Original Research Article

BIOMARKER AND GENETIC VARIATION AMONG HEPATITIS B VIRUS POSITIVE PATIENTS ATTENDING SELECTED HEALTH FACILITIES IN BAUCHI METROPOLIS, NIGERIA

ABSTRACT

Aim of the study: The aim of this study is to determine biomarker and genetic variation among hepatitis B virus positive patients in Bauchi metropolis, Nigeria.

Study design: This is a cross-sectional and laboratory based study.

Place and duration of the study: The study involved patients attending two tertiary hospitals and one primary health care centre in Bauchi metropolis, from January 2024 to July 2024.

Methodology: A total of 200 samples were collected via vein from random participants, structured questionnaire and consent forms were issued to each participant for signing and data collection. The sample were screened for Hepatitis B surface antigen using one step rapid diagnostic test, the positive samples were further investigated for other hepatitis B virus biomarkers using Hepatitis B combo cassette.DNA of the positive samples were extracted and amplified using nested multiplex polymerase chain reaction(PCR), the product was ran on Gel electrophoresis and the DNA bands were visualized using Gel documentation system.

Results: The overall prevalence rate of Hepatitis B virus infection according to this study was 9%(18), the rate is higher in males 55.6% (10) than females 44.4%(08) within the age range 21-30 (33.3%), followed by 27.2% and 5.6% for 41-50 and 51-60 years ranges. The risk factors analysis showed those that have infected family members have the highest prevalence 42.3%(11) followed by those that have the history of barbing tools sharing 38.4(10) and lowest was observed among vaccinated participants (3.8%). Among the other biomarkers detected, HBcAb has the highest rate 72.3%(13) of occurrence followed by 66.7%(12), 55%(10) and 5.5%(01) for HBeAb, HBsAb and HBeAg respectively. Genotype A frequency of appearance was the highest among the genotypes detected with prevalence rate 38.5%(05) followed by 30.8%(04) for both genotype E and B, while genotype C and D were not detected in this study. Mixed and Mono infections account for 50%(04) and 37.5%(03) respectively. The combinations of the mixed infection were ABE(25%), BE(12.5%), and AE(12.5%). Statistically, the biomarkers, genotypes and

risk factors have no significant difference within their parameters.
Conclusion: The overall prevalence portrayed in this research was 9% mostly among male participants within the age range 21-30, the infection is less common among vaccinated participants.

Key words. Hepatitis, virus, Genetic, biomarkers

1. INTRODUCTION

Hepatitis B virus (HBV) infection is a global public health problem presence in almost every part of the world but more pronounce in low and middle income countries, out of almost 8 billion world population about 297 million people are living with hepatitis B virus infection, 68% of infected people are in Africa and West pacific Asia, in low income countries across the globe 5-15% of the population are chronically infected [1,2]. The infection is hyper endemic in Africa, being covered 12% of the world population, 18% of people living with HBV are from Africa, and 2% of the total death is related to liver related problems such as cirrhosis and liver cancer [3,4]. Despite availability of antiviral medication that shows efficacy and effective vaccine, hepatitis B virus infection is world major health problem especially in low income communities due lack of proper personal hygiene and nutrition [5,6].Nigeria is among the countries with high HBV rates regarded as hyper-endemic area, the prevalence differs across demography and geographical area, the prevalence rate in Nigeria is put at 13.6% averagely. Different data analysis showed average of 14.1% prevalence among pregnant women and 14% among blood donors[7]. Hepatitis B virus (HBV) belongs to family Hepadnaviridae, genus orthohepnavirus, DNA virus with 42nm diameter, encoat by lipoprotein hence hepatitis B surface antigen(HBsAg) [8,9]. The incubation period of hepatitis B virus is 30-180 days [10]. Hepatitis B virus has capacity to cause liver cirrhosis, hepatic decomposition and hepatocellular carcinoma, in those that acquired the virus early in life, estimated life time risk is 25%-40% [11]. Hepatitis B virus infection is very contagious easily transmitted via body fluids such as blood, semen, saliva, hugging [12]. Blood transfusion, needle sticks and sexual contact are part of the transmission line but the efficiency of the transmission depend on the number of factors which include virus concentration, volume of the inoculums, duration of the exposure, type of transmission and susceptibility of the contact[13]. Those at risk are infants born to mothers with hepatitis B, people born in certain countries where hepatitis B is common, unvaccinated people, people with hepatitis C, people who have been incarcerated, people who inject drugs or share needles, syringes, and other types of drug equipment, sex partners, people with HIV infection, gays, those living with infected people, health care and public safety workers exposed to blood on the job and people on dialysis [14]. Perinatal transmission from Hepatitis B e antigen(HBeAg) positive mothers lead to chronic infection, neonates frequently clear the virus when the transmission is from HBeAg negative mother [15].

