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Original Article

Prevalence of hepatitis B virus infection among antenatal attendees in a university teaching hospital in Southern Nigeria

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ABSTRACT

Objectives: The aim of the study was to determine the prevalence of HBV infection among pregnant women receiving antenatal care at the University of Uyo Teaching Hospital, Nigeria.

Material and Methods: This was a cross-sectional study of 291 antenatal attendees over a period of 3 months. Blood samples from all consenting pregnant women were tested for hepatitis B surface antigen (HBsAg) and antibody, hepatitis B envelop antigen and antibody, and hepatitis C antibody serum markers for HBV infection. A structured questionnaire was used to obtain sociodemographic data and the presence of risk factors. Data obtained were analyzed using SPSS, version 17.0.

Results: Twenty-three women (7.9%) tested positive for Hepatitis B core antibody, while 6 (2.1%) tested positive for HBsAg. None tested positive for Hepatitis B envelop antigen. Forty-one (14.1%) women showed evidence of post vaccination immunity while 8 (2.8%) had evidence of post-infection immunity. Two hundred and twenty-seven (78.0%) women were susceptible to HBV infection. Higher educational attainment correlated significantly with less risk of acquiring HBV infection ($P = 0.003$).

Conclusion: Our study revealed an intermediate prevalence and low infectivity of HBV infection among our pregnant women. Government and non-governmental organizations should intensify efforts to create awareness about the disease through public enlightenment campaigns, as well as recall systems through systems of mobile communication.

Keywords: Hepatitis B virus infection, Prevalence, Pregnant women, Hepatitis surface antigen

INTRODUCTION

Hepatitis B virus (HBV) infection is a fatal disease and constitutes a serious public health problem globally.[1] Two billion people are infected with HBV worldwide, with 350–400 million having chronic infection and 600,000–1.2 million dying from infection related liver diseases and hepatocellular carcinoma (HCC) each year.[1,2] In 2015, an estimated 4.5 million new cases occurred and about 887,000 people died globally from chronic HBV infection with the major cause of mortality being liver cancer (38%) and cirrhosis (52%).[3]

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Sub-Saharan Africa is reported to harbor 60 million chronically infected people.[1,2] In Nigeria, available evidence indicates that about 21 million people are infected with the HBV while 12.2% of the total population are chronic carriers.[4] Nigeria has remained a hyper-endemic area for HBV infection, despite the availability of a safe and effective vaccine.[5]

The prevalence of hepatitis B surface antigen (HBsAg) in the general Nigerian population ranges from 2.7% to 13.3%[6] while the prevalence of the infection among pregnant Nigerian women ranges from 2% to 15%.[7] Pregnancy does not alter the natural history of HBV and infection in pregnancy is not known to be teratogenic. However, viral hepatitis during pregnancy could result in spontaneous miscarriage, intrauterine growth restriction, and pre-term delivery as well as a higher incidence of low birth weight, low intelligence quotient, liver cirrhosis, and HCC among offsprings of sufferers as they attain adulthood.[1,8] Vertically HBV-infected children have a 200 times higher risk of developing HCC than the general population.[9]

HBV can be transmitted from mother to child, through sexual intercourse, exchange of saliva during kissing with an infected person, unsafe drug injections, use of contaminated or inadequately sterilized instruments, sharing of sharps objects, and blood transfusion.[1]

Pregnant mothers who test positive for both HBsAg and hepatitis B envelop antigen (HBeAg) have a 70–90% risk of transmitting the infection to their newborn and 10–49% risk if they test positive for only HBsAg.[1] It has been shown that vertical transmission occurs in up to 10% of neonates of infected mothers when the infection occurs in the first trimester of pregnancy and in 60–90% of babies when it occurs in the third trimester or during labor with perinatal/neonatal and childhood infection significantly contributing to the population of carriers.[9] It is generally accepted that at least 50% of people with chronic hepatitis, acquired their infections either perinatally or in early childhood, especially in countries where the virus is endemic.[9] Perinatal transmission is the major route of HBV infection in Nigeria probably because of the absence of routine screening in pregnancy and low vaccination rates.[5]

The prevalence of HBV infection among women receiving antenatal care (ANC) is a reliable indicator of its prevalence in the general population.[7] Hence, screening of women attending antenatal clinic for HBV infection can give a reliable prevalence of the disease in a population and in addition, provide an avenue for preventing mother to child transmission of the virus.[7]

Although studies have been carried out on HBV infection in other parts of Nigeria, information on its pattern in our environment is lacking. The aim of this study was to determine the prevalence and pattern of HBV infection among pregnant women receiving ANC in our center. The outcome of this study will assist in the development of guidelines and policies on the prevention and control of HBV in our setting and similar settings within and around Nigeria.

