Perinatal transmission of hepatitis B virus: an Australian experience

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ABSTRACT

Objective: To determine the rate of perinatal hepatitis B virus (HBV) transmission in an Australian setting and to identify maternal virological factors associated with highest risk of transmission.

Design, participants and setting: A prospective, observational study of perinatal transmission of HBV. Participants were pregnant women attending Sydney South West Area Health Service antenatal clinics who tested positive for hepatitis B surface antigen (HBsAg), and their babies. All babies were routinely offered hepatitis B immunoglobulin (HBIG) and HBV vaccination. Babies positive for HBsAg at 9-month follow-up underwent further virological testing, including HBV DNA sequencing. The study was conducted between August 2002 and May 2008.

Main outcome measures: HBV DNA levels and demographic characteristics of HBsAg-positive pregnant women; proportion of their infants with active HBV infection at 9-month follow-up; maternal characteristics affecting transmission rate; HBV DNA sequencing of infected infants and their mothers.

Results: Of 313 HBsAg-positive pregnant women, 213 (68%) were HBV DNA-positive and 92 (29%) were positive for hepatitis B "e" antigen (HBeAg); 138 babies born to HBV DNA-positive mothers were tested for HBV infection (HBsAg positivity) at about 9 months of age. Four cases of transmission were identified. All four mothers had very high HBV DNA levels ($> 10^8$ copies/mL) and were HBeAg-positive. Three of the four infants were infected with wild-type HBV strains, with identical maternal/infant isolates. The fourth mother–infant pair had an S gene variant, HBV D144E, which has been previously reported in association with vaccine/HBIG escape. (Unfortunately, HBIG was inadvertently omitted from the immunisation schedule of this infant.) Transmission rates were 4/138 (3%) from HBV DNA-positive mothers overall, 4/61 (7%) from HBeAg-positive mothers, and 4/47 (9%) from mothers with very high HBV DNA levels. No transmission was seen in 91 babies of mothers with HBV DNA levels $< 10^8$ copies/mL.

Conclusion: In this cohort, HBV perinatal transmission was restricted to HBeAg-positive mothers with very high viral loads.

MJA 2009; 190: 489–492

Perinatal transmission is the predominant mode of hepatitis B virus (HBV) transmission in areas of high disease prevalence, and still occurs despite immunoprophylaxis with hepatitis B immunoglobulin (HBIG) (passive immunisation) and infant HBV vaccination (active immunisation). Reported rates of transmission from mothers who are positive for hepatitis B "e" antigen (HBeAg) vary from 7%[1] to 28%.[2,3] Several studies have implicated high maternal viraemia as the most important factor associated with failure of neonatal vaccination.[4]

In Australia there is a low prevalence of HBV infection (0.49%–0.87%),[5] the majority of cases being among immigrants from North and South-East Asia. Perinatal transmission in Australia has not been reported and is not routinely monitored. The *Australian immunisation handbook*[6] has recently recommended that infants born to mothers positive for hepatitis B surface antigen (HBsAg) be screened for HBsAg and antibodies to HBsAg (anti-HBs) after completion of vaccination.

The aim of our study was to determine the rate of perinatal HBV infection from a cohort of HBsAg-positive, HBV DNA-positive women in Australia.

METHODS

Study population

From August 2002 to May 2008, 313 asymptomatic, HBsAg-positive pregnant women from Sydney South West Area Health Service antenatal clinics were assessed in the Liverpool Hospital hepatitis clinic. Their initial clinical and biochemical assessment included tests for liver enzymes, HBV DNA and HBeAg, and hepatitis C and HIV serology. The study was approved by the Sydney South West Area Health Service ethics committee, and all participants gave informed consent for participation.

Passive and active immunoprophylaxis was given to babies according to the Australian HBV vaccination schedule.[6] Within 12 hours of delivery, infants were given 100 IU HBIG by intramuscular injection (human hepatitis B immunoglobulin-VF; CSL Bioplasma) and a dose of hepatitis B vaccine (either H-B-VAX II [thiomersal-free, 5 µg recombinant HBsAg protein; CSL Biotherapies/Merck Sharp & Dohme] or ENGERIX-B [10 µg recombinant HBsAg protein; GlaxoSmithKline]). Vaccination was completed with doses at 2, 4 and 6 months of age. Completion of the vaccination schedule was assessed for each infant using child health records.

