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Patterns of serologic markers of hepatitis B virus infection and the risk of transmission among pregnant women in southwestern Nigeria

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ABSTRACT

Hepatitis B virus (HBV) infection is a major health concern in developing countries that has a high morbidity and mortality rate. Vertical transmission of HBV from mother to child has been identified as a major factor leading to chronicity with attendant liver conditions, especially in poor socioeconomic settings. This study aims to evaluate the prevalence of serological HBV markers among pregnant women in Ibadan southwestern Nigeria and to determine the implications for perinatal HBV transmission. This study revealed the presence of varied HBV serological patterns of infection or immunity among pregnant women in Ibadan, Nigeria, and thus the risk of mother to child transmission.

KEYWORDS

HBV; Serologic markers; Pregnant women; Ibadan; Nigeria

Introduction

Hepatitis B virus (HBV) is a global public health concern that has affected more than two billion people with an estimated 248 million of these people as chronic carriers of hepatitis B surface antigen (HBsAg). An estimated 686,000 people die annually from HBV-related liver disease complications.[1] The prevalence of HBV infection varies worldwide with approximately half of the world's population living in regions where HBV infection is endemic; these areas include Asia, the Pacific Islands, Africa, and the Middle East.[2] In regions in which HBV infection is endemic, it is acquired mostly through perinatal transmission or in early childhood.[3] Chronicity is much more likely to develop in individuals infected as infants and young children compared to individuals infected during adulthood.[4,5]

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Hepatitis B virus specific markers (such as HBsAg, an antibody to HBsAg [anti-HBs]; hepatitis B core antigen (HBcAg), an antibody to hepatitis B core antigen [anti-HBc]; and hepatitis B 'e' antigen (HBeAg), an antibody to HBeAg [anti-HBe]) are present in the serum during the different phases of HBV infection;[6] thus, the presence of one of these antibodies can be used to determine an individual's infectivity or immune status.[7] Specifically, HBsAg is used to screen for ongoing HBV infection and to estimate the prevalence of acute or chronic HBV infection in the population.[8] Antibodies to Hepatitis B core (anti-HBc)IgG and -IgM indicate previous or recent infection respectively, while anti-HBs show immunity after either infection or vaccination.[7] Although Hepatitis B antigen (HBeAg) is not essential for virus replication, it acts as an immune modulator and typically correlates with higher levels of HBV DNA.[9] Seroconversion of HBeAg to anti-HBe and a significant decrease of HBsAg are considered a good sign for a spontaneous or therapy-induced improvement.[10] Progression to chronic hepatitis in HBsAg-positive individuals with detectable anti-HBe occurs quite often with HBV genotypes D and A1, which is prevalent in North and South Africa, respectively.[11]

In Nigeria, all pregnant women are expected to undergo HBsAg testing, and neonates born to HBsAg-positive women should receive a hepatitis B immune globulin (HBIG) and vaccine within 12 hr of birth. In most centers, however, funding for medical screening programmes is limited. In addition, there are issues of home deliveries and problematic logistics of vaccine supplies and storage in the remote areas. Also, despite the efficacy of high titer HBIG and HBV vaccinations as post-exposure prophylaxis (PeP) in newborns,[12] approximately 3% PeP failure rates in general and 9% from mothers with very high levels of HBV-DNA have been reported.[13]

The goal to prevent mother-to-child-transmission (MTCT) of HBV makes antenatal identification and management of HBV carriers critical so that combined neonatal prophylaxis can be administered in a timely fashion. The Center for Diseases Control and Prevention has recommended that HBsAg screening be performed as part of routine prenatal testing in all pregnant women.[14] Consequently, we had previously screened our participants for HBsAg, and subsequently examined isolated HBV DNA for molecular characterization.[15] As a follow up, this study was designed to screen the samples for other serological HBV infection markers with the aim of describing HBV serological profiles in the population and the proportion of pregnant women who are still susceptible to new HBV infection.

Methodology

Study location, enrolment of participants, and sample collection

As previously described, a cross-sectional study was carried out among consenting pregnant women attending ante-natal clinics in two different locations in Ibadan, southwestern Nigeria.[15] The consenting participants were enrolled between September, 2012 and June, 2013 from Ade-Oyo State Hospital (ASH) and University College Hospital (UCH), Ibadan. Demographic information was obtained from the study participants using a structured questionnaire. Ethical approval for the study was granted by the UI/UCH Ethics Committee (UI/EC/11/0058) and Oyo State Ministry of Health (AD3/479/349).