MATERIAL AND METHODS

Study design

This was a cross-sectional study which sought to determine the prevalence of HBV infection among pregnant women receiving ANC at the University of Uyo Teaching Hospital (UUTH), Uyo, Nigeria.

Background/location of study

The University of Uyo Teaching is a 500-bed capacity health-care facility and the only tertiary health institution in the state. The institution receives referrals from peripheral health facilities in the state which has a population of about 4.0 million people.

Study population

The study population was drawn from women who registered for ANC during the study period and those sampled included all categories of pregnant women who consented to participate in the study.

Sample size

A study on prevalence of HBsAg among pregnant women carried out in Port-Harcourt, Rivers State, in South-South, Nigeria, revealed a prevalence of 4.3%.[10] This was the prevalence used to calculate the sample size.

The sample size was determined using the formula[10]

$$n^{\circ} = \frac{Z^2 pq}{d^2}$$

Where

n° = minimum sample size

Z = 1.96 (A value of standard deviation corresponding to 1.96)[11]

p = prevalence of HBsAg in an area in the same zone as Uyo. = 4.3% or 0.043

q = (1–p) = 0.957

d = degree of accuracy desired 2.5% = 0.025

$$n^{\circ} = \frac{(1.96)^2 \times 0.043 \times 0.957}{(0.025)^2} = \frac{3.8416 \times 0.043 \times 0.957}{0.000625}$$

n° = 252.93709.

This was rounded off to 253.

The study population was <10000 (3763). The true sample size *n* was further determined by the equation: $n = n°/1 + (n°-1)/N$

Where n=desired sample size for population <10000
$n°$ = desired sample size for population >10000 i.e. 253
N = total estimated study population = 3763
$n = 253/1 + (253-1/3763)$
$= 237.1205$

Rounded off to 237 and 10% of this number was added to cater for attrition.

Total minimum sample size was 237 + 24 = 261

Sampling technique

The total population that registered for ANC during the period under study was 942 ("*N*!"). The desired sample size was 261. A systematic sampling technique was used. Every k^{th} number from the sample frame was selected every week on Wednesdays during the booking clinic where "*K*" is the sampling interval.

$$K = N!/n$$

N! = total population
n = proposed size of the sample
K = 942/261=3.6

Sampling interval was therefore 3

The starting number was selected by simple random technique through lottery.

Specimen collection and preparation

All women who registered at the antenatal clinic of the hospital during the period of study were counseled on the purpose of the study during the health talks. Every third incident pregnant woman was recruited into the study and willingness to participate was asked. Consenting women were informed of their right to opt-out any time that they desired. They were also assured of the confidentiality of any information obtained from them in the course of the research. A pre-test of the questionnaires at the antenatal clinic was carried out and necessary corrections were made before the commencement of the study. The questionnaires were self-administered by the consenting pregnant women and information obtained from the questionnaires included, the mother's demographic data as well as information on knowledge and risk factors for HBV infection. A sample of blood was collected from each participant in the side laboratory attached to the antenatal clinic and tested with the OnSite HBV 5-Parameter Rapid Test kit for HBsAg, hepatitis B surface antibody (HBsAb), HBeAg, hepatitis B envelop antibody (HBeAb), and hepatitis B core antibody (HBcAb).

The onsite HBV 5–parameter rapid test is a lateral flow chromatographic immunoassay consisting of five test panel strips assembled in one cassette. Each strip of the panel member is composed of a sample pad, colloid gold conjugate pad, nitrocellulose membrane strip pre-coated with control band, and absorbent pad. The onsite HBV 5–parameter CTK BIOTECH INC has a sensitivity accepted as weak positive +, medium positive ++, and strong positive +++. It has specificity of > 98% acceptable and flow rate completed in 5 min.

Inclusion criteria

All pregnant women that consented to participate in the study and were selected by systematic sampling were included in the study.

Exclusion criteria

Patients who were ill were excluded from the study.

