Research Article

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Mother-to-infant transmission of hepatitis B virus infection: Significance of maternal viral load and strategies for intervention

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Background & Aims: Immunoprophylaxis reduces but does not completely eradicate hepatitis B virus (HBV) transmission. This prospective study aims at assessing the rate and risk factors of maternally transmitted HBV infection.

Methods: We enrolled 303 mother-infant pairs with positive maternal hepatitis B surface antigen (HBsAg) under current immunization program. Maternal viral load was determined by a real-time PCR-based assay. The children were tested for HBsAg at 4–8 months and/or 1–3 years of age. Rates of HBV infection were estimated using a multivariate logistic regression model.

Results: HBeAg-positive mothers (81/303, 26.7%) had higher viral loads than HBeAg-negative mothers (7.4 ± 1.9 *vs.* 2.7 ± 1.4 $\log_{10}$ copies/ml, p <0.0001). Ten children, born to HBeAg-positive mothers with high viral load (median, 8.4; range, 6.5–9.5 $\log_{10}$ copies/ml), were chronically infected. After adjustment for maternal age, birth type, factors related to maternal-fetal hemorrhage, gestational age, infant gender, birth weight, timeliness of vaccination, and feeding practice, maternal viral load was significantly associated with risk of infection (adjusted odds ratio for each $\log_{10}$ copy/ml increase, 3.49; 95% confidence interval (CI), 1.63–7.48; p = 0.001). The predictive rates of infection at maternal viral load levels of 7, 8, and 9 $\log_{10}$ copies/ml were 6.6% (95% CI, 0.5–12.6%; p = 0.033), 14.6% (95% CI, 5.6–23.6%; p = 0.001), and 27.7% (95% CI, 13.1–42.4%; p <0.001), respectively.

Conclusions: Additional strategies to further reduce transmission should be considered in mothers with a viral load above 7–8 $\log_{10}$ copies/ml.

Keywords: HBV DNA; Perinatal transmission; Immunoprophylactic failure.
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Abbreviations: HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HBeAg, hepatitis B e antigen; HBIG, hepatitis B immunoglobulin; HBsAg, hepatitis B surface antigen; PCR, polymerase chain reaction; SD, standard deviation; OR, odds ratio; CI, confidence interval; ALT, alanine aminotransferase; ULN, upper limit of normal; BF, breast feeding; CS, Cesarean section; CVS, chorionic villus sampling; FF, formula feeding; GA, gestational age; n.a., not available.

Introduction

Hepatitis B virus (HBV) infection is a serious health problem and a major cause of liver cirrhosis and hepatocellular carcinoma (HCC) worldwide. In regions highly endemic for HBV infection, the infection is usually acquired perinatally or in early childhood. HBV infection early in life is likely to cause chronic disease and subsequent complications [1]. Vaccination is a safe and effective measure to prevent HBV infection. In many countries, mass hepatitis B immunization programs have successfully decreased the transmission and diseases associated with acute and chronic infection, including HCC [2–5].

Despite effective immunoprophylaxis, breakthrough HBV infection does occur, and mother-to-infant transmission causes the majority of cases with immunoprophylactic failure [5–7]. Mother-to-infant HBV transmission usually occurs in the perinatal period [8]. Intrauterine infection and postnatal maternal transmission are also possible [9–11]. Mothers positive for the e antigen (HBeAg) and with a high viral load are most likely to transmit the virus [12], even with immunoprophylaxis [10,13–15]. In the era of universal HBV vaccination, transmission from highly viremic mothers remains a major challenge in eradicating the HBV-related diseases [2].

A limited number of studies have reported the efficacy of antiviral therapy in highly viremic HBV-infected pregnant women to reduce maternal viral load and thus reduce transmission [16–19]. However, the optimal cut-off level of maternal viral load for antiviral therapy in pregnancy is still under debate [20–22]. Various cut-off levels have been used as criteria for antiviral therapy [17–19]. In the present study, we prospectively investigated a

cohort of HBV-infected women and their children to assess the rate and risk factors of maternally transmitted HBV infection despite immunoprophylaxis. We discuss the testing and intervention protocols to identify and treat mothers at high risk of transmitting HBV to infants.

Patients and methods

Patients

From April 2007 to March 2011, 347 hepatitis B surface antigen (HBsAg)-positive mothers with 359 deliveries, from National Taiwan University Hospital, Cardinal Tien Hospital, and Tzu-Chi General Hospital Taipei Branch, were enrolled. These mothers were routinely tested for HBsAg and HBeAg in the early third trimester according to the national screening program for pregnant women. The mothers positive for HBsAg were asked to join the study at the time of prenatal visits or delivery. All mothers were negative for human immunodeficiency virus.

Protocol

After obtaining informed consents, the mothers' serum specimens were collected in the third trimester of pregnancy or within 2 months postpartum. Serum alanine aminotransferase (ALT), HBV genotype, and viral load were tested. In addition to the data obtained from prenatal screening, maternal HBeAg was rechecked at the same time when viral load was tested. All infants received 3 doses of hepatitis B vaccine, either H-B-Vax II (5 μg/0.5 ml; Merck Sharp & Dohme) or ENGERIX-B (20 μg/1 ml; GlaxoSmithKline Biologicals, Rixensart, Belgium) within the first week of birth, at 1 month, and at 6 months. For newborns of HBeAg-positive mothers, 0.5 ml (100 IU) HBIG was administered within 24 hours of birth. For newborns of HBsAg-positive but HBeAg-negative mothers, administration of self-paid HBIG was optional [5,15]. The on-schedule rates of vaccination were defined as the administration of the first, second, and third dose of HBV vaccines no longer than 7 days, 1.5 months, and 7 months after birth, respectively.

