the spin column, it was then centrifuged at a speed of 10,000 x g for a period of one minute. After adding 500 uL of g-DNA Wash Buffer to the spin column, it was then subjected to one minute of centrifugation at 10,000 x g. After that, the spin column was moved to a microcentrifuge tube that had been cleaned. The spin column received an additional 50 uL of DNA Elution Buffer. After that, it was allowed to sit at room temperature for two to five minutes before being centrifuged at maximum speed (10,000xg) for thirty seconds in order to extract the DNA. The eluted DNA was kept frozen at a temperature of -20 degrees Celsius for later usage.

DNA Quantification

Using the Nanodrop 1000 spectrophotometer, the genomic DNA that had been extracted was measured and analyzed. The software of the apparatus was activated by performing a double click on the Nanodrop icon on the desktop. The apparatus was calibrated using 2 ul of sterile distilled water, and then it was blanked with normal saline solution. Following the transfer of two microliters of the extracted DNA to the lower pedestal, the DNA on the lower pedestal was brought into contact with the DNA on the upper pedestal by lowering the upper pedestal. The amount of DNA in the sample was determined by selecting the "measure" button on the toolbar. While the level of DNA purity was assessed by the 260/280 absorbance ratio, DNA concentration was quantified in ng/ul (ranging from 5 to 100 ng/ul) (1.5 – 2.0).

Multiplex nested polymerase chain reaction (PCR)

This was 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: Hepatitis B virus DNA detection.

During the initial round of the nested PCR, the total volume of the reaction that was carried out was 20 L. The sample ID was written on the label of each of the premix tubes. After the DNA was extracted, it was put into a Master Mix, which consisted of a cocktail of 16 uL of deionized water [D.H2O] and a premix of 250 M of each dNTP, 1X PCR buffer, 15 mM of MgCl2, and 1U of thermostable Taq polymerase, along with 1uL of P1 (forward) and S1 2 (reverse) outer primers in equal amounts. The PCR was carried out with a thermal cycler, and the reaction conditions were as follows: initial activation at 95 degrees Celsius for five minutes; denaturation at 94 degrees Celsius for twenty seconds; annealing at 60 degrees Celsius for thirty seconds; and extension at 72 degrees Celsius for one minute. The temperature of the first six cycles was lowered by a total of –0.50 C, while the temperature of the subsequent 29 cycles was maintained at 570 C. There were a total of 35 whole cycle sets that were observed, going from denaturation through extension. The third and final extension was carried out at 72 degrees Celsius for five minutes.

Second round polymerase chain reaction: Hepatitis B virus genotyping

The second round of PCR was carried out in two separate tubes for each sample. The first tube contained the common universal sense primer (B2) and type specific primers for the genotypes A, B, and C. The second tube contained the common universal anti sense primer (B2R)

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

Primer	Sequence (5'-3')	Specificity	Position	Polarity
1st round PCR				
P1	TCACCATATTCTTGGGAACAAGA	Universal	2823- 2845	Sense
S1-2	S1-2 CGAACCACTGAACAAATGGC	Universal	685- 704	Antisense
2nd round PCR: Mix A				
B2	GGCTCCAGTTCCGGAACAGT	Type A-E	67- 86	Sense
BA1R	CTCGCGGAGATTGACGAGATGT	Type A	113- 134	Antisense
BB1R	GGTCCTAGGAATCCTGATGTTG	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	CACCAGAAATCCAGATTGGGACCA	Type E	2955- 2978	Sense
BF1	GTTACGGTCCAGGGTTACCA	Type F	3032-3051	Sense

and genotype specific primers for the genotypes D, E, and F. The second round of PCR was carried out in two different tubes for each sample. Each tube containing premix 'A' and 'B' received an additional 17 uL of water that had been distilled. The mixes each received an additional 0.5 ul of each of the four primers, which brought the total volume to 2 ul of primers total. 1 u of the product of the first PCR round should be added to each tube of the premix. After being stirred carefully, the mixture was centrifuged. The PCR conditions were kept the same as before. After running 20 uL of each of the negative control, samples, and the ladder across an agarose gel containing 2%, the gel was then electrophoresed in a buffer containing 1 x TAE 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 a period of 30 minutes. We were able to determine the size of the bands as well as the fragment size by comparing the bands to a DNA ladder that was 100 kb or longer.