The HBV biomarkers which include immunoglobulin M antibody, hepatitis B core antigen (IgM anti-HBc), immunoglobulin G antibody, hepatitis B core antigen (IgG anti-HBc);Hepatitis B e antigen(HBeAg) and its corresponding antibody, anti-HBe, hepatitis B surface antigen(HBsAg) and its corresponding antibody, that can be detected serologically and HBV DNA and recently HBV RNA are possible markers helps to improve the general management and understanding of HBV prevention and treatment [16]. Currently there is lack of novel and predictive makers of Hepatitis B virus, the detection of each of the available marker is significant in number of ways but has limitation in prediction of treatment response, risk and disease outcome, for better understanding of nature and pathogenesis of the disease, source of the infection and critical patient information and effective way of management need to be explored [17].

Hepatitis B virus is highly heterogeneous, this is due to rapid mutation that include precore/ core promoter mutation and S/S deletion mutation in the genome of the virus, the

heterogeneity influences the pathogenicity of the virus as well as the response to antiviral action, immune response and complete clinical outcome [18]. Genotypes, subgenotypes, HBV mutation in preS regions and pre core regions are the genomic variations in hepatitis B virus and are associated to development of cirrhosis and cancer of the liver [19,20]. So far ten genotypes of HBV named A to J each prevalent in certain geographic location have been recognized [21]. Differences in function and structure among genotypes can influence the severity and clinical outcomes of HBV infection as well as complications associated with differences in response to antiviral therapy, infection by HBV genotypes A and D is more likely to progress to the chronic phase than infection by genotypes B and C, whereas genotypes A and B have higher rates of HBeAg seroconversion than genotypes C and D [22]. Genotype E dominated west African region including Nigeria [23,24,25].The genotypes have approximately 8% difference in their genomic nucleotides sequence, and genotype C is highly associated to development of liver diseases. Genotype A predominates western and northern Europe and more prevalent in people who have sex with other people in USA, in Asia and among the Asian that lived in developed countries, genotype B and C is more prevalent [26].

In chronic situation of hepatitis B, mild and gradual liver inflammation occurs silently which may be later transform to life threatening, leading to development of fibrosis and eventually cirrhosis of the liver and hepatacellular carcinoma [27]. Complete treatment of chronic HBV infection is not attainable most of the times, even though it is proved that number of antiviral agent drastically reduced replication of the virus and minimize the possibility of development of liver cirrhosis and cancer, hence the effective way of hepatitis infection eradication is by taking preventive measures such as vaccine [28,29]. Detection and determination of prevalence of HBV in the study area can significantly contribute to overall management, prevention and control of hepatitis B virus infection. The genotype and biomarker help in predicting the eventual outcome of the infections, treatment response and resistance observation, risk and media of transmission. This research will therefore help researchers and public health agencies in addressing the challenges related to hepatitis B virus management

2. MATERIAL AND METHODS

2.1 Study Design

This is a cross-sectional laboratory based study involving patients attended Abubakar Tafawa Balewa University Teaching Hospital (ATBUTH), Specialist Hospital Bauchi (SHB), and Federal Low-cost Primary Health Care Centre all in Bauchi metropolis, from January 2024 to June 2024.

2.2 Inclusion and Exclusion Criteria

All patients attended the selected health facilities across all the age and gender, married and single, vaccinated and non-vaccinated within the period of January 2024 to June 2024 were included until the sample size is reached. Those that refused to sign consent form were excluded.

2.3 Sample and Data Collection

From 200 randomly selected patients 3ml of whole blood was collected via vein and transferred in to EDTA container. Before collection a consent form was issued and signed by each patients and confidentiality was maintained during the process. A structured questionnaire was administered and interviews were conducted with the help of health assistants to collect relevant clinical information and demographic details of the participants.