Data analysis

Data were statistically described in terms of mean ± standard deviation, frequencies, and percentages when appropriate. Multivariate logistic regression analysis was done to determine the preferential effect of important independent variables on the occurrence of HBV infections. Probability ($P < 0.05$) was considered statistically significant. All statistical calculations were done using computer programs (Statistical Package for the Social Science; SPSS Inc. Chicago, IL, USA) version 17 for Microsoft Windows.

Ethical considerations

The participation of patients in this research study was voluntary. The principle of patient confidentiality was strictly adhered to. Each participant was duly counseled and a written informed consent form signed. The study was approved by the UUTH ethics review committee.

RESULTS

A total of 291 pregnant women were systematically recruited and screened, out of these 64 had serological markers for HBV infection. Their ages ranged from 16 to 43 years, (mean 28.3 ± 4.9 years).

[Table 1] shows the frequency distribution of seromarkers among the pregnant women. Out of the 291 pregnant women tested, 23 were positive to HBcAb with a point prevalence rate of 7.9% (95% confidence interval [CI] 4.79–11.02). Six women tested positive to HBsAg resulting in a prevalence of current infection of HBV of 2.1% (95% CI 0.42–3.70%). Fifty-four women tested positive to HBsAb, giving a prevalence rate of 18.6%, HBeAg was not detected in any pregnant woman whereas HBeAb was detected in 13 women (4.5%).

[Table 2] shows the categories of HBV. Twenty-three women (7.9%) tested positive to HBcAb, which is considered the most representative marker for HBV exposure and represents the proportion of women with past and recent infections to HBV. The criteria for the diagnosis of high infectivity were set for pregnant women who tested positive to HBsAg, HBcAb and HBeAg but negative to HBeAb and HBsAb. No individual met this criterion. Forty-one (14.1%) women tested positive for HBsAb alone indicating individuals with immunity from vaccination. The detection of HBcAb and HBsAb in 8 (2.8%) individuals suggest past infection with conferred immunity. Six (2.1%) women tested positive to HbsAg, indicating those with acute or chronic HBV infection.

Two hundred and twenty-seven pregnant women (78%) lacked serological markers for HBV and were thus susceptible to infection.

Two hundred and seventy-seven married women participated in the study, out of which 21 (7.6%) tested positive while out of 14 unmarried women, 2 (14.3%) tested positive to the infection. The relationship between marital status and HBV was not statistically significant ($P = 0.4$). Eight primigravidae (5.9%) were HBV positive, whereas 15 (9.7%) of the remaining 155 multigravidae were HBV positive. When the two were compared against the risk of having infection with HBV, the difference was not statistically significant. Two women (18.2%) who tested positive had positive history of blood transfusion whereas 9 (81.8%) who tested positive had not received any blood transfusion. However, this was not statistically significant as a risk for acquiring HBV infection. Only 27 (9.3%) respondents practiced some form of contraception and there was no difference in the rate of HBV infection when those who used contraceptives were compared with none users [Table 3].

One hundred and eighty-three women (62.9%) presented in the second trimester. However, 3.1%, 5.5%, and 15.8% of the women who tested positive to HBcAb were in the first, second, and third trimester, respectively. Using Chi square, the proportion of increase in the rate of the HBV infection with trimester of pregnancy was found to be statistically significant ($P = 0.01$).

Higher academic attainment was found to be associated with low risk of HBcAb positivity compared to low level of education and this was statistically significant ($P = 0.001$). Employment as a risk factor for acquiring HBV infection was not statistically significant ($P = 0.82$). Increase parity though associated with increase rate of HBV infection, was

Table 1: Seromarkers of all respondents.

Seromarker	Number	Percentage
Hepatitis B core antibody		
Positive	23	7.9
Negative	268	92.1
Hepatitis B surface antigen		
Positive	6	2.1
Negative	285	97.1
Hepatitis B surface antibody		
Positive	54	18.6
Negative	237	81.4
Hepatitis B envelope antigen		
Positive	0	0
Negative	291	100
Hepatitis B envelope antibody		
Positive	13	4.5
Negative	278	95.5

Table 2: Categories of HBV infection among the respondents.

