Hepatitis B core IgM detection in neonates born to HBsAg and HBeAg positive mothers in Maiduguri, Nigeria

Abstract

Hepatitis B virus is a serious global public health problem and is endemic in Africa, including Nigeria. Infection of pregnant women during the second and third trimester poses a threat of 10 and 90% respectively for vertical transmission. A total of ninety two blood samples from consenting pregnant women were screened for HBsAg and HBeAg while cord blood of five babies born to women that were HBsAg and HBeAg positive were screened for HBcIgM using ELISA test kit (Cortez Diagnostic Inc, USA). Questionnaire was used to collect data on demography, history of blood transfusion and presence of tribal mark of pregnant women. An overall prevalence of 8.7% (8/92) and 5.4% (5/92) for HBsAg and HBeAg seromarkers respectively were obtained. Mean age of the pregnant women was 25.75 with 27.2% within 20–24 years and 28.3% within 25–29 years, however this distribution was not statistically significant (p= 0.6840). Of the 8/92 HBsAg positives, 50% (4/8) were within 25–29 years while 80% (4/5) of 5/92 HBeAg positives were within 20 - 24 years. Blood transfusion (p= 0.002791) and tribal mark (p=0.00265) were associated with acquisition of infection. The cord blood of 4/5 (80%) of babies born to five HBsAg and HBeAg positive women were reactive to HBcIgM. The prevalence of surface antigen in this study highlights the endemicity of hepatitis B virus in Nigeria while the presence of both surface and envelope antigens in pregnant women portends infectivity and these results suggest the utmost need for establishment of a sustainable intervention measure that would protect not only pregnant women but women of childbearing age in order to mitigate spread of the virus. Screening for other hepatitis B virus seromarkers besides HBsAg before blood transfusion is also advocated.

Key words: Hepatitis B surface antigen, Hepatitis B envelop antigen, pregnant women, cord blood, Maiduguri.

Introduction

Hepatitis B virus (HBV) is a member of the hepadnaviridae family. It is a DNA virus with partially circular double stranded DNA and a core antigen surrounded by a shell containing hepatitis B surface antigen (HBsAg), hepatitis B envelop antigen (HBeAg) and hepatitis B core antigen (HBcAg) (Zuckerman *et al*., 1996; Gasim *et al*., 2013). Corresponding antibodies to each of the antigen are hepatitis B surface antibody (anti-HBs or HBsAb), hepatitis B envelope antibody(anti-HBe or HBeAb) and hepatitis B core IgM and IgG antibody (anti-HBc or HBcAb) (Eke *et al*., 2011)

Infection with hepatitis B virus (HBV) is a serious public health problem worldwide and a major cause of chronic hepatitis, cirrhosis, and hepatocellular carcinoma (HCC). It was estimated that there are over 350 million hepatitis B virus (HBV) carriers worldwide. The majorities of them reside in the developing countries of South East Asia and sub Saharan Africa, where the lifetime risk of infection is estimated to be greater than 60%, and carriage rates are in excess of 8% (World Health Organization., 2012). Over 600,000 persons die each year worldwide from complications of HBV infection including liver cirrhosis and hepatocellular carcinoma (Michael *et al*., 2013).

Transmission of HBV from carrier mothers to their babies can occur during the perinatal period, and appears to be the most important factor in determining the prevalence of infection in high endemicity areas. Nigeria is classified among the countries endemic for HBV infection and currently 18 million Nigerians are infected (Mbaawuaga *et al.,* 2008; Olokoba *et al*., 2011). When a pregnant woman is infected with HBV, there is a chance she may infect her fetus. It has been reported that 10 -20% of women seropositive for HBsAg transmit the virus to their neonates, but in women who are seropositive for both HBsAg and HBeAg; vertical transmission is approximately 90% (Vranckx *et al,* 1999; Ugwuja, 2010).Vertical transmission from infected

mother to infants is thought to be partially responsible for high prevalent of infection in certain high risk group (Ndako *et al*., 2012).The risk of transmission depends on the degree of maternal infectivity and the genomic type of the virus (Ezegbudo *et al.*, 2004).