Analyses of statistical data

The Statistical Package for the Social Sciences (SPSS) version 21 was utilized in order to perform the analysis on the data that was gathered from this study. Chi-square tests were used to make comparisons between the variables. A statistically significant p-value was determined to be lower than 0.05. Tables, pie charts, and percentage breakdowns were used to show the findings.

RESULTS

Demographic presentation of participants

Of a total of 656 subjects screened for HBsAg, 475 (72.4%) were females while their male counterpart had 181 (27.6%) participants. 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 less than 15 respectively.

Of the 656 participants, 66 (10%) were positive to HBsAg with females having 36 (5.4%) positive subjects and 439 (66.9%) negative. Male participants had 30 (4.6%) positive subjects with 151 (23.0%) negative which were not included for further studies.

Prevalence of HBsAg by age.

Of the 656 participants, 37 (5.6%) subjects within the age group 26-35were HBsAg positive, 14 (2.1%) within age group 36-45, 9 (1.4%) in age group 16-25,1 (0.1%) in age group >56, 3(0.5%) in 46-55 and finally 2 (0.3%) in age group > 15 were positive to HBsAg respectively.

Using the P-value of <0.05 indicating significant variations, there is no significant association between the age groups and HBsAg positivity (P = 0.097).

Prevalence of HBsAg by gender.

Of the 656 subjects in this study, female population were 36 (5.5%) while male was 30 (4.5%) positive HBsAg participants.

Table 3.1: *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

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Table 3.2: *Prevalence of HBsAg by gender and age*

		MALE		FEMALE		
Age groups	TOTAL N.E (%)	N.E(%)	N.I(%)	N. E (%)	N.I(%)	TOTAL N.I (%)
<15	30 (4.6)	11 (1.7)	1 (0.2)	19 (2.9)	1 (0.2)	2 (0.3)
16-25	118 (18.0)	28 (4.3)	4 (0.6)	90 (13.7)	5 (0.8)	9 (1.4)
26-35	301 (45.9)	50 (7.6)	12 (1.8)	251 (38.3)	25 (3.8)	37 (5.6)
36-45	120 (18.3)	40 (6.1)	10 (1.5)	80 (12.2)	4 (0.6)	14 (2.1)
46-55	38 (5.8)	25 (3.8)	2 (0.3)	13 (2.0)	1 (0.2)	3 (0.5)
≥56	49 (7.5)	27 (4.1)	1 (0.2)	22 (3.4)	-	1 (0.2)
TOTAL	656	181 (27.6)	30 (4.6)	475 (72.4)	36 (5.5)	66 (10)

N.E: Number Examined

N.I: Number Infected

Table 3.3: *Prevalence of HBsAg by age.*

Age group	Total No. Examined (%)	Negative (%)
<15	30 (4.6)	28 (4.3)
16-25	118 (18.0)	109 (16.6)
26-35	301 (45.9)	264 (40.2)
36-45	120 (18.3)	106 (16.2)
46-55	38 (5.8)	35 (5.3)
≥56	49 (7.5)	48 (7.3)
Total	656	590 (90.0)
Chi square value	4.667	-
p-value	0.097	-

Using the P-value of <0.05 indicating significant variations, there is no significant association between the age groups and HBsAg positivity (P = 0.097).

Table 3.4: *Prevalence of HBsAg by gender*

Gender	Positive (%)	Negative (%)	Total (%)
Male	30 (4.5)	151 (23.0)	181 (27.6)
Female	36 (5.5)	439 (66.9)	475 (72.4)
Total	66 (10.0)	590 (90.0)	656
Chi square value	0.091	-	-
p-value	0.763	-	-

Using the P-value of <0.05 indicating significant variations, there is no significant association between gender and HBsAg positivity (P = 0.763).

