

Protective effect of an improved immunization practice of mother-to-infant transmission of hepatitis B virus and risk factors associated with immunoprophylaxis failure

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Abstract

Although routine immunoprophylaxis has been known to reduce hepatitis B virus (HBV) transmission, immunoprophylaxis failure still occurs. The study aimed to investigate the protective efficacy of an improved immunoprophylaxis protocol to prevent mother-to-infant transmission of HBV and to explore the potential risk factors associated with immunoprophylaxis failure and low antibody response.

A prospective observational cohort study was conducted from July 2012 to April 2015. A total of 863 HBsAg-positive mothers and their 871 infants (8 pairs of twins) were included in the study. Two different hepatitis B vaccine doses (20 or 10 µg) were administered to the infants based on the hepatitis B e-antigen (HBeAg) status of their mothers. Simultaneously, hepatitis B immunoglobulin (HBIG) was administered to the infants. Initial injections of HBIG and the hepatitis vaccine were given within 2 hours after birth. Rates of HBV infections among the infants were evaluated at 7 months of age. Factors associated with immunoprophylaxis failure and low responses to vaccination were analyzed by unconditional logistic regression.

At 7 months of age, no immunoprophylaxis failure was observed in the 565 infants born to HBeAg-negative mothers. Among the 306 infants born to HBeAg-positive mothers, immunoprophylaxis failed in 16 infants (5.2%) of the infants and they were found to be HBsAg-positive. Further analysis showed that HBV DNA levels $\geq 10^8$ IU/mL [odds ratio (OR) = 4.53, 95% confidence interval (95% CI): 1.19–17.34], delayed vaccination (OR = 4.14, 95% CI: 1.00–17.18), and inadequate initial injections (OR = 7.69, 95% CI: 1.71–34.59) were independently associated with immunoprophylaxis failure. Adequate titers of antibody to HBsAg (anti-HBs, ≥ 100 mIU/mL) were present in 96.5% of immunoprophylaxis-successful infants. For full-term infants, birth weights <3000 g were correlated with low immune responses to vaccination.

This improved immunoprophylaxis protocol is effective in preventing perinatal transmission of HBV. Among infants with HBeAg-positive mothers, high HBV viral loads and inadequate and delayed initial injections were associated with immunoprophylaxis failure. The majority of the infants in our study produced adequate levels of protective anti-HBs titers after immunoprophylaxis. Additional efforts to further reduce perinatal transmission should be considered, especially for HBeAg-positive mothers.

Abbreviations: HBV = hepatitis B virus, HBsAg = hepatitis B surface antigen, HBeAg = hepatitis B core antigen, HBIG = hepatitis B immunoglobulin, OR = odds ratio, CI = confidence interval, MTCT = mother-to-child transmission, CMIA = chemiluminescent microparticle immunoassay, ALT = alanine aminotransferase, ULN = upper limit of the normal, SD = standard deviation, SAEs = serious adverse events.

Keywords: HBIG, hepatitis B virus, immunoprophylaxis, mother-to-infant transmission, vaccine

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1. Introduction

Hepatitis B virus (HBV) infection is a serious global health problem with approximately 240 million people persistently infected with HBV and 780,000 HBV-related deaths annually worldwide.^[1] The risk of becoming an HBV carrier is inversely related to the age of the infected patient. Chronic HBV infections persist in 1% to 5% of infected adults, 20% to 30% of young children, and up to 90% to 95% of perinatally infected infants. HBV infection early in life is likely to cause chronic disease and subsequent complications.^[2]

A national survey conducted in China in 1992 showed that the prevalence of hepatitis B surface antigen (HBsAg) carriers was 9.67% among children <5 years old.^[3] Approximately 20 years ago, China implemented an HBV immunoprophylaxis strategy, which led to a 90% reduction (to 0.96%) in HBsAg carriers <5 years old by 2006.^[4,5] Approximately 90% of infants born to both HBsAg-positive and hepatitis B e-antigen (HBeAg)-positive mothers will become HBsAg carriers if no immunoprophylaxis is given.^[6] The most effective way to prevent mother-to-child transmission (MTCT) of HBV infection is by immunizing all susceptible individuals with adequate hepatitis B immune globulin (HBIG) and hepatitis B vaccines at birth, especially newborn infants born to HBV-positive mothers.^[2,7-9] Although administration of HBIG and the hepatitis B vaccine at birth has significantly reduced HBV infection rates, 0.75% to 9.66% of vertical HBV transmissions have not been eliminated by this combined intervention strategy.^[10-13] It has been suggested that providing antiviral therapy during the third trimester of pregnancy to mothers with high HBV viral loads would reduce the risk of perinatal transmission, but data regarding when and for how long this therapy should be administered are lacking.^[14]