2.4 Hepatitis B virus surface antigen screening

The blood samples were spun for 5min at 3000rpm, the serum was obtained and used to screen for hepatitis B surface antigen (HBsAg) using strips (Micropoint technologies Inc), the result was read based on manufacturers protocol(two line indicating positive, single line negative and none invalid)[1].

2.5 Detection of Hepatitis B virus Biomarkers

Hepatitis B virus diagnostic cassette kit (Micropoint technologies inc USA)was used to detect hepatitis B virus biomarkers which are hepatitis B virus surface antigen (HBsAg), Hepatitis B virus surface antibody(HBsAb), hepatitis B virus E antigen(HBeAg), hepatitis B virus E antibody (HbeAb) and hepatitis B virus core antibody (HBcAb), the method is immunochromatographic and qualitative in nature, with a small pipette the whole blood sample was taken and drops into the five wells of the cassette, the drop of buffer was added into the wells. The interpretation of the result was performed according to the manufacturer's description. The positive blood samples were centrifuged at 5000rpm for 15 minutes; the serum was collected and immediately transported to molecular laboratory for further analysis[42].

2.6 DNA Extraction

The DNA of the hepatitis B virus was extracted using BIONEER kit (Accu prep Genomic DNA extraction kit from Bioneer) for DNA extraction. In summary, 200ul aliquot of serum was incubated with protease and buffer at 70^0C for 10 minutes, potentially infectious agents was inactivated by incubation at 95^0C for 15minute. The lysate was applied to a spin column, spin and wash three times with buffer and finally elute with 50ul of elution buffer, the extracted DNA was used for further analysis [3].

2.7 Hepatitis B virus DNA amplification by Nested multiplex PCR

2.7.1First round PCR

Two microlitre (2ul) of extracted DNA was added into master mix. (the master mix is cocktail of 16ul of deionised water and premix of 250uM of each dNTPs, 1X PCR buffer, 15Mm of Mgcl and 1U of thermostable taq polymerase), 1ul each of the universal forward and backward primers was used. The PCR condition is set at initial activation 95^0C for 5min, denaturing at 94^0C for 20s, annealing at 54^0C for 20s and extension at 72^0C for 1min. complete set of 30 cycles were observed from denaturing to extension and final extension at 72^0C for 5min.

2.7.2 Second round PCR

Two tubes were used, tube A harbored 17ul of deionized water and 2ul of cocktail of common universal sense primer and type specific antisense primers of genotype A, B and C, 1ul of each were added, 1ul of first round PCR product was added and mixed gently. For the tube B, 17ul of deionised water and 2ul each of the cocktail of common universal antisense primer and type specific sense primers for genotype D, E and F were mixed, 1ul of first round PCR product was added and mixed gently. The PCR was set at 94^0C for initial activation for 3min, followed by 30 cycles of denaturation at 94^0C for 1min, annealing at 50^0C for 1min and extension at 72^0C for 1min for both tube A and B, with the final extension at 72^0C for 5min.The reaction was carried out in PTC programmable thermal cycler[23].

table 1: Characteristics of primer used in the amplification of pre S gene of genotype A to F

Gene	Primer	Oligonucleotides Sequence	Amplicon size	Reference
Universal	PI	F:TCACCATATTCTTGGGAACAAGA	1063	Ahmad *et al*[23]
	SI-2	R:CGAACCACTGAACAAATGGC		
A	BAIR	F:GGCTCCAGTTCCGGAACAGT	68	"
		R:CTCGCGGAGATTGACGAGATGT		
B	BBIR	F:GGCTCCAGTTCCGGAACAGT	281	"
		R:CAGGTTGGTGAGCTGGAGA		
C	BCIR	F:GGCTCCAGTTCCGGAACAGT	122	"
		R:GGTCCTAGGAATCCTGATGTTG		
D	BD1	R:GGAGGCGGATTTGCTGGCAA	119	"
		F:GCCAACAAGGTAGGAGCT		
E	BE1	R:GGAGGCGGATTTGCTGGCAA	167	"
		F:CACCAGAAATCCAGATTGGGACCA		
F	BF1	R:GGAGGCGGATTTGCTGGCAA	97	"
		F:GTTACGGTCCAGGGTTACCA		

2.8 Detection of the PCR products by Gel electrophoresis

Twenty microliters of each of negative control, samples and the standard ladder were run on 1.5% agarose gel (2% w/v in 1 × TAE buffer) and electrophoresed in 1 X TAE buffer for 45 min at 100V. The bands were visualised under gel documentation system (BioRad Gel Doc-XR, USA) and screenshots captured.

