Original Article

Seroprevalence and Genotype profile of Hepatitis B Virus Infection Among Antenatal Attendees in ABU Teaching Hospital Zaria, Northwestern Nigeria

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Abstract

Nigeria like other sub-Saharan Africa countries falls among WHO`s hyperendemic region for hepatitis B virus infection and with prevalence greater than 8%. It's been found out that in hyperendemic regions, the major route of acquisition of this virus is by mother-to-child transmission at the time of birth or infancy. Newborns that contract the infection have about 90% risk of developing chronic HBsAg carriage and 25% risk of chronic liver diseases. The genotype of Hepatitis B virus determines its mode of transmission, geographic distribution, clinical outcome, and response to antiviral therapy. **AIM:** This study was conducted to determine the seroprevalence and genotype profile of hepatitis B virus infection among pregnant women attending antenatal clinic in Ahmadu Bello University Teaching Hospital, Zaria. **Material and Method**: A hospital based cross sectional study was conducted among 192 pregnant women at the antenatal booking clinic of ABUTH, Zaria from August 2017 to January 2018. Their blood samples were collected and tested for HBsAg with a third generation ELISA kit (Monolisa HBsAg ULTRA, BIORAD-France). The positive samples were analysed using multiplex polymerase chain reaction and agarose gel electrophoresis to determine the virus genotypes **Result**: Twenty-nine of the women were positive for HBsAg. Mean age was 26.0±6.1years. About a quarter of them 53(27.6%) were pregnant for the first time and approximately half of them 100(5.2.1%) were in their second trimester. About a half of the women were housewives 91(47.4%), while 39(20.3%) were employed in the formal sector. The genotypes determined were all recombinant genotypes of A/B/E (75.86%), B/E (17.24%) and B/C/E (3.45%). **Conclusion**: The seropositivity of hepatitis B virus was high in the studied participants; signifying a potential risk to the unborn generation. This study found multiple recombinant genotypes which are because of mixed infections and may be associated with poor prognosis. Further research is needed in the larger society to confirm the significance of these recombinant genotypes.

Keywords: Hepatitis B virus (HBV), genotype, antenatal clinic, Ahmadu Bello University Teaching Hospital (ABUTH), Zaria

INTRODUCTION

Hepatitis B virus infection is a very important global public health problem.[1] The global burden of the disease showed that an estimated two billion people were affected, over 360 million have chronic liver infections, 240 million have active infection and 10-30 million will become infected each year.[2] The chronicity of HBV infection is responsible for about 57% of cases of liver cirrhosis and 78% of cases of

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primary liver cell cancer worldwide.[3] HBV-related end stage liver disease are responsible for over 1 million deaths per year globally and currently represent 5−10% of cases of liver transplantation.

In hyperendemic region for HBV infection, the usual mode of transmission is mother-to-child transmission (MTCT) at the time of birth from a chronically infected mother. Mother-to-child transmission signifies hepatitis B surface antigen (HBsAg) positivity at 6 to 12 months of life in an infant born to an HBsAg carrier mother.[4] The risk of MTCT is related to maternal and viral factors including antepartum haemorrhage, premature rupture of membranes, hepatitis B e antigen (HBeAg) positivity, viral load {HBV DNA level $\geq 10^6$ copies/mL (>200 000 IU/mL)} etc.[5,6] Depending on the region of study, up to 38% of pregnant women in Africa with chronic hepatitis B viral infection are positive for HBeAg and can transmit HBV to their infants.[7] Approximately 90% of the infants of HBsAg and HBeAg seropositive mothers become HBsAg chronic carriers, while for HBeAg negative mothers, the rate is between 10-40%.[8] Almost 25% of individuals who become chronically infected during infancy and childhood die from HBV-related liver cancer or cirrhosis later in life.[9] Nigeria falls within the highly endemic zone with a pooled prevalence of 12.2% for the general population and 11.5% for pregnant women attending antenatal clinics.[10, 11]

The natural course of HBV infection in the host is complex and highly variable, depending on the interplay of the virus replication rate, host immune responses, duration of infection, route of acquisition, race, infecting HBV genotype, associated mutations, socioeconomic status and the occurrence of co-morbidities.[12] Hepatitis B virus reverse transcriptase lacks proofread functions as such, several HBV genotypes, sub-genotypes, mutants, and recombinants emerge.[13] The genotypes of HBV have impact on its mode of transmission, global distribution, pathogenicity, serologic reactivity, virulence, response to treatment and rate of progression to complications,[14, 15]There have been ten (10) genotypes (A-J) HBV identified to date.[16, 17]

Studies regarding the genotypes of HBV in this part of the world are scarce, and imperative to determine the genotypic variants which could influence transmission, pathogenesis, disease progression and response to antiviral therapy.

The present study aimed to determine the prevalent hepatitis B virus genotype(s) among pregnant women positive for HBsAg in Ahmadu Bello University Teaching Hospital Zaria.

MATERIAL AND METHOD

Study Area

This study was carried out in Ahmadu Bello University Teaching Hospital (ABUTH), a tertiary health care centre located in Zaria, one of the major towns in Kaduna state in Northwestern Nigeria. It is a 500 beds hospital which serves as a referral centre for patients within and from neighbouring states. The Department of Obstetrics and Gynaecology runs antenatal clinics on Mondays to Fridays and booking of new cases is done every Wednesday of the week. The clients load on every booking day was between 40-70 pregnant women. At booking, every pregnant woman is screened for HBsAg with rapid kits and immunoprophylaxis is provided for the newborns of seropositive mothers.