HBV serological markers identified by ELISA screening

A total of 272 samples were screened for HBsAg, anti-HBs, HBeAg, anti-HBe, total anti-HBc, and anti-HBc-IgM using enzyme-linked immunosorbent assay (ELISA) kits (Diagnostic Automation/Cortez Diagnostic, California, USA). The assays were performed according to the manufacturer's instructions. The optical density was read using the Emax endpoint ELISA microplate reader (Molecular Devices, California, USA), and the results were interpreted according to the manufacturer's instructions.

Results

Overall, 15 (5.5%) of the participants had detectable HBsAg of which three (20.0%) and one (6.7%) had detectable anti-HBc-IgM and HBeAg, respectively. A total of 27 (9.9%) of the participants had detectable anti-HBe out of which 13 (48.1%) were HBsAg positive and 14 (51.9%) were HBsAg negative. Altogether, 120 (44.1%) participants had detectable total anti-HBc, while anti-HBs was detected in 50 (18.4%) (Table 1).

The participants were subsequently grouped into two serological profiles A (HBsAg positive) and B (HBsAg negative) as shown in Tables 2 and 3, respectively. Profile A was subdivided into three subgroups and B into five

Table 1. Serological markers of HBV infection by age among pregnant women in Ibadan.

Age range (yrs)	No. tested	HBsAg (%)	HBeAg (%)	Anti-HBe (%)	Anti-HBc (%)	Anti-HBc-IgM (%)	Anti-HBs (%)
15–20	6	1 (16.7)	0 (0.0)	1 (16.7)	2 (33.3)	1 (100.0)	0 (0.0)
21–25	27	0 (0.0)	0 (0.0)	3 (11.1)	8 (29.6)	0 (0.0)	4 (14.8)
26–30	96	5 (5.2)	1 (20.0)	9 (9.4)	42 (44.0)	1 (20.0)	22 (22.9)
31–35	93	6 (6.5)	0 (0.0)	9 (9.7)	43 (46.2)	1 (16.7)	13 (14.0)
36–40	42	3 (7.1)	0 (0.0)	4 (9.5)	22 (52.0)	0 (0.0)	9 (21.4)
41–45	8	0 (0.0)	0 (0.0)	1 (12.5)	3 (37.5)	0 (0.0)	2 (25.0)
Total	272	15 (5.5)	1 (6.7)	27 (9.9)	120 (44.1).	3 (20.0)	50 (18.4)

Table 2. Serological profile of HBsAg positive pregnant women in Ibadan.

Profile	Additional marker(s)	Number positive	Virological interpretation
A1	Anti-HBc, HBeAg	1	Probably chronic infection with active viral replication; one case with anti-HBc-IgM may have an acute resolving infection
A2	Anti-HBc Anti-HBe	13	Probably chronically infected with low viral replication
A3	Anti-HBc only	1	Chronic inactive stage
Total		15	

Table 3. Serological profiles of HBsAg-negative pregnant women in Ibadan.

Profile	Positive marker	No. (%)		Interpretation
B1	Anti-HBc, anti-HBe	14 (13.3%)	105 (40.9%)	Resolved or occult HBV infection
B2	Anti-HBc only	64 (61.0 %)		Resolved or occult HBV infection
B3	Anti-HBc, anti-HBs (with or without anti-HBe)	27 (25.7%)		Resolved HBV infection, immunity
B4	Anti-HBs only	23	8.9%	Immunity due to vaccination (?)
B5	No marker	129	50.2%	Susceptible to HBV infection (?)
Total		257	100%	

subgroups based on detected serological markers. Only one participant within profile A had detectable anti-HBc and HBeAg; she was categorized as subgroup A1. A total of 13 participants with HBsAg, anti-HBe, and anti-HBc were categorized as subgroup A2, while one participant who had HBsAg and anti-HBc was placed in subgroup A3 (Table 2). Within profile B, 105 (40.9%) of the 257 HBsAg negative participants had anti-HBc. Among this number, 14 participants had detectable anti-HBc and anti-HBe and were categorized as subgroup B1. Sixty-four (61%) participants who had only anti-HBc were classified as subgroup B2; 27 (25.7%) others with both anti-HBs and anti-HBc were classified as B3. Subgroup B4 has 23(8.9%) participants with detectable anti-HBs only, while 129 (50.2%) participants without detectable HBV serological markers were placed into subgroup B5 (Table 3).