2.9 Statistical analysis

R statistical software version 4.1.0 with the ggstatsplot" package was used to create visualizations including chi-square test details and to determine relationship between identified HBV genotype across demographic profile.

3. RESULTS AND DISCUSSION

3.1 Occurrence of Hepatitis B Virus Infection based on Demographic Profile of the patients

Hepatitis B virus infection has connection with different demographic factors such as gender, age and occupation according to several studies across the globe. This study successfully determined the seroprevalence rate of HBV infection based on certain demographic profile (Gender, age, marital status, occupation and location) of the participants in the study population, the overall prevalence recorded was 9%. The range of prevalence of hepatitis B in Nigeria is 4.3% to 23.3% and that of sub-Saharan Africa is 9% to 20% as reviewed and reported by Eustache *et al.* [30].The findings of this research fall within both indicating it to be within the expected index. The result of this study is comparable to report of 12.2 % prevalence by Thomas *et al.* [31] in the same study population but 14.6% was reported by Jibrin *et al.* [32] which is slightly higher than the finding of this research. Similar studies in the same study population were conducted by Ndako *et al*. [33] which indicated 12.45% prevalence among pregnant women, Gambo *et al.* [34] reported 12.2% among nomadic Fulani and Alkali *et al.* [35] reported 7% among blood donors. The prevalence of other north eastern state of Nigeria were in concordance to this study while few reported relatively higher, in Borno metropolis Oyinloye and Bukbuk [36]reported 8.3% prevalence, in Gombe Precious *et al.* [37] reported 14.6% prevalence and higher prevalence of 15% was reported by Keneth *et al.* [38] in Taraba. Presidential committee on North east initiative reported 17.5% prevalence in Yobe which is the highest ever reported in north eastern Nigeria. The cause of the highness may be attributed to method employ, ELISA was used in those studies which were more sensitive than the method employed in this study and the time of conducting the research may be major factor because of late adoption of Hepatitis B vaccine in routine vaccination by health facilities in Nigeria.

Many studies on HBV proved that gender influences the distribution of HBV infection within the population, the differences in the life style which led to difference in exposure to risk factors such as sharing barbing tools that is hardly experienced by female may be the influencing factor. The observation in this research shows that Infection rate among the male is higher than that of female even with the fact the population of female participants is much higher than for male participants, the difference in the prevalence is not significance, this may be attributed to the earlier stated reason or as a result of other influencing life style engaged by males. This finding is similar to 8.5% for male and 4.9% for the female reported by Oyinyole and Bukbuk [36] in Borno metropolis.There is no relationship between the HBV infection and the marital status of the participant according to this study this opposes the report of significance relationship between the HBV infection and general social status by Iliyasu *et al*., [41].

The performance of immune system is greatly influences by the age of the subject which in turn affect the survival of HBV in the patient, this explain why those that got infected at early age have the highest chance of developing chronic infection, therefore age is the most important demographic factor [39]. The age range of 21-30 has higher prevalence followed by age 31-40 years range, and age group of 50-60 and 60 and above have smallest prevalence, the rejection and late adoption of hepatitis B virus vaccine into routine over the last 30 years may have contributed to the higher rate of infection seen within age group 21-30 and 31-40, because they were born within the said period and they may have not receive the HBV vaccine, the low rate observed within the age group 1-10 may as a result of increased vaccine collection over the last 10 years. For the age of 40 and above the infection is trace, this is in conformity with research conducted by Danwafor*et al.* [40] which reported the highest prevalence among similar age group and lowest in age group of above 60.

