

Prevalence of Hepatitis B Viral Infection Using Nucleic Acid Test Amongst Pregnant Women in Port Harcourt, Nigeria.

Theodora Phiemueki Etu-Efeotor¹, Justina Omoikhefe Alegbeleye², Hannah Emmanuel Omunakwe³

Institution: Department of Haematology¹

Niger Delta University Teaching Hospital, Okolobiri, Bayelsa State, Nigeria

Department of Obstetrics and Gynaecology²

University of Port Harcourt Teaching Hospital, Port Harcourt, Rivers State, Nigeria

Department of Haematology³

River State University Teaching Hospital, Port Harcourt, Rivers State, Nigeria

Corresponding Author: Justina Omoikhefe Alegbeleye

Department of Obstetrics and Gynaecology, University of Port Harcourt Teaching Hospital, Port Harcourt, Rivers State

ABSTRACT

Background: Hepatitis B virus (HBV) infection is a serious global public health problem and is endemic in Sub-Saharan Africa. Vertical transmission during delivery and breastfeeding contributes significantly to the high transmission rate in the region.

Objectives: To determine the prevalence and risk factors for hepatitis B virus in pregnant women at the University of Port Harcourt Teaching Hospital (UPTH) and to determine the association between serological and nucleic acid testing methods.

Materials and Methods: This was a cross-sectional study conducted at the Obstetric unit of the UPTH, Port Harcourt from July 1, 2016 to December 31, 2016. Five hundred pregnant women who presented in labour were recruited into the study. The socio-demographic characteristics and risk factors for hepatitis B virus infection were documented in a pre-designed structured questionnaire for each participant. Five milliliters of blood was drawn from each pregnant woman into EDTA bottle for HBV testing. Screening for HBsAg was done by chromatographic immunoassay kit and nucleic acid testing was done using polymerase chain reaction method. Data was analyzed with SPSS version 22.0. Statistical significance was set at P value <0.05. Results are presented in tables and percentages.

Results: The prevalence of hepatitis B infection in pregnant women was 6.2%. Termination of pregnancy (TOP) was significantly associated with increased risk of HBV infection. Multiple sexual partners, marriage in a polygamous setting and HIV infection were associated with high odds of HBV infection while blood transfusion and previous surgery were associated with reduced odds of HBV infection. Three women that tested positive with serological method were negative using the PCR technique.

Conclusion: The prevalence of HBV infection among pregnant women at UPTH is of intermediate prevalence. Termination of pregnancy, multiple sexual partners, and HIV infection remain major risk factors for HBV infection. There is good concordance between serological and PCR testing methods for HBV infection.

Keywords: Hepatitis B, Pregnancy, Prevalence, Risk Factors, Port Harcourt, Nigeria.

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I. Introduction

Hepatitis B virus infection is a serious global public health problem and is endemic in Africa, including Nigeria.¹ Hepatitis B virus (HBV) is the prototype member of the *Hepadnaviridae* (hepatotropic DNA virus) family. Hepadna viruses have strong preference for infecting liver cells, but a few can be found in kidney, pancreas, and mononuclear cells. Infection at these sites is not linked to extra-hepatic disease.² Globally, over 2 billion people are infected with the virus and over 350 million have a chronic infection.³ It is estimated that 12% of Nigerian are asymptomatic carriers of the virus and are capable of transmitting to others. Disease spectrum associated with HBV infection ranges from acute hepatitis to chronic hepatitis, asymptomatic carrier, cirrhotic liver disease and hepatocellular carcinoma.

Hepatitis B virus (HBV) is transmitted horizontally through unsafe sex, infected blood products, percutaneous inoculation, and contact of non-intact skin or mucous membrane of infected persons among

others.⁴ Perinatal and early childhood transmissions from infected mothers are a major route of HBV infection in endemic areas.

The prevalence rate of HBV infection in pregnant women in Nigeria is variable from one geographical location to another. It is estimated to range from 2 – 15%.^{1, 5 - 7} Majority of these studies were based on serological testing. There is paucity of studies on hepatitis B viral DNA in pregnant women in Nigeria thus the need for a more specific testing method to precisely define the burden of HBV infection in pregnancy. The purpose of this study is to determine the prevalence of HBV infection among pregnant women receiving care at the UPTH, to identify possible risk factors associated with the infection and also to determine an association between serological testing and nucleic acid testing methods.

