



Maternal Seroprevalence of Hepatitis B Virus Serologic Markers among Attendees of a Secondary Health Facility in Maiduguri, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Authors SOO, MO and BTM participated in the design of the research and compilation of manuscript. Authors BBA and MAL supervised the analysis of sample, while author SOO serving as the corresponding author coordinated participation of all authors. All authors read and approved the final manuscript.

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ABSTRACT

The profile of four hepatitis B virus markers [Hepatitis B surface antigen (HBsAg), antibodies of the immunoglobulin M (IgM) class against the hepatitis B core antigen (IgM anti-HBc), Hepatitis B surface antibody (Anti-HBs) and Hepatitis B envelop antigen (HBeAg)] among pregnant women (n=91), with mean age of 25.96 years, were determined using Enzyme linked immunosorbent assay kit. A significant (p=0.00001) overall sero-prevalence of 8.79%, 36.26%, 6.59% and 7.65% were observed for HBsAg, Anti-HBs, IgM anti-HBc and HBeAg respectively. Forty seven point three percent were susceptible (HBsAg^{-ve}, IgM anti-HBc^{-ve} and anti-HBs^{-ve}) to hepatitis B virus infection. One of thirteen pregnant women tested positive for HBsAg and HBeAg; this portends high risk of transmission to the fetus. This result portrays the unreliability of using presence of HBsAg as the

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sole marker for hepatitis B virus infection, high susceptibility to hepatitis B virus infection and the risk of transmission of HBV to fetuses.

Keywords: Hepatitis B surface antigen; antibodies of the immunoglobulin M (IgM) class against the hepatitis B core antigen; hepatitis B surface antibody; hepatitis B envelop antigen; seroprevalence.

1. INTRODUCTION

Hepatitis B Virus (HBV) is a member of the *hepadnaviridae* family. It is a DNA virus with partially double-stranded DNA and a core antigen surrounded by a shell containing hepatitis B surface antigen (HBsAg). HBV has numerous antigenic components such as HBsAg, hepatitis B core antigen (HBcAg), Hepatitis B surface antibody (anti-HBs) and hepatitis B envelope antigen (HBeAg). The HBV genome harbors 3200 nucleotides and it can encode four groups of protein and their regular components by shifting the reading frame over the same genetic matter [1]. Despite availability of a vaccine and antiviral treatment, HBV infection is still a major health problem causing considerable morbidity and mortality [2-4]. The World Health Organization (WHO) estimates the burden of HBV infection to be approximately 2 billion, including more than 350 million chronically infected, and 500,000 – 700,000 patients die annually as a result of HBV related liver disease, or hepatocellular cirrhosis with most of these deaths in developing countries [5].

The endemicity of hepatitis B is defined according to the prevalence of the hepatitis B surface antigen (HBsAg) in the general population of geographical areas, and it varies considerably globally: HBsAg prevalence of greater than 8% is typical of highly endemic areas, prevalence of 2-8% are found in areas of intermediate endemicity, whereas in areas with low endemicity less than 2% of the population is HBsAg positive [6]. The prevalence of Hepatitis B Virus Infection in Nigeria is estimated to be 18.4 – 24% of the population [7,8] and is classified among the group of countries endemic for HBV infection with a current infected population of 18 million [9,10].

Hepatitis B virus is transmitted primarily through parenteral and sexual exposure to HBsAg positive blood or other body fluid [11]. Perinatal transmission of HBV occurs if the mother has had acute HBV infection during late pregnancy, or if she is a chronic carrier. Neonatal infection does occur during labour and delivery. Vertical

transmission from chronic carriers exceeds 90% and accounts for up to 40% of the world carriers in endemic areas [12,13]. If infected, the neonate becomes a chronic carrier himself in 50% to 90% of cases and is prone to cirrhosis and hepatocellular carcinoma in adult life. When the mother is positive for viral DNA in her serum, transmission rate is estimated at 90%; if the mother is negative for viral DNA in the serum, transmission rate is about 10% to 30%. Similarly, a positive test for HBeAg viral protein, which is a marker for patient at high risk for transmission of the disease, indicates that the individual has a high level of virus. However, the absence of HBeAg does not necessarily exclude active viral replication because some patients have mutated viruses that do not give rise to e-antigen. The disappearance of HBeAg and rise in anti-HBe is associated with a decline in viremia [14-17].

Although studies have been carried out on HBV serological markers in different part of Nigeria, there is dearth of information regarding HBV serological markers in a single study in North-eastern region of the country. This preliminary, simple descriptive study was therefore designed to determine the seroprevalence of four maternal HBV serological markers among pregnant women attending antenatal care at a secondary health facility in Maiduguri, Borno state, Nigeria.

2. MATERIALS AND METHODS

2.1 Study Design

A descriptive hospital-based cross-sectional design was utilized. A structured questionnaire was administered to each pregnant woman to obtain demographic data which included age, pregnancy term, occupation/grade-level of husband, tribal mark and history of blood transfusion.