Information relating to age distribution of participants' serological profiles are presented in Table 4. The highest risk of HBV infection (66.7%; B5) was

Table 4. Age distribution of serological profiles of HBV infection among pregnant women in Ibadan.

		HBsAg positive			HBsAg negative				
Age range	No tested	A1	A2	A3	B1	B2	B3	B4	B5
15–20	6	0 (0.0)	1 (1.7)	0 (0.0)	0 (0.0)	1 (16.7)	0 (0.0)	0 (0.0)	4 (66.7)
21–25	27	0 (0.0)	0 (0.0)	0 (0.0)	3 (11.1)	4 (14.8)	1 (3.7)	3 (11.1)	16 (59.3)
26–30	96	0 (0.0)	4 (4.2)	1 (1.0)	5 (5.2)	20 (20.8)	12 (12.5)	10 (10.4)	44 (45.8)
31–35	93	1 (1.1)	5 (5.4)	0 (0.0)	4 (4.3)	25 (26.0)	8 (8.6)	5 (5.4)	46 (49.5)
36–40	42	0 (0.0)	3 (7.1)	0 (0.0)	1 (2.4)	12 (28.6)	7 (16.7)	3 (7.1)	16 (38.1)
41–45	8	0 (0.0)	0 (0.0)	0 (0.0)	1 (12.5)	2 (25.0)	0 (0.0)	2 (25.0)	3 (37.5)
TOTAL	272	1	13	1	14	64	27	23	129
			15				257		

observed in the age group 15–20 years while the lowest (37.5%) was found in the age group 41–45 years. The highest rates in profiles B4 (25%) and B1 (12.5%) were also observed in age group 41–45 years. Profile B3 had the highest and lowest rates (16.7% and 3.7%, respectively) in age groups 36–40 and 21–25 years, respectively. Also, the highest rate (28.6%) for profile B2 was recorded in the age group 36–40 years and the lowest (14.8%) in the age group 21–25 years. In profile A (HBsAg-positive), in which a majority of the participants were categorized into A2, the highest rate (7.1%) was found in the age group 36–40 years while the lowest rate (1.7%) was observed in the age group 15–20 years (Table 4).

Discussion

In this study, the observed prevalence of 5.5% for HBsAg was comparable with the previously reported range of 3.9%–16.5% in the southwest and northern Nigeria[16–19] but higher than the 2.2%–4.3% range reported in other regions of the country[20–22] in similar cohorts. The reason for this difference is not clear; however, variations in population, sample sizes, and test kits among others may be contributory factors. The rate of 6.7% (1/15) for HBeAg reported in this study (Table 2) was lower than 26.7% (4/15) and 30.3% (10/33) reported by Anaedobe et al.[19] and Bayo et al.[23] among pregnant women, respectively. However, this rate falls within the range of 6.4%–8.9%,[2,24,25] but was much lower than the 3% found among blood donors in Nigeria.[26] It has been reported that HBsAg carriers with genotype E, which is predominant in Nigeria, have often seroconverted to anti-HBe before they reach adulthood and that HBsAg positive women are less frequently HBeAg-positive than men.[27] Studies have shown that HBeAg is a marker of high infectivity; thus, the participant with profile A1 tends to be highly infectious.[28,29] She also indicates a high risk of possible transmission not only to her unborn child but also to her sexual partners and household contacts. Behrouz et al. showed that about 90% of HBeAg-positive mothers transmit HBV infection to their offspring compared to only 10%–20% of HBeAg-negative mothers.[30]

Studies have shown that HBeAg can transverse the placenta and elicit HBe/HBcAg specific Th cell tolerance *in utero*, leading to high chronicity rates in babies born to HBeAg positive mothers.[31–33] MTCT can be prevented by immediate passive-active immunization of the newborn within 24 hr after birth. According to a recent large clinical trial in newborns from mothers with very high viraemia that exceeds one million international units of HBV DNA per mL, transmission may still occur in about 18% of cases.[33] Treatment of these mothers with the highly effective antiviral drug tenofovir for three months before birth and continued for another three months post-delivery reduced the HBV risk to 5%.[33] In line with this new information,

screening of pregnant women for HBsAg followed by HBeAg and thereafter for HBV DNA levels appears advisable. In cases of high viraemia exceeding 10^6 IU/mL, antiviral therapy for the mother beginning at the third trimester is indicated.[33]