II. Materials and Methods

2.1 Study site

This study was carried out at the Obstetric unit of the University of Port Harcourt Teaching Hospital (UPTH) from July 1, 2016 to December 31, 2016. The UPTH is an 882-bed hospital located at Port Harcourt in Obio-Akpor local government area of Rivers State, South-South Nigeria. An average of 2,500 deliveries are conducted annually. The unit has a total of 40 beds in the postnatal ward, 40 beds in the unbooked ward, 13 beds in the first stage room, and 8 beds in the private/semi-private rooms. There are five units; each unit has five consultant obstetricians, five specialist senior registrars and five registrars with many experienced midwives.

2.2 Methods

This was a prospective cross-sectional study conducted at the labour ward of the department of Obstetrics and Gynaecology of the University of Port Harcourt Teaching Hospital from July 1, 2016 to December 31, 2016. Five hundred pregnant women receiving care at the hospital were recruited for the study, after fulfilling the inclusion criteria. All participants gave informed consent prior to recruitment. At presentation, a bio-demographic and clinical data were obtained. The socio-demographic characteristics including maternal age, parity, educational level, booking status, gestational age at booking, haemoglobin genotype, risk factors, mode of delivery and perinatal outcome obtained were entered into a spread sheet. Excluded from the study were women who have been immunized against HBV infection, those on treatment for HBV infection or had earlier known they were positive for HBV.

Five milliliters of blood sample was collected from the antecubital vein or dorsum of the non-dominant hand by venipuncture. Aseptic techniques were applied. Ethylenediamine tetra-acetic acid (EDTA) bottles were used to collect the blood sample which was centrifuged at 3000g for 5 minutes. The supernatant (plasma) was used for the HBsAg test. Screening for hepatitis B surface antigen (HBsAg) was done by chromatographic immunoassay using hepatitis B ACON test kit with Ref number IHBsAg-301 and Lot number BSG 2110040 (ACON laboratories, USA). The cells of HBV positive women were stored at -80°C and at the completion of sample collection (8 months). The samples were transported to Research Laboratory of University of Nigeria Enugu Campus for hepatitis B viral DNA testing by real time polymerase chain reaction. The data generated was coded and analyzed using SPSS 22.0.

2.3 Statistical Analysis

Statistical analysis was done using Statistical Package for Social Sciences (SPSS) version 22 (SPSS Inc. Chicago, Illinois, U.S.A.). The results are presented in tables. The risk for HBV infection was estimated using odd ratios. Statistical significance was considered at $p < 0.05$. The results are presented as percentages and odd ratios.

2.4 Ethical Approval

All authors declare that the study was approved by the ethical review of the hospital and was carried out in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

III. Results

Five hundred women were enrolled into the study during the six-month period. Of these, 31 tested positive to HBsAg using both ELISA and PCR, giving a sero-prevalence rate of 6.2% in the study population. The age range of the women studied was 15-45 years. The mean age of the pregnant women was 27.68 ± 4.59 years, with majority of the women in the 20-35 years age group constituting 90.2% of the total population. Two hundred and ninety (58%) of the women had tertiary level of education while 194 (38.8%) and 16 (3.2%) had secondary and primary level of education respectively. Only 13.2% of the women were not married. Over one-third 188 (37.6%) of the women in the study population were business women (Table 1). Table 2 shows the sero-prevalence rate for HBV against age in the study population, the prevalence was highest among the teenage age group, where 1 out of the 12 (8.3%) women tested positive to HBV and the least prevalence was seen in the

≥35years age group where none of the women tested positive to HBV. The women's occupation as a risk factor to the acquisition of HBV was also reviewed, the highest prevalence was seen in business women where 15 of the 188 women in this group were positive for HBV infection (8%) and the least prevalence was seen in public servants (3.4%). However, there was no significant relationship between infection and the occupation of the women in the study. (Table 3) Regarding type of marriage, 424 (97.7%) were in monogamous and 10 (2.3%) in polygamous marriages. Seventeen of the women in polygamous marriages were positive for HBV infection while only one woman in a polygamous marriage tested positive. The remaining positive cases were seen in single women and those that were divorced. However, there was no significant association between type of marriage and positivity for HBV. Two hundred and seventy-nine (55.8%) of the women gave a history of termination of pregnancy in the past, while 221 (44.2%) had no termination of pregnancy. Among those who had a history of termination of pregnancy, 22 (8%) tested positive to HBsAg. This was statistically significant (P value 0.04). Table 4 highlights the relationship between the risk factors and HBV infection. Fifteen out of the 31 women that were positive to HBV infection had ≥ 2sexual partners in the past but this was not statistically significant. Of the 37 women with a previous history of blood transfusion, only 1 tested positive to HBsAg, this was not a statistically significant (P value 0.3). One hundred and forty-five women gave a history of previous caesarean section and surgical procedures, of these 7 were positive to HBV, both were not significant route of HBV transmission in the study (P value 0.5 and 0.4 respectively). It was noted from this study that scarification marks or tattoos was not significantly associated with HBV infection. (P value 0.2).

