

Research Article

Molecular Characterization of Hepatitis B Virus among HIV Positive and HIV Negative Pregnant Antenatal Women in Federal Capital Territory Abuja Hospital

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Abstract

A significant contributor to chronic hepatitis, cirrhosis and hepatocellular carcinoma is the Hepatitis B Virus (HBV). Hepatitis B Virus (HBV) is a major cause of Chronic Hepatitis, cirrhosis and Hepatocellular Cancer (HCC). The incidence of HBV-related HCC cases is projected to increase for at least two decades due to the high prevalence of chronic HBV infection throughout the world. The overall aim of this study is to determine the seroprevalence and molecular characterization of hepatitis B virus among HIV positive and HIV negative antenatal women attending Federal Capital Territory Abuja hospitals. A total number of 302 samples were collected from 302 participants between the ages of 18-55 years. The samples were screened for HIV, HBV, HBV serology markers, using rapid test kits, HBV Viral load and HBV genotyping was carried out using multiplex PCR method and data was analyzed using appropriate tools. Nine tested positive for HIV, 34 tested positive for HBV and 2 tested positive for both HIV and HBV, corresponding to 2.9 % (95% CI; 1.2 - 3.8) prevalence for HIV, 11.3% (95% CI; 8.7 - 13.6) for Hepatitis B and 0.7% for HIV/HBV co-infection. Age 25-35 years old had the highest prevalence of HBV 22(7.3%) followed by those of less than 25 years old 8(2.8%) and 1(0.7%) each for age range 36-45 years, 2(0.7%) and 46-55 years. HBeAb and HBcAb were expressed highest among subject within the age range of 25 - 35 year 14 (41.2%) and 22 (64.7 %,) participants respectively, followed by the age range of less than 25 years 5 (14.7%) and 8 (36.4%), for HBeAb and HBcAb respectively. There was no Hepatitis B envelop antigen (HBeAg) nor Hepatitis B surface antibody (HBsAb) among the study group. Of the 34 samples, 20 (62%) had undetectable viral load, while 14 (38%) had detectable viral load. Genotype E was three times more prevalent among those of 25 years old and above than those less than 25 years old detected (75% and 25%). However, genotype B/E mixed was more expressed (60% of cases) among those less than 25 years old, than those greater than 25 years of age (40%). There

was no significant difference in the prevalence of genotype E and B/E serotype between HBV+/HIV-patients and their HBV+/HIV+ counterpart (P=0.08 and P=0.15, respectively). HBV Genotype E single infection viral load was significantly (P=0.01)

more expressed among those 25 years of age and above than less than 25 years old ($4,354,703 \pm 346,865$ IU/ml versus 55.6 ± 28.1 IU/ml). Mixed infection genotype B/E was significantly ($P=0.04$) more expressed among those less than 25 years of age than those 25 years old and above (730.9 ± 238 IU/ml versus 91.1 ± 48 IU/ml). There was no significant difference between the mean of HBV Viral load HBV+/HIV- patients and their HBV+/HIV+ counterpart (T-test =0.009; $P=0.92$). In conclusion FCT is 11.3% prevalence rate of HBV and genotypes E and B/E are in circulation among pregnant women among the pregnant women attending antenatal clinic in FCT hospital in Abuja.

Keywords: Molecular; Hepatitis; HIV; Pregnant; Women

Introduction

Cirrhosis, hepatocellular carcinoma and chronic hepatitis are all mainly caused by the Hepatitis B Virus (HBV). Nearly two billion people globally suffer from HBV infection; of them, 360 million are thought to be chronically infected and nearly one million of this pass away each year [1]. About half of the estimated 360 million critically ill individuals contract the disease during pregnancy or infancy [2]. It is believed that this is a major contributing cause to the high prevalence of HBV infection in sub-Saharan Africa and other regions with high rates of HBeAg-positive infections in women of childbearing age [3]. Regardless of the evolution of antiviral interventions, the majority of those with chronic HBV infection are located in regions where these medications are not cost-effective nor easy to find [4]. Given to the substantial rate of chronic HBV infection around the globe, the number of HCC cases associated with HBV is expected to rise for at least two decades. In Nigeria, regional investigations have revealed a range of sero-prevalence rates, from 4.7% to 15.8% [5].

Hepatitis B Virus poses a serious threat to global public health as it is the commonest cause of Chronic Liver Disease in Nigeria. This places an enormous burden on the healthcare system and leads to a loss of productivity in the active population [5]. HBV is vaccine-preventable yet Nigeria is hyper-endemic having 14% for HBV with about 24 million infected people, the majority of who are children and young adults. This estimate, places Nigeria as one of the most HBV affected countries in Africa [6]. A recent meta-analysis showed that the pooled prevalence of HBV in pregnant women attending Antenatal Care (ANC) in Nigeria was 12% and represents only a fraction of all pregnant women, as many women of about 40% still do not attend ANC. According to other research, between 8 and 11% of pregnant women have high levels of HBV [7]. Based on Bierhoff et al., [8]. Mother-to-Child Transmission of HBV (MTCT-HBV) accounts for a large portion of HBV cases in Nigeria. According to certain investigations, children with HBV had an incidence rate that is between 12 and 17% [9]. Surveys across Nigeria have revealed high HBeAg-positivity rates of 30 to 63% and vertical transmission rates of 40 to 53%, demonstrating that the threat of vertical transmission (MTCT), is significant and cannot be disregarded in Nigeria [10]. The primary cause of liver cancer is thought to be 60% HBV, which is the second most common toxin cause after tobacco use [11]. The incidence of HBV infection ranges globally, with over half of the earth's residents living in areas where HBV infection is prevalent, which include most of Asia and Africa, with Sub-Sahara Africa having the second highest overall burden of persistent HBV infection behind the Asian continent [11].