2.2 Study Population

The population of the study constituted of pregnant women attending ante natal clinic at a secondary health facility in Maiduguri, Borno State, Nigeria.

2.3 Ethical Issue

The Ethics committee of the hospital gave approval for the study.

2.4 Inclusion Criteria

Only pregnant women, confirmed by routine pregnancy test, were included in the research.

2.5 Exclusion Criteria

Women who did not give their consent for the study were excluded from the research.

2.6 Sample Collection

A total of ninety one blood samples were collected. Three millilitre of venous blood was taken aseptically in plain dry sterile vacutainer tubes and serum was separated by centrifugation at 4000 rpm for five minutes. The supernatant (serum) was collected in cryovials, labelled, and stored at -20°C until needed for analysis as described by Obi et al., in 1993 [18].

2.7 Sample Analysis

The sera were brought out of the refrigerator for its equilibrating with room temperature before testing. After this, each sample was tested for HBsAg, anti-HBs, IgM anti-HBc and HBeAg using Enzyme Linked Immunosorbent Assay kit (Cortez Diagnostics Inc., U.S.A) for each marker. Positive and negative controls were run alongside test samples. The entire procedure with result interpretation was according to the manufacturer's recommendation.

2.8 Data Analysis

Chi square statistical calculator was employed to compare the overall prevalence of four HBV seromarkers, and the prevalences among different age distribution and trimester of pregnancy.

3. RESULTS

3.1 Distribution of Hepatitis Markers

From Table 1, this study obtained a seroprevalence of 8.79% for HBsAg, while 36.26% of the pregnant women had anti-HBs. IgM anti-HBc was positive among 6.59% of the tested individuals and 7.65% of them were seropositive for HBeAg. Comparism of the

prevalence by chi-square analysis showed that they were significant ($p=0.00001$) at $P<0.05$.

3.2 Susceptibility to Hepatitis B Virus Infection

The result of the assay revealed an alarming 47.3% susceptibility to hepatitis B virus infection among the pregnant women tested as forty three of them were negative for HBsAg, anti-HBs and IgM anti-HBc (Table 1).

3.3 Distribution of Hepatitis B Virus Markers According to Age of Pregnant Women

During the study period ninety one women aged 17- 46 years attending ante natal care were enrolled. The highest prevalence (31 individuals, 34.1%) of the pregnant women fell within the 17-21 year group with a significant ($P=0.00001$ at $P=0.05$) high proportion (54.84% or 17/31) positive to all the four seromarkers (Table 2). Other seroprevalences of hepatitis B virus serologic markers of the pregnant women according to age are also presented in Table 2.

3.4 Trimester Based Distribution of Hepatitis Markers

Out of a total of ninety one pregnant women, 53.8% of them were found to be in the first trimester of pregnancy. Those in the 2nd trimester were 26.4% while the least proportion of 19.8% was in the 3rd trimester (Table 3). A significant prevalence of the four sero-makers according to trimester was found ($P=0.00001$) (Table 3).

4. DISCUSSION

In the present study, ninety one subjects were tested for four hepatitis B virus serologic markers. The overall prevalence of HBsAg, anti-HBs and IgM anti-HBc was 8.79% 36.26% and 6.59%, respectively (Table 1). Subjects who tested positive for HBsAg and IgM anti-HBc were re-tested for presence of HBeAg. We recorded one of thirteen positive for HBeAg (7.69%) (Table 1).

HBsAg is the main serologic marker recommended by the guidelines for the detection of maternal HBV infection [19-21]. It is the first detectable serologic marker of infection to appear and is found in almost all infected persons [22]. The 8.79% seroprevalence obtained in this study at Maiduguri is comparatively lower than the earlier recorded

11.6% in 1994 and 15.8% in 1999 [23,24]. It is also lower than 12.3% and 16.6% prevalence among pregnant women in Minna and Vom respectively [25,26]. Our record is however higher than the prevalence of 6.0% in Taraba state [17], 5.7% in Ilorin [27], 2.19% in Benin City [28] and 4.3% in Port Harcourt [29]. With particular reference to the observed decline of prevalence in Maiduguri from 15.8% in 1999 to 8.79% in the present study, this can be attributed to the increased awareness of the disease which has led to improved medical and paramedical practices and in turn has reduced likelihood of exposure to the predisposing risk factors. Also, the inclusion in the national immunization program by the government, albeit partial, of immunization of neonates and children against hepatitis may have contributed to the observed reduction.

While the high positive proportion (54.84% or 17/31) to all the four hepatitis B seromarkers was

in the youngest group (17-21 years), the highest prevalence (37.5%) was found among the 32-36 year group. This is similar to the high frequency of HBsAg among 30-39 age group reported in Makurdi, Nigeria [30,31]. These results indicate that the vaccination program, besides the neonate targeted ones, should be extended to include young women within 17-21 years age bracket and perhaps even less in order to protect them against infection thereby forestalling any possible vertical transmission of the virus.