Out of the 19 women who tested positive to the Human immunodeficiency virus (HIV), 2 also tested positive to HBV, giving a HIV/HBV co-infection rate of 0.4% in this study.

Table 5 shows that seventeen out of the 31 babies born to HBV-positive mothers were also positive to HBV, giving a neonatal sero-prevalence rate of 3.4% (17/500) and a vertical transmission rate of 54.8% (17/31). This study also showed that the PCV, MCV, MCH and MCHC were significantly lower in women with HBV infection while platelet count was significantly higher. However, there was no significant difference in the WBC count between the two groups. (Table 6).

Regarding the neonates, it was only the PCV that was significantly lower in babies that were HBV positive. All other haematological indices were within the same range. (Table 7).

Table 1: Socio-demographic characteristics of the women

Characteristics	Number (n=500)	Percentage (%)
Age (years)		
<20	12	2.4
20-34	451	90.2
≥35	37	7.4
Educational status		
Primary	16	3.2
Secondary	194	38.8
Tertiary	290	58.0
Occupation		
Business woman	188	37.6
Housewife	96	19.2
Professional	73	14.6
Public servant	58	11.6
Student	54	10.8
Civil servant	31	6.2
Parity		
Para 0	158	31.6
Para 1-4	334	66.8
Para ≥ 5	8	1.6

Table 2: HBV prevalence according to age group

Age group	Number (n=500)	HBsAg (+)	HBsAg (-)
<19	12 (2.4)	1 (8.3)	11 (91.7)
20-34	451 (90.2)	28 (6.2)	423 (93.8)
35 and above	37 (7.4)	0 (0.0)	37 (100.0)

Table 3: HBV prevalence according to occupation

Occupation	Number examined (n=500)	Number (%) of positive cases (n=31)	Prevalence (%)
Business woman	188	15 (48.4)	8
Civil servant	31	2(6.5)	6.6
Housewife	96	6(19.4)	6.3
Student	54	3(9.6)	5.6
Professional	73	3 (9.6)	4.1
Public servant	58	2(6.5)	3.4

Table 4: Prevalence of HBV associated with risk factors

Risk factors	Number (%)	HBsAg (+)	HBsAg (-)	OR (95% CI)	p-value
Blood transfusion	37 (7.4)	1 (2.7)	36 (97.3)	0.43 (0.02-3.15)	0.349
Termination of Pregnancy	279 (55.8)	22 (7.9)	257 (92.1)	2.62 (1.04-6.88)	0.04
One sexual partner	286 (57.2)	14 (4.9)	272 (95.1)	1.46 (0.65-3.32)	0.419
Two or more sexual partners	214 (42.8)	15 (7.0)	199 (93.0)		
Previous c/s	78 (15.6)	4 (5.1)	74 (94.9)	0.86 (0.24-2.73)	0.517
Tattooing / tribal marks	29 (5.8)	3 (10.3)	26 (89.7)	1.97 (0.44-7.56)	0.232
Previous surgeries	67 (13.4)	3 (4.5)	64 (95.5)	0.73 (0.17-2.67)	0.438
Type of marriage					
Monogamous	424 (97.7)	17 (4.0)	407 (96.0)	2.66 (0.00-23.15)	0.348
Polygamous	10 (2.3)	1 (10.0)	9 (90.0)		
HIV status					
Positive	19 (3.8)	2 (10.5)	17 (89.5)	1.98 (0.00-9.75)	0.303
Negative	481 (96.2)	27 (5.6)	454 (94.4)		

p-value <0.05 is considered significant

Table 5: Mother-to-Child transmission rate

Status tested	Number tested	No of positive babies (%)	No of negative babies (%)
HBsAg (+) mothers	31 (5.8)	17 (54.8)	14 (45.2)
HBsAg (-) mothers	469 (94.2)	0 (0.0)	469 (100.0)
*Chi square + 1.82	P value+ 0.189		

Table 6: Haematological Indices of the mothers

Characteristics	HBsAg Mother		t-test	p-value
	Positive Mean ± SD	Negative Mean ± SD		
Hgb (g/dl)				
PCV (%)	34.81 ± 3.79	36.18 ± 2.99	2.36	0.02*
WBC (x 10 ⁹ /L)	11.18 ± 1.98	12.85 ± 2.74	1.54	0.122
PLT (x 10 ⁹ /L)	217.59 ± 67.94	191.98 ± 456.18	2.86	0.04*
MCV (fl)	76.44 ± 4.80	79.07 ± 4.59	2.98	0.003*