Motherly diagnosis, post-exposure prophylaxis including hepatitis B vaccination commencing at birth and the administration of updated anti-viral drugs for high-risk pregnant women diagnosed with HBV are all approaches for decreasing the entire highest prevalence of emerging chronic HBV illnesses [12]. HBV is most commonly obtained in the prenatal stage or early childhood in endemic areas [13]. Chronic infection is considerably more likely to occur in newborns (90%) and youngsters (30%) [14]. Despite adults having the maximum incidences of fresh infection and acute illness, the progress rate between acute through prolonged HBV infection is less than 5% [15]. Given that 90 percent of HBeAg-reactive mothers pass the virus to their kids while only 10 to 20 percent of HBeAg-non-reactive mothers do, this incidence correlates with the mother's viral multiplication status [16]. The overall prevalence of positive HBeAg among these nations' procreative women is likely a contributing factor to the specific rate of perinatal transmission in endemic regions [16]. Several women of reproductive age who acquired HBV infection in their early childhood are still extremely contagious to their infants since studies have demonstrated that the frequency of HBeAg seroconversion within the initial 20 years after birth is quite slow [17].

A number of these transferable diseases are only routinely screened for in pregnant women and blood donors. It has had a negative impact on the prevention, detecting and early treatment of the illness during pregnancy [18]. While few studies on the seroprevalence of HBV infection in pregnancy have been conducted in Nigeria, only a few of them took place in secondary

healthcare centres in the country's southern area [19]. Perhaps there is a lack of understanding and information about the subject, particularly in FCTA. The overall aim of this study is to determine the molecular characteristics of the hepatitis B virus in HIV-positive and negative antenatal mothers accessing hospitals in the Federal Capital Territory of Abuja.

Material and Methods

Study Population

The group was made up of women who are pregnant that are from different parts of Nigeria who were attending prenatal clinics at FCTA hospitals in FCT, North Central. Pregnant women from various regions of Nigeria who were visiting antenatal clinics at FCTA hospitals in FCT, North Central, made up the study population.

Ethical Approval

Informed consent was gotten from all participants who enrolled for the research procedure in all the hospitals and request was sort for ethical approval and this was granted by the Health and Human Services Secretariat.

Sample Size

The test size for this study was obtained using Araoye of 2004 formula at a 95% confidence level. Therefore, the calculated sample size = 100.

However, when an attrition rate of 10% was added, the sample size is determined as follows: calculated sample size + attrition rate = 110. 302 subjects between the childbearing age of 18 -55 years and above were recruited for the study.

Sample Collection

Five millilitres of venous blood were drawn and allowed to coagulate in a plain blood container with clear labelling. After being carefully divided into cryovials, the serum was kept for long-term storage below -20°C and for 3 days at 2-8°C. Before testing, specimens were allowed to come to ambient temperature. To obtain demographic information and other relevant details, a comprehensive survey was created and distributed to each of the volunteers at the different facilities in FCTA.

Sample Analysis/Methods

HBsAg Screening: One-step Hepatitis B was used to detect the HBsAg in the specimen using whole blood. The test samples were allowed to reach ambient environment. The test strips were taken out of the enclose packet. The strips were submerged vertically into the specimen carefully for 15 seconds, ensuring that it did not cross the limit line on the strip. The strips were removed and placed on a clean smooth surface. The results were read after 15 minutes.

Negative: One red line at control region. No red line in the test region.

Positive: One red line each at both control and test region.

HBV Serological Screening (5 Parameter Rapid Test)

Using rapid test kits (Acon Laboratories, USA) to detect the hepatitis markers both antigens and antibodies. It is a quantitative test that makes use of chromatographic immunoassay. Every component on the strip of the panel consists of a specimen pad, a colloid gold conjugate pad, an absorbent pad and a strip of nitrocellulose membrane (NC membrane) with pre-applied control and test bands. The HBsAg strip performs a sandwich immunoassay with the aid of antibodies. The NC membrane has a monoclonal anti-HBsAg pre-coated on it and the conjugate pad has polyclonal anti-HBsAg antibodies coupled to colloid gold.

Procedure 5 Parameter Rapid Test

Specimen and reagents strip were allowed to get to ambient temperature. The pack was cut open to remove the test kit. Once opened the test instrument was placed on a flat, smooth surface. The specimen ID number was put on the gadget. Using a pipette dropper 2-3 drops of the specimen was added to each sample well, making sure no air bubble was added. After 15 minutes the result was read as follows.

Interpretation of Assay Result

Negative Result: Negative result showed only the C band develops on the HBsAg, HBsAg strip or both the C and T bands appear on the HBeAb or HBcAb strip.

Positive Result: The test reveals the existence of the component being examined shows the HBsAg, HBsAb or HBeAg strip forms both the C and T bands or the HBeAb or HBcAb strip showed only on the C band.

HIV Screening (Using the National Algorithm)

Determine, Unigold and Stat Pak. The test strip was removed from the pack and placed on a flat smooth surface and 50nl of the sample was introduced into the test strip following the direction of the arrow mark on the test pad. A drop of chase buffer was added to the test pad after 60 seconds. The result was read after 15 minutes.

Positive: (Two Bars) at the control window and the patient window.

Negative: (One Bar) red bar control window and no appears in the patient.

Viral Load Using (HBV PCR Fluorescence Detection Test)

Following the direction on the package insert, HBV DNA was extracted from the samples utilizing the Bioneer AccuPrep genetic DNA extraction reagent.

Procedure

To the washing buffer was added 17 ml of absolute ethanol. Then 48 ml of absolute ethanol was added to the washing was added to the washing buffer and mixed. 48 ml absolute ethanol was added to washing buffer w and mixed well.