Even though studies have been carried out on HBV infection in different parts of Nigeria, and in different cohorts, the prevalence of both HBsAg and HBeAg among pregnant women and especially information regarding the vertical transmission rate is scanty from the north-east region of the country. Therefore, this study was aimed at assaying for HBsAg and HBeAg in pregnant women; HBcIgM in cord blood of babies born to HBsAg and HBeAg positive mothers and to determine the contributory effect of tribal mark and history of blood transfusion to maternal infection in the study area.

Materials and Methods

Study area

This research was carried out at General Mamman Shuwa Memorial Hospital, Maiduguri, Borno State, Nigeria. Maiduguri, also called Yerwa by locals, lies on the geographical coordinates of 11° 50' 42" N, 13° 9' 35" E. It is the capital and the largest city of Borno State in north-eastern Nigeria. The city sits along the seasonal Ngadda River which disappears into the Firki swamps in the areas around Lake Chad. The indigenes are predominantly Kanuri by tribe.

Ethical clearance

Ethical approval for the study was obtained from the Ethical Board of the hospital. Informed oral consent was obtained from all subjects recruited into the study.

Study design

A cross-sectional, hospital –based design was utilized. Ninety two pregnant women whose consent was obtained participated in the research. A structured questionnaire was administered to obtain necessary clinical and demographic data which included age, tribal mark, history of blood transfusion and trimester.

Exclusion criteria

Non pregnant women were excluded from the research.

Inclusion criteria

Only pregnant women were included in the research.

Specimen Collection, Processing and Storage

Five milliliter (5mls) of blood sample was aseptically collected by venipuncture from pregnant women; cord blood was collected by midwives on duty. The blood samples were transferred into clean plain tubes and allowed to clot at room temperature before centrifuging at 300rpm for at least 5 mintues. The serum which was separated from the whole blood was aseptically aspirated into a labeled sterile container and kept or stored frozen at -20^{o}c until needed.

Determination of HBsAg and HBeAg

Procedure

The assay for HBsAg and HBeAg was carried out according to manufacturer's (Cortez Diagnostic Inc, USA) instructions. The reagents and samples were allowed to reach room temperature (18-30^{o}) for at least 15-30 minutes. The wash buffer concentration was checked for the presence of salt crystals. The solution was re-solubilized by warming at 37^{o}C until the crystal was properly dissolved. The strips were set in a strip-holder and sufficient number of wells were numbered which include three Negative Control (e.g. B1, C1, D1) two Positive

Control (e.g. E1, F1) and one Blank (e.g. A1). Fifty microlitres (50 µl) of Positive Control, Negative Control and Specimen were added into their respective wells using separate disposable pipette tip in order to avoid cross-contamination. Fifty microlitres (50 µl) HRP conjugate was added to each well except in the Blank well and mixed by tapping the plate gently.

The plate was then covered with the plate cover and incubated for 60 minutes at 37^{o} by using thermostat-controlled water tank in order to assure the temperature stability as well as humidity. At the end of the incubation, the plate cover was discarded and each well was washed 5times with diluted wash buffer and each time, the microwells was allowed to soak for at least 30-60 seconds. After the last wash the plate was turned down onto blotting paper, and then taped in order to remove the remaining solution.

Fifty microlitres (50µl) of chromogen A and B solution were dispensed into each well including the Blank well and then mixed by tapping the plate gently and the plate was then incubated at 37^{o}C for 15 minutes and light was avoided. At the end of 15 minutes incubation, it was observed that the enzymatic reaction between chromogen A and B solutions and the HRP-conjugate produced blue color in the control and HBsAg/HBeAg Positive Sample wells. Fifty microlitre (50µl) of stop solution was added into each well and properly mixed. The plate reader was calibrated with the Blank well and the absorbance was read at 450nm. The reference wavelength of the dual filter instrument was set at 630nm. The cut-off value was calculated. The absorbance was read within 5minutes after stopping the reaction.

HBsAg Interpretation of result

The results were calculated by relating each specimen absorbance (A) value to the cut-off value (C.O) of the plate.

Calculation of the cut-off value (C.O) = NC + 0.06 (NC = the mean absorbance value for three negative controls).

