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ORIGINAL ARTICLE

Seroprevalence of hepatitis B infection during pregnancy and risk of perinatal transmission

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Abstract
Objectives To investigate the seroprevalence of hepatitis B surface antigen (HBsAg) in pregnant women and possible risk factors for perinatal hepatitis B virus (HBV) transmission.
Methods Four thousand pregnant women were evaluated using history, examination, and test for serum HBsAg using commercial enzyme immunoassay kits. For HBsAg positive women, liver function tests and a test for hepatitis B e antigen (HBeAg) was done. HBV DNA analysis was done by polymerase chain reaction (PCR).
Results Of 4,000 women studied, 37 (0.9%) tested positive for HBsAg. Of these 37 women, 6 (16%) presented with acute hepatitis and 31 (84%) were asymptomatic. The highest HBsAg positivity rate was seen in the age group of 21–25 years (1.15%) followed by 26–30 years (0.86%). Assessment of risk factors revealed history of tattooing in 29/37 (78.4%) women. HBeAg was positive in 21 of 37 (56.8%) women. Of the 16 HBeAg negative women, 5 were positive for HBV DNA and anti-HBe antibody, 6 had only anti-HBe antibody and 5 had neither HBV DNA nor anti-HBe. Vertical transmission was seen in 65% (13/20) of babies born to mothers who were positive for HBeAg and HBV DNA. In contrast, it was only 9.1% (1/11) for babies born to mothers who were negative for both HBeAg and HBV DNA. Of the 25 babies delivered vaginally, 15 (60%) developed vertical transmission. None of the four babies delivered by elective cesarean section had evidence of vertical transmission.
Conclusions Seroprevalence of HBsAg in antenatal women was found to be 0.9%. HBe-antigen and HBV DNA positivity was associated with a higher chance of vertical transmission

Keywords Diagnosis · HBeAg · HBsAg · HBV DNA · Hepatitis B · Perinatal transmission · Prevalence · Virus

Introduction

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). 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, particularly in China and Southeast Asia. Before HBV vaccine was integrated into the routine immunization program, the proportion of babies that became HBV carriers was about 10% to 30% for mothers who were HBsAg positive but HBeAg negative. However, the frequency of perinatal infection was higher, i.e. 70% to 90%, when the mother was also HBeAg positive [1, 2]. There are three possible routes of transmission of HBV from infected mothers to infants: transplacental transmission of HBV *in utero*, natal transmission during

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delivery or postnatal transmission during care of infant or through breast milk [3–5].

In endemic areas, most individuals are infected by vertical transmission or in early childhood [6]. Viral hepatitis during pregnancy is associated with a high risk of maternal complications, has a high rate of vertical transmission causing fetal and neonatal hepatitis and has been reported as a leading cause of maternal mortality [7–10]. Epidemiological data on HBV infection are important to program managers and health planners, to plan vaccination and other preventive strategies. Though several studies on epidemiology of viral hepatitis in pregnancy are available from Africa [11–15], there is paucity of such data from India.

India has intermediate endemicity of HBV infection, with population prevalence rate of around 4% [16]. Vertical and horizontal transmission in the perinatal period and early childhood are the major routes of propagation of this infection in India. It is important to collect data on HBV infection in pregnancy to determine morbidity and mortality due to HBV infection in pregnant woman, its effect on parturition and transmission of infection to the newborn children. This study investigated the seroprevalence of hepatitis B surface antigen (HBsAg) among pregnant women and possible risk factors for perinatal transmission of HBV infection.

Methods

Study population and procedures

The study was carried out at M L N Medical College, Allahabad during 2006 and 2007. Women in any trimester of pregnancy with or without jaundice attending the antenatal clinic were included. After a complete general, systemic and obstetrical examination, 4 mL blood was collected after consent. Sera were tested for HBsAg using ELISA (Span Diagnostic Ltd, Surat, India).