Study Design

This was a cross-sectional study in which pregnant women that presented for booking every Wednesday at the antenatal clinic of ABU teaching hospital Zaria were recruited. At every booking clinic, selected pregnant women were administered an interviewer based, semi-structured questionnaire and blood samples were subsequently collected from consenting participants that had neither been diagnosed of HBV infection nor received hepatitis B virus vaccine in the past.

Sample Size Estimation

The sample size was estimated based on the Cochran formula: $n=Z^2pq/d^2$; where Z is the standard normal deviate at 1.96 corresponds to the 95% confidence level; p is the prevalence, taken as 13.3%[18] based on previous studies among pregnant women; q is 1-p, d is the degree of accuracy desired set at 0.05. A sample size of 174 was arrived at which was raised to 192 to allow for 10% attrition for participants that had to leave the study.

Sampling Technique

Recruitment of pregnant women for this study was based on systematic random sampling technique on every booking day over a period of 24 weeks from the month of August 2017 to January 2018. The sample frame was estimated from the ABUTH antenatal booking register for an average weekly turnover of 60 clients over a period of 24 weeks.

Sample interval was estimated from k = N/n, where k is the sampling interval; N is the total population; n is the calculated sample size; k is taken

as 8. A number was randomly selected between 1 and 8 to pick a participant who met the inclusion criteria as the starting point. Then for the randomly chosen participant, a sampling interval of 8 was used to arrive at the next participant. For any client that refused to participate in the study, the next 8th client that met the inclusion criteria and agreed to participate was chosen. This pattern was continued till the minimum sample size was reached.

Specimen Collection

About 4 milliliter of blood sample was aseptically collected by venepunture from each participant into a labeled sterile plain bottle and subsequently transported to the laboratory. These were left at room temperature for about an hour for clot formation and then centrifuged at 1000 rpm for 5 minutes. The serum from each sample was separated, pooled then screened for HBsAg at every week. The HBsAg positive sera were stored in the freezer at -20 degree Celsius for subsequent genotyping test.

Biosafety Consideration

Most procedures were carried out in biosafety cabinet 2. Standard precaution was ensured during sample handling. Sterile disposable needles and syringes used to collect blood samples were disposed of in a safety box. Decontamination of surfaces during and after procedures was done with 10% hypochlorite solution. All waste generated were autoclaved before final disposal.

Laboratory Procedures

All test kits manuals and inserts were followed strictly according to the manufacturer's instructions.

Screening

All samples collected were pooled and screened for HBsAg with a third generation ELISA kit (Monolisa HBsAg ULTRA, BIORAD- France). The positive samples for HBsAg were subjected to PCR for the virus genotypes.

Assay Procedure for HBsAg

One hundred (100) µl of negative and positive controls were dispensed into the respective wells according to instructions provided in the manual. One hundred (100) µl of serum sample was dispensed into each of the labeled wells according to sample number. Fifty (50) µl of conjugate was added to each well; the plate was covered with adhesive film and then incubated for 1hour and 30 minutes. The wells were then aspirated of the contents and washed 5 times manually and then dried. The development solution (substrate + chromogen) was prepared and 100µl was dispensed into each well, then covered and incubated in the dark for 30min at room temperature. Then 100µl of stop solution was added to each well, allowed to stand for about 7min and the optical density (OD) then read at 450nm in an EIA plate reader.

Calculation/Interpretation of Result

The Cut off value (Co) of the assay was determined with Negative Control (NC) as: Cut Off (Co) = Mean of NC + 0.050. The ratio of the sample(s) OD to cut-off value (S/Co) was calculated. In accordance with the manual, a sample with S/Co ratio more than 1.0 was taken as positive, while that with S/Co result of less than 1.0 was considered negative, S/Co of 0.9- 1.0 was considered equivocal.

Quality Control

Negative control OD at 450nm was < 0.080 and OD of positive control was > 1.000.

Hepatitis B Virus Genotyping

DNA Extraction

DNA was extracted from 200µl serum of each sample using Bioneer *Accu Prep* (Munpyeongse-ro, Republic of Korea) genomic DNA extraction mini kit according to the manufacturer's instructions.

Extraction Procedure

The sera were brought to room temperature for a few minutes to thaw and then vortexed. Two hundred (200) µl of serum was pipetted into a new Eppendorf tube. Two hundred (200) µl of binding buffer was added into the tube. Twenty (20) µl of proteinase K was added to the mixture. The tube was closed and vortexed for 15 seconds. It was then incubated at 60°C for 10 minutes and vortexed for about 5 minutes. One hundred (100) µl of isopropanol was added to the mixture and then inverted 3 times. The mixture was then decanted into a DNA-binding column and centrifuged at 12,000rpm for 1 minute. Five hundred (500) µl of wash buffer I was added to each mixture and centrifuged at 12,000rpm for 1 minute. The flow-through was then discarded. Five hundred (500) µl of wash buffer II was then added and centrifuged at 12,000rpm for 1 minute. The flow-through was discarded and re-centrifuged for another 3 minutes at 13,000 rpm to remove excess ethanol from the column.