Positive result (A/C.O ≥1): Specimens with an absorbance equal to or greater than the cut-off value were considered reactive, which indicates that hepatitis B virus surface antigen has been detected.

Negative result (A/C.O ≤1): Specimens with an absorbance less than or equal to the cut-off value were considered reactive, which indicates that hepatitis B virus surface antigen was not detected using HBsAg ELISA.

***HBeAg Interpretation of results*:**

Negative Results (S/C.O. < l): Samples with an absorbance less than cut-off value were considered negative, which indicates that no Hepatitis B virus "e" antigen has been detected with this HBeAg ELISA kit.

Positive Result (**S/C.O**. **≥ 1**): Samples with an absorbance greater than, or equal to the cut-off value are initially reactive, which indicates that Hepatitis B virus "e" antigen was detected with this HBeAg ELISA kit.

Determination of HBcIgM in cord blood

144 **Procedure:** Same as HBsAg and HBeAg but with slight modification: One hundred microliter
145 (100µl) of samples, positive and negative controls was added to respective wells.

146 **Interpretation of result**

147 Calculation of cut-off value (C.O) = Nc x 2.1, Nc = the mean absorbance value for three negative
148 controls.

149 **Negative result**: (S/C.O <1). Samples with an absorbance less than the cut-off value
150 were considered negative, which indicates that no IgM-class antibodies to hepatitis B core
151 antigen have been detected with this anti HBcIgM ELISA kit.

152 **Positive Result**: (S/C.O>1) Samples with an absorbance greater than, or equal to the cut-
153 off value were considered reactive, which indicates that IgM-class antibodies to hepatitis B core
154 antigen have probably been defected with this anti HBcIgM ELISA kit.

155 **Statistical analysis**

156 Data were analysed using online chi-square calculator with $p<0.05$ at 95% confidence interval.
157 Also, any sample reactive for either of the seromarker was considered positive.

158 **Results**

159 A total of 92 pregnant women who consented were enrolled in this study. An overall prevalence
160 of 8.7% (8/92) and 5.4% (5/92) for HBsAg and HBeAg were obtained (Table 1). Mean age of
161 the pregnant women was 25.75 with 27.2% within 20 – 24 years and 28.3% of them within 25 –
162 29 years, however this distribution was not statistically significant ($p= 0.6840$; Table 1). Of the
163 8/92 HBsAg positives, 50% (4/8) were within 25 – 29 years while 80% (4/5) of 5/92 HBeAg
164 positives were within 20 - 24 years. Also, for HBsAg, one (1) pregnant woman each within 15-

19; 20-24; 30-34; and 35-39 years age group was positive while four (4) pregnant women within 25-29 years were positive. The assay for HBeAg show that one (1) woman within 20-24 years and four(4) within 25-29 years were positive (Table 1). Table 2 shows that blood transfusion (p= 0.002791) and tribal mark (p=0.00265) were associated with acquisition of infection. The cord blood analysis for the detection of HBcIgM reveals 4/5 (80%) of babies born to five HBsAg and HBeAg as positive.

Table 1: Distribution of HBVseromarkers according to age

		Positive seromarkers		
Age (Years)	**n**	HBsAg	HBeAg	p-value
15 – 19	15	1	0	0.684091
20 – 24	25	1	1	
25 – 29	26	4	4	
30 – 34	14	1	0	
35 – 39	12	1	0	
>40	00	0	0	
Total	92	8(8.7%)	5(5.4%)	

Table 2: Predisposing factors for HBV maternal infection unique to study area

		Positive seromarkers		
Predisposing factors	**n**	HBsAg	HBeAg	p-value
Tribal Mark				
Yes	67	5	0	0.00265
No	25	3	5	
Blood Transfusion				
Yes	2	2	2	0.002791
No	90	6	3	

Table 3: Hepatitis B seromarkers in mothers and detection of HBcIgM in cord blood

Sample No.	**Trimester**	HBsAg	HBeAg	HBcIgM in cord blood
3	3rd	+ve	+ve	Reactive
29	3rd	+ve	+ve	Reactive
40	3rd	+ve	+ve	Reactive
72	3rd	+ve	+ve	Reactive
81	3rd	+ve	+ve	Non-Reactive