Table 2: Distribution of Hepatitis B virus infection according to patients' demographic profile

Demographic profile	No. of sample collected(n=200)	No. of positive sample(n=18)	Percentage positive (%)
Gender			
Male	76	10	55.6
Female	124	08	44.4
Marital status			
Single	107	09	50
Married	93	09	50
Age(years)			
(0-10)	29	02	11.1
(11-20)	42	03	16.7
(21-30)	51	06	33.3
(31-40)	41	05	27.7
(41-50)	18	00	0.0
(51-60)	10	01	5.6
(above60)	09	01	5.6
Health Facility			
PHCC FLC	70	06	33.3
SHB	90	08	44.4
ATBUTH	40	04	22.2
Occuaption			
Health worker	09	01	5.6
Non health worker	191	17	94.4

Keys:
ATBUTH: Abubakar Tafawa Balewa University Teaching Hospital
PHCC FLC: Primary Health Care Centre Federal Lowcost
SHB: Specialist Health Bauchi

3.2 Occurrence of hepatitis B virus infection based on the risk factors and clinical profile of the participants

This research studied risk factors that are known to enhance transmission of HBV infection, the result shows that the participants with infected family members have the highest prevalence, this implies mother to child transmission is the most common means of HBV transmission and this form of infection mostly result in development of chronic infection because it occurred when the patient immune system was weak (at early age), some of the infection within the group may not be as a result of mother to child transmission but as a result of day to day domestic sharing such as sharing spoons, cloth and toothbrush that usually happened within the people in the same home. Barbing tools is probably most shared shaped object within our communities, as indicated by this study all the males participants are sharing or have the history of sharing barbing tools, the prevalence rate within this group is high making it the group with second highest prevalence, even though

the positive participants within this group have one or more additional risk factors.Blood transfusion and surgery are important risk factors as far as HBV infection is concerned, by this research percentage positive within these groups are the same and very low, two participant from each group representing 7.7% each were positive, the low record was probably achieved due to increased adoption of screening exercise before blood transfusion and aseptic technique during surgery and proper management of surgical wounds. Based on the findings of this research vaccination remained the effective means of HBV infection prevention, only 1 participant representing 3.8% was positive out of 25 vaccinated participants, the positive case may be due to incomplete dose case or noncompliance of proper time interval between one dose to another. The risk of transmission from mother to baby is very high at perinatal, such transmission can be prevented by vaccination and provision of hepatitis B immune globulin within 12hours of delivery [32].

Table 3: Distribution of hepatitis B virus infection based on the risk factors and clinical profile of the participants

Risk factors	No.of participant	No. of positive participants(n=26)	Percentagepositive(%)
History of blood transfusion	11	02	9.5
History IVDrug abuse	00	00	0.0
History Vaccination	22	01	4.7
Infected family member	15	11	28.6
Barbing tools sharing	85	10	47.6
History of surgery	12	02	9.5

3.3 HBV Biomarkers distribution among Hepatitis B virus surface antigen positive participants

There is currently lack of biomarkers that can sufficiently allow proper diagnosis and prediction of eventual outcome of the hepatitis B virus infection, making determination of available biomarker very indispensible in HBV infection investigation. Out of 18 hepatitis B surface antigen positive patients representing 9% prevalence of the total population, the highest prevalence was observed in HBsAb within the age range 31-40, and males dominated, this is expected because the maturity of immune system is reaching peak within this age range, low prevalence was observed within age range of 1-10 and above 60 years, these are age ranges of weak immune system, the biomarker with low prevalence is HBeAg, presence in only 1 participant indicating the infection in the study population is more of latent. Different studies reported values that are in concordance with the findings of this research. The prevalence 7.1%, 2%, 0%, 47.1% and 45.6% for HBaAg, HBsAb, HBeAg, HBeAb and HBcAb respectively was reported in Borno Oyinyole*et al.* [36] which agrees with this study findings, similar research conducted by Butt *et al.* [42] in Kaduna state among

blood donor indicated opposing result, the research founds 7.3%, 3.0%, 7.3% and 10.9% prevalence for HBsAg, HBsAb, HBeAb and HBcAb respectively, the result shows the same prevalence between HBsAg and HBeAb while in this research the prevalence of HBsAg is higher than that of HBeAb, also HBcAb was higher than both HBsAg and HBeAb unlike in this research which indicated relatively lower HBcAb.