Fifty (50) µl of pre-warmed (60ºC) elution buffer was added to release the DNA. It was allowed to stand at room temperature for 5 minutes and then centrifuged at 13,000 rpm for 3 minutes to collect the eluted DNA into the tube along with the flow-through. The flow-through was recollected back into the column to 'double elute' and re-centrifuged at 13,000 rpm for 3 minutes to increase the DNA yield. The column was discarded and the tube containing the DNA was kept in the freezer for subsequent PCR for HBV genotyping.

The genotyping Procedure

The genotyping of HBV was done based on rapid and specific genotype system corresponding to six genotypes A through F by nested polymerase chain reactions, using type specific primers for HBV according to the method described by Naito *et al.*[14] The sequences of PCR primers that were used in this study are shown in table 1 below.

Hepatitis B Virus DNA Amplification

The nested PCR primers were designed based on the conserved nature of the nucleotide sequences in regions of the pre-S1 through S genes. P_1 and S1-2 being the universal outer primers and B2 the inner sense (forward) primer with a combination of BA1R, BB1R and BC1R as anti-sense (reverse) inner primers for genotypes A, B and C respectively in a multiplex system tagged 'Mix A'. For genotypes D, E and F, an anti-sense primer B2R was used in combination with BD1, BE1 and BF1 as sense (forward) primers, also in a multiplex system tagged 'Mix B'.

First round PCR: Detection of HBV DNA

The HBV was detected by amplification of pre- S1 through S genes using universal primers; (P1) sense primer, (S1-2) antisense primer. The total reaction mixture for the first round PCR was 20µl. The premix tubes were labeled with the samples ID numbers. Two (2) µl of extracted DNA was added to a mixture {16µl of deionized water ($D.H_2O$), premix of 250µM of each dNTP, 1X PCR buffer, 15mM of $MgCl_2$ and 1U of thermostable Taq polymerase} and 1µl each of P_1 (forward) and S1-2 (reverse) outer primers. The PCR was performed using thermal cycler (PTC-100™ Programmable thermal controller, MJ Research, Inc.) and reaction condition was set as: initial activation at 95ºC for 5 minutes; denaturation at 94ºC for 20 seconds; annealing at 55ºC for 20 seconds and extension at 72ºC for 1 minute. Total cycles of 30 from denaturation to extension were observed. Then final extension was set at 72ºC for 5 minutes.

Second Round PCR: Hepatitis B Virus Genotyping

The second round PCR was performed in two different tubes for each sample, one with the universal sense primer (B2) and type specific primers for genotypes A, B, C in "Mix- A" and the other with the universal anti-sense primer B2R and type specific primers for genotypes D, E, F in "Mix- B". Seventeen (17) µl of $D.H_2O$ was added into each tube of premix 'A' and 'B'. Two (2) µl of the cocktail primers (containing 0.5µl each of the four primers) were added into the mixtures. One (1) µl of the first round PCR product was also added into each tube of the premix. The mixture was gently agitated and then centrifuged at 3000rpm for 5 minutes. The PCR condition was set as: initial activation at 94ºC for 3 min, followed by 30 cycles of denaturation at 94ºC for 1 minute, annealing at 50ºC for 1 minute and extension at 72ºC for 1 minute for both "Mix A" and "Mix B", with final extension at 72ºC for 5 minutes.

Agarose Gel Electrophoresis

From the negative control, sample, and the ladder mixes, twenty (20) µl of each was set on 2% agarose gel (2% w/v in 1x TAE buffer) and electrophoresed in 1x TAE buffer for 45 minutes at 100V. The bands were visualized under gel documentation system (BioRad Gel Doc-XR, USA) and screenshots captured. The size of the separated bands (DNA fragments) was compared with GeneRuler™ 100bp+ DNA ladder (MBI Fermentas, Life Sciences, Canada).

RESULT

Sociodemographic Characteristics of Study Participants

A total of 192 pregnant women participated in this study. The mean age was 26.0 ± 6.1 SD years. The predominant tribe was Hausa/Fulani 155(80.7%). The dominant religion was Islam 171(89.1%). One hundred and ninety of the respondents (99.0%) were married and those in a monogamous setting constituted 159(83.2%).

Table 1: Primer Sequence Used for Hepatitis B Virus Genotyping by Nested Pcr[15]

Primer	Sequence (5'-3')	Specificity	Position	Polarity
First Round PCR				
P_1	TCACCATATTCTTGGGAACAAGA	Universal	2823-2845	Sense
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