Although many studies on perinatal HBV prevention have been carried out using various prophylaxis protocols, the hepatitis B vaccine doses used in these studies have been ambiguous or inconsistent and ranged anywhere from 5 to 20 µg. Although high maternal HBV viral loads and HBeAg-positivity have been shown to be the predominant risk factors for immunoprophylaxis failure, only a few studies have evaluated the use of larger hepatitis B vaccine doses for infants born to HBeAg-positive mothers. Also, the initial injection time for HBIG and hepatitis B vaccine administration has not yet been strictly defined.^[15-18]

In the present study, we conducted a prospective cohort of HBsAg-positive mothers and their infants to assess the protective efficacy of a modified immunoprophylaxis protocol. This modified protocol uses 2 different vaccine doses based on the maternal HBeAg status in combination with HBIG, and the injections are administered within 2 hours after birth. The HBV infection rate in infants and the potential risk factors associated with immunoprophylaxis failure and low protective antibody titers were evaluated.

2. Methods

2.1. Study design

This is a prospective, observational cohort study of HBsAg-positive mothers and their corresponding infants. On the basis of the status of HBeAg of mothers, different hepatitis B vaccine doses (20 or 10 µg) were given to the infants. Therefore, we limited our study to a nonrandomized, double-blind control design (with no appropriate control in the same HB vaccination group). For this reason, this study was not registered in the

clinical trial registry. The study was approved by the Medical Ethics Committee of First Hospital of Jilin University (approval number 2012-098). Participants were informed of the purpose of the study and of their rights to keep information confidential. Written informed consent was obtained from each subject before participation. Patient records/information was anonymized and de-identified before analysis.

2.2. Study populations

For this study, all pregnant women were screened for HBV infection to improve the prevention and control of vertical HBV transmission. Pregnant women who tested positive for HBsAg at antenatal clinics in all of the districts and counties of Changchun were encouraged to go to the First Hospital of Jilin University for further HBV transmission prevention. Each pregnant woman was then given an immunization schedule that obstetricians and midwives at 25 cooperative birth hospitals would follow.

Pregnant women would not be included if they met any of the following exclusion criteria: having received antiviral medications or immune-modifying therapies during pregnancy; having coviral infections; and having any immunologically compromised conditions. Infant exclusion criteria were as follows: birth weight lower than 2500g; and born before 37 weeks of gestational age. The study was carried out from July 2012 to April 2015, and infants were followed-up at 7 and 12 months of age. In total, 918 HBsAg-positive mothers registered and participated in the study, and 28 mothers and 27 infants were excluded due to the various reasons mentioned above. As a result, 871 infants were included in the final analysis. The flow chart depicting the study participants is summarized in Fig. 1.

2.3. Data collection

Two doctors were trained to complete the unified questionnaires when the mothers visit at the outpatient clinic. The questionnaires were administered face-to-face; the following sections were covered: socio-demographic data, gestational weeks, smoking and drinking status, family history of hepatitis B, antiviral therapy, and whether they were aware of HBV infection. Mothers were followed-up after delivery through telephone call, including delivery mode, feeding pattern, infant gender, infant weight, gestational age, and HBV vaccination dose with the time of injection. In addition, infants were continued 4 times of follow-up after birth (24 hours, 14 days, 1, and 6 months of age) for HBV vaccination information, and HBV serologic markers were measured at 7 and 12 months of age.