Women who tested HBsAg positive were enrolled in the follow up study after an informed consent. Personal history, history of risk factors, chronic hepatitis and obstetric history was obtained. At the time of admission for delivery, again a detailed history was taken, and general, systemic and obstetrical examination was done. Data on ultrasound findings, mode of delivery, indication for cesarean section if done, weight and maturity of babies at delivery, and results of neonatal physical and neurological examination were recorded. Cord blood was collected at the time of delivery and tested for HBsAg, HBeAg and HBV DNA; presence of HBV DNA in cord blood was taken as evidence of vertical transmission of HBV.

Biochemical and serological tests

One aliquot of serum was used for liver biochemical tests including bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (Autospan) and enzyme immunoassays for HBsAg and HBeAg (EIAgen kit; Adaltis Italia SPA, Milano, Italy). All HBsAg positive specimens were tested further for HBV DNA, anti-HBe antibody and anti-HBc IgM using Autospan Diagnostic kit as manufacturer instruction (Span Diagnostic Ltd, Surat, India). Infants born to HBsAg positive mothers received HBV vaccine (10 μg) and hepatitis B immunoglobulin (HBIG; 0.5 mL) were administered, one in each thigh, within 12 h of birth.

HBV DNA analysis

From blood specimens, serum was separated and stored immediately at −20°C until use. From one aliquot, total DNA was extracted using QIAamp DNA Blood Mini Kits (Qiagen, GmbH, Germany). HBV DNA was amplified using primers corresponding to S gene (5′-TCACCA TATTCTTGGGAACAAGA-3′ and 5′-TTCCTGAACTG GAGCCACCA-3′); cycling conditions were as have been reported previously [17]. Amplified PCR products were analyzed using 2% agarose gel electrophoresis and visualized under ultraviolet light.

Statistical analysis

Inter-group comparisons were done using Student's t test and χ^2-test with and without Yates' correction as applicable; p values below 0.05 were taken as significant. All analyses were done using Statistical Program for Social Sciences (SPSS 10.0 for Windows). The study was approved by ethics committee of M L N Medical College, Allahabad.

Results

Demographic details

Of 4,000 women screened, 1,800 (45%) were from urban areas and 2,200 were from rural areas (55%). A majority of screened women were from low (2,600/4,000; 65%) and middle (1,200/4,000; 30%) socioeconomic groups, as per the revised Kuppuswamy's socioeconomic status scale [18]; 200/4,000 (5%) women belonged to high socioeconomic group.

HBsAg prevalence rate

Seroprevalence of HBsAg was found to be 0.9% (37/4,000). The highest prevalence rate was observed in the age group of

Table 1 Prevalence of HBsAg in pregnant women in different age groups

Age group (years)	Number of women studied	HBsAg positive
15–20	269	–
21–25	1,824	21 (1.15%)
26–30	1,753	15 (0.86%)
31–35	154	1 (0.65%)
Total	4,000	37

21–25 years (21/1,824; 1.15%) followed by the 26–30 year age group (15/1,753; 0.86%) (Table 1). The difference in HBsAg prevalence rates in different age groups was not significant. The prevalence rates increased with increase in parity. It was 2/1,373 (0.15%) in primigravida, 11/1,403 (0.78%) in 2nd gravida and 15/715 (2.10%) in 3rd gravida women. Rest of the 9/509 (1.76%) females had more than 3 parity. The prevalence rates in the first three parity groups were significantly different ($p<0.05$).

Out of two primigravida one each belonged to high and middle socioeconomic group. Of the 11 2nd gravida 5 (45.4%) belonged to middle socioeconomic group and 6 (54.5%) were from lower socioeconomic group. Four out of 15 (26.6%) third gravida were from middle socioeconomic group and 11(73.3%) were from lower socioeconomic group. All the 9 (100%) females with more than 3 parity belonged to lower socioeconomic group).

Of the 37 HBsAg positive antenatal women, six women had acute hepatitis B; of these, one was in early 2nd trimester, 5 in 3rd trimester. The remaining 31 women were asymptomatic, and remained so throughout the pregnancy.