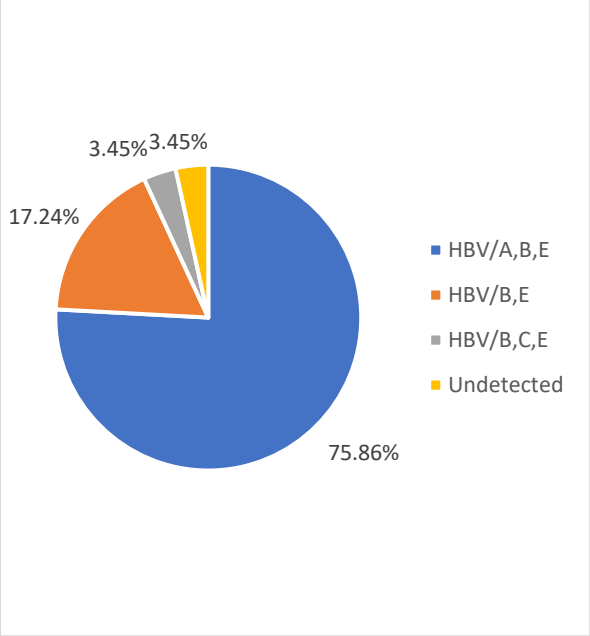

Figure 1: Hepatitis B virus genotype profiles ANC attendees at ABUTH Zaria

Variables	Frequency, n=192	%
Age		
16-20	25	13
21-25	71	37
26-30	47	24.5
31-35	21	16.1
□▤	18	9.4
Ethnic Group		
Hausa/Fulani	155	80.7
Yoruba	8	4.2
Igbo	3	1.6
Others	26	13.5
Religion		
Christianity	21	10.9
Islam	171	89.1
Marital status		
Married	190	99
Single	2	1
Employment Status		
Employed	39	20.3
Unemployed	22	11.5
self employed	12	6.3
house wife	91	47.4
Student	27	14.1
Others	1	0.5
Family Setting		
Monogamous	107	82.3
Polygamous	22	16.9
single mother	1	0.8
Residence		
Within Zaria	172	89.6
Outside Zaria	20	10.4
Gravidity		
1	53	26.6
2	36	18.7
3	55	17.2
4	24	12,5
5 or more	46	24
Gestational Age		
0-1	21	10.9
4-6	100	52.1
7-9	71	37

Table 2: Sociodemographic characteristics of study participants at ANC of ABUTH Zaria

Those with tertiary education constituted about a half 87(45.5%) of the study participants while 7(3.7%) had non-formal "Quranic" education. About a half of the women were housewives 91(47.4%), while only 39(20.3%) were employed in the formal sector. Almost all the participants were residents of Zaria 172(89.6%). About a quarter of them 53(27.6%) were pregnant for the first time and approximately half of them 100(52.1%) were in their second trimester (Table 2).

Table 3: Seroprevalence of HBV among ANC attendees in ABUTH Zaria

	Frequency	%
Ag		
Negative	163	84.9
Positive	29	15.1*
Total	**192**	**100**

*Seroprevalence

Seroprevalence of HBsAg among the Studied Participants

Among the 192 participants, HBsAg was detected in 29(15.1%) of them. This is shown in Table 3

Hepatitis B Virus Genotypes Among the Studied Participants

Of the 29 samples that tested positive for HBV DNA on PCR, 28(96.6%) showed genotype specific bands on gel electrophoresis. The pattern showed mix

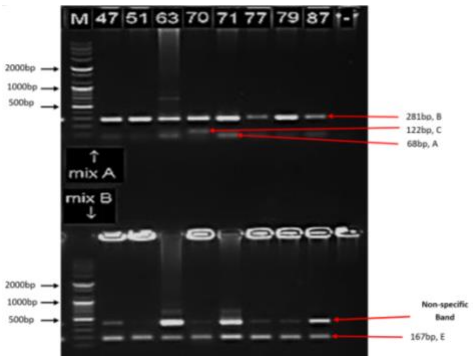

Figure 2: Agarose gel electrophoretogram of PCR products for specific HBV genotype. Mix A contains primers for genotypes A, B, C and Mix B contains primers for genotypes D, E, F.; M: 100bp+ DNA ladder. 47-87: Samples representative. (-): Negative control.

genotypes and distributed as follows: 22(75.86%) samples contained mix genotypes A+B+E, 5(17.24%) samples contained mix B+E and 1(3.45%) sample contained mix B+C+E. One sample was untypeable, i.e showed no band on electrophoresis. Figures 1 & 2 further depict these.

DISCUSSION

The seroprevalence of HBsAg positivity obtained from this study was 15.1%. This prevalence was comparable to 13.3% reported by Jatau *et al*[19] among pregnant women attending antenatal clinic in Ahmadu Bello University Medical Centre Zaria in 2014. However, this finding was higher than 8.3% reported by Luka *et al*[19] in 2008 among antenatal clients in the same facility where this study was carried out. It was also higher than the prevalence reported from other geographic regions of this country: 7.9% reported in Kano by Yakasai *et al*,[20] 12.3% reported in Minna by Ndams *et al*,[21] 8.2% reported by Olokoba *et al*[22] in Yola, 8.3% reported in Ibadan by Anaedobe *et al*[23] and 6% reported by Lu *et al* in Nnewi. All studies were conducted among antenatal clients in public hospitals in Nigeria. The disparities in prevalence obtained from these studies may be due to the assay methods employed, study period and geographical location. Most of these studies mainly used rapid diagnostic kits[24] which are not as sensitive or as specific compared to the ELISA that was used in our study.

The seroprevalence obtained showed that this area like other parts of the country is highly endemic for hepatitis B virus. This agrees with the WHO [1990] report that countries in sub-Saharan Africa are hyperendemic for hepatitis B virus, with seroprevalence greater than 8%.[25] This finding was also in conformity with reports by Kirre *et al*,[26] that sub-Saharan Africa has HBV carrier rate between 9-20%. However the prevalence was higher than findings from studies done in some other sub-Saharan Africa countries including Uganda,[27] northern Cameroon [28] and eastern Ghana.[29] The results from this study compared to studies carried out in other parts of the world, doubled the figure reported from parts of China[30]and the United states.[31]and was much higher than reports from Hong Kong[32] and Taiwan.[33] This prevalence was however, lower than reports of a study conducted in Burkina Faso,[34] and even much lower than that obtained in a subgroup of individuals such as HBV infected female sex workers in Nigeria[35] and among intravenous drug abusers in Pakistan.[36] The endemic pattern of HBV in different geographical locations and sample sizes analyzed might contribute to the disparities in prevalence reported from these studies.