Serostatus	Point prevalence (%)	95% confidence interval
Past or recent infection (HBcAb Positive)	7.9	4.79–11.02
Current/Chronic infection (HBsAg Positive)	2.1	0.42–3.70
Immunity from vaccination (HBsAb only)	14.1	10.07–18.11
Post-infection immunity (HBsAb+HBcAb)	2.8	0–1.6

HBV: Hepatitis B virus, HBcAb: Hepatitis B core antibody, HBsAg: Hepatitis B surface antigen, HBsAb: Hepatitis B surface antibody, HBcAb: Hepatitis B core antibody

Table 3: Association of sociodemographic data with HBV.

Sociodemographic data	Negative	%	Positive	%	*P*-value
Marital status					
Married	256	92.4	21	7.6	0.4
Not married	12	85.7	2	14.3	
Gestation					
1st Trimester	31	96.9	1	3.1	0.011*
2nd Trimester	173	94.5	10	5.5	
3rd Trimester	64	84.2	12	15.8	
Gravidity					
1	128	94.1	8	5.9	0.20
2–4	129	90	14	10	
≥5	11	75	1	25	
Blood transfusion					
No	258	92.5	21	7.5	0.42
Yes	9	81.8	2	18.2	
Contraception					
No	243	92.1	21	7.9	0.92
Yes	25	92.6	2	7.4	
State of origin					
Akwa Ibom	220	92.8	17	7.2	0.33
Non AKS	48	88.9	6	11.1	

*$P<0.05$ considered statistically significant. HBV: Hepatitis B virus

not found to be statistically significant ($P = 0.48$). Six women volunteered a positive history of tattoo or body piercing and one tested positive to HBcAb while 285 did not give a history of scarification but 22 of them tested positive to HBcAb. Seventeen women volunteered a history of previous sexually transmitted infection and one of them tested positive to HBcAb. Of the 274 women that did not have a history of sexually transmitted infection, 22 were HBcAb positive. Two hundred and eighty-nine women denied contact with a jaundiced patient but 22 (7.6%) of them tested positive to HBcAb. Two persons (0.7%) admitted having had contact with jaundiced patients and one tested positive to HBcAb.

Twenty-two (7.5%) of pregnant women tested positive for HBV infection out of the 269 (92.4%) who did not have more than one sexual partner, whereas one (4.5%) out of the 22(95.5%) women who volunteered information about having more than one sexual partner tested positive to HBV infection. The risk of acquiring HBV infection among pregnant women with multiple sexual partners was not statistically significant. ($P = 0.55$) Whereas 8 (2.8%) women had a positive history of intravenous drug use, only one was positive to HBcAb. Majority (91.9%) of the HBcAb negative women were HIV negative while only 8.1% of the HBcAb seronegative women were HIV positive. Twenty women were HIV positive but only one HIV positive pregnant woman tested positive to HBcAb.

The application of multivariate logistic regression analysis to educational status demonstrated that attainment of higher educational level correlated significantly with less risk of acquiring HBV infection among pregnant women in this study as shown in [Table 4] ($P = 0.003$).

DISCUSSION

This study revealed that the prevalence of HBV infection among pregnant women attending antenatal clinic in our center was 7.9%. This result is very low compared to studies in Jos, Nigeria,[12] and Brazil[13] which reported 63.3% and 42.2% prevalence rates, respectively.

HBsAg assay is the most commonly used marker to indicate ongoing infection with HBV which may be completely asymptomatic. In this study, 2.1% of the patients tested positive to HBsAg. This value is similar to the 2.2% reported from Onitsha[14] but higher than 1.6% reported from Saudi Arabia.[15] It is however lower than the 11.0% found among pregnant women in Makurdi,[16] 13.3% in Zaria, Northern Nigeria,[17] 38.0% in Maiduguri,[3] 9.3% reported from Kenya,[18,19] and 9.2% reported from Brazil.[13] Higher rates of false positive results are observed during pregnancy than in the general population. False negative or atypical results are also observed under several circumstances, including S (Surface) gene mutants and variants in HBV, and Hepatitis C virus co-infection which may interfere with HBV replication and/or HBsAg expression.[20]

The wide variations in the seroprevalence of Hepatitis B seromarkers among pregnant women from the literature may be due to geographical variations, differences in cultural practices, sexual behavior, and practices as well as differences in methods employed to detect HBV infection.[21] The finding in this study may also be influenced by the study population where more than 60% of the patients had tertiary level education and as such were less likely to be infected with HBV.