2.4. Immunization schedule

A combined passive and active immunization was administered to neonates born to HBsAg-positive mothers. Infants born to HBsAg-positive, HBeAg-negative mothers were injected with 100 IU of HBIG (Hualan Biological Engineering Inc., Xinxiang, China) and 10 µg of the hepatitis B vaccine (Hansenua polymorpha yeast-derived recombinant Hepatitis B vaccine; Dalian Hissen Bio-pharm Co., Dalian, China) within 2 hours after birth, followed by administration of 10 µg of the hepatitis B vaccine at 1 month and 6 months of age. Infants born to HBsAg-positive, HBeAg-positive mothers were injected with 100 IU of HBIG and 20 µg of the hepatitis B vaccine within 2 hours after birth, followed by administration of 20 µg of the hepatitis B vaccine at 1 month and 6 months of age. Administration of HBV

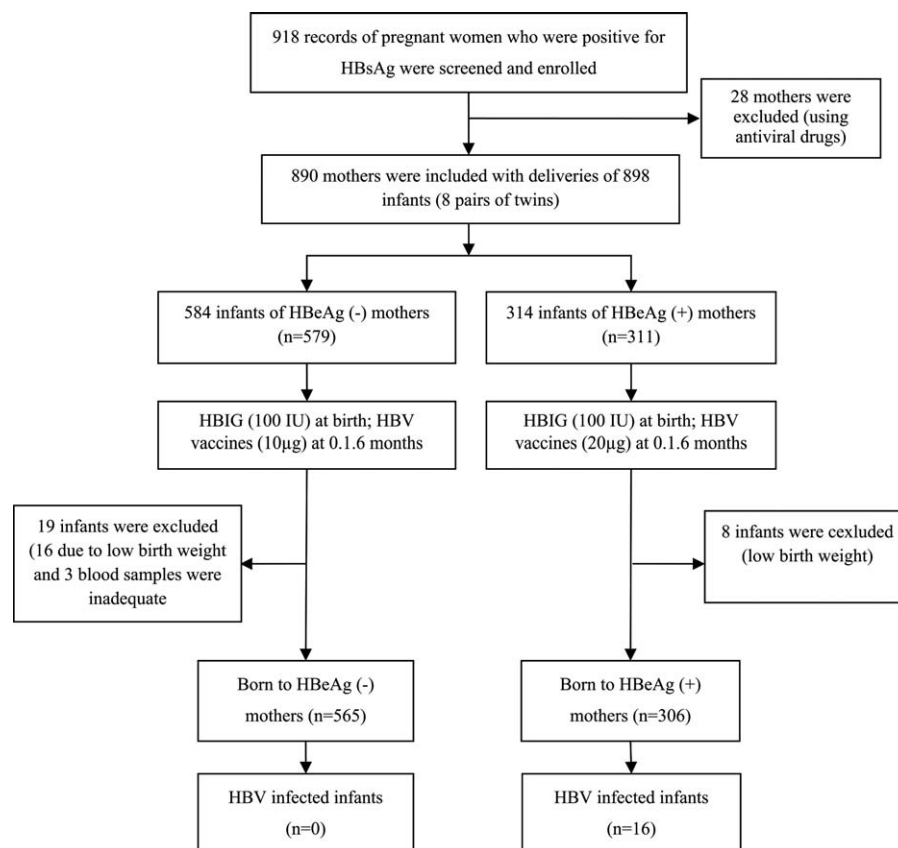


Figure 1. Flowchart depicting the study participants.

vaccine and HBIG in accordance with the described protocol is defined as a timely and adequate injection.

2.5. Laboratory examinations

Venous blood samples were collected from pregnant women at approximately 1 month before delivery. Blood samples from infants were collected at the 7-month follow-up visit. All laboratory tests were performed in the central laboratory of the First Hospital of Jilin University. Serum samples from the mother and the infant were examined for 5 markers of HBV, including HBsAg, anti-HBs, HBeAg, anti-HBe, and anti-HBc, by chemiluminescent microparticle immunoassay (CMIA) with Abbott ARCHITECT i2000SR (Abbott Laboratories, North Chicago, IL). HBV DNA was assessed by the Roche Taqman HBV test (Roche Diagnostics, Grenzach, Germany) with a detection range from 8.9 to 109 IU/mL. Liver function tests were performed with a Synchron LXH20 autoanalyser (Beckman Coulter, Brea, CA).

2.6. Definition of immunoprophylaxis outcome

Seropositivity for HBsAg was used as the marker of HBV infection in infants. If an infant tested HBsAg-positive, the HBV DNA test was performed later to confirm immunoprophylaxis failure and HBV infection. The nonresponders, low responders, medium responders, and high responders were defined as HBsAg-negative infants with anti-HBs < 10 mIU/mL, anti-HBs from 10 to 100 mIU/mL, anti-HBs from 100 to 1000 mIU/mL, and anti-HBs ≥ 1000 mIU/mL, respectively. We usually take anti-HBs ≥ 10 mIU/

mL as a seroprotective response to vaccine. In this study, enough anti-HBs titer is still needed to prevent the babies from close contact infection with mothers, so anti-HBs titer ≥ 100 mIU/mL was considered a protective response, whereas a titer ≤ 100 mIU/mL was considered a low protective response.