Hepatitis B Virus First Round PCR.

Samples ID were written on the premix tubes. Master mix was prepared by adding 16µl of deionized water (DH₂O) into 250 µm of each of dnTP, IX PCR buffer, 15 Mm of MgCl₂ and IU of thermostable Taq polymerase, 2µl of extracted DNA and 1µl of P1 (forward) and 1µl of S1-2 (reverse) outer primers. Two (2) µl of the master mix (cocktail) was add into the sample id labelled premix tubes. The tubes were place in a programmed thermal controller at various temperatures and time as follows Initial activation at 95°C for 5 min, denaturation at 94°C for 20 sec, annealing at 55°C for 20 sec and extraction at 72°C for 1 min were the reaction's operating conditions. Making 30 cycles, from denaturation to extension, that were observed. The last extension was set for five minutes at 72°C.

Second Round PCR; Hepatitis B Virus Genotyping.

Two separate tubes were labelled for each sample one for the common universal sense primer (B2) and type specific primer for genotypes A, B, C in mix A the other tube for the common anti universal primer B2R and type specific primer for genotypes D, E, F in mix B. Into each of the mix A and mix B was tubes added 17 ml of DH₂O and 2 ml of master mix (cocktail) primer and one (1) ul of first-round PCR product. It was mixed gently and centrifuged for 5 minutes at 3000 rpm. The tubes were place in a programmed thermal controller at various temperatures and time as follows for PCR; 3 minutes for initial activation at 94°C, followed by 1 minute for 30 cycles of denaturation at 94°C, 1 minute at 50°C for and 1 minute at 72°C for extension for both "mix A" and "mix B," with a final extension at 72°C for 5.

Agarose Gel Preparation and Casting Procedure

Into a conical flask containing 100 ml of TAE buffer was added 2 gram of agarose powder and it was mixed gently, heated in a microwave for 2 minutes to dissolve the mixture completely. The mixture was allowed to cool to about 50-55°C for about 15 minutes. Five µl Ethidium bromide, which serve as stain was added and gently mixed. The gel chamber's ends were sealed with tape. Combs were set on the gel tray; the molten gel was poured onto the casting tray. The combs were removed, TAE buffer was applied into the gel. The gel's wells were filled with the DNA and the standard (ladder). The gel was electrophoresed in for 45 minutes at 100V with the negative control, sample and ladder. Images of the bands were captured after 45 minutes via a gel documentation device (Bio-Rad gel Doc-XR, USA). The measurement of the different bands was determined using the Gene Ruler TM 100bp+DNA LADDER (Table 1,2).

First-round PCR				
Primer	sequence (5'-3')	specificity	position	Polarity
P1	TCACCATATTCTTGGGAACAAGA	Universal	2823-2845	Sense
S1-2	CGAACCACTGAACAAATGGC	Universal	685-704	Anti-sense
Second round PCR: Mix A				
B2	GGCTCCAGTTCGGAACAGT	Type A-E	67-86	Sense
BAIR	CTCGCGGAGATTGACGAGATGT	TYPE A	113-134	Anti-sense
BBIR	GGTCCTAGGAATCCTGATGTTG	TYPE B	165-186	Anti-sense
BCIR	CAGGTTGGTGAGCTGGAGA	TYPE C	2979-2996	Anti-sense
Second round PCR: Mix B				
B2R	GGAGGCGGATTTGCTGGCAA	Type D-F	3078-3097	Anti-sense
BD1	GCCAACAAGGTAGGAGCT	Type D	2979-2996	Sense
BE1	CACCAGAAATCCAGATTGGGACCA	Type E	2955-2978	Sense
BF1	GTTACGGTCCAGGGTTACCA	Type F	3032-3051	Sense

Table 1: Primer sequences for multiplex-nested PCR utilized in HBV amplification and genotyping.

Primer Mix	HBV Genotype	Band Size (bp)
Mix A	A	68
	B	281
	C	122
Mix B	D	119
	E	167
	F	97

Table 2: Viral Genotypes and Their Band Sizes in Base Pairs (Bp).

Results

Socio-demographic Characteristics of the Pregnant Women Attending Antenatal Clinics in FCTA Hospitals

Table 3 reveals the socio demographic characteristics of antenatal women. From the Table, a total number of 302 participants were recruited at random for the study. Social demographic characteristics of the participants showed that the age interval of participants was 18 to 65 years. The most frequent age interval was 26 to 35 years 115 (51.3%), followed by 18 to 25 years 70 (23.2%). In terms of marital status, majority of the participants were married 224(74.2%). Educationally, almost all the participants 293 (97%) had completed at least primary school education. In terms of the number of children each participant had, 160 (53%) of the participants had at least one child. The distribution by gestational age revealed that about half of the participants were in their first 67(22.2%) and second trimester 78 (25.8).

Variables	Number Observed	Percentage (%)
Age (years)		
18 - 25	70	23.2
26 - 35	155*	51.3 *
36 - 45	56	18.5
46 - 55	17	5.6
56 - 65	4	1.5
Total	302	100
Marital status		
Divorced	19	6.3
Married	224*	74.2*
Single	59	19.5
Total	302	100

Education Status		
College	179*	59.3*
Junior Secondary	6	2.0
Master or above	45	14.9
Never went to school	6	2.0
Primary	9	3.0
Senior Secondary	48	15.9
Total	293	97
Number of Children		
More than one	83	27.5
Zero	124*	41.1*
One	77	25.5
Total	284	94.0
Gestational period (age)		
First	67*	22.2*
Second	78*	25.8*
Third	37	12.3
Total	182	60

Table 3: Socio demographic characteristics of pregnant women attending antenatal clinics in hospitals in FCT Abuja.

