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# Presence of hepatitis B virus markers in umbilical cord blood: Exposure to or infection with the virus?

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ABSTRACT

*Background:* We aimed to clarify whether presence of hepatitis B virus (HBV) markers in cord blood indicates exposure to or infection with HBV.
*Methods:* We prospectively recruited HBsAg-positive pregnant women and their neonates 2012 through 2015. All neonates received postnatal immunoprophylaxis. The infants were followed up at 7–14 months of age.
*Results:* Totally 329 HBsAg-positive pregnant women and 333 neonates were enrolled. No cord blood was anti-HBc IgM positive. A total of 290 (87.1%) neonates were followed up at 7–14 months of age and 6 (2.1%) of them were infected with HBV. Of 146 neonates born to HBeAg-negative mothers, 38 (26.0%) and 30 (20.5%) had detectable HBsAg and HBV DNA in cord blood respectively, but none of 126 infants followed up was infected. Of 187 neonates born to HBeAg-positive mothers, 92 (49.2%) and 79 (42.2%) had detectable HBsAg and HBV DNA in cord blood respectively; 6 (3.7%) of 164 infants followed up were infected. Of seven neonates with HBV DNA > $10^5$ IU/ml in cord blood, four had no infection and three others were infected.
*Conclusion:* Presence of HBsAg and/or HBV DNA, even at high levels, in cord blood just indicates exposure to, but not infection with HBV. Presence of HBV markers in cord blood cannot define intrauterine infection.


## 1. Introduction

Hepatitis B virus (HBV) infection remains a global health problem, with approximately 250 million individuals being chronically infected [1]. Mother-to-infant transmission (MTIT) is a major cause of chronic HBV infection in endemic countries. The failure of active-passive immunoprophylaxis mostly occurs in infants born to mothers with positive hepatitis B e antigen (HBeAg) or with high viral load (HBV DNA $\geq 10^6$ copies/ml, or $2 \times 10^5$ IU/ml). The transmission is considered to occur before (in utero), during, or after birth. It has been assumed that the main reason for hepatitis B immunoprophylaxis failure in infants born to HBV infected mothers is intrauterine infection [2]. Some scholars considered that the presence of hepatitis B surface antigen (HBsAg) and/or HBV DNA in umbilical cord blood or peripheral blood of newborn infants collected within several days after birth could define intrauterine infection, resulting in the rate of MTIT as high as 30–60% [3–6]. This rate is substantially different from the overall HBsAg positive rate of 0.8–3.7% in children born to HBV infected mothers after passive-active immunoprophylaxis [7–11]. Longitudinal observations showed that the presence of HBV markers in cord or peripheral blood samples could not predict MTIT [12,13].

Numerous reports showed that, after passive-active immunoprophylaxis, almost no children born to HBeAg-negative carrier mothers were infected with HBV, while the chronic infection still occurred in 4–12% children born to HBeAg-positive mothers [7–11]. More recent studies demonstrated that the chronic HBV infection rate was nearly zero in infants born to HBeAg-

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positive or highly viremic (HBV DNA $\geq 10^6$ copies/ml) mothers who received antiviral therapy during late pregnancy [14–16]. As postnatal immunoprophylaxis cannot prevent intrauterine infection, abovementioned nearly zero infection in infants had us to question whether intrauterine HBV infection really exists. In the present study, we aimed to clarify whether the presence of HBsAg and/or HBV DNA in cord blood could define intrauterine HBV infection. Additionally, since maternal IgM cannot transfer into fetus and the presence of pathogen-specific IgM in cord blood may indicate intrauterine infection, such as cytomegalovirus [17], we tested IgM antibody against hepatitis B core antigen (anti-HBc IgM) in cord blood to add more evidence to elucidate whether intrauterine infection is frequent or rare.

## 2. Subjects and methods

### 2.1. Subjects and sample collection

A prospective cohort study was conducted April 2012 through March 2015. The pregnant women attending Nanjing Drum Tower Hospital, Taixing People's Hospital, and Zhenjiang Fourth People's Hospital received the routine prenatal examinations, including hepatitis B serologic markers. The women with positive HBsAg were invited to participate in this study. The pregnant women were excluded if they were ongoing or had history of antiviral therapy, or were co-infected with hepatitis C virus or human immunodeficiency virus, or had other immunocompromised diseases. During April 2012 through March 2013, we invited all eligible HBsAg positive pregnant women to participate in this study. Considering that MTIT of HBV scarcely occurs in infants of HBeAg-negative mothers but may still occur in infants of HBeAg-positive mothers after the passive-active immunoprophylaxis [7–9], we intentionally invited pregnant women positive for both HBsAg and HBeAg from April 2013 to March 2015.