Table 4: Biomarkers observed among HBV positive participants in the study area based on demographic profile

Demographic profile	No. and percentage of positive cases based on the biomarkers				
	HBsAg (n=18)	HBsAb (n=10)	HBeAg (n=1)	HBeAb (n=12)	HBcAb (n=13)
Gender					
Male	10(55.6)	05(50)	01(100)	08(66.7)	07(53.8)
Female	08 (44.4)	05(50)	00(00)	04(33.3)	06(46.2)
Age(years)					
(0-10)	02(11.1)	01(10.0)	00(0.0)	01(8.3)	01(7.7)
(11-20)	03(16.7)	02(20.0)	00(0.0)	02(16.7)	02(15.4)
(21-30)	06(33.3)	04(40.0)	00(0.0)	02(16.7)	05(38.5)
(31-40)	05(27.8)	02(20.0)	01(100)	05(41.7)	03(23.0)
(41-50)	00(0.0)	00(0.0)	00(0.0)	01(8.3)	01(7.7)
(51-60)	01(5.6)	01(10.0)	00(0.0)	01(8.3)	01(7.7)
(60 and above)	01(5.5)	00(0.0)	00(0.0)	00(0.0)	00(0.0)

3.4 Occurrence of HBV genotypes among the Hepatitis B surface antigen positive participants

HBV genotypes have proven to be significant in the determination of the pathogenesis and resolution of viral hepatitis and it has also helped to shed more light on the evolution and geographic distribution of the virus [36]. In this study HBV DNA assay was conducted to 18 hepatitis B positive samples, in which 8(44.4%) has shows detectable HBV DNA. Genotype A prevailed followed by E and B while genotypes C and F were not detected. This study finding opposes adoption of genotype E as prevalent genotype in Nigeria. Mixed infection was very pronounce in the study accounting for half of the result, the combination were three types, ABE(25%), AE(12.5) and BE(12.5%), one sample was negative and might belong to genotype I and J that were not employed in this research. The genotype E been prevalent in Nigeria and West Africa according different researchers, recently different genotypes other than E and mixed infection have been reported in Nigeria. The findings in this research corroborates with the similar research conducted in Lagos by Uche *et al.* [43] which shows that genotype A was most prevalent in study population with prevalence of 46.6% followed by genotype B(44.7%), then genotype E(23.8%) but as oppose to this study mixed infection account for less than half(33.9%) and genotype C and D were detected with 20.9% and 11.2% respectively, similar research conducted by Ahmed *et al.* (2019), in Zaria shows mixed infection account for 82.6% but reported genotype E(97%) as most prevalent followed by B(82.6%) then A (24.6%), and genotype C(17.4) and D(0.7%) were detected. Mixed infection of B and E in all sample ran was detected by Oyinloye and bukbud [36] in research conducted in Borno metropolis, while the other genotypes were negative. This study

conclusively found that the infection rate is significance in the study area especially among males who have infected siblings and have the history of sharing barbing tools, genotype A predominantly prevailed followed E and B.

Table 5: Distribution of HBV genotypes across Hepatitis B surface antigen (HBsAg) positive participants

Genotypes	No. of positivegenotypes (n=8)	Percentage positive (%)
Mono genotypes infection		
A	02	25.0
B	01	12.5
Total	03	37.5
Mixed genotypes infection		
AE	01	12.5
BE	01	12.5
ABE	02	25.0
Total	04	50.0
Negative sample	01	7.1
Frequency		
A	05	38.5
B	04	30.8
C	00	00
D	00	00
E	04	30.8
F	00	00

KEYS:
A: Genotype A
B: Genotype B
AE: Genotype A and E
AB: Genotype A and B
ABE: Genotype A, B and E