Table 3.5: *Distribution of HBsAg among subjects by age and gender (using 5 panel kit).*

Age groups	MALE		FEMALE		
	N.E (%)	N.I (%)	N.E (%)	N.I (%)	TOTAL
<15	1 (1.5)	1 (1.5)	1 (1.5)	1 (1.5)	2 (3.0)
16-25	8 (12.1)	8 (12.1)	3 (4.6)	3 (4.6)	11 (16.7)
26-35	12 (18.2)	12(18.2)	26 (39.4)	26(39.4)	38 (57.6)
36-45	7 (10.6)	7 (10.6)	3 (4.6)	3 (4.6)	10 (15.2)
46-55	3(4.6)	3 (4.6)	-	-	3 (4.6)
≥56	1 (1.5)	1 (1.5)	1 (1.5)	1 (1.5)	2 (3.0)
TOTAL	32 (48.5)	32 (48.5)	34 (51.5)	34(51.5)	66

N.E: Number Examined

N.I: Number Infected

Table 3.6: *Distribution of HBsAb among the subjects by age and gender.*

Age groups	MALE		FEMALE		
	N.E (%)	POSITIVE (%)	N.E (%)	POSITIVE (%)	TOTAL
<15	1 (1.5)	-	1 (1.5)	-	2 (3.0)
16-25	8 (12.1)	-	3 (4.6)	-	11 (16.7)
26-35	12 (18.2)	-	26 (39.4)	-	38 (57.6)
36-45	7 (10.6)	-	3 (4.6)	-	10 (15.2)
46-55	3 (4.6)	-	0	-	3 (4.6)
≥56	1 (1.5)	-	1 (1.5)	-	2 (3.0)
TOTAL	32 (48.5)	-	34 (51.5)	-	66

N.E: Number Examined

Table 3.7: *Distribution of HBeAg among the subjects by age and gender*

Age groups	MALE		FEMALE		
	N.E(%)	Positive (%)	N.E(%)	Positive (%)	TOTAL
<15	1 (1.5)	-	1 (1.5)	-	2 (3.0)
16-25	8 (12.1)	-	3 (4.6)	-	11 (16.7)
26-35	12 (18.2)	-	26 (39.4)	-	38 (57.6)
36-45	7 (10.6)	-	3 (4.6)	-	10 (15.2)
46-55	3 (4.6)	-	0	-	3 (4.6)
≥56	1 (1.5)	-	1 (1.5)	-	2 (3.0)
TOTAL	32 (48.5)	-	34 (51.5)	-	66

N.E: Number Examined

Table 3.8: *Distribution of HBeAb among the subjects by age and gender*

Age groups	MALE		FEMALE		
	N.E (%)	Negative (%)	N.E (%)	Negative (%)	TOTAL
<15	1 (1.5)	-	1 (1.5)	-	2 (3.0)
16-25	8 (12.1)	1 (1.5)	3 (4.6)	2 (3.0)	11 (16.7)
26-35	12 (18.2)	1(1.5)	26 (39.4)	3(4.5)	38 (57.6)
36-45	7 (10.6)	1 (1.5)	3 (4.6)	-	10 (15.2)
46-55	3 (4.6)	1(1.5)	-	-	3 (4.6)
≥56	1 (1.5)	-	1 (1.5)	1 (1.5)	2 (3.0)
TOTAL	32 (48.5)	4 (6.1)	34 (51.5)	6 (9.1)	66

N.E: Number Examined

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Table 3.9: *Distribution of HBcAb among the subjects by age and gender*

Age groups	MALE		FEMALE		
	N.E (%)	Positive (%)	N.E(%)	Positive (%)	TOTAL
<15	1 (1.5)	1 (1.5)	1 (1.5)	1 (1.5)	2 (3.0)
16-25	8 (12.1)	8 (12.1)	3 (4.6)	3 (4.6)	11 (16.7)
26-35	12 (18.2)	12 (18.2)	26 (39.4)	25 (37.9)	38 (57.6)
36-45	7 (10.6)	7 (10.6)	3 (4.6)	3 (4.6)	10 (15.2)
46-55	3 (4.6)	3 (4.6)	-	-	3 (4.6)
≥56	1 (1.5)	1 (1.5)	1 (1.5)	1 (1.5)	2 (3.0)
TOTAL	32 (48.5)	32 (48.5)	34 (51.5)	33 (50.0)	66