2.7. Statistical analysis

A database of the mother-to-infant HBV transmission prevention cohort was established by the CapitalBio Life Science Data Management System (CB-LDMS-Sample). When the descriptive values were of normal distribution, *t* tests or nonparametric tests were used and data were expressed as mean ± standard deviation (SD). The Chi-square or Fisher exact test was used for categorical variables expressed in terms of *n* (%). Risk factors for immunoprophylaxis failure and low protective vaccine responses were analyzed by unconditional logistic regression with adjustment for possible confounders. The odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to estimate risk. All statistical analyses were performed with the Statistical Package for Social Science (SPSS) for Windows version 18.0 (SPSS Inc., Chicago, IL). All *P* values were 2-tailed and a *P* value < 0.05 was considered statistically significant.

3. Results

3.1. Basic characteristics of mothers and infants

A total of 863 mothers and their corresponding 871 infants (8 pairs of twins) were included in the final analysis. Of these,

306 infants (35.1%) were born to HBsAg-positive, HBeAg-positive mothers, and 565 (64.9%) were born to HBsAg-positive, HBeAg-negative mothers. HBeAg-positive mothers were younger (27.71 ± 3.89 vs 28.98 ± 3.95 years old, $P < 0.001$) and had higher HBV DNA levels (7.75 ± 1.06 vs 2.71 ± 1.16 \log_{10} IU/mL, $P < 0.001$), but had a lower rate of breastfeeding (30.1% vs 63.2%, $P < 0.001$) than HBeAg-negative mothers. Infants born to HBeAg-positive mothers and to HBeAg-negative mothers were similar in terms of gender distribution, birth weight, gestational age, and percentage of delayed vaccination. The basic characteristics of mothers and infants are summarized in Table 1.

3.2. The immunization outcome of the infants

Among 871 infants in this study, there were 16 infants who failed immunoprophylaxis and were HBsAg positive after the completion of hepatitis B vaccination schedule; the infant HBV infection rate was 1.84% (16/871). No immunoprophylaxis failure was observed in the 565 infants born to 560 HBeAg-negative mothers at 7 months. Among the 306 infants born to 303 HBeAg-positive mothers, the immunoprophylaxis failure (HBsAg-positive) rate was 5.2% (16/306). The HBV marker test results for infants at 7 months are summarized in Table 2. Four infants (3 in the 10 μ g of HBV vaccine group and 1 in the 20 μ g of HBV vaccine group) were HBeAg-positive at 7 months of age, but were HBeAg-negative at 12 months of age. All of the infants with immunoprophylaxis failure were born to HBeAg-positive mothers with HBV DNA $>4 \times 10^7$ IU/mL. Detailed information regarding the 16 infants with immunoprophylaxis failure and their mothers is summarized in Supplementary Table 1, <http://links.lww.com/MD/B215>.

3.3. Factors associated with immunoprophylaxis failure

As HBV transmission to infants born to HBeAg-negative mothers was all successfully prevented, we further analyzed factors related to immunoprophylaxis failure in infants born to HBeAg-positive mothers. The results of the univariate and multivariate logistic regression analyses are summarized in Table 3. After adjustment for other risk factors, multivariate regression analysis showed that mothers with higher HBV DNA levels ($\geq 10^8$ IU/mL; Adjusted OR = 4.53, 95% CI: 1.19–17.34) and inadequate initial injections

(Adjusted OR = 7.69, 95% CI: 1.71–34.59) were independently associated with an increased risk for immunoprophylaxis failure. Besides, nontimely initial injections (over than 2 hours) reached a borderline *significant* (Adjusted OR = 4.14, 95% CI: 1.00–17.18, $P = 0.050$). Other factors, including delivery mode, feeding pattern, infant gender, and birth weight, were not significantly associated with maternal HBV transmission.