Table 4: Univariate and multivariate logistic regression models for independent factors associated with HBV infection.

Risk factor	Univariate model Odds ratio (95% CI) *P*-value	Multivariate model Odds ratio (95% CI) *P*-value
Age (years)	1.02 (0.93–1.11) 0.72	1.02 (0.91–1.13) 0.76
Educational status		
Primary	1	1
Secondary	0.10 (0.02–0.42) 0.002‖	0.09 (0.01–0.43) 0.003‖
Tertiary	0.24 (0.08–0.72) 0.01‖	0.24 (0.07–0.87) 0.03‖
Occupation		
Employed	1	1
Unemployed	1.20 (0.43–3.37) 0.72	1.07 (0.33–3.40) 0.91
Self-employed	1.06 (0.36–3.15) 0.92	1.13 (0.31–4.05) 0.86
Parity	1.17 (0.83–1.67) 0.37	1.04 (0.67–1.62) 0.85
Scarification	2.39 (0.27–21.38) 0.44	1.03 (0.02–48.10) 0.99
Presence of STDs*	0.72 (0.09–5.65) 0.75	0.65 (0.06–6.63) 0.72
History of Contact with jaundiced person	12.14 (0.73–200.72) 0.08	11.47 (0.16–805.62) 0.26
Multiple sexual partners	0.53 (0.07–4.17) 0.55	0.90 (0.10–8.09) 0.92
IV injection abuse**	1.69 (0.20–14.40) 0.63	2.97 (0.30–29.26) 0.35
HIV status***	1.01 (0.22–4.60) 0.99	0.79 (0.15–4.09) 0.78

HBV: Hepatitis B virus, *STD: Sexually transmitted disease, **IV: Intravenous, ***HIV: Human immunodeficiency virus, CI: Confidence interval. ‖$P<0.05$ considered statistically significant

Out of the six pregnant women who tested positive to HBsAg, none tested positive for HBeAg. This result is unlike what was reported in Kano, Nigeria[22] and Zimbabwe[23] where the prevalences of HBeAg were 7.6%, and 0.8%, respectively. This suggests that the risk of vertical transmission and chronic infection of HBV from the mother to child is low our environment.[24] Nevertheless, determination of HBV DNA in HBsAg positive but HBeAg negative pregnant women, though an expensive analysis, may be considered useful to detect the very few remaining proportion of these women who are infectious and are likely to transmit the infection to their babies.[25]

The genotype of HBV infection B and C is found to be prevalent in the Asian continent whereas in Sub-Saharan Africa where Akwa Ibom State is located, the A1 or E HBV genotype predominates.[26] HBV A1 and E genotype is associated with seroconversion to HBeAb before 15 or 16 years with a consequent low rate of vertical transmission.[24] The pre-core and core mutation suppresses the production of HBeAg and hence the development of HBeAb[27] although an HBV carrier may still remain infective.[25] It is therefore recommended for future research to ascertain the HBV genotype among pregnant women in Uyo and evidence of mutation in the pre-core and core region.

Forty-one women (14.1%) had markers for prior vaccination against HBV infection. This is similar to findings in Zaria, Nigeria[17] where 15.4% of the study population showed evidence of vaccination against HBV infection but below the 22.4% among health workers in Enugu, Nigeria[28] and 74.5% in Brazil.[29] This study also showed that 78% of the pregnant women had non-immune status, making them susceptible to HBV infection. This is the same as the 79.9% reported from Saudi Arabia[30] but higher than 55.3% reported from Brazil.[13] This suggests that the full impact of hepatitis B vaccination program has not yet reached all women in their reproductive period in our environment. This large negative result is not surprising since the vaccine only became widely available <17 years ago in Nigeria. In the UUTH, every child born is vaccinated with Hepatitis B vaccine before discharge. Women of child bearing age who are seronegative for HBV infection should be encouraged to embrace the vaccination program to prevent the propagation of the disease.

Eight patients (2.8%) had markers for post infection immunity. This is low compared to the results of a study in Brazil[13] where 27.3% of the patients tested positive to both HBsAb and HBcAb, suggesting past infection with conferred immunity. The findings in this study may be influenced by the fact that the population comprised mainly women with high educational attainment; this has been shown to be associated with low risk of HBV infection. This may likely be due to awareness of the risk factors and routes of transmission of the disease by these groups of women.