N.E: Number Examined

Table 3.10: *Interpretation of 5 panel test result*

Marker	Result	Interpretation
HBsAg **HBcAb** **HBsAb**	Negative Negative Negative	Susceptible
HBsAg **HBcAb** **HBsAb**	Negative Positive Positive	Immune due to natural/ resolved infection
HBsAg **HBcAb** **HBsAb**	Negative Negative Positive	Immune due to hepatitis B vaccination
HBsAg **HBcb** **HBsAb**	Positive Positive Negative	Acute/chronic infection
HBsAg **HBcAb** **HBsAb**	Negative Positive Negative	1. Resolved infection 2. False-positive HBcAb
HBeAg	Positive	Active viral replication
HBeAb	Positive	Seroconverted

Table 3.12: *COMPARISION OF HBV serology (HBsAg) and PCR*

	HBsAg		PCR	
	N.E (%)	POSITIVE (%)	N.E (%)	POSITIVE (%)
MALE	32 (48.5)	32 (48.5)	32 (48.5)	19 (28.8)
FEMALE	34 (51.5)	34 (51.5)	34 (51.5)	15 (22.7)
TOTAL	66	66	66	34 (51.5)

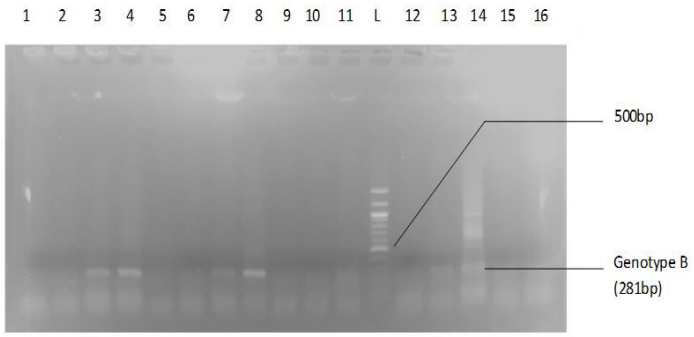

Plate 1: Agarose gel electrophoresis showing the hepatitis B genotype B. Lanes 3, 4 7,8, and 14 showing the genotype B bands at 281 bp while lane L represents the 100bp molecular ladder.

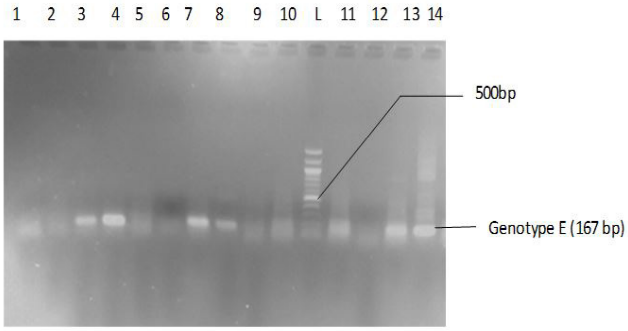

Plate 2: Agarose gel electrophoresis showing the hepatitis B genotype B. Lanes 3,4, showing the genotype B bands at 281 bp while lane L represents the 100bp molecular ladder.

Table 3.11: *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

Using the P-value of <0.05 indicating significant variations, there is no significant association between gender and HBsAg positivity (P = 0.763).

Distribution of HBV using the 5-panel testing kit

The median age for the 66 positive HBsAg participants were 31 (26-35) years of which 36 (54.6%) were female while males were 30 (44.5%). Age group 26-45 had 37 (5.6%) positive participants followed by 36-45 with 14 (2.1%), then 16-25 with 9 (1.4%). 0-15 had 2 (0.3%) followed by ≥56 with 1 (0.1%).

Of the 66 positive HBV patients, 34 (51.5%) were female

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while male was 32 (48.5%). Age group 26-35 had 38 (57.6%) followed by 16-25 with 11 (16.7%) then 36-45 with 10 (15.2%). Age groups 16-25 and 36-45 both had 11 (16.7%) and 10 (15.2%) respectively while 0-15 and ≥56 had 2 (3.0%) each.

All the 66 were negative for HBsAb as well as HBeAg. For HBeAb age group 26-35 had 4 (6.1%) negative followed by age group 16-25 with 3(4.6%), ≥ 56 with 1(1.5%) and then 0-15 with 46-55 both had zero respectively.

For HBcAb only age group 26-35 had one negative with 1.5%.

HBV genotypes.