Infants born to women with detectable HBV DNA were tested at about 9 months of age for HBsAg, anti-HBs and antibodies to hepatitis B core antigen (anti-HBc) (total anti-HBc and, if positive, anti-HBc IgM, if adequate serum was available) to determine rates of perinatal transmission. HBsAg-positive infants were further assessed for HBV DNA and viral sequencing.

Laboratory methods

Before November 2006, HBV serology was performed using the AxSYM microparticle enzyme immunoassay (Abbott Laboratories). Subsequently, serology testing was

Abbreviations

Anti-HBc	Antibodies to hepatitis B core antigen
Anti-HBs	Antibodies to hepatitis B surface antigen
HBeAg	Hepatitis B "e" antigen
HBsAg	Hepatitis B surface antigen
HBIG	Hepatitis B immunoglobulin
HBV	Hepatitis B virus

1 HBeAg status and viral load in 213 HBsAg-positive pregnant women with detectable HBV DNA

HBV DNA level	HBeAg-negative mothers ($n = 122$)	HBeAg-positive mothers ($n = 91$)
$< 10^5$ copies/mL	106 (87%)	9 (10%)
10^5–10^8 copies/mL	16 (13%)	13 (14%)
$> 10^8$ copies/mL	0	69 (76%)

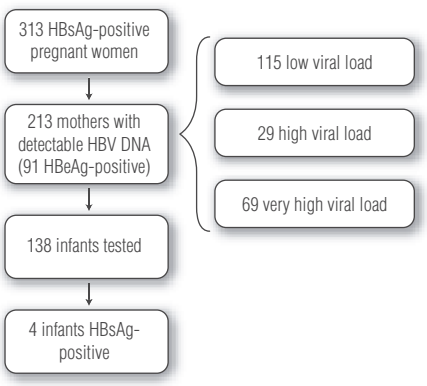
HBeAg = hepatitis B "e" antigen. HBsAg = hepatitis B surface antigen. HBV = hepatitis B virus. ◆

performed by chemiluminescent microparticle immunoassay using the ARCHITECT system (Abbott Laboratories).

HBV DNA quantitation was performed from August 2002 to October 2005 using the COBAS AMPLICOR HBV monitor test (Roche Molecular Systems). Viral loads above the linear range were determined by dilution, as recommended by the manufacturer. In October 2005, the method of HBV DNA quantitation changed to the COBAS TaqMan HBV test with high pure extraction (Roche Diagnostics), and from November 2007, the COBAS AmpliPrep–COBAS TaqMan HBV test (Roche Diagnostics) was used.

For HBsAg-positive infants, HBV DNA was extracted from the sera of each mother-and-baby pair using a QIAamp DNA mini kit (QIAGEN). The region encompassing the HBV surface gene "a" determinant was amplified and sequenced, as described elsewhere.[7] HBV mutational analysis and genotyping were performed using the SeqHepB viral genome analysis program (Evivar Medical).[8] Sequence alignments of mother-and-baby pairs were performed using BioEdit sequence alignment editor software (Ibis Biosciences, Carlsbad, Calif, USA).[9]

2 Flow diagram of pregnant women and their infants included in our study*



HBeAg = hepatitis B "e" antigen. HBsAg = hepatitis B surface antigen. HBV = hepatitis B virus. * Viral load (HBV DNA level): low, $< 10^5$ copies/mL; high, 10^5–10^8 copies/mL; very high, $> 10^8$ copies/mL. ◆

Statistical analysis

Fisher's exact test was used to test for associations between grouped maternal viral load, maternal HBeAg positivity, infant anti-HBc positivity and transmission rates. Statistical significance was set at $P < 0.05$. Statistical calculations were performed using SAS, version 9.1 (SAS Institute, Cary, NC, USA).

RESULTS

Maternal characteristics

Of the 313 HBsAg-positive pregnant women, 92 (29%) were HBeAg-positive and 213 (68%) had detectable HBV DNA. The HBV DNA levels in viraemic mothers were arbitrarily stratified into three groups: 115 (54%) had levels $< 10^5$ copies/mL (low viral load), 29 (14%) had levels between 10^5 and 10^8 copies/mL (high viral load), and 69 (32%) had levels $> 10^8$ copies/mL (very high viral load). Of those with detectable HBV DNA, 91 (43%) were HBeAg-positive. HBeAg positivity was strongly correlated with a very high viral load ($P < 0.001$), and all patients with HBV DNA levels $> 10^8$ copies/mL were HBeAg-positive (Box 1).