The 7.9% prevalence rate of lifetime infection with HBV and 2.1% prevalence rate of HBsAg suggests that Uyo is in the intermediate endemic area for HBV infection. This would seem to conform to the pattern of transmission of HBV in Sub-Saharan Africa, which is predominantly horizontal and in childhood.[31]

Those aged between 20 and 29 years had the highest rate of infection. This is similar to findings in Onitsha,[14] Ibadan[32] and Port Harcourt,[33] Nigeria but is in contrast to the observations in Middle East[34] where most of the patients were between 30 and 40 years of age. The high rate infection observed among the 20–29 age group may be influenced by the population under study being predominantly women of reproductive age. The mean age of women who tested positive for HBV when compared with that of HBV negative women was not statistically significant suggesting that age in not associated with increased risk of HBV infection.

Women in their 3rd trimester of pregnancy had the highest prevalence of infection similar to the observations in Eastern Orissa[35] but contrary to findings from Zaria[17] and Kano[22] Nigeria, where women in the second trimester had the highest prevalence. This high prevalence in the 3rd trimester suggests increased risk of transmission of HBV to the fetus especially when maternal antibodies to HBcAg are lacking,[36] as seen in 33% of HBsAg positive patients in this study.

There was an inversely proportional relationship between educational level of the women and HBV infection. Women with higher prevalence of infection had primary level of education while those with secondary and tertiary level of education had lower prevalence. This was statistically significant and is similar to the findings in Minna[6] Egypt[37] Jos, Nigeria[38] but contrary to the findings at Nnewi.[7] Our result may be due to the fact that the educated women are more likely to be aware of the risk factors and route of infection of the virus as well as the means of avoiding them. Besides, educated women are usually those who obtain ANC. It is possible therefore, that if the test was done among a cohort that contained more women of low educational background the prevalence may have been higher.

In our study, there was no statistically significant relationship between HBV infection and blood transfusion. This is in agreement with a study in Saudi Arabia[39] and Mexico.[40] However, other studies have revealed that blood transfusion is an important risk factor for acquiring HBV infection.[41] The reason could be that since the emergence of HIV infection, all blood transfusions in our center and indeed most centers throughout the country are subjected to rigorous screening measures for HIV and HBV infections and thus reducing the risk of HBV transmission to recipients of donated blood.

Other risk factors for HBV infection include scarification and tattooing, presence of sexually transmitted diseases,

history of contact with jaundiced patients, multiple sexual partners, intravenous drug use and HIV coinfection.[4] These risk factors were not found to be significantly associated with an increased rate of HBV infection. This finding is similar to the findings in Enugu[28] and Port Harcourt,[33] but different from the findings in Mexico.[40] The difference is likely due to the fact that this study population involved only pregnant women obtaining ANC. Moreover, our study population was likely to have received intensive health education on unhealthy sexual and other practices which are re-enforced during each antenatal visit.

This study had some limitations. The sensitivity and specificity of the test kit used may be different from other test devices. This study relied on results obtained from the laboratory procedures and errors may arise from the human element or from the equipment to influence the results adversely. HBcAb immunoglobulin M could not be assayed to accurately diagnose acute HBV infection. The study population consisted of women who obtained ANC in the Teaching Hospital, so the prevalence obtained may be an underestimate of the true prevalence of HBV infection among women of reproductive age in Uyo. Bias on self-reported accounts of possible risk factors like risky sexual behavior and injection by drug-users may lead to under estimation of their true rates since most of these activities are not culturally acceptable in our environment.

CONCLUSION

This study revealed an intermediate prevalence and low infectivity status of HBV infection among pregnant women receiving ANC our center. The intermediate prevalence and low infectivity status in this study supports infant, early childhood, and adult means of transmission in Uyo. Government and non-governmental organizations should intensify efforts to create awareness about the disease. This can be done through public enlightenment campaign using town criers, churches, and media and recall system through global systems of mobile communication. The determination of the genotype and core/pre-core mutant in the HBV predominant in our environment is strongly advocated as future research to address the findings of low infectivity of HBV in this study. Large community based and multicenter studies in Nigeria are urgently required on this subject to be able to draw a firm conclusion.

Declaration of patient consent

Institutional Review Board (IRB) permission obtained for the study.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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