3.4. Factors correlated with the infant immune response to vaccination

We divided 855 HBV-negative infants into 2 groups. One group, the responder group, included 825 (96.5%) infants with anti-HBs titers ≥ 100 mIU/mL after completion of a series of 3 hepatitis B vaccine injections. The second group consisted of infants whose anti-HBs titers did not reach the protective level. Possible factors related to low immune responses to vaccination are summarized in Table 4. Infant birth weights <3000 g were correlated with low immune responses to vaccination (Adjusted OR = 2.47, 95% CI: 1.02–5.99; $P = 0.045$), whereas other factors such as the hepatitis B vaccine dose, maternal HBV DNA, delivery mode, feeding pattern, infant gender, gestational age, and initial injection time did not significantly correlate with a low response to vaccination. Besides, no difference was observed between different HepB injection dose and infants immune response distribution (Supplementary Table 2, <http://links.lww.com/MD/B215>).

3.5. Adverse events and safety

All of the infants who received the recombinant hepatitis B vaccine and HBIG were followed-up. No serious adverse events (SAEs) were reported, and only 36 (4.1%) of the newborns had temporary redness and fever following the first dose of the vaccine, but the symptoms resolved on their own without special treatment. No difference in the number of adverse reactions was observed between the 10 and 20 μ g hepatitis B vaccine dose groups (4.4% vs 3.6%, $P = 0.557$).

4. Discussion

In this cohort study, infants born to HBsAg-positive mothers with different HBeAg statuses were given different immunization

Table 1

Basic characteristics of mothers and infants in the HBeAg-negative and HBeAg-positive groups.

Characteristics	HBeAg (+) mothers	HBeAg (–) mothers	P
Maternal data	N=303	N=560	
Age of delivery (y, mean \pm SD)	27.71 \pm 3.89	28.98 \pm 3.95	<0.001
HBV DNA levels (mean \pm SD)*	7.75 \pm 1.06	2.71 \pm 1.16	<0.001
ALT >1 ULN, no. (%)†	28 (11.2)	17 (6.4)	0.051
Gestation weeks (w, mean \pm SD)	39.18 \pm 0.96	39.08 \pm 1.03	0.185
Cesarean, no. (%)	243 (79.4)	428 (75.8)	0.220
Infant data	N=306	N=565	
Male infant, no. (%)	145 (47.4)	256 (45.3)	0.557
Birth weight (g, mean \pm SD)	3396 \pm 407	3412 \pm 444	0.604
Breast feeding, no. (%)	92 (30.1)	357 (63.2)	<0.001
Delayed vaccination, no. (%)	25 (8.2)	39 (6.9)	0.494
Insufficient vaccination, no. (%)	16 (5.2)	14 (2.5)	0.050
Singleton, n (%)	303 (99.0)	560 (99.1)	1.000

ALT = alanine transaminase; SD = standard deviation; ULN = upper limit of the normal.

* The maternal HBV DNA was measured as \log_{10} IU/mL.

† Only mothers with available ALT data were included.

Table 2**HBV marker test results for infants at 7 months of age.**

HBV markers	No. (%) of infants born to HBeAg-negative mothers who were positive for this marker (N=565)	No. (%) of infants born to HBeAg-positive mothers who were positive for this marker (N=306)	<i>P</i>
HBsAg	0 (0%)	16 (5.2%)	<0.001
Anti-HBs	562 (99.5%)	290 (94.8%)	<0.001
HBeAg	3 (0.5%)	17 (5.6%)	<0.001
Anti-HBe	110 (19.5%)	17 (5.6%)	<0.001
Anti-HBc	162 (28.7%)	115 (37.6%)	0.007

* Chi-square.

schedules, and the results were encouraging. None of the infants born to HBeAg-negative mothers were infected with HBV. All the immunoprophylaxis failure infants were born to mothers positive for both HBsAg and HBeAg, with a immunoprophylaxis failure rate of 5.2% observed. Maternal high HBV DNA levels and HB vaccine dose insufficiency along with delayed first injection were significantly associated with MTCT. Furthermore, our results showed that the majority of infants (96.5%) achieved protective level of antibodies at 7 months of age. Relatively low birth weight <3000g in full-term babies was associated with weak response to vaccination.