Antibodies to HBcAg (anti-HB_c) have been reported to be indicative of infection: IgM anti-HBc signifies recent infection and usually disappears within six months whereas IgG anti-HBc persists for life and indicates past infection [32]. In this study we recorded 6.59% (6/91) IgM anti-HBc positive cases. This might as well indicate recent infection and signifies that the populace in the study area is still being infected with hepatitis B virus.

Table 1. Overall prevalence of hepatitis B virus markers among pregnant women

Markers	No. tested	+ve (%)	-ve (%)	P-value
HBsAg	91	8(8.79)	83(91.21)	0.00001
Anti-HBs	91	33(36.26)	58(63.74)	
IgM anti-HBc	91	6(6.59)	85(93.41)	
HBeAg	91	1(7.69)	12(92.31)	

$$\chi^2=46.28; P<0.05$$

Table 2. Age seroprevalence of hepatitis B virus serologic markers

Age	N (%)	Positive HBV seromarkers				HBV seromarkers -ve	p-value
		HBsAg n=8 +ve(%)	Anti-HBs n=33 +ve(%)	IgM anti-HBc n=6 +ve(%)	HBeAg n=1 +ve(%)		
17-21	31(34.1)	1(12.5)	11(33.3)	4(66.7)	1(7.7)	14	0.00001
22-26	22(24.2)	1(12.5)	9(27.3)	1(16.7)	0(0.0)	11	
27-31	17(18.7)	1(12.5)	8(24.2)	0(0.0)	0(0.0)	08	
32-36	14(15.4)	3(37.5)	4(12.1)	1(16.7)	0(0.0)	06	
37-41	06(6.6)	2(25)	1(3.0)	0(0.0)	0(0.0)	03	
42-46	01(1.1)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	01	

$$\chi^2=138.126; P<0.05$$

Table 3. Trimester based distribution of HBV sero-markers

Trimester	No. tested (%)	Positive HBV seromarkers				p-value
		HBsAg n=8 +ve(%)	Anti-HBs n=33 +ve(%)	IgM anti-HBc n=6 +ve(%)	HBeAg n=1 +ve(%)	
1 st	49(53.8)	2(25)	21(63.6)	4(66.7)	1(7.7)	0.00001
2 nd	24(26.2)	4(50)	7(21.2)	2(33.3)	0(0.0)	
3 rd	18(19.8)	2(25)	5(15.2)	0(0.0)	0(0.0)	

$$\chi^2=64.79; P<0.01$$

Of particular interest was the case of a 19 year old pregnant woman (one of the six IgM anti-HBc positive persons) in the first trimester (Table 3) whose serum sample tested positive for IgM anti-HBc, negative for HBsAg but had high titre of anti-HBs. The result is of dual importance. Firstly, it demonstrates the unreliability of using HBsAg positivity as a sole indicator for hepatitis B virus infection. Secondly, since the presence of antibody against HBsAg (anti-HBs) appears after clearance of HBsAg or after immunization [1] and the subject had no history of any recent immunization, we hypothesize that the patient is undergoing window period of re-infection. It is also pertinent to state that serum of the patient was positive for HBeAg. The HBeAg positivity further indicates a high level viremia and a high risk for transmission of the disease to the developing fetus. If this patient continued the same situation to the 3rd trimester, her fetus would be 80% - 90% prone to cirrhosis and hepatocellular carcinoma in adulthood [15,16].

The remaining five women who tested positive for IgM anti-HBc were also negative for HBsAg. Such cases might result in liver cirrhosis, hepatocellular carcinoma and possibly eventual death. Also, the unreliability of dependence on presence of HBsAg as sole marker for infection is once again brought to the fore.

In this study, we report an alarming 47.3% susceptibility rate to HBV infection in the study area. The high susceptibility rate might explain why Africa, Nigeria in particular remains endemic for hepatitis virus. This is because the result we present being a microcosm of the larger society indicates that there might yet be more uninfected persons than the infected.

5. CONCLUSION

The seroprevalence of HBV serologic markers reported in this study is various, as discussed earlier. However, the 8.79% HBsAg seroprevalence obtained shows that hepatitis B infection remains of high endemicity in the study area. Also HBsAg positive status alone should not be relied upon as the only marker for HBV infection. The result of study has equally demonstrated that the number of people yet susceptible to HBV infection remains high, and this indicates a potential source of economic loss due to the debilitating effect of infection with hepatitis.

It is imperative to state that due to financial constraint, hepatitis B virus DNA and IgG anti-

HBc were not assayed. Therefore we recommend a more comprehensive research on prevalence of HBV serological markers in Nigeria. Routine screening and prevention of perinatal transmission is also recommended. Government should invigorate its vaccination campaign strategies.

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COMPETING INTERESTS

As authors, we declare that we have no competing interest.

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