All newborn infants received hepatitis B immunoglobulin (HBIG) (100 IU) and birth dose of hepatitis B vaccine (10 μg recombinant yeast HBsAg) as soon as possible after birth, preferably <24 h after birth. The infant received two additional doses of hepatitis B vaccine at the age of 1 and 6 months respectively.

Blood samples of the pregnant women were collected just before or during delivery. The cord blood was collected by direct umbilical vein venipuncture after the umbilical cord was thoroughly rinsed with sterilized normal saline solution three times to avoid maternal blood contamination. In the follow-up, the peripheral blood was collected from infants at the age of 7–14 months. Each serum was separated and aliquoted; one aliquot was used to measure alanine transaminase (ALT) level in each hospital and other aliquots were stored at −30 °C and transported in the ice box to Nanjing Drum Tower Hospital for detection of HBV markers.

This study was approved by the institutional review board of each hospital. All methods were performed in accordance with the protocols set up based on the relevant guidelines and regulations. Written informed consent was obtained from each pregnant woman; the infant's consent was assigned by his/her mother.

### 2.2. Detection of HBV markers

Hepatitis B serologic markers, including HBsAg, total anti-HBc, HBeAg and anti-HBs were qualitatively tested by enzyme-linked immunosorbent assay (ELISA) kits (Kehua Biotech, Shanghai, China). The anti-HBc IgM was examined with ELISA reagents (Yingkexinchuang Biotech, Xiamen, China), in which anti-human IgM (μ chain specific) is used to coat microplates. HBsAg, HBeAg, and anti-HBs were further quantitatively measured by microparticle enzyme immunoassay (ARCHITECT, Abbott, USA). Based on the manufacturer's instructions, the sample was considered to be HBsAg positive when the level was ≥0.05 IU/ml, and HBeAg positive when the sample/cut off (S/CO) was ≥1.0. When HBsAg level was beyond the upper detection limit (250 IU/ml), the sera were retested by 100–1000-fold dilution.

HBV DNA levels were detected by fluorescent quantitative polymerase chain reaction (PCR) (Shenyou Biotech, Shanghai, China), with the lower detection limit of 100 IU/ml.

### 2.3. Assessment of MTIT of HBV

Infants were considered to be infected with HBV when they were HBsAg positive at the age of 7–14 months. HBV S sequences were amplified by PCR, and determined by directly sequencing on an ABI Prism 3130 sequencer (Applied Biosystems, Hitachi, Tokyo, Japan) as described previously [18,19]. Genotype analysis was performed on the obtained sequences and compared with S sequences of different HBV genotypes from the NCBI website (URL: http://www.ncbi.nlm.nih.gov/projects/genotyping/view.cgi?db=2). MTIT of HBV was confirmed by homology comparison of the S sequences between the infants and their mothers.

Since maternal anti-HBc in infants may persist up to 24 months, we defined anti-HBc status in infants based on comparison of antibody titers between umbilical cord blood and follow-up samples. When anti-HBc titer in infants at follow-up was >4-fold lower than that in cord blood, anti-HBc was considered to be derived from mothers. Otherwise, anti-HBc was considered to be actively produced after infection.

### 2.4. Statistical analysis

Statistical analysis was performed using software SPSS 11.0 (SPPS Inc., Chicago, IL, USA). The levels of HBsAg, HBeAg, and HBV DNA were expressed by logarithm of measured values. Continuous variables normally distributed were expressed as mean ± standard deviation. Quantitative data non-normally distributed were presented as median and range (min–max), and nonparametric tests were used for statistical calculations. Enumeration data were described by rate or proportion and compared by Chi-square tests. $P < 0.05$ was considered statistically significant.