Different from previous studies, we used 2 different doses of hepatitis B vaccine (20 and 10 µg) to the newborns based on their mothers' HBeAg status instead of administered with 1 same dose of vaccine. Although an appropriate postnatal hepatitis B vaccine

combined with HBIG was developed to prevent MTCT in 1992 in China, however, before the year of 2014, the HBV vaccination policy in Changchun city recommends that all newborn infants receive only 5 µg hepatitis B vaccine within 12 hours of birth and again at 1 and 6 months of age, and no additional prevention measures were implemented to mothers who were HBsAg carriers. This old strategy did not take the HBeAg status and HBV DNA level of the pregnant women into consideration. Given this background, it is very important to provide an improved immunoprophylaxis protocol to prevent MTCT of HBV. This benefit engineering was unique for that it provided an individual schedule to protect these in high-risk infants of HBV infection.

This study showed that immunoprophylaxis was successful for the majority of infants with only an overall MTCT rate of 1.84%

Table 3**Factors influencing immunoprophylaxis failure in infants born to HBeAg-positive mothers (N=306).**

Factors	Failure (N=16), n (%)	Nonfailure (N=290), n (%)	Univariate	Multivariate	<i>P</i> [†]
			Crude OR (95% CI)	Adjusted OR (95% CI)	
Maternal HBV DNA [*]					
<10 ⁸ IU/mL	3 (2.3)	126 (97.7)	Reference	Reference	0.027
≥10 ⁸ IU/mL	13 (8.1)	148 (91.9)	3.69 (1.03–13.24)	4.53 (1.19–17.34)	
Delivery mode					0.140
Vaginal	5 (7.9)	58 (92.1)	Reference	Reference	
Cesarean	11 (4.5)	232 (95.5)	0.55 (0.18–1.65)	0.41 (0.12–1.35)	
Feeding pattern					0.749
Formula feeding	11 (5.1)	203 (94.9)	Reference	Reference	
Breast or mix feeding	5 (5.4)	87 (94.6)	1.06 (0.36–3.14)	0.83 (0.26–2.63)	
Infant sex					0.083
Female	5 (3.4)	140 (96.6)	Reference	Reference	
Male	11 (6.8)	150 (93.2)	2.05 (0.70–6.06)	2.78 (0.87–8.82)	
Infant weight					0.855
≥3000 g	14 (5.3)	248 (94.7)	Reference	Reference	
<3000 g	2 (4.5)	42 (95.5)	0.84 (0.19–3.85)	0.86 (0.18–4.23)	
Infant gestational age					0.433
≥38 wk	14 (5.0)	267 (95.0)	Reference	Reference	
<38 wk	2 (8.0)	23 (92.0)	1.66 (0.36–7.75)	1.93 (0.37–10.02)	
Initial injection time					0.050
Within 2 h	13 (4.6)	268 (95.4)	Reference	Reference	
Above 2 h	3 (12.0)	22 (88.0)	2.81 (0.75–10.61)	4.14 (1.00–17.18)	
Initial injection dose [‡]					0.008
Sufficient	13 (4.55)	277 (95.5)	Reference	Reference	
Insufficient	3 (18.8)	13 (81.3)	4.92 (1.25–19.41)	7.69 (1.71–34.59)	

CI = confidence interval, HBV = hepatitis B virus, OR, odds ratio.

^{*} This category excludes 16 mothers with insufficient samples for the HBV DNA test.[†] Adjusted ORs (95% CI) were assessed using logistic regression analysis adjusted for all variables.[‡] Nonsufficient vaccination refers to administration of the first dose of vaccine lower than 20 µg.

Table 4
Possible factors related to low immune responses (anti-HBs \leq 100 mIU/mL) to vaccination (N = 855).