Awareness of the mode of transmission of Hepatitis B Virus (HBV)

Table 4 presents the level of awareness of the mode of transmission of Hepatitis B Virus among the study participants. Majority of the participants knew that Hepatitis B is caused by a virus 253 (83.8%), transmitted through blood transfusion 235 (77.8%), through unprotected sex 235 (77.8%), from mother to child 234 (77.5%), using unsafe needles and sharps 253 (83.8%) and that co-infection (HIV and HBV) do exist 250 (82.8%). Also, more than half 217 (71.9%) of the participants understand that HBV infection could result to liver cancer.

Variables	Number Observed	Percentage (%)
Hepatitis B is caused by a virus		
Don't know	24	7.9
No	16	5.3
Yes	253*	83.8*
HBV can be transmitted Through blood transfusion		
Don't know	37	18.2
No	6	2.0
Yes	235*	77.8*
HBV can be transmitted through unprotected sex		
Don't know	55	18.2
No	6	2.0
Yes	235*	77.8*
HBV can be transmitted from mother to fetus		
Don't know	10	3.3
No	58	19.2
Yes	234*	77.5*
HBV can be transmitted through use of unsafe needles or sharps		

Don't know	40	13.2
No	3	1.0
Yes	253*	83.8*
An individual can be infected by both HBV and HIV		
Don't know		
No	40	13.2
Yes	6	2.0
HBV infection can lead to liver cancer	250*	82.8*
Don't know	64	21.2
No	15	5.0
Yes	217*	71.9*
Have you ever been operated upon?		
No	240*	79.5*
Yes	44	14.6
Are you on any antiretroviral drug?		
No	248*	82.1*
Yes	39	12.9

Table 4: Awareness of the mode of transmission of Hepatitis B Virus (HBV) among the study participants.

Evaluation of Exposure to Risk Factors by Study Participants

Table 5 presents the evaluation of exposure to risk factors by the study participants. More than half (62.9%) of the participants knew that HBV can infect an individual without having any symptoms of the infection. Majority of the participants (84.9% and 79.5%) were willing to get their babies vaccinated and had never received blood transfusion. Also, a majority of the participants do not have multiple sexual partners 172 (57.0%) and 9 (3%) exchange needles and sharps. More than half of the participants had not been vaccinated 264 (87.7%) and 274 (90.7%) were not on Anti-retroviral drugs.

Variables	Number observed	Percentage (%)
A person can be infected with HBV and not have symptoms of the disease		
Don't know		
No	74	24.5
Yes	19	6.3
	190*	62.9*
PIB		
Don't know	81	26.8
No	12	4.0
Yes	187*	61.9*
Are you willing to get your baby vaccinated against HBV		
Don't know	22	
No	24	7.3
Yes	256*	7.9
Share sharp objects		
No	223*	84.8*
Yes	64	73.8
Have domestic staff		
No	200*	21.2*
Yes	84	66.2*

Are they tested for HBV		
No	172*	27.8
Yes	90	57.0*
Do you have multiple partners		
No	264*	29.8
Yes	23	87.4*
Have you ever been vaccinated with HBV vaccine		
No		7.6
Yes		
Have you ever been transfused?		
No	240*	79.5*
Yes	44	14.6
Have you ever been operated upon?		
No	248*	82.1*
Yes	39	12.9
Are you on any antiretroviral drug?		
No	274*	90.7*
Yes	13	4.3
Are you on any regular injectable?		
No	277*	91.7*
Yes	6	2.0
If yes, do you reuse needles		
No	275*	91.1*
Yes	9	3.0

Table 5: Evaluation of exposure to risk factors by study participants.

Prevalence of HBV / HIV Co-infection, HIV, HBV Among Participants

Fig. 1 illustrates that 302 participants were tested for both HIV and HBV. Nine tested positive for HIV, 34 tested positive for HBV and 9 tested positive for both HIV and HBV, corresponding to 2.9 % (95% CI; 1.2 - 3.8) prevalence for HIV, 11.3% (95% CI; 8.7 - 13.6) for Hepatitis B and less than 0.7 % for HIV/HBV co-infection.