Risk factors for HBV infection in HBsAg positive women

Twenty nine of the 37 (78%) HBsAg positive women had history of tattooing, and 11 (30%) gave history of previous surgical procedures, including dilatation and curettage for miscarriage in six and lower segment cesarean section (LSCS). Tattooing (3%) and history of previous surgical procedure (1.5%) were infrequent among HBsAg negative women. None of HBsAg positive antenatal women reported known HBsAg positivity in their sibling(s).

History of blood transfusion within 3–5 years was present in 4 (10.81%) antenatal HBsAg positive women. All the blood transfusions were for obstetric indications. One (2.7%) HBsAg positive woman was a health care worker. None of the 37 HBsAg positive mothers had history of drug abuse.

Information on HBsAg status of husband was available for 17 of the 37 HBsAg positive women; of these, 5 were HBsAg positive. Husbands of the remaining 20 HBsAg positive women declined HBsAg testing; of these, three reported history of jaundice in the past.

One pregnant woman died of acute liver failure with hepatic encephalopathy, coagulopathy and renal failure at 32 weeks of gestation. She had history of blood transfusion for severe anemia 3 months back.

HBeAg, anti-HBe and HBV DNA status

Twenty one (57%) of the 37 HBsAg positive women tested positive for HBeAg. Of these 21 HBeAg positive mothers, 6 (28.6%) had presented with acute hepatitis and 15 (71.4%) were asymptomatic. Sixteen (43.2%) HBeAg negative patients were tested for HBV DNA and anti-HBe antibody; of these, five (31%) women were positive for both HBV DNA and anti-HBe, 6 (38%) were positive for anti-HBe alone, and five (31%) were negative for both these markers.

Vertical transmission rate and factors influencing it

One woman died during follow up; therefore these data were available for only 36 seropositive women. Of 5 women with acute hepatitis B, 3 (60%) transmitted infection to their babies (Table 2). Of the 31 infants born to mothers with asymptomatic HBsAg positivity, 14 (45.2%) showed evidence of vertical transmission (Table 2); this included 10/15 (67%) newborns of HBsAg positive, HBeAg positive asymptomatic mothers and 4/16 (25%) HBeAg negative but HBsAg positive mothers. Among

Table 2 Maternal HBV infection status and vertical transmission

Maternal serological status for HBV infection	Number of mothers	Number with vertical transmission
Acute hepatitis B (HBsAg +ve, HBeAg +ve, IgM anti-HBc +ve)	5	3 (60%)
HBsAg +ve, HBeAg +ve chronic HBV infection	15	10 (67%)
HBsAg +ve, HBeAg −ve, anti-HBe +ve, HBV DNA +ve	5	3 (60%)
HBsAg +ve, HBeAg −ve, anti-HBe +ve, HBV DNA −ve	6	0 (0%)
HBsAg alone	5	1 (20%)
Total	36	17 (47%)

+ve: positive; −ve: negative

HBeAg negative women, vertical transmission was seen in 3 of 5 mothers with detectable HBV DNA, 0 of 6 with detectable anti-HBe but no HBV DNA and 1 of 5 with neither anti-HBe nor HBV DNA.

A significant difference in vertical transmission was found in babies born to HBeAg positive and HBeAg negative mother 76.4% vs. 23.6% (*p=0.02)*. HBV DNA positivity along with HBeAg was also associated with higher rate of vertical transmission in babies than those born to HBeAg positive but HBV DNA negative mother 94.1% vs. 5.9% (*p=0.001)*. Only 1/11 (9%) babies showed evidence of vertical transmission, when both HBeAg and HBV DNA were negative in mothers. Maternal e-antigen + HBV DNA in combination are more sensitive for predicting vertical transmission than e-antigen alone (sensitivity 94.12% for e-antigen + HBV DNA compare to 76.47% for e-antigen alone). Negative predictive value for e-antigen + HBV DNA combination was 90.91% compared to 75% for e-antigen alone for vertical transmission.