In this study we found that one of the 13 pregnant women in profile A2 (Table 2) was also positive for anti-HBc-IgM and thus, could be a case of resolving acute infection. Early reports in HBV endemic area have shown an increase in mortality and development of fulminant hepatitis with acute HBV infection during pregnancy.[34] However, several studies have shown that many individuals with chronic hepatitis B have low to moderate titers of anti-HBc-IgM,[35] especially during acute exacerbation of chronic HBV infection.[36] The presence of anti-HBe may suggest good prognosis and is indicative of controlled viral replication in these individuals in profile A2 since persons with anti-HBe tend to have lower viraemia.[29] One might assume that participants in this subgroup are mostly inactive carriers and may not transmit the infection to their offspring, but laboratory tests suggest the opposite. Materno-fetal transmission of HBV DNA through the cord blood was reported in 72% of HBsAg-positive mothers.[37] Candotti et al. studied paired samples from HBV DNA positive mothers and found 55% transmission if the mother had >10,000 IU/mL HBV DNA, but only 3.3% transmission at lower viraemia.[38] Both studies tested only the HBV DNA in the cord or neonatal blood but did not follow whether the HBV infection (the ability of the virus to replicate) was transmitted to the child, which appeared unlikely.

The participant in subgroup A3 (Table 2) with detectable HBsAg and anti-HBc but no HBeAg or anti-HBe was one of the HBsAg-positive individuals that could not be typed in our previous study due to abundant substitutions in the major hydrophilic region of the HBV S gene.[15] She may be in the acute resolving or chronic inactive stage of infection. This finding suggests that circulatory anti-HBe might have waned since HBeAg is a much weaker antigen compared to HBcAg or that anti-HBe never developed. Anaedobe et al. reported 3/15 HBsAg-positive women without HBeAg or anti-HBeAg during pregnancy.[19] However, the significance of this serological pattern is not clear. Normally, the absence of HBeAg is the more relevant finding.

Among the 257 subjects without HBsAg, 40.9% showed evidence of previous exposure to HBV since they were anti-HBc positive (Table 3). This is also evident in a study by Olotu et al. who reported a rate of 70.5% anti-HBc and 5.4% HBV DNA among 502 HBsAg negative individuals in Nigeria indicating previous exposure to the virus.[39] This rate is consistent with the status of Nigeria as highly endemic for HBV.[40,41] and much lower than for regions of low endemicity like Germany in which a rate of 3.5% has been reported for women in the age range of 20 to 39 years.[42] Since the great majority of the anti-HBc positive participants were anti-HBc-IgM negative, it

has to be assumed that most of the participants had been infected many years ago, possibly in early childhood, as has been shown in other sub-Sahara African countries.[3]

The 78 participants of profiles B1 and B2 (Table 3) could be considered to possibly be in the window period in which anti-HBs is yet to develop but have cleared the HBsAg below detectable limit of the test kit.[43] However, this is unlikely in view of the large proportion of these patterns and the fact that they were all anti-HBc-IgM negative.[9] They could be candidates for occult HBV infection; and this raises the question of whether this portends a risk of MTCT. Studies have shown that individuals with detectable anti-HBc with or without anti-HBs but negative for HBsAg can be viraemic at a low level. [44–46] Zahn et al. found 1.5% occult HBV infection (OBI) among 1368 pregnant women from West Africa and a similar rate in blood donors.[47] A much higher rate of OBI (17%) although with low viraemia (<50 IU/mL) was reported by Oluyinka et al.,[26] and 33% of the OBI carriers were anti-HBc negative. Whether these low levels are really relevant for MTCT remains uncertain (or even unlikely) because the majority of HBV particles in occult-infected subjects are not infectious even in the setting of blood transfusions.[48] Alexander et al. reported that women with such a profile can transmit HBV to their infants, but this has not yet been confirmed by other groups and is currently not acknowledged in official guidelines.[49]

The rate of recorded isolated anti-HBc (B2) among those who were exposed to HBV was 61% (64/105) (Table 3). Isolated anti-HBc may represent a resolved infection with the loss of anti-HBs, occult chronic HBV infection with levels of HBsAg below detectable limit, or a false positive reaction.[49] However, it has been shown that false positivity is less likely to be the cause of positive anti-HBc in areas of high HBV endemicity.[50] The significance of this serological pattern among pregnant women and the risk it poses for the unborn babies is still unclear, but reactivation during immune deficiency is a known occurence.