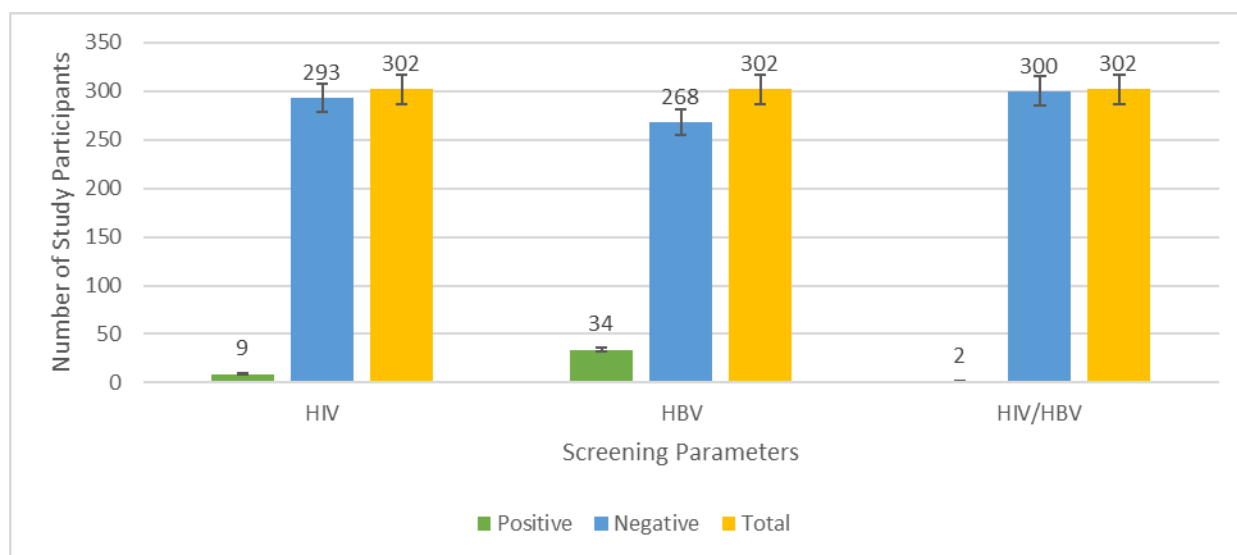


Figure 1: Prevalence of HBV, HIV and HBV/HIV co-infection among pregnant women attending antenatal clinic in hospitals in FCT Abuja.

Age Distribution of HIV and HBV Positive Pregnant Women Attending Antenatal Clinic in Hospitals in FCT, Abuja

Fig. 2 shows the age distribution of HIV and HBV positive pregnant women attending antenatal clinic in hospitals in FCT Abuja. From the figure, 34 were positive for HBV and 9 for HIV. The figure reveals that participants within the age group that are less than 25 years is 8 (2.6%), 25-36 years, 22(7.3%), 36-45 years, 2(0.7%) and 46-55 years, 2 (0.7%). These findings shows that pregnant women within the age group of 25-36 years old are more susceptible to HBV Infection, followed by those below the age of 25 years old.

Age Related Prevalence of Circulating HBeAb and HBcAb Among HBV Positive Study Participants of Different Age Groups

Fig. 3 presents the age-related prevalence of circulating Hepatitis B envelop antibody (HBeAb) 34 (11.3%) and Hepatitis B core antibody (HBcAb) 22(7.3%) among HBV positive study participants of different age groups. HBeAb and HBcAb were expressed highest among the age group of 25 - 35-year-old, 14 (4.6%) and 22 (7.3%), respectively, followed by the interval less than 25 years old, 5 (1.7%) and 8 (2.6%), respectively). Others, 36 -45 years old, 1 (0.3%) 2(0.7%) and 46 -55 years old, 2 (0.7%) 2 (0.7%) respectively. From the table there was no Hepatitis B envelop antigen (HBeAg) nor Hepatitis B surface antibody (HBsAb) in all the pregnant women with HBV infection.

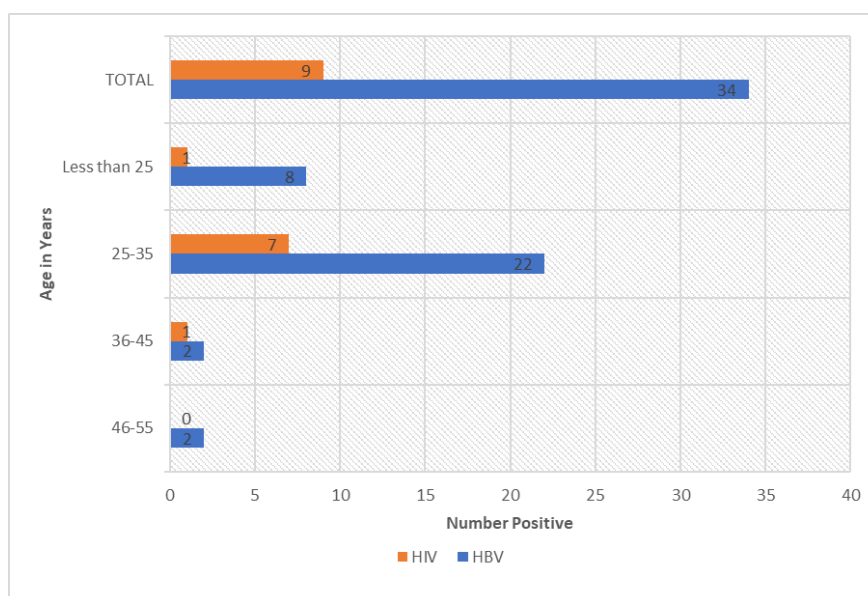


Figure 2: Age distribution of HIV and HBV positive pregnant women attending antenatal clinics in FCT, Abuja.

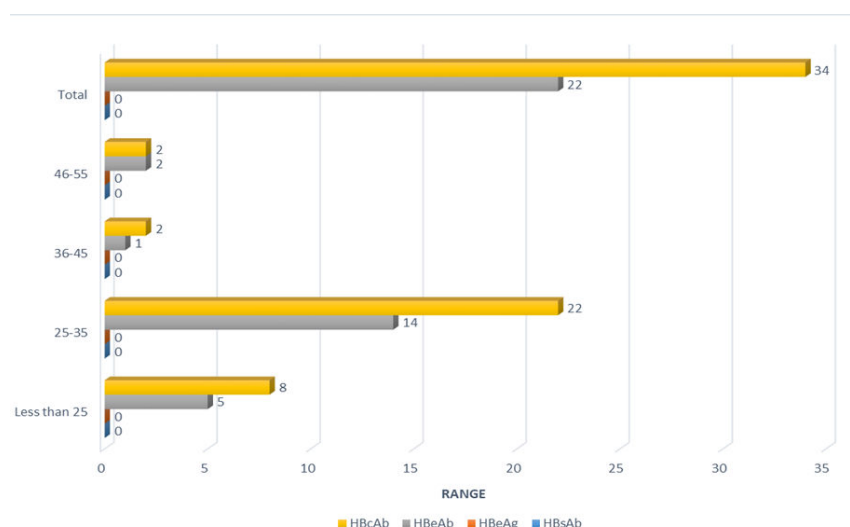


Figure 3: Age related prevalence of circulating HBeAb, HBcAb HBsAb and HBeAg among HBV positive participants by age groups.