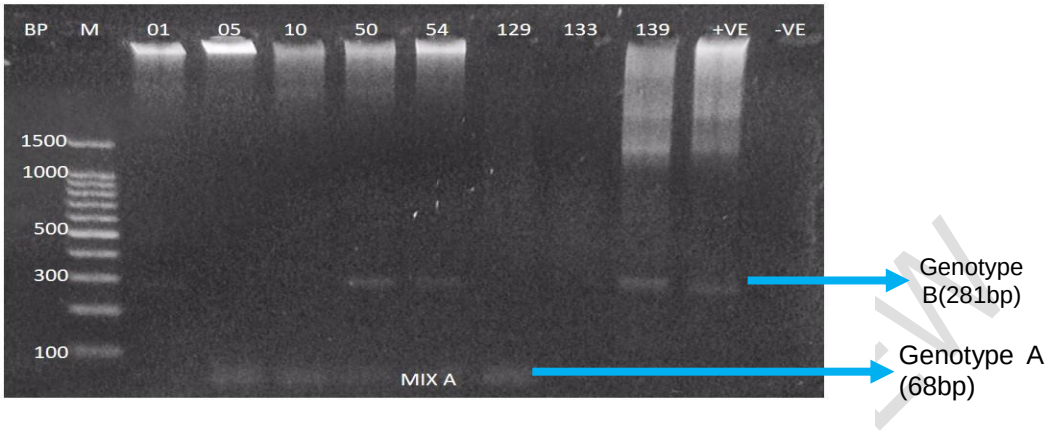

Figure1**:** Agarose gel electrophoretogram showing Hepatitis B Genotypes, Mix A identifying HBV Sample number 01,50,54 and 139 positive to Genotype B (281bp) and sample no. 05,10 and 129 positive to genotype A (68bp).

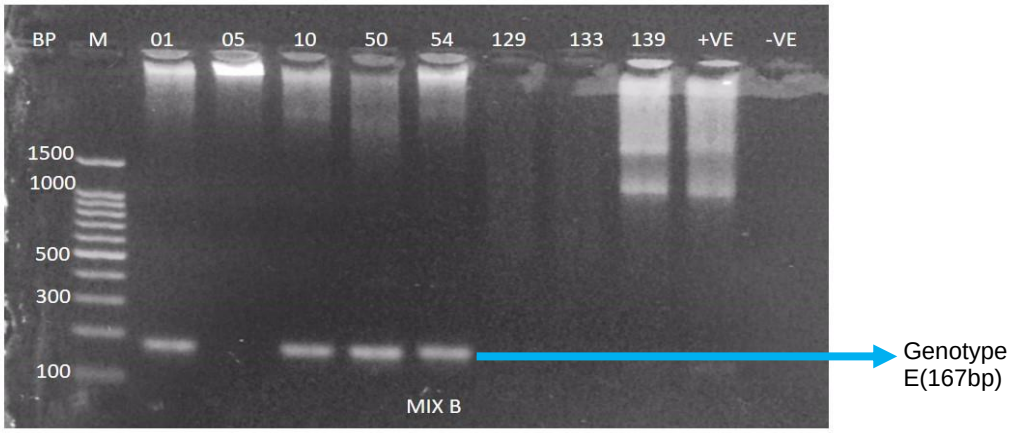

Figure 2**:** Agarose gel electrophoretogram showing Hepatitis B Genotypes, Mix B identifying HBV Sample number 01, 10, 50 and 54 positive to genotype E (167bp).

4. CONCLUSION

The study portrayed the overall prevalence of HBV biomarker and genotypes among the patient attended selected health facilities in Bauchi metropolis. The HBV infection prevalence in the study area was high and of public health importance, the biomarker results revealed low transmission possibility as only 5.5%, were positive to HBeAg, a biomarker that signifies the active replication of the HBV virus. The genotypes of HBsAg positive sample were molecularly determined and mixed infection account for 50% of the total infection which implies increasing liver disease complication. The studies confirmed presence of genotype A with the highest prevalence followed by B and E in the study population. This findings opposes the adoption of Nigeria within genotype E index by many studies, further population studies is need to be done in order to either confirm or refute our findings. Genotypes investigation involving all the ten HBV genotypes should be conducted in the study population because six genotypes were employed in this study.

CONSENT

All authors declare that 'written informed consent was obtained from the patient (or other approved parties) for publication of this case report and accompanying images. A copy of the written consent is available for review by the Editorial office/Chief Editor/Editorial Board members of this journal.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki." Ethical clearance was obtained from health research and ethic committee of the abubakartafawabalewa university teaching hospital with reference number ATBUTH/ADM/42/VOL.i and bauchi state ministry of health with reference number MOH/GENS/S/1409/ii.

Disclaimer (Artificial intelligence)

Authors hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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