Factors	Anti-HBs (mIU/mL)		Univariate	Multivariate	P [†]
	<100	\geq 100	Crude OR (95% CI)	Adjusted OR (95% CI)	
Hepatitis B vaccine dose					
10 μ g	22 (3.9)	543 (96.1)	Reference	Reference	0.743
20 μ g	8 (2.8)	282 (97.2)	0.70 (0.31–1.59)	1.37 (0.21–9.01)	
Maternal HBV DNA*					
<10 ⁶ IU/mL	23 (4.0)	555 (96.0)	Reference	Reference	0.385
\geq 10 ⁶ IU/mL	6 (2.3)	255 (97.7)	0.57 (0.23–1.41)	0.42 (0.58–3.01)	
Delivery mode					
Vaginal	8 (4.1)	187 (95.9)	Reference	Reference	0.991
Cesarean	22 (3.3)	638 (96.7)	0.81 (0.35–1.84)	1.00 (0.41–2.45)	
Feeding pattern					
Formula feeding	14 (3.4)	397 (96.6)	Reference	Reference	0.769
Breast or mix feeding	16 (3.6)	428 (96.4)	1.06 (0.51–2.20)	0.89 (0.40–1.96)	
Infant sex					
Female	15 (3.8)	381 (96.2)	Reference	Reference	0.776
Male	15 (3.3)	444 (96.7)	0.86 (0.41–1.78)	1.12 (0.53–2.36)	
Infant weight					
\geq 3000 g	21 (2.9)	711 (97.1)	Reference	Reference	0.045
<3000 g	9 (7.3)	114 (92.7)	2.67 (1.19–5.98)	2.47 (1.02–5.99)	
Infant gestational age					
\geq 38 wk	24 (3.1)	739 (96.9)	Reference	Reference	0.420
<38 wk	6 (6.5)	86 (93.5)	2.15 (0.85–5.40)	1.52 (0.55–4.20)	
Initial injection time					
Within 2 h	28 (3.5)	766 (96.5)	Reference	Reference	0.844
Above 2 h	2 (3.3)	59 (96.7)	0.93 (0.22–3.99)	0.86 (0.20–3.80)	
Initial injection dose [‡]					
Sufficient	29 (3.5)	799 (96.5)	Reference	Reference	0.823
Insufficient	1 (3.7)	26 (96.3)	1.06 (0.14–8.08)	1.27 (0.16–9.84)	

CI = confidence interval, HBV = hepatitis B virus, OR, odds ratio.

* This category excludes 16 mothers with insufficient samples for the HBV DNA test.

[†] Adjusted ORs (95% CI) were assessed using logistic regression analysis adjusted for all variables.

[‡] Nonsufficient vaccination refers to administration of the first dose of vaccine lower than 20 μ g.

(16/871), which was lower than previous reports with failure rates that ranged from 2.46% to 5.44%.^[18–20] Larger hepatitis B vaccine doses and timely vaccination as well as high cesarean rate might contribute to the low infection rate in our study. However, a higher immunoprophylaxis failure rate (5.2%) was observed in infants born to HBeAg-positive mothers. In a study by Kubo et al,^[9] among 624 infants born to 481 HBeAg-positive mothers, the overall infant HBV infection rate was 3.37%, which is lower than our results. Use of antiviral medications for parts of higher-risk populations in their study explains their low infection rates. In our study, to evaluate immunoprophylaxis efficacy only, we excluded all the mothers who had taken antiviral drugs during pregnancy.

The analysis of factors related to immunoprophylaxis failure in infants born to HBeAg-positive mothers revealed that high HBV DNA levels, insufficient hepatitis B vaccine doses, and delayed first injections were significantly associated with immunoprophylaxis failure. In our cohort, all the 16 blocking failure infants were born to HBeAg-positive mothers with HBV DNA $>4 \times 10^7$ IU/mL. After adjusting for other factors, HBV DNA was shown to be an independent risk factor for MTCT. This result is in accordance with previous data, which suggested that maternal HBV DNA $>7\text{--}8 \log_{10}$ copies/mL was significantly correlated with perinatal HBV transmission.^[17,21] Maternal HBV DNA $>2 \times 10^7$ IU/mL level had been recommended by AASLD (2009) and APASL (2012) for antiviral treatment during pregnancy.^[22,23] Our results showed that combined HBIG and hepatitis B

vaccine immunoprophylaxis strategy is effective for HBeAg-negative mothers and for HBeAg-positive mothers with HBV DNA $<4 \times 10^7$ IU/mL. Additional prophylaxis measures, such as late-pregnancy antiviral treatments, may be needed for HBeAg-positive mothers with high HBV viral load.