Age Distribution of HBV Genotypes Expressed Among the HBV Positive Participants

Table 6 shows the age distribution of HBV genotypes expressed among the HBV positive participants. Genotype E was three times more prevalent among those 25 years and above, than those less than 25 years old (3 and 1). However, genotype B/E mixed was more expressed (3 of cases) among those less than 25 years, those than greater than 25 years [2]. Genotype B single infection was not expressed in any of the pregnant women with HBV infection. This implies that genotype E is more predominant among HBV infected pregnant women who attended antenatal clinics in FCT Abuja hospitals.

Genotypes	Age (Less than 25 years)	Age (25 years and above)	X ² ; P-value
Genotype E Single infection	1	3	1.06; 0.30
Genotype B Single infection	0	0	None
Genotype B&E Mixed Infection	3	2	0.21; 0.64
P= probability; Chi-square=X ²			

Table 6: Age distribution of HBV genotypes expressed among the HBV positive participants.

Viral Load of HBV Genotypes Expressed Among Participants of Different Age Groups

Table 7 shows viral load of HBV genotypes expressed among participants of different age group. Genotype E single infection was significantly (P=0.01) more expressed viral load among those 25 years old and above, compare to less than 25 years old (4,354,703 ± 346865 IU/ml versus 55.6 ± 28.1 IU/ml). Mixed infection genotype B/E has significantly (P=0.04) more expressed viral load among those of less than 25 years old than those 25 years old and above (730.9 ± 238 IU/ml versus IU/ml versus 91.1 ± 48 IU/ml). Genotype B single infection was not identified in any of the participants so no mean viral load value.

Viral Load of HBV Genotypes	Age (less than 25 years)	Age (25 years and above)	P-value
Genotype E single infection	55.6 ± 28.1 IU/ml	4,354,703 ± 346865 IU/ml	0.01*;
Genotype B single infection	0	0	None
Genotype B&E Mixed infection	730.9 ± 238 IU/ml	91.1 ± 48 IU/ml	0.04*
P-value < 0.05 (significant)			

Table 7: Viral loads of HBV genotypes expressed among participants of different age groups.

Comparing the Viral Load of HBV+ / HIV- and HBV+ / HIV+

Table 8 shows that there was no statistically significant difference between the Mean of HBV Viral Load of HBV+/HIV- antenatal pregnant women attending hospital in FTC Abuja and their HBV+/HIV+ counterpart (T-test = 0.009; p=0.92).

Variable	HBV+/HIV- (n = 32)	HBV+/HIV+ (n = 2)	T-Test	P-value
Mean HBV Viral Load ±SEM	382236 ± 21950	227059 ± 32108	0.009	0.92
SEM= Standard Error of the Mean; T tabulated at p=0.05 with 33 degree of freedom = 2.037.				

Table 8: Comparing the viral load of HBV+ / HIV- and HBV+ / HIV+ for any significant difference.

Comparing the Genotype Identified Among HBV+ / HIV- and HBV+ / HIV+ Study Participants

Table 9 compares the genotype identified among HBV+/HIV- and HBV+/HIV+ wish shows that there was no significant difference in the type of HBV genotypes expressed in both categories of antenatal pregnant women attending clinics in FCT hospital in Abuja.

Variable	HBV+/HIV- (n=32)	HBV+/HIV+ (n=2)	Chi-Square (MH)	P-value
Prevalence of genotype E	3	1	2.9	0.08
Prevalence of genotype B/E	4	1	2.0	0.15

Table 9: Comparison between the genotype of HBV+ / HIV- and HBV+ / HIV+ study participants.

Proportion of Detectable and Undetectable Viral Load Among HBV Positive Participants

Fig. 4 shows the proportion of undetectable viral load among HBV positive participants. Genotype E had 4(4.12%) participants having detectable viral load, genotype B/E, 5(5.14%) participants and unclassified 5(5.15%) participant genotypes could not be identified by the kit used though they had detectable viral load. and undetectable 20(20.59%) while these participants did not have any antigen detected by the kit used.

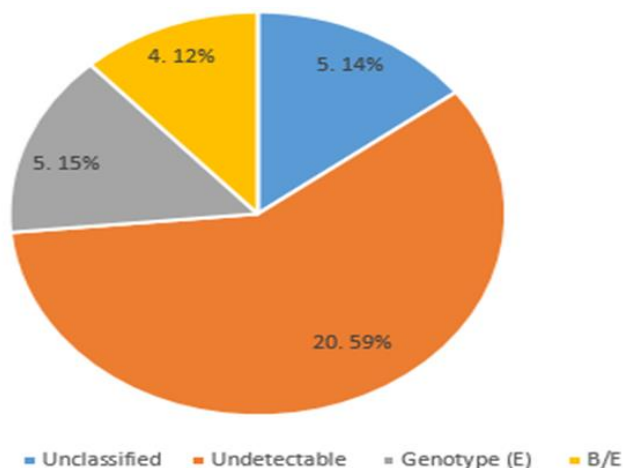


Figure 4: It shows the proportion of undetectable viral load among HBV positive participants.

Agarose Gel Electrophoresis Plate of HBV DNA Genotype Using mix A and mix B to Identify Genotype B and E

Fig. 5 shows the agarose gel electrophoresis plate of HBV DNA genotypes using mix A and mix B using two tubes for each reaction (multiplex mix A identifies genotyped A (68 bp), B (281 bp) and C (122 bp). We saw bands on the 281 bp (relative to the molecular ladder) in mix A, hence genotypes B identified. Mix B identifies genotypes D (119 bp), E (167 bp) and F (97 bp). There were bands on the 167 bp (relative to the molecular ladder) in mix B hence genotype E identified using Gene Ruler TM 100 bp+DNA LADDER.