Levels of alanine transaminase were elevated (> 20 U/L) in 100 women (32%), and of these women, 27 (9%) had levels > 40U/L. Two women (< 1%) had concurrent hepatitis C infection, but none had concurrent HIV infection.

Demographics of a randomly selected subgroup of women

A random subgroup of 75 consecutive mothers (24% of the total) was further assessed. Of these, 72 were born overseas and 36 required interpreter services for their clinic visits; 62 were from South-East Asian countries, most commonly Vietnam (44) and Cambodia (12). For 22 women, HBV infection was diagnosed for the first time during their antenatal care visits. This suggests that a significant number of people in Australia have unrecognised chronic HBV infection.

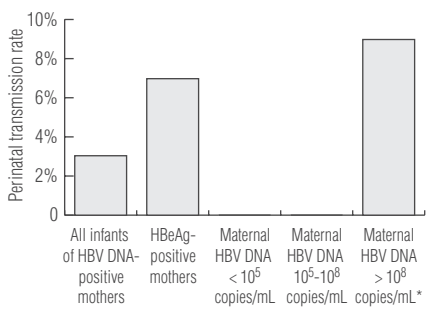
Perinatal transmission rates

Among the original 313 HBsAg-positive pregnant women, there were 213 HBV DNA-positive mothers from whom 138 babies (65%) had been born, reached 9 months of age, and returned for follow-up. These infants were the subject of our studies of perinatal transmission (Box 2). Five pregnancies did not proceed to term and 70 babies have not been tested, either because they have not reached the age of 9 months or were lost to follow-up.

Perinatal transmission was identified in 4/138 infants (3%), indicated by HBsAg-positive and anti-HBs-negative tests. All four were born to HBeAg-positive women with HBV DNA levels $> 10^8$ copies/mL. The transmission rate was 4/47 (9%) from mothers with HBV DNA levels $> 10^8$ copies/mL and 4/61 (7%) from HBeAg-positive mothers. Perinatal transmission was not seen in babies born to mothers with HBV DNA levels $< 10^8$ copies/mL or mothers who were negative for HBeAg (Box 3). The risk of transmission was significantly higher from HBeAg-positive mothers compared with HBeAg-negative mothers (4/61 v 0/77; $P = 0.039$), and from mothers with a very high viral load compared with mothers with high or low viral loads (4/47 v 0/18 v 0/73; $P = 0.031$).

Anti-HBs testing of infants at about 9 months of age revealed that 130/134 (97%) had titres greater than 10 IU/mL, indicating development of immunity (four infants could not be tested because of insufficient serum). All of these infants were negative for HBsAg.

3 Perinatal transmission rates



HBeAg = hepatitis B "e" antigen. HBV = hepatitis B virus. * $P = 0.031$ from mothers with low or high compared with very high HBV DNA levels. ◆

Seventy-four of 125 infants (59%) with sufficient serum for testing were positive for anti-HBc. Of 40 who had adequate serum for subsequent anti-HBc IgM testing, all were negative. There was no relationship between the presence of anti-HBc in infants and maternal viral load ($P = 0.072$).

Perinatal transmission cases

Four infants were HBsAg-positive and anti-HBs-negative, consistent with active HBV infection.

Three of the infants were born after uncomplicated pregnancies and vaginal deliveries. All three were breastfed. In each case, their mothers were HBeAg-positive and had very high viral loads (HBV DNA levels 10^9 copies/mL, $> 6.31 \times 10^8$ copies/mL and 1.58×10^8 copies/mL, respectively). HBIG injection and HBV vaccination were completed according to the recommended schedule.

HBV DNA in each mother and baby was sequenced over the 800 base pairs and assessed for known vaccine escape mutations. In each case, the HBV DNA was identical in the mother and her infant, and no mutations within the S gene of HBV (the gene coding the viral surface antigen) were identified. One of the three infants developed neonatal jaundice 48 hours after delivery. At the time, serological testing of this infant showed detectable anti-HBs (173 IU/mL) but was negative for HBsAg. However, retesting at 10 months of age revealed that anti-HBs was no longer detectable and the infant was HBsAg-positive, with an HBV DNA level $> 6.31 \times 10^8$ copies/mL.