The prevalence of anti-HBs (18.4%) in this study (Table 1) was low compared to the prevalence of anti-HBc (44.1%). This rate is slightly higher than 15.2% anti-HBs reported by Japhet et al.[24] among blood donors but lower than 35% detected among similar population as documented by Oluyinka et al.[26] It is possible that the anti-HBs test kit used for diagnosis does not detect natural anti-HBs against the circulating strain so well and this may account for the variation. In our earlier report we demonstrated that the circulating variant of HBV is E/ayw4 and the existence of immune escape mutant amongst our population;[15] thus, antibodies to these untypable mutants in cases in which they represent a new genotype may be more difficult to detect. Reports have shown that substantial number of test kits from around the world has impaired sensitivity for genotype D/ayw3, E/ayw4, and F/adw4.[51–53] Anti-HBs

indicates protection against HBV and may be acquired either through infection or vaccination.[9] Although vaccinated individuals with anti-HBs are protected against clinically apparent hepatitis B, asymptomatic breakthrough infections in vaccinated population have been demonstrated.[54] Precisely, Stramer et al.[54] showed that low levels of anti-HBs (<100IU/mL) do not protect against infection with HBV genotypes that are heterologous to the vaccine strain. Hence, our inability to quantify anti-HBs in this study makes it difficult to establish how many of these anti-HBs positive pregnant women are truly protected. However, although breakthrough infections caused by immune escape mutants have been demonstrated, they have not been shown to represent a major problem to date.[55,56] Consequently clinically silent virus breakthrough in vaccinated persons may be considered insignificant but such an infection might create problems as it may persist in occult form in the liver and reactivate with subsequent serious consequences if such an individual becomes immunodeficient.[57]

In this study we observed that 47.4% (129/272) of the population belonging to subgroup B5 (Table 3) had no detectable marker of exposure to HBV or vaccination against HBV infection. This is of public health importance as these individuals are pregnant and sexually active. Thus, they pose a challenge to successful control of HBV infection because if they become infected there is risk of vertical transmission to their offspring or horizontal transmission to their sexual partners (during the incubation phase). Considering the current HBV vaccine is safe even in pregnancy,[58] many countries have been able to reduce the rate of chronic HBV infection by a factor of three to ten.[2] It is recommended that strategies to lessen HBV burden should incorporate targeted methods to also reduce vertical transmission of the virus by vaccinating pregnant women.

The lowest and highest risks of HBV infection (B5) recorded in the age groups 41–45 years and 15–20 years, respectively (Table 4) were expected as studies have shown correlation between HBV infection and age,[59,60] Thus, it can be assumed that the longer one lives in an endemic environment the higher the chances of being exposed to the virus and subsequently develop immunity. Age distribution of profiles B2 and B3 also support this correlation between HBV infection and age, and when combining this trend together with profile A2, one can suggest that these adults might have been infected early in life, hence the reason for the highest rate of chronicity being observed in the age group 36–40 years.[59] Nigeria introduced HBV childhood immunization in 2004; thus, most of our participants might not have benefitted from the programme as children. Though we do not have the immunization history of the participants the fact that our study detected lowest rate of evidence of protection against the virus by immunization (B4)

in the age group 15–20 years might be of public health concern. This indicates that there may be high prevalence of susceptible young mothers at risk of HBV infection. Thus, identifying such persons and targeting vaccination of such individuals will ultimately reduce the rate of HBV transmission.[58]

Conclusion

This study has described various HBV serological profiles among pregnant women in Nigeria. It has also revealed that a significant proportion (47.4%) of the study population is at risk of being infected with HBV since they have no detectable marker of infection or immunity against the virus. Therefore, there is need for regular and prompt screening of pregnant women for HBV infection and vaccinate those at risk.

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Declaration of interest

No potential conflict of interest was reported by the authors.

Authors' contributions

(a) Study Design (IIM, BAS, AMO, FTOC, OEC)
(b) Sample collection (AMO)
(c) Reagent acquisition and Laboratory and Data analysis (All authors)
(d) Wrote first draft of the manuscript (IIM)
(e) Revised the manuscript (All authors)
(f) Read and approved the final draft (All authors)

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