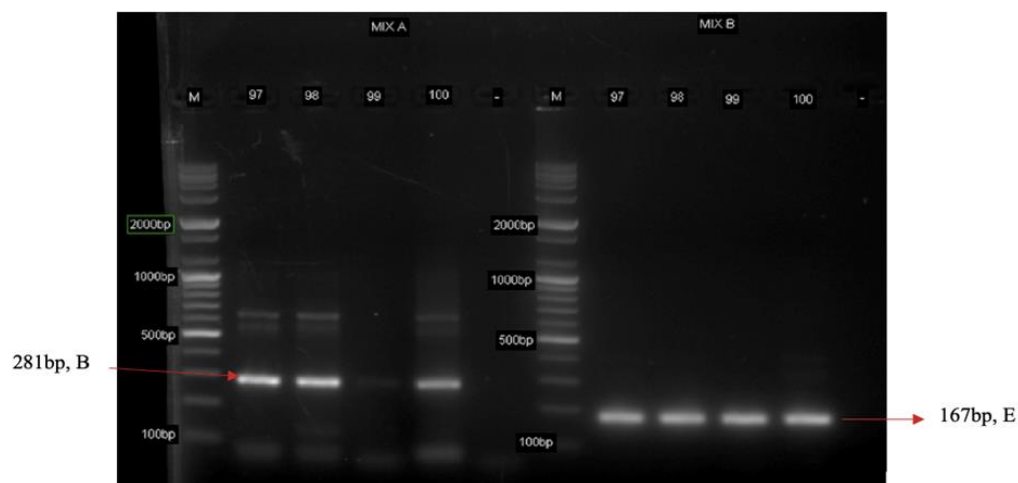


Figure 5: It shows the agarose gel electrophoresis plate of HBV DNA genotypes using mix A and mix B using two tubes for each reaction (multiplex mix A identifies. Agarose gel electrophoresis plate of, HBV DNA standard + samples. Mix A and B identifying HBV genotypes B (281 bp) and E (167 bp) using Gene Ruler TM 100 bp+DNA LADDER.

Discussion

The overall aim of the study was to establish the molecular characterization of hepatitis B virus among pregnant women attending antenatal clinics in Hospitals in FCT, Abuja. Our findings showed that the prevalence of HIV was 2.9%. This prevalence was far below the 26.4% prevalent rate recorded among pregnant women attending antenatal clinic in University College Hospital, Ibadan [20]. This prevalence rate of HIV among pregnant women attending antenatal clinic in hospitals in FCT, Abuja, was remarkably lower, compared with the previous reports of pregnant in previous years; 4.5% in 2001, 11.2% in 2006 and 12.3% in 2009 [21]. This could be attributed to increase in awareness of HIV and its mode of infection. The prevalence of 2.9% recorded among pregnant women, also correspond with the overall prevalence of HIV in Nigeria, in 2016 [20], for women attending antenatal clinic in 2014 and 2018, as reported by the federal Government of Nigeria in 2018 and UNAIDS in 2018 [22].

From our findings, 34 of the pregnant women were positive for HBV out of the 302-participant screened, corresponding to 11.3% (95% CI; 8.7 - 13.6). The highest prevalence was observed among the age range 25-35 years, 22 (7.3%). These findings corresponded with highest prevalence of HBV infection observed in pregnant women in the age range of 25-35 years among the study population [23]. Ranges of 2% to 15% prevalence of HBV among pregnant women have been reported in Nigeria [24]. In contrast to our findings on similar research studies carried out in other part of Nigeria, the following reported a high prevalence rate of HBV infection (19.8%), among pregnant women attending antenatal care clinic at Federal Medical Center, Keffi, North-Central Nigeria 16.6% among patients attending a tertiary hospital in Jalingo at Ibadan 16.3% and 14.6% in Bauchi, North-Eastern Nigeria [19,25-27]. A pooled prevalence of 13.6% for adults and 11.5% for children from another study conducted in Nigeria [28]. Similarly, some investigators found a high HBV prevalence of 25.7% among blood donors 23.4% among surgeons and infants 16.3% [29]. Umego, et al., have reported 12.7% and 12.8% prevalence rate of HBV in Kwara and Niger respectively, among pregnant women [28]. Lower rates of 4.0%, 6.08% and 7.1%, were recorded in Studies conducted on pregnant women attending antenatal clinics in Ekiti, Lagos and Osun states in South western Nigeria respectively [30]. In South eastern Nigeria, 3.4%, 6.5% 7.1% and 7.6% prevalence rates were observed among pregnant women in Enugu, Ebonyi, Abia and Anambra states [28].

The 11.3% prevalence rate recorded in this study, depicts a trend that follows a low prevalence from the Southern parts of the country and increasing to its highest of 15.8% in the Northern parts. This could be attributed to the level of health education on prevention practices, early seeking of health-care assistance and effective utilization of these health-care facilities may play a role in this trend.

All the 34 pregnant women positive for HBV had HBcAb, these antibodies appear in circulation at the acute stage of HBV infection and it remains circulation for life [31]. The presence of HBcAb most likely indicates previous or ongoing infection with HBV [32]. Therefore, the women who had no immunity to the virus were likely having chronic hepatitis B infection but had reduced risk of vertically transmitting the virus to their offspring. Twenty-two HBV positive participants had both HBcAb and HBeAb. The presence of HBeAb correlates with the decreased infectivity, as HBeAb replaces HBeAg in the resolution of the disease [33]. The presence of HBeAg indicates active viral replication in hepatocytes with high risk for developing hepatocellular carcinoma [34]. In this study, there was no HBeAg nor HBsAb in all the pregnant women with HBV infection (0.0%).