Mode of delivery and vertical transmission

The rates of vertical transmission were 15/25 (60%) among babies delivered by the vaginal route, 0/4 among those delivered by elective LSCS and 2 of 7 among those delivered by emergency LSCS (p=0.02). Among women with vaginal delivery, mean (SD) duration of labor was 13.1 (1.0) hours in mothers who transmitted infection to their babies (n=15) and 10.6 (0.5) hours for those who did not transmit infection (n=10) (p=ns).

Discussion

In the present study, seroprevalence of HBsAg among pregnant women was found to be 0.9%. Sero-epidemiological studies of different populations show variations and differences (Table 3). This difference may be because of the type of population studied, different geographical regions, genetic factors and socioeconomic status.

We observed a slight non-significant decline in HBsAg positivity rate with increasing age. This finding is similar to the data from a previous study (3.8% in 20–24 years, 3.4% in 25–29 years, 2.7% in 30–34 years and 1.8% above 35 years) of antenatal women [19]. However, other studies have reported an increase in seropositivity with increasing age of antenatal women [20, 21]. We also found a higher frequency of HBsAg positivity in multigravida women.

In our study, the most significant risk factor for HBV infection was tattooing.

HBeAg positivity rate among HBsAg positive antenatal women have been shown to vary widely in different geographical regions around the world [22, 24–33]. HBeAg positivity in our study (57%) was similar to that previously reported in a study by Biswas et al. (48%) from Kolkota, India [22]. Shenoy et al. have reported geographical variations in HBeAg positivity rate among HBsAg positive pregnant women in different parts of India [23].

Rate of HBV infection to baby was higher if mother had acute hepatitis in third trimester as compared to those that

Table 3 Comparison of seroprevalence rates of HBsAg and HBeAg among pregnant women in different populations

Author, year and references	Population studied	Number of persons screened	HBsAg +ve (%)	HBeAg +ve (% of HBsAg positive)
Mohammed et al. 2008 [20]	Saudi Arabia		1.60	
Batayneh et al. 2002 [24]	Jordan		4.30	0.1
Bertolini et al. 2006 [21]	Brazil	3,188	1.50	
Makuwa et al. 2008 [25]	Gabon	1,186	9.20	10.1
Okoth et al. 2006 [26]	Kenya	2,241	9.30	8.8
Khalil et al. 2005 [27]	Saudi Arabia		2.44	0.15
Al Awaidy et al. 2006 [28]	Oman	1,694	7.10	0.5
	Qatar		1	
	United Arab Emirates		1.50	
Madzime et al. 1999 [33]	Zimbabwe			3.3
Nayak et al. 1987 [29]	Northern India	8,575	3.70	7.8
Panda et al. 1991 [30]	India	8,431	2.6	12.5
Gill et al. 1995 [31]	India	2,000	5.0	12.0
Biswas et al. 1989 [22]	India	1,000	2.3	48
Mittal et al. 1996 [32]	India	850	6.3	18
Present study 2008	Northern India	4,000	0.9	57

suffered in 2nd trimester. Similar finding was also shown by Reinus et al. who showed transmission rate of 80% to 90% in infants, if mother suffered from acute hepatitis B during 3rd trimester of pregnancy [34].

This study also showed a significant association between e-antigen in HBsAg positive women and transmission of infection to their babies, suggesting that the presence of e-antigen is related to HBV vertical transmission and infectivity. Combination of e-antigen and HBV DNA is a more sensitive marker of vertical transmission than e-antigen alone.

Transmission was significantly higher in babies delivered by vaginal route than by cesarian section. This finding also corresponds with Sharma et al., who showed higher transmission in babies delivered per vaginally (69%) as compared to cesarian section babies (31%) [35]. This can be explained by least micro transfusion during elective cesarian section.

In conclusion, seroprevalence of HBsAg in antenatal women was found to be 0.9%. HBeAg and HBV DNA positivity was associated with a significantly higher chance of vertical transmission.

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