The fourth infant who had acquired HBV perinatally was born to a mother who also had a very high viral load (HBV DNA level 1.58×10^9 copies/mL) and was positive for HBeAg. The baby was delivered vaginally after an episiotomy and was bottle-fed. Unfortunately, HBIG was not administered in this case, but the infant did undergo hepatitis B vaccination according to schedule. At 10 months of age, the child was HBsAg-positive and anti-HBs-negative. Examination of the sequences of the HBV envelope region showed an S gene variant, D144E, which has been associated with HBIG or vaccine escape.[10] This mutation was detected in the viral sequence from both mother and baby.

DISCUSSION

Perinatal transmission of HBV still occurs in infants despite passive and active immunoprophylaxis. Rates of perinatal transmission have not previously been described in Australia. Our study revealed an overall rate of perinatal transmission from HBsAg- and HBV DNA-positive mothers of 3% and from HBeAg-positive mothers of 7%. In a Dutch study of 705 infants born to HBsAg-positive mothers,[1] the rate of transmission was 1.1%, but their HBV DNA status was not disclosed. In contrast, alarmingly high rates of transmission (23%–28%)[2,3] have been reported in other countries such as China, despite passive and active immunoprophylaxis. Explanations for these reported differences are unclear and may reflect variation in HBIG efficacy, varying adherence to immunisation protocols, or possibly different prevalences of vaccine escape mutations.

In our study, perinatal transmission only occurred when the mother's viral load was $> 10^8$ copies/mL. Canho et al[1] also reported that transmission only occurred when the mother's viral load was high (> 150 pg/mL or about 3.16×10^7 copies/mL). In contrast, Ngui et al[11] reported that, although transmission was more likely from mothers with a higher viral load, only 7/12 reported cases of transmission came from mothers with a viral load $> 10^8$ copies/mL. Fluctuations in viral loads over time may reduce the predictive value of the HBV DNA level.

The HBV transmission rate from HBeAg-positive women was 4/47 (7%) in our study. Although an HBV DNA level $> 10^8$ copies/mL identified a smaller cohort at risk for transmitting infection (transmission rate, 4/47 [9%]), the HBeAg test is more readily available and may reduce the possibility of missing infants at risk. This is important if we are to identify a cohort appropriate for therapeutic intervention to reduce transmission.

Administration of lamivudine to highly viraemic women during the third trimester of pregnancy could be beneficial, as reported in a small case–control trial.[3] When highly viraemic mothers were treated with 150 mg lamivudine daily during the last month of pregnancy, perinatal transmission fell from 7/25 (28%) in historical controls to 1/8 (13%). Other interventions, such as administering HBIG to the mother in the third trimester or increasing the dose of HBIG to the newborn, have also been tried, but with variable results.[2,12,13] Although caesarean section is not generally recommended as a means of preventing HBV transmission, one study reported a reduction in HBV transmission from 96/385 (20%) to 6/62 (10%).[14]

Variations in the S gene have been associated with failure of the HBV vaccine. Most mutations identified are amino-acid substitutions or insertions within or immediately upstream of the "a" determinant, the main target of the vaccine[15] and the antibody neutralisation domain of the virus. The reported frequency of vaccine escape mutation in cases of perinatal transmission despite vaccination is 12%–39%.[15-17] In our study, HBV DNA variation in the HBsAg region was identified in one of four cases of transmission. Unfortunately, this infant was not offered HBIG at the time of delivery and thus the role of the mutation in this case remains unclear.

A high proportion of infants in our study (59%) had detectable anti-HBc without anti-HBc-specific IgM. The presence of antibodies was unrelated to maternal viral load. Maternal IgG antibodies are able to cross the placenta, but the larger IgM antibodies are not. Wang et al[18] reported that infant anti-HBc levels fell gradually from 100% to zero during the first 2 years of life. The source of anti-HBc is likely to be transplacental maternal immunoglobulin.

In conclusion, perinatal transmission of HBV was observed in 7% of infants from HBeAg-positive mothers. Maternal viral load correlated with infection, with all transmissions occurring from women with very high viral loads. Interventions such as use of antiviral therapy during the third trimester of pregnancy should be considered for further study. The significance of HBsAg gene mutations needs to be further analysed, with particular reference to determining the prevalence of vaccine escape mutations in the Australian HBV-infected population.

ACKNOWLEDGEMENTS

Our study was supported by a Sydney South West Area Health Research Foundation grant. We thank Raymond Chan for help with initial virological assessment of one case.

COMPETING INTERESTS

None identified.

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(Received 26 Sep 2008, accepted 27 Jan 2009) ❑