34 (51.5%) of the 66 samples that tested positive for HBsAg also tested positive for HBV-DNA. Participants in the study who tested positive for HBsAg and HBV-DNA have bands representing their genotypes visible on the electrophoretogram. The primers' Mix 'A' bands were displayed below, and Mix 'B' bands were displayed on the second gel. In Mix "A," genotypes B were found with band sizes of 281, and in Mix "B," genotypes E were found with band sizes of 167 bp. The marker for identification was a molecular ladder (L) of 100 plus base pairs (100 bp+).

Distribution of HBV genotype by age and gender

Amongst the 34 HBV-DNA positive samples it could be seen that in Yenagoa 28 (82.4%) subjects had HBV E genotype while HBV B and HBV B + E mixed infection had 4 (11.8%) and 2 (5.9%) respectively. There were more male participants with 19 (55.9%) to females with 15 (44.1%).

Comparison of HBV serology (HBsAg) and PCR

It can be seen from the table below, that not all samples that were positive for HBsAg were positive for HBV PCR. Out of 66 positive HBsAg samples only 34 (51.5%) were HBV-DNA positive out of which males were 16 (28.8%) while females were 15 (22.7%).

Discussion

Nigeria has achieved great strides in each of the four areas of the WHO's four-pronged strategy, which it adopted in 2010 to acknowledge viral hepatitis as a problem of international health. These include keeping tabs on cancer cases linked to hepatitis, creating national guidelines for healthcare workers' infection control, enforcing the vaccine requirement, and screening all donated blood. The strategy was adopted to acknowledge viral hepatitis as a global health problem. On the other hand, there are no national policies in place to prevent infections from being passed down from mothers to children or to eradicate HBV; this is perhaps reflected in the findings of Musa et al., 2015, in which it was revealed that between 2000 and 2013, 14% of Nigerians were exposed to HBV. According to this estimation, Nigeria is among the nations with the highest prevalence of the disease, making it extremely endemic not just in Africa but also worldwide. This study places the prevalence of HBV in Yenagoa at 10% (table 4.2), and this result is consistent with that of Olayinka et al., (2016), who estimated that 12.2% of people have HBV. This high incidence may have certain explanations, some of which include the facts that HBV infection is not a disease that is widely identified in Africa, that infections are subclinical, and that there is a substantial length of time before the effects of chronic carrying become apparent.

An infection with hepatitis B is a condition that can be prevented by receiving a vaccination. When Nigeria's national vaccination schedule was updated in 1995, the vaccine was added, and it became available to the general public in 2004. As can be seen in table 4.1, the participants in this research project ranged in age from 1 year to 31.0 years, with the median age falling somewhere in the range of 31.0 to 35.0 years.

According to the findings of this research, the prevalence of HBV was higher among female participants than among male participants. This contradicts the findings of past study that suggested men were more prone to infection than women because of the qualities associated with being a man. According to Vilibic et al., 2014, this finding is another evidence that sexual activity and HBV infection are not related to one another.

According to Frank-Peterside and Ayodele in 2016, the higher female to male ratio could be explained by the fact that more females than men visit hospitals for medical care. This may be connected to the high proportion of female participants found in this study as it may be related to the fact that hospital visits by patients are more common among women than men. Despite the fact that there was no significant correlation between gender and HBsAg positivity, sexual orientation has been demonstrated to be a significant risk factor for HBsAg positivity (Frank-Peterside and Ayodele, 2016).

The highest prevalence of HBsAg to age group as shown in this study was between age group 26-35 with frequency of 37 (5.6%) followed by 36-45 with 14 (2.1%) as shown in Table 4.3, this is also in agreement with Frank-Peterside and Ayodele in 2016 where the highest frequency was recorded in age group 31-40 and 20-30 years and attributed to high and unsafe sexual activities among people within these age groups (Frank-Peterside and Ayodele, 2016). Additionally, it has been stated that Nigerians in their third decade of life use intravenous drugs at high rates, this could be another

reason for the high prevalence of HBV infection within this age group. Needle-stick injuries and reusable razor blades can also be a major predisposing factor.