The genotypes of hepatitis B virus obtained in this study were mainly a mix of A+B+E (75.9%), B+E (17.2%) and B+C+E (3.5%). Further analysis showed that genotypes B and E were virtually present in every mix accounting for 96.6% while genotype A was

found in one of the mix and C in the other. The finding of mix genotypes of HBV was comparable to reports of studies conducted in other places that also employed the same multiplex PCR technique. In Pakistan, 75% of the samples analyzed contained mix genotypes A+B+C+D and 25% contained mix genotypes B+C+D.[15] In Sistan and Baluchestan province, mix genotypes C+D (5.5%) were reported in addition to genotype D (94.5%) in HBV infected patients.[37] In eastern China, HBV genotypes among chronic carriers showed B, C, D, B+C, and B+D in proportion of 19.21%, 64.75%, 1.49%, 13.63%, and 0.92% respectively.[38] The findings of mix genotypes in this study differed from reports of a study conducted in Ghana that employed a different HBV genotyping method (RFLP) among chronic carriers in which a single genotype E was found as predominant, followed by genotype A then genotype D.[39] The mix genotypes may be due to the techniques used, or may probably be a reflection of the genotypes present in this region. Further research will be needed to elucidate this.

The presence of HBV genotypes A and E in this study though in a mix was in conformity with earlier documented reports of several studies on the geographical distribution of the genotypes of this virus that are prevalent in West Africa.[39,40,41,42,] However, the discovery of HBV genotypes B and C was incidental as they had not been reported in West Africa but were known to be prevalent in Europe, Australia, China, Vietnam and Asia.[41] These genotypes might have been introduced to this area due to migration of people among different regions for business and other services. Studies have shown that the global migratory flow of people could alter the normal geographic distribution of HBV genotypes with the appearance of some genotypes in areas where they were not previously dispersed.[43,44] In HBV hyperendemic regions, infection with multiple genotypes commonly result in recombinant strains and chances of co-infection or super-infection with other genotypes in an individual is very high. The occurrence of mix genotypes in a chronic carrier compared to infection with a single genotype result in a higher viral load and acceleration of HBV replication in vitro.[37] The presence of co-infection or super-infection with several genotypes in a particular host is associated with poor prognosis.[45]

The untypeable band on gel electrophoresis of one of the HBV DNA may represent a minor subgenotype or a recombinant genotype which was not detected by the Multiplex PCR HBV genotyping technique that was used in this study.[39] Multiplex PCR techniques for HBV has a sensitivity and specificity of 94–100% and of 92–97.5% respectively, it can precisely determine mix genotypes but sensitivity for detecting subgenotypes of the virus is as low as 10%.[46]

Studies have shown that the clinical course of the disease and response to antiviral therapy can be influenced by the HBV genotypes. The seroconversion to anti HBeAb is faster with genotypes A and B than those infected with genotype C.[47] For response to intravenous antiviral therapy, patients infected with genotype A and B respond to standard interferon-alpha or pegylated interferon-alpha better than those infected with genotypes C.[48,49] In the case of oral antiviral therapy, genotype B is more commonly associated with lamivudine-resistant variants than genotype C.[50] Other studies found that HBeAg negative patients infected with genotype B and C responded better to PEG-IFN than other genotypes.[51,52]

CONCLUSIONS

The seroprevalence of HBV obtained in this study among pregnant women attending ANC ABUTHZaria was 15.1%. The high prevalence of the virus in this environment is a significant risk to newborns. The major genotypes of the virus identified were mainly a mix of A+B+E (75.9%), B+E (17.2%) and B+C+E (3.5%). This study incidentally found in the mix, genotypes B and C in addition to genotypes A and E that are known to be prevalent in this part of the world. Further research is needed to determine the significance of these recombinant genotypes in the larger community.

REFERENCES

1. Ikobah J, Okpara H, Elemi I, Ogarepe Y, Udoh E, Ekanem E. The prevalence of hepatitis B virus infection in nigerian children prior to vaccine introduction into the national programme onimmunization schedule. *Pan Afr Med J* [Internet]. 2016 [cited 2018 Jul 19];**23**. Available from: http://www.panafrican-med-journal.com/content/article/23/128/full/
2. Stanaway JD, Flaxman AD, Naghavi M, Fitzmaurice C, Vos T, Abubakar I, et al. The global burden of viral hepatitis from 1990 to 2013: findings from the Global Burden of Disease Study 2013. *Lancet*. 2016;**388** (10049).
3. WHO. WHO | Hepatitis [Internet]. 2015. Available from: http://who.int/topics/hepatitis/en/
4. Chasela CS, Kourtis AP, Wall P, Drobeniuc J, King CC, Thai H, et al. Hepatitis B virus infection among HIV-infected pregnant women in Malawi and transmission to infants. *J Hepatol* [Internet]. 2014 Mar [cited 2018 Jul 21];**60**(3):508–14. Available from: http://linkinghub.elsevier.com/retrieve/pii/S016882781300