Infants born to these HBsAg-positive mothers were asked to test for HBsAg at 4–8 months and at 1–3 years of age. HBsAg-positive children were further assessed for HBV genotype. HBV subtypes and mutants of the *a* determinant of HBsAg in the infected children and corresponding mothers were also determined. The institutional review board of the above-mentioned hospitals approved the protocol.

Laboratory methods

The hepatitis B serologic markers were tested using the AxSYM microparticle enzyme immunoassay (Abbott Laboratories, Abbott Park, IL, USA). The serum hepatitis B viral load was quantified by a real-time polymerase chain reaction (PCR) assay (Abbott RealTime HBV assay, Abbott Molecular Inc., Des Plaines, IL, USA). The lower and upper limits of quantification are 15 IU/ml (51.2 copies/ml) and 10^9 IU/ml (3.4×10^9 copies/ml), respectively.

The HBV genotype was determined by real-time PCR with subsequent melting curve analysis (LightCycler hybridization probes assay system, Roche Diagnostics). This assay determines the HBV genotype when viral load is not less than 1000 copies/ml [23]. HBV subtypes and mutants of the *a* determinant of HBsAg were determined by direct sequencing of the nucleotide sequences encoding the *a* determinant of HBsAg (amino acids 110–160) and subsequent sequence comparison. Detailed procedures were described previously [24].

Definition of HBV infection

Because the seropositivity of the HBV core antibody at age 0–24 months mainly results from the passive transfer of the maternal antibody [25], we used seropositivity of HBsAg as the marker of HBV infection in children. If a child tested HBsAg-positive, HBsAg testing was repeated 6 months later to confirm chronic infection.

Statistical analyses

Statistical analyses were performed using Stata 11.2 software (Stata Corporation, College Station, TX, USA). Two-sided $p \leqslant 0.05$ was considered statistically significant. The group differences were examined using the Wilcoxon rank-sum test for continuous variables or the Chi-square test or Fisher's exact test for categorical variables. HBV DNA levels were $\log_{10}$-transformed for subsequent analyses. For statistical comparisons, we assigned a value of 51.2 copies/ml, the lower detection limit of the quantification assay, to samples with detectable HBV DNA levels less than 51.2 copies/ml.

Univariate and multivariate logistic regression analyses were performed to identify predictive factors of maternally transmitted HBV infection. The predictive variables examined included maternal age, maternal HBeAg status, maternal viral load, maternal HBV genotype, factors related to maternal-fetal hemorrhage (threatened abortion, threatened preterm labor, chorionic villus sampling, amniocentesis, forceps/vacuum delivery, and emergent Cesarean section after any period of labor) [26], type of birth [27], gestational age, infant gender, birth weight, timeliness of HBV vaccination, and feeding practice [28,29]. The odds ratio (OR) and 95% CI of each factor were derived using logistic regression analyses. The goodness of fit of the multivariate logistic regression model was determined using the Hosmer-Lemeshow statistics. Using the multivariate logistic regression model, predictive rates of maternally transmitted HBV infection were estimated at various maternal viral load levels.

Results

The general data for the participants are shown in Fig. 1. Eighty-five percent (222/262) of infants with HBeAg-negative mothers and 83.5% (81/97) of infants with HBeAg-positive mothers returned for HBsAg testing. The main reasons for infants not returning for testing were moving away and parental unwillingness to let their children receive painful blood sampling. The infants tested for HBsAg and the infants dropping off the study had comparable maternal age, maternal ALT levels, maternal HBV genotype distributions, maternal HBV viral loads, sex distributions, rates of Cesarean section, birth weights, and rates of preterm birth (Supplementary Table 1).

Maternal characteristics

Table 1 lists the characteristics of the mothers whose children returned for tests. HBeAg-positive mothers (81/303, 26.7%) were younger (31.0 ± 4.9 years *vs.* 33.9 ± 4.0 years, p <0.0001) and had higher HBV DNA levels (7.4 ± 1.9 *vs.* 2.7 ± 1.4 $\log_{10}$ copies/ml, p <0.0001) than HBeAg-negative mothers. Fig. 2A and B depict the viral load distributions in HBeAg-negative mothers and HBeAg-positive mothers, respectively. All the 81 HBeAg-positive mothers and 98.6% (219/222) of the HBeAg-negative mothers had concordant HBeAg results with prenatal screening. The HBeAg-positive mothers tested before or on the day of delivery had comparable viral loads to those tested after delivery (7.3 ± 1.9 *vs.* 7.5 ± 2.0 $\log_{10}$ copies/ml, $p = 0.3950$), as did the HBeAg-negative mothers (2.6 ± 1.6 *vs.* 2.7 ± 1.3 $\log_{10}$ copies/ml, $p = 0.9234$).

Immunization and follow-up of infants

All 81 infants with HBeAg-positive mothers and 85.1% (189/222) of infants with HBeAg-negative mothers were administered HBIG. All infants with HBeAg-positive mothers received HBIG within 24 hours after birth and 95.1% (77/81) received HBIG within 12 hours. The HBV vaccine completion rate (3 doses) was 100%. The on-schedule rates for the first, second, and third doses of vaccine were 93.1%, 93.1%, and 93.7%, respectively.

Of the 303 children undergoing HBsAg testing, 250 (82.5%) were tested at 4–8 months of age, and 53 (17.5%) were tested at 1–3 years of age. Seventy-three percent (183/250) of children with the first HBsAg test at 4–8 months underwent follow-up