The disappearance of HBeAg or appearance of HBeAb in serum does not completely rule out chronic HBV carrier state or infectivity. From this study and the above table, it can be seen that all the patients recruited for this study were in the chronic phase of the infection

HBcAb is the first detectable antibody in the course of HBV disease a positive reaction is an indication of acute, past or recurrent infection and usually persist for life (Kahila et al., 2012). In this study only one subject within age group 26-25 was negative for HBcAb which is an indication of an already cleared HBV infection.

Despite PCR being the second-gold standard to sequencing 51.5% (34/66) of the samples used in this investigation were able to show the presence of HBV infection and have its genotype determined. This might be the result of sample storage (deterioration) prior to analysis, sensitivity of the primers, or something unrelated to the technique. No matter the method of detection, Ahmad et al., 2019 found that the different methodologies applied maintained the variability in HBV-DNA isolation in relation to HBsAg positivity, in that not all HBsAg-positive samples yielded positive results for HBV-DNA detection, irrespective of the method of detection. This is due to the fact that HBV is an unencapsulated virus, its DNA tends to break down quickly. A contributing element might also be the stage of the illness, as in the case of individuals who have been long-term carriers of an inactive infection. Additionally, it might be brought on by sporadic viraemia or relatively low and undetectable HBV-DNA levels as a result of prior therapy or natural clearance.

Multiplex PCR was used because it has higher accuracy (93.2%) as compared to the RFLP method (87%), it is rapid, simple and cost effective. It can be used on large population of study/samples. This method can detect mixed genotypes with sensitivity for detecting minor species as low as 10%.

Sequencing, INNO-LiPA, multiplex PCR, oligonucleotide microarray chips, restriction fragmentation polymorphism, reverse dot blot, serotyping, invasion assay, and real time PCR are a few of the genotyping techniques for HBV that have been developed. Each is unique from the others in terms of sensitivity, specificity, cost, and time.

Out of the 34HBsAg-positive samples, 32 (94.1%) had a single genotype infection, while the remaining 2 (5.9%) had a mixed HBV genotype of E and B infection. According to the trend of infections, genotype E was more common, with 27 infections (79.4%), while genotype B mono infections were less common, with 4 infections (11.7%).

Utilizing the multiple-nested PCR technology, which has a high accuracy rate of 93%, increased sensitivity for the detection of mixed genotypes, cost-effectiveness for large populations, and simplicity of use(Hamida et al., 2021), we concentrated on the six primary genotypes (A-F) among patients in this investigation.

Single genotype was found to have a 94.1% (32/34) to 5.9% (2/34) advantage over mixed infection. This is in consistent with Hamida's findings from Eritrea, where single genotype D infection was most prevalent, and with a study from Egypt, where 87% of patients were found to have single-genotype infection (Khaled et al., 2011). Contrary to a study conducted in Zaria, Nigeria, where multiple mixed infections with genotype E combination comprised 82.6% of the population, this study shows that single genotype accounts for a larger proportion of the population. Chronic hepatitis B patients with multiple genotype infections had greater viral levels as compared to people with a single genotype. According to Coa, 2009, they also have greater in vitro HBV replication rates (Coa, 2009).

Furthermore, this study was able to establish that genotype E, genotype B, and combination HBV/B + E infection were the most common genotypes in this part of Nigeria. This validates research by Ahmad et al., 2019 showing genotype E was the more prevalent genotype in Nigeria.

Patients with HBV B infections who are young and have fulminant hepatitis are more likely to experience a recurrence of HCC. The HBV B genotype also exhibits sluggish seroclearance, although seroconversion is more frequent and is associated with a better response to interferon-based therapy in chronic hepatitis (Coa, 2009). The therapeutic significance of genotype E is little understood, but it has been claimed that this genotype is the most challenging to cure and necessitates a longer term of medication (Mustafa, 2014).

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 all children and adults who have not yet contracted the disease with a universal immunization. This study also illustrates the prevalence of HBsAg overall and the genotype that predominates in Yenagoa. Yenagoa 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%.

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Recommendation

Therefore, it is reasonable to predict that the efficient application of interventions like early immunization and screening of high-risk people might considerably lower the burden of HBV while simultaneously improving Nigeria's socioeconomic indicators.

Contribution to knowledge

This study has portrayed the overall prevalence of HBsAg in Yenagoa as well as its predominant genotype.

It has also portrayed the importance of HBV genotypes which should be incorporated into testing algorithm for HBV screening and treatment.

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