It was also found that vaccine doses and injection time are another 2 risk factors that are associated with maternal HBV transmission among infants born to HBeAg-positive mothers. The Chinese Society of Hepatology and Chinese Society of Infectious Diseases and Chinese Medical Association recommended that newborns of HBsAg positive mothers administering 10 μ g recombinant yeast derived HB vaccine combined with HBIG 100 IU within 24 hours after birth (preferably within 12 hours), followed by 2 additional HB vaccine at 1 and 6 months, respectively.^[24] In most studies, a range of 5 to 20 μ g of hepatitis B vaccine was given to newborns.^[17,25] In our study, the hepatitis B vaccine dose recommended for infants born to HBeAg-positive mothers was 20 μ g, which was larger than the previous. Our results showed that this immunoprophylaxis was successful with a low infection rate and HB vaccination was well tolerated. However, immunoprophylaxis failure still occurred despite the use of combined HB vaccine and HBIG. Our data demonstrated that insufficient hepatitis B vaccine doses ($<20 \mu$ g) were significantly associated with immunoprophylaxis failure (OR = 7.69, 95% CI: 1.71–34.59). Similarly, the Centers for Disease Control and Prevention (CDC) and the Advisory Committee on Immunization Practices (ACIP) also recommend that the HBV

vaccine and HBIG be administered to at-risk infants within 12 hours of delivery.^[26] If their mothers are positive for both HBsAg and HBeAg, HBIG should be administered to newborns as soon as possible.^[27,28] However, there have been a variety of schedules for the initial passive-active immunization of the newborn, which vary anywhere from 3 to 24 hours after birth.^[15,17,29–32] Previous data from liver biopsies indicated that more than 2 hours is required for HBV to infect healthy liver cells^[33]; therefore, the first injection of HBIG and the hepatitis B vaccine must be administered as early as possible after birth.^[11] In our study, the first dose of hepatitis vaccine and HBIG was required to be administered simultaneously within 2 hours after birth. The result showed that delayed initial injection of vaccine was correlated with immunization failure, which reach a borderline significant.^[34]

HBeAg, anti-HBe, and anti-HBc pass through human placenta from mother to fetus and gradually disappear over time.^[35] In our study, 4 infants were HBeAg-positive at 7 months of age, but by 12 months of age, HBeAg was not detectable in these infants. The presence of anti-HBe before 12 months of age and the presence of anti-HBc before 24 months of age in infants may represent transplacental maternal antibodies rather than indicators of HBV infectious status.^[35] Therefore, HBV markers should not be evaluated in newborns immediately after birth, rather the HBV infection status of infants should be evaluated at 7 months of age and should continue thereafter to determine whether a chronic infection persists.

Because adequate primary vaccination provides long-term protection,^[36,37] it is necessary and important for infants to obtain adequate hepatitis B surface antibody titers at primary immunization to protect them from HBV infections and HBV-related diseases caused by potential future exposures. In this study, all the infants received the corresponding planned immunoprophylaxis protocol with the same brand of vaccine, the majority of the infants achieved a protective level, while still 3.5% of the infants became low immune responders. In this study, some factors that may be associated with low responsiveness to the HBV vaccine were analyzed, such as maternal HBV DNA, delivery mode, feeding pattern, infant gender, gestational age, birth weight, and vaccine dose. Besides, genetic factors such as the HLA group were also reported to be associated with non/low response to HB immunization.^[38,39] We found that only birth weights <3000g was an independent predictive factor for weak response. Because the hepatitis B vaccine doses used in this study were greater than the doses used in most of the previous studies, and infants born prematurely weighing <2500g may lead to incomplete immune function development,^[40] the vaccine-related safety may not be guaranteed; for this reason, only full-term infants with birth weights ≥ 2500 g were included in our study. The above results may be more significant if pre-term infants weighing <2500g were also included. Therefore, it is essential to pay close attention to those low birth weight infants for the anti-HBs level, and hepatitis B booster vaccinations should also be considered when necessary.

This study is more convincing by the large sample size and accurate data collection. However, some limitations should be acknowledged. First, our observational cohort study was limited by the evaluation of the efficacy of immunization strategies; therefore, rigorous designed randomized controlled trials are needed in the future. Second, we can define the MTCT of our objects, but we have no evidence to confirm when the transmission actually occurred (intrauterine infection, natal transmission, or postnatal transmission).

In conclusion, our study showed that this improved immunization is effective for preventing perinatal transmission of HBV, particularly for mothers who are HBeAg-negative. Maternal HBV DNA level and inadequate and delayed initial injections were the independent risk factors that predicted immunoprophylaxis failure. The majority of the infants in this study reached adequate protective antibody titers after immunoprophylaxis. A relatively low birth weight of <3000g for full-term infants was associated with a weak response to vaccination. It is noteworthy that we are keeping on following up these children until 3 years old, and their long-term immunological outcome will be evaluated in the further research.

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