Discussion

The result of this study shows 8.7% hepatitis B infection rate among pregnant women in the study area and this, according to the World Health Organization classification for hepatitis B infection, indicates an endemic infection rate (Chen and Chang, 2010). The prevalence is higher than some previous studies in Nigeria (Ndam *et al*., 2008; Pennap *et al*., 2011 and Alegbeleye *et al*., 2013). The implication of this result is the likelihood of transmission of the virus to the newborn especially by mothers who were both HBsAg and HBeAg positive. In endemic countries, mother-to-child transmission accounts for most cases of infections and is, therefore,

the main mechanism that perpetuates the infection in the population (Borgia *et al*., 2012; Coppola *et al*., 2010). HBV causes an acute hepatitis that becomes chronic in a percentage that is highly dependent on age of acquisition of the infection. (Coppola *et al*., 2010; Coppola *et al*., 2013a; Coppola *et al*., 2013b). The rate of chronicity is about 90% in infants infected at birth or during the first year of life, 30%–50% in children aged 1–6 years, and 5%–10% in children above 6 years of age and in adults (WHO, 2013; Borgia *et al*., 2012). Once chronic hepatitis is established, a percentage ranging from 15%–40% evolve to liver cirrhosis and hepatocellular carcinoma (Sagnelli *et al*., 2012; Coppola *et al*., 2014).

Age-based distribution of sampled pregnant women shows that they enter marriage/pregnancy as early as 15 years of age however only those within 20 – 24 years (27.2%) and 25 – 29 (28.3%) years were positive for HBsAg and HBeAg. This is similar to results obtained in Port Harcourt, Nigeria (Alegbeleye *et al*., 2013). Also, this outcome has some significance. First, it highlights the necessity of targeting not only pregnant women but all women of childbearing age including those younger than 15years old during vaccination against hepatitis with the view to protecting them even before marriage. Secondly, the sum of the pregnant women who were positive for both seromarkers in the two age groups above is more than half (55.4%) of the total sample therefore their babies have high chances of being infected at birth. This was the case observed in this study as 80% rate of vertical transmission was recorded. This may have occurred either by intrauterine transmission or transmission during delivery. While intrauterine transmission accounts for only a minority of cases of HBV transmission, transmission of HBV during delivery is the most frequent method of vertical transmission. It is mostly due to newborn contact with the mother's infected secretions or blood at the time of delivery. (Piratvisuth, 2013). Also, a high viral load and positivity of HBeAg have been associated with an increased risk of transmission

through this route (Xu *et al.,* 2002; Xu *et al.* 2013). Therefore, it implies that such infection, where it is not managed properly, will progress to hepatocellular carcinoma later in life.

With respect to tribal mark, statistical analysis (p=0.00265) shows that it was a significant factor in hepatitis B transmission. This may not be unconnected to the methods employed in the incision. Also, the low economic status of the patients makes the reuse of incision instrument quite possible. Therefore, since tribal mark incision is a common cultural practice among the populace, the need to enlighten the population on the expediency of using sterile instruments is very essential with the view to preventing possible spread of the virus through contaminated instruments.

In most pre-transfusion blood screening exercises in Nigeria, only HBsAg is routinely tested for without assaying for other seromarker(s) such as the rare HBcAg or the readily assessable anti-HBc (where present) which indicate window phase of infection. Therefore, the reason for the statistical significance of blood transfusion as an important factor in the spread of hepatitis B virus in the study population in this research can premised on the possible transfusion of infected blood. Hence it is advised that any blood meant for transfusion should be subjected to comprehensive screening in order to prevent transmission of the virus.

Conclusion

The prevalence of HBsAg (8.7%) and HBeAg (5.4%) in this study corroborates results of previous studies in Nigeria that classify hepatitis B infection as endemic. The age groups (20-24 years and 25-29 years) that recorded highest rate of infection bring to the fore the at-risk-population that should be targeted during vaccination exercise even though younger women may be included. Also, 80% (4/5) detection rate of HBcIgM in cord blood of babies born to infected

mothers is an indication of either transplacental immunoglobulin transfer or infection of the newborn during delivery. This exposes such babies to risk of complications if infection is not remedied promptly.

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