7551
5. Flores A, Marrero JA. Emerging trends in hepatocellular carcinoma: focus on diagnosis and therapeutics. *Clin Med Insights Oncol* [Internet]. 2014 Jan [cited 2016 Apr 18];**8**:71–6. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4039215&tool=pmcentrez&rendertype=abstract
6. Nelson NP, Jamieson DJ, Murphy T V. Prevention of Perinatal Hepatitis B Virus Transmission. *J Pediatric Infect Dis Soc* [Internet]. 2014 Sep 1 [cited 2016 May 1];**3 Suppl 1**(suppl_1):S7–12. Available from: http://jpids.oxfordjournals.org/content/3/suppl_1/S7.abstract
7. Andersson MI, Rajbhandari R, Kew MC, Vento S, Preiser W, Hoepelman AIM, et al. Mother-to-child transmission of hepatitis B virus in sub-Saharan Africa : time to act. *Lancet Glob Heal* [Internet]. 2013;**3**(7):e358–9. Available from: http://dx.doi.org/10.1016/S2214-109X(15)00056-X
8. Li Z, Hou X, Cao G. Is mother-to-infant transmission the most important factor for persistent HBV infection? *Emerg Microbes Infect* [Internet]. 2015 May [cited 2016 May 1];**4**(5):e30. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4451268&tool=pmcentrez&rendertype=abstract
9. Franco E, Bagnato B, Marino MG, Meleleo C, Serino L, Zaratti L. Hepatitis B: Epidemiology and prevention in developing countries. *World J Hepatol* [Internet]. 2012 Mar 27 [cited 2016 May 25];**4**(3):74–80. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3321493&tool=pmcentrez&rendertype=abstract
10. Nasidi A, Olayinka AT, Gidado S, Sha'aibu S, Balogun MS, Oyemakinde A, et al. Seroprevalence of Hepatitis B Infection in Nigeria: A National Survey. *Am J Trop Med Hyg* [Internet]. 2016 Oct 5 [cited 2018 Jul 2];**95**(4):902–7. Available from: http://www.ajtmh.org/content/journals/10.4269/ajtmh.15-0874
11. 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. 2015;**18**(2).
12. Kim BK, Revill PA, Ahn SH. HBV genotypes: Relevance to natural history, pathogenesis and treatment of chronic hepatitis B. *Antivir Ther*. 2011;**16**(8):1169–86.
13. Sunbul M. Hepatitis B virus genotypes: global distribution and clinical importance. *World J Gastroenterol* [Internet]. 2014 May 14 [cited 2016 Mar 19];**20**(18):5427–34. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4017058&tool=pmcentrez&rendertype=abstract
14. Naito H, Hayashi S, Abe K. Rapid and Specic Genotyping System for Hepatitis B Virus Corresponding to Six Major Genotypes by PCR Using Type-Specic Primers. *Society*. 2001;**39**(1):362–4.
15. Muhammad P, Rashid A, Salih GF. IDENTIFICATION AND GENOTYPING OF HEPATITIS B VIRUS BY PCR ASSAY USING GENOTYPE SPECIFIC PRIMERS Peshnyar Muhammad Atta Rashid , Msc Molecular genetic. *Eur Sci J*. 2014;**10**(9):424–33.
16. Guettouche T, Hnatyszyn HJ. Chronic hepatitis B and viral genotype: the clinical significance of determining HBV genotypes. *Antivir Ther* [Internet]. 2005 Jan 1 [cited 2016 Apr 11];**10**(5):593–604. Available from: https://www.researchgate.net/publication/7609449_Chronic_hepatitis_B_and_viral_genotype_The_clinical_significance_of_determining_HBV_genotypes
17. Lien JM, Aldrich CE, Mason WS. Evidence that a capped oligoribonucleotide is the primer for duck hepatitis B virus plus-strand DNA synthesis. *J Virol* [Internet]. 1986 Jan [cited 2016 Apr 10];**57**(1):229–36. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=252719&tool=pmcentrez&rendertype=abstract
18. Jatau E., Yabaya A. Sero prevalance of Hepatisis B Virus in pregnant women attending a clinic in Zaria, Nigeria. *Sci World J* [Internet]. 2010 Feb 25 [cited 2018 Mar 28];**4**(2). Available from: http://www.ajol.info/index.php/swj/article/view/51837
19. Luka SA, Ibrahim M, Iliya S. Sero-prevalence of hepatitis B surface antigen among pregnant women attending Ahmadu Bello University Teaching Hospital, Zaria, Nigeria. *Niger J Parasitol* [Internet]. 2008 May 12 [cited 2016 Apr 21];**29**(1):38–41. Available from: http://www.ajol.info/index.php/njpar/article/view/37901
20. Yakasai I, Ayyuba R, Abubakar I, Ibrahim S. Sero-prevalence of Hepatitis B Virus Infection and its Risk factors among Pregnant Women Attending Antenatal Clinic at Aminu Kano Teaching Hospital, Kano, Nigeria [Internet]. Vol. 1, Journal of Basic and Clinical Reproductive Sciences. Nigerian Medical Association, Enugu State; 2012 [cited 2016 Apr 22]. p. 49–55. Available from: http://www.ajol.info/index.php/jbcrs/article/view/115359
21. Ndams I., A. JI, A. LS, O. SH. EPIDEMIOLOGY OF HEPATITIS B INFECTION AMONG PREGNANT WOMEN IN MINA, NIGERIA. *Sci World J* [Internet]. 2006 [cited 2018 Aug 1];**3**(3). Available from: http://www.scienceworldjournal.org/article/view/4710/3262
22. Olokoba AB, Salawu FK, Danburam A, Olokoba LB, Midala JK, Badung LH, et al. Hepatitis B virus infection amongst pregnant women in North-Eastern Nigeria - A call for action. *Niger J Clin Pract*. 2011;**14**(1):10–3.
23. Anaedobe CG, Fowotade A, Omoruyi CE, Bakare RA. Prevalence, sociodemographic features and risk factors of Hepatitis B virus infection among pregnant women in Southwestern Nigeria. *Pan Afr Med J* [Internet]. 2015 Jan [cited 2016 Apr 15];**20**:406. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4524914&tool=pmcentrez&rendertype=abstract
24 O E, IK K, AM B, ZI I, I D, I S. Comparison of ELISA and Rapid Screening Techniques for the Detection of HBsAg among Blood Donors in Usmanu Danfodiyo University Teaching Hospital Sokoto, North Western Nigeria. *J Blood Lymph* [Internet]. 2014 Jul 31 [cited 2018 Jul 6];**04**(02):1–5. Available from: https://www.omicsonline.org/open-access/comparison-of-elisa-and-rapid-screening-techniques-for-the-detection-of-hbsag-among-blood-donors-in-usmanu-danfodiyo-university-teaching-hospital-sokoto-north-western-nigeria-2165-7831.1000124.php?aid=30523
25 Ott JJ, Stevens GA, Groeger J, Wiersma ST. Global epidemiology of hepatitis B virus infection: new estimates of age-specific HBsAg seroprevalence and endemicity. *Vaccine* [Internet]. 2012 Mar 9 [cited 2015 Sep 21];**30**(12):2212–9. Available from:

http://www.ncbi.nlm.nih.gov/pubmed/22273662
26. Kiire CF. The epidemiology and prophylaxis of hepatitis B in sub-Saharan Africa: a view from tropical and subtropical Africa. *Gut* [Internet]. 1996 [cited 2018 Mar 28];**38 Suppl 2**(Suppl 2):S5-12. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8786055
27. Bayo P, Ochola E, Oleo C, Mwaka AD. High prevalence of hepatitis B virus infection among pregnant women attending antenatal care: a cross-sectional study in two hospitals in northern Uganda. *BMJ Open* [Internet]. 2014 Jan 1 [cited 2016 Apr 5];**4**(11):e005889. Available from: http://bmjopen.bmj.com/content/4/11/e005889.abstract
28. Frambo AAB, Atashili J, Fon PN, Ndumbe PM. Prevalence of HBsAg and knowledge about hepatitis B in pregnancy in the Buea Health District, Cameroon: a cross-sectional study. *BMC Res Notes* [Internet]. 2014 Jan 25 [cited 2016 Apr 14];**7**(1):394. Available from: http://bmcresnotes.biomedcentral.com/articles/10.1186/1756-0500-7-394
29. Luuse A, Dassah S, Lokpo S, Ameke L, Noagbe M, Adatara P, et al. Sero-prevalence of Hepatitis B surface antigen amongst pregnant women attending an antenatal clinic, Volta region, Ghana. *J Public Health Africa* [Internet]. 2016 [cited 2018 Apr 26];**7**(7). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5345407/pdf/jphia-2016-2-584.pdf
30. Ding Y, Sheng Q, Ma L, Dou X. Chronic HBV infection among pregnant women and their infants in Shenyang , China. *Virol J*. 2013;**17**(10):1–5.
31. Dunkelberg JC, Berkley EMF, Thiel KW, Leslie KK. Hepatitis B and C in pregnancy: a review and recommendations for care. *J Perinatol* [Internet]. 2014 Dec [cited 2016 Apr 14];**34**(12):882–91. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4777346&tool=pmcentrez&rendertype=abstract
32. Shuping Tong, Kyun-Hwan Kim, Charles Chante, Jack Wands JL. Hepatitis B Virus e Antigen Variants [Internet]. Int J Med Sci. 2005 [cited 2016 Apr 22]. p. 2–7. Available from: http://www.medsci.org/v02p0002.htm
33. Lin H-H, Kao J-H, Chang T-C, Hsu H-Y, Chen D-S. Secular trend of age-specific prevalence of hepatitis B surface and e antigenemia in pregnant women in Taiwan. *J Med Virol* [Internet]. 2003 Apr [cited 2016 Apr 22];**69**(4):466–70. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12601752
34. Collenberg E, Ouedraogo T, Ganamé J, Fickenscher H, Kynast-Wolf G, Becher H, et al. Seroprevalence of six different viruses among pregnant women and blood donors in rural and urban Burkina Faso: A comparative analysis. *J Med Virol* [Internet]. 2006 May 1 [cited 2018 Mar 30];**78**(5):683–92. Available from: http://doi.wiley.com/10.1002/jmv.20593
35. Forbi JC, Onyemauwa N, Gyar SD, Oyeleye AO, Entonu P, Agwale SM. High prevalence of hepatitis B virus among female sex workers in Nigeria. *Rev Inst Med Trop Sao Paulo* [Internet]. 2008 [cited 2017 Aug 18];**50**(4):219–21. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18813761
36. Alam MM, Zaidi SZ, Shaukat S, Sharif S, Angez M, Naeem A, et al. Common genotypes of Hepatitis B virus prevalent in injecting drug abusers (addicts) of North West Frontier Province of Pakistan. *Virol J* [Internet]. 2007 Jan 28 [cited 2016 Apr 11];**4**(1):63. Available from: http://virologyj.biomedcentral.com/articles/10.1186/1743-422X-4-63
37. Yoosefi K, Taheri M, Khosravi S, Saneie-Moghadam E, Mohagheghi-Fard AH. Genotyping of Hepatitis B Virus by Multiplex PCR in Sistan and Baluchestan Province. *Zahedan J Res Med Sci* [Internet]. 2016 Feb 28 [cited 2018 Dec 27];**In Press**(InPress). Available from: http://zjrms.com/en/articles/5986.html
38. Zhong J, Gao YQ, Sun XH, Zhu XJ, Li M. High prevalence of the B2+C2 subgenotype mixture in patients with chronic hepatitis B in Eastern China. *Acta Pharmacol Sin* [Internet]. 2012;**33**(10):1271–6. Available from: http://dx.doi.org/10.1038/aps.2012.78
39. Dongdem AZ, Dzodzomenyo M, Asmah RH, Nyarko KM, Nortey P, Agyei A, et al. Hepatitis B virus genotypes among chronic hepatitis B patients reporting at Korle-Bu teaching hospital, Accra, Ghana. *Pan Afr Med J* [Internet]. 2016 [cited 2018 Apr 11];**25**(Suppl 1):5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28210373
40. Madan K, Tyagi P. Hepatitis B virus genotyping: is the time ripe for routine clinical use? *J Clin Exp Hepatol* [Internet]. 2012 Jun [cited 2016 Apr 12];**2**(2):174–80. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3940128&tool=pmcentrez&rendertype=abstract
41. Guirgis B, Abbas R, Azzazy H. Hepatitis B virus genotyping: current methods and clinical implications. *Int J Infect ...* [Internet]. 2010 [cited 2016 Apr 10];**14**(11):e941–e593. Available from: http://www.sciencedirect.com/science/article/pii/S1201971210024239
42. Valente F, Lago BV do, Castro CAV de, Almeida AJ de, Gomes SA, Soares CC. Epidemiology and molecular characterization of hepatitis B virus in Luanda, Angola. *Mem Inst Oswaldo Cruz* [Internet]. 2010 Dec [cited 2018 Apr 12];**105**(8):970–7. Available from: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0074-02762010000800004&lng=en&tlng=en
43. Mbaawuaga E., Enenebeaku M, Okopi J, Okopi J. Hepatitis b virus (hbv) infection among pregnant women in makurdi, nigeria. *African J Biomed Res* [Internet]. 2010 Feb 8 [cited 2018 Mar 28];**11**(2). Available from: http://www.ajol.info/index.php/ajbr/article/view/50700
44. Malagnino V, Salpini R, Maffongelli G, Battisti A, Fabeni L, Piermatteo L, et al. High rates of chronic HBV genotype E infection in a group of migrants in Italy from West Africa: Virological characteristics associated with poor immune clearance. Bonino F, editor. *PLoS One*. 2018 Mar;**13**(3):e0195045.
45. Cao G-W. Clinical relevance and public health significance of hepatitis B virus genomic variations. *World J Gastroenterol* [Internet]. 2009 Dec 14 [cited 2016 Jun 9];**15**(46):5761–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19998495
46. Liu W-C, Lindh M, Buti M, Phiet PH, Mizokami M, Li H-H, et al. sssGenotyping of hepatitis B virus--genotypes a to g by multiplex polymerase chain reaction. *Intervirology* [Internet]. 2008 [cited 2016 Jun 7];**51**(4):247–52. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18812698
47. Livingston SE, Simonetti JP, Bulkow LR, Homan CE, Snowball MM, Cagle HH, et al. Clearance of hepatitis B e antigen in patients with chronic hepatitis B and genotypes