MCH (pg)	25.52 ± 2.10	26.76 ± 2.19	2.95	0.003*
MCHC	32.40 ± 2.04	32.90 ± 1.37	1.84	0.01*

*Significant ($p < 0.05$)

Table 7: Haematological Indices of the Neonates

Characteristics	HBsAg Baby		t-test	p-value
	Positive Mean ± SD	Negative Mean ± SD		
Hgb (g/dl)				
PCV (%)	43.087 ± 3.67	45.26 ± 3.35	2.64	0.01*
WBC ($\times 10^9/L$)	11.82 ± 4.01	11.17 ± 2.93	0.89	0.374
PLT ($\times 10^9/L$)	203.24 ± 60.63	204.96 ± 54.28	0.13	0.89
MCV (fl)	100.49 ± 8.98	102.58 ± 7.26	1.55	0.248
MCH (pg)	31.91 ± 2.08	32.89 ± 2.25	1.77	0.07
MCHC	31.72 ± 1.40	32.19 ± 1.63	1.15	0.247

*Significant ($p < 0.05$)

IV. Discussion

Hepatitis in pregnancy may contribute to increase maternal and fetal/neonatal morbidity. There are several studies on hepatitis B virus in pregnancy in Nigeria, however most of these studies were based on serological methods using enzyme linked immunoassay kits. There are limited studies using nucleic acid testing and the degree of concordance between serological assay and nucleic based testing have not been adequately investigated especially in the context of HBV infection in pregnancy.

This study using nucleic acid testing method found a prevalence rate of hepatitis B infection to be 6.2% among women receiving antenatal care and delivery. The current prevalence rate is slightly higher than previous reports in the region which were based mainly on rapid kit testing using serological methods. Earlier studies of HBsAg in Port Harcourt had prevalence rates ranging from 4.3 - 6%.⁸ The variability in rapid kit sensitivity and specificity may in part account for the variation, also variation in subject selection criteria may be responsible. However, it is not impossible that there is an actual increase in incidence in the region during the period under review. The implication of an actual increase is enormous. An increase despite increased awareness through health education and advocacy may suggest the need to evaluate the effectiveness of existing programmes. The study found significantly increased odds of HBV infection in women who had termination of pregnancies in the past. This was consistent with the reports from previous studies.¹⁰ Termination of pregnancy is an illegal practice punishable by the laws of Nigeria with jail sentence of up to 14 years in correctional service.¹¹ However, this practice is still rampant in the country. Sadly, because of the criminal nature of the act, women may patronize outlets willing to offer the services sometimes without regard to their hygiene practices. Also, a large proportion of unqualified medical practitioners are involved in rendering this service.¹⁰ There is a need for increased surveillance by regulatory agencies to supervise and ensure that medical service centers practice within their limits and comply with the ethics of the profession. The finding may also account for the increased advocacy for the review of the existing abortion laws.¹² Multiple sexual partners, tattooing, marriage in a polygamous setting and HIV infection were found to have increased odds of HBV infection in the women though this association was not statistically significant.^{9, 10} This is consistent with existing publications on the risk factors for HBV infection. Blood transfusions and previous surgeries including caesarean section were associated with reduced odds of HBV infection. The mandatory screening of blood for transmissible infections including HBV may contribute to the reduced risk associated with transfusion. More recently, HBV screening is mandatory for all blood and blood products prior to use and the screening modality has improved significantly to the use of highly sensitive detection methods capable of detecting viral particles few days after exposure.¹³⁻¹⁵ With regard to surgeries, routine screening of surgical patients for hepatitis and dedication of special packs to patients with HIV and hepatitis infection contributed to the reduced risk of hepatitis observed in the study.¹⁶

Prevalence rate for Hepatitis B viral infection using HBsAg rapid test kit and PCR technique differ. Expectedly, nucleic acid detection method is more specific than serological methods that can give false positives. Misclassification of an individual as positive using the serological methods has huge psychological implications on the affected persons and their family. While screening with rapid kits is acceptable for the general population due to its cost effectiveness, a standard protocol that will ensure that all who tested positive to serological testing should be subjected to nucleic acid testing using PCR method should be enforced by all institutions before reporting an individual as positive.

V. Conclusion

The HBV infection rate is intermediate in this study, thus routine screening of all pregnant women during antenatal services should be sustained. Strategies to sustain HBV immunization should be put in place so

that the girl child grows into the reproductive age with some protection against HBV. Also, modalities should be put in place to improve availability of sensitive testing facility such as PCR machine in the region and testing protocol for diagnosis which includes nucleic acid testing of viral particle for samples testing positive to serological screening should become a routine.

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