HBV Co-infection with HIV (HBV/HIV) increases the rate of transmission of viral hepatitis substantially [35]. It also increases the risk for hepatotoxicity of HAART and likelihood of onset of an AIDS-defining illness [35]. The HIV/HBV co-infection rate in this study was 0.7%. This is far lower compared to a study done in Jos, Plateau State, with a prevalence rate of 12.6% among pregnant women [36]. Studies conducted in Africa showed that the rates of HBV co-infection among HIV-positive individuals vary from 6.1% in Kenya [37]. to 25.9% in Nigeria [23] in 2018. From our study, two (0.7%) participants were both HBV/HIV positive accounting for HIV/HBV co-infection in this study. Similar rates have been recorded in similar studies. Prevalence rates of HIV/HBV co-Infection was 0.24% in National Hospital, FCT, Abuja, Nigeria [38]. A 0.7% prevalent rate of HBV/HIV co-infection among pregnant women was reported in Anambra State, Nigeria [39].

Although HBV infection is widely reported to be endemic in Nigeria and the prevalence of HBV genotypes, the molecular epidemiology of the virus is poorly understood across the country with limited available data [40]. Our study revealed that Nine (9) out of the 34 positive HBV samples, only two HBV genotypes (B and E), were confirmed to be circulating in the study population. This finding corroborates with the finding of a similar study carried out in Zaria, that genotypes B and E are prevalent

in Nigeria [41]. Other Authors have reported the predominance of genotypes A, B and E in different population groups [41]. Genotypes of HBV show special geographical distribution worldwide [42]. In this current study, genotype E was found to be the predominant genotype with a prevalence of 75% and genotype B (25%). This finding is in agreement with the research work reported in Philippines and Nigeria [40]. However, this finding was contrary to the study conducted in the South-eastern part of the country as genotype E was not detected but HBV genotype A sub-genotype its sub-genotype A1 were predominate in the study. This could be as a result of the limitation of the test kit used in this research, where it only detects HBV genotype A to J. Also observed in this study, was the mixed infection with genotypes B/E among HBV infected pregnant women in the study. This finding agreed with earlier reports in Nigeria [40]. In Sudan and Pakistan also reported mixed infection with different HBV genotypes among hepatitis B carriers. In this study, Genotype B was express as a co-infection and not as single infection [43].

HBV viral load of Genotype E single infection was significantly ($p=0.01$) more expressed among the HBV positive pregnant women within the age range of 25years and above, compared with those that are less than 25 years of age ($4,354,703 \pm 346865$ IU/ml versus 55.6 ± 28.1 IU/ml). This report is in agreement with the report of Kramvis, who reported that the HBV infected subjects had significantly higher viral load genotype E-infection than HBVB and HBVB/E [44]. A similar group of Sudanese revealed that patients infected with genotype E had higher viral loads compared with those patients with HBV that were infected with genotype D [44]. The proportion of undetectable viral load among HBV positive participants were for Genotype E, 4(4.12%), genotype B/E, 5(5.14%), Unclassified 5(5.15%) and as undetectable 20(20.59%). This corresponds to the study of Ibrahim et al. [45]. Certain individuals have persistent viral count elevations and persistent compensatory hepatocyte proliferation (necro-inflammation), which can lead to cirrhosis or liver cancer in the long run. The relationship between the level of viral growth and the immune system reaction of the host determines the clinical presentation of hepatitis B [46]. Many sufferers exhibit continuously raised viral load and persistent hepatocyte compensatory growth (necro-inflammation), which over time develop to cirrhosis or liver cancer [47].

This indicate that positive HBsAg are at various phase of the infection. For the ones that had detectable viral load and no envelop antigen detected it is most likely that the infection has moved to the inactive stage or immune reactive phase with the envelop antigen absent in the system. While those that the viral load was undetectable, is possible that the virus must have been cleared either with anti-retroviral drug or naturally. So, the infection has entered the inactive or carrier stage which is characterized by very low or undetectable HBV DNA. It is important to regularly monitor the viral load of this class of infected person as they can revert to the active stage with the presence of envelope antigen.

Conclusion

The prevalence of HBV, HIV and co-infection of HBV/HIV among pregnant women attending antenatal clinics in hospital in FCT, Abuja in this study was 11.3%, 2.9% and 0.7% respectively. The study revealed that HBV infection was not at the active viral replication stage. Single HBV genotype E (5.15%) and mixed infection with genotype B/E (4.120) were prevalent among the study population. Other HBV genotypes (15.15%) not detected in this study are likely to exist in the study population. Viral load of genotype E single infection was significantly more expressed among age range of 25 years and above, whereas the viral load of the mixed infection of HBV genotype B/E was more significantly expressed among the study participants less than 25 years of age. This study therefore hopes on more attention to be directed on the screening of HBV among women of child bearing age before pregnancy.

1. Government should set aside fund for more study into hepatitis its diagnosis treatment and monitoring of patient
2. There is need to carry out further study on hepatitis genotypes that could be in circulation which we were not able to identify due to the kit used
3. Policy should be put in place for the vaccination of all child bearing age women and measures should be put in place to monitor the efficiency and implementation of the policy
4. Hepatitis vaccines should be made available for women of child bearing age. The vaccine should be given free or highly subsidized and should be accessible to the women even to the primary health where antenatal services are available
5. There should be deliberate and well-targeted constant awareness program from the urban setting down to the very remote community

Conflict of Interests

The authors declare no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

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Authors Contribution

The entire study procedure was conducted with the involvement of all writers.

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