A, B, C, D, and F. *Gastroenterology* [Internet]. 2007 Nov [cited 2016 Apr 11];**133**(5):1452–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17920063

48. Erhardt A, Blondin D, Hauck K, Sagir A, Kohnle T, Heintges T, et al. Response to interferon alfa is hepatitis B virus genotype dependent: genotype A is more sensitive to interferon than genotype D. *Gut* [Internet]. 2005 Jul [cited 2016 Jun 5];**54**(7):1009–13. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1774609&tool=pmcentrez&rendertype=abstract
49. Janssen HLA, van Zonneveld M, Senturk H, Zeuzem S, Akarca US, Cakaloglu Y, et al. Pegylated interferon alfa-2b alone or in combination with lamivudine for HBeAg-positive chronic hepatitis B: a randomised trial. *Lancet (London, England)* [Internet]. Jan [cited 2016 Jun 5];**365**(9454):123–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15639293
50. Hsieh T-H, Tseng T-C, Liu C-J, Lai M-Y, Chen P-J, Hsieh H-L, et al. Earlier, Hepatitis B virus genotype B has an C, emergence of lamivudine resistance than genotype [Internet]. Antiviral Therapy. 2009 [cited 2016 Jun 6]. p. 1157–1153. Available from: http://www.intmedpress.com/serveFile.cfm?sUID=4f98a1b5-7947-4061-abd7-4040f37059f0
51. Guirgis BSS, Abbas RO, Azzazy HME. Hepatitis B virus genotyping: current methods and clinical implications. *Int J Infect Dis* [Internet]. 2010 Nov [cited 2016 Feb 24];**14**(11):e941-53. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20674432
52. 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* [Internet]. 2014 Jun 21 [cited 2016 Apr 11];**20**(23):7152–68. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4064061&tool=pmcentrez&rendertype=abstract