## 3. Results

### 3.1. Participant characteristics

Totally 426 women were invited for the study and 358 (84.0%) of them signed the consent forms. Of them, 29 (8.1%) pregnant women did not deliver their neonates in our hospitals. Finally 329 HBsAg-positive pregnant women and their 333 newborn infants (including four twin pairs) were enrolled (Fig. 1). The mean age of these women was 26.1 ± 3.6 years. Of them, 145 (44.1%) were HBeAg-negative and 184 (55.9%) were HBeAg-positive. Of total 333 neonates, 290 (87.1%) were followed up at the age of 10.5 ± 3.4 months (7–14 months), including 164 of 187 (87.7%) infants (three twin pairs) of 184 HBeAg-positive mothers and 126 of 146 (86.3%) infants (one twin pair) of 145 HBeAg-negative mothers (Fig. 1).

### 3.2. HBV markers in newborn infants of HBeAg-negative and −positive mothers

Table 1 shows the detectable proportions of HBsAg (≥0.05 IU/ml) and HBV DNA (≥2 $\log_{10}$ IU/ml) in cord blood samples of neonates born to HBeAg-negative and −positive mothers respectively. The detectable proportions of HBsAg, HBV DNA, or both in neonates born to HBeAg-positive mothers were much

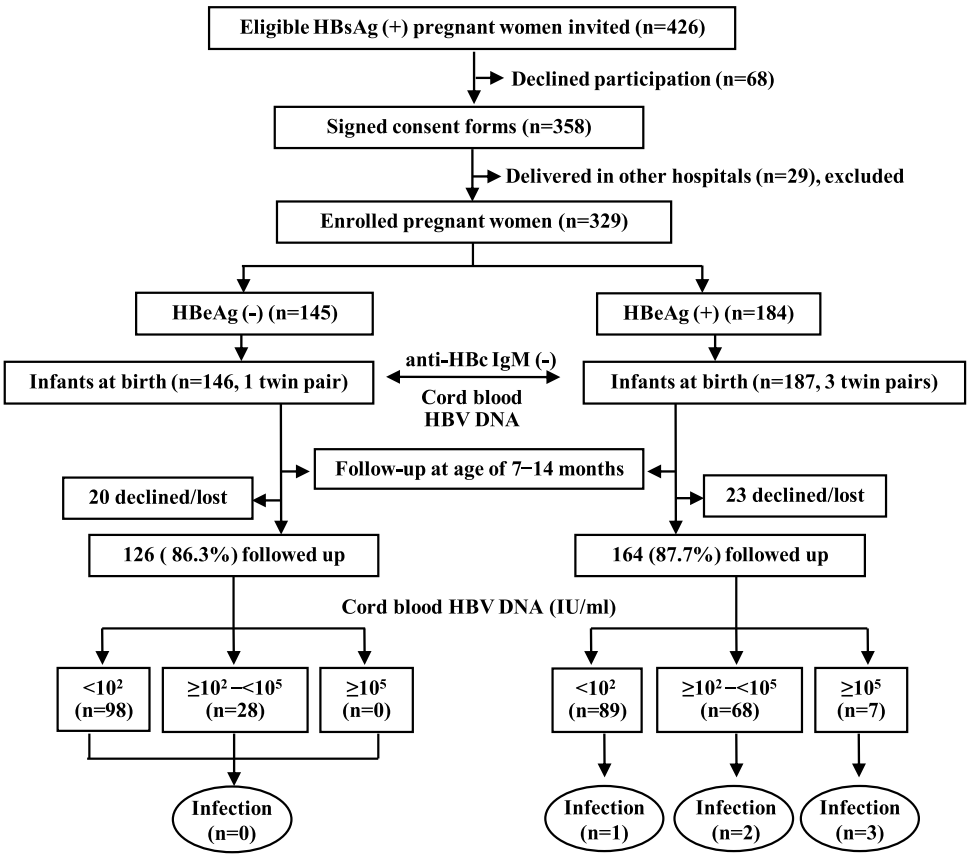


**Fig. 1.** Flow diagram of participants. HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; HBeAg, hepatitis B e antigen; anti-HBc IgM, IgM antibody against hepatitis B core antigen.

**Table 1**
Detectable proportion of HBV markers in 333 cord sera of neonates born to HBeAg-negative or -positive carrier mothers.

| Cord blood (reference value) | Neonate born to HBeAg(−) mother, n = 146 (%) | Neonate born to HBeAg(+) mother, n = 187 pairs (%) | $\chi^2$ | P |
|---|---|---|---|---|
| HBsAg(+) (≥0.05 IU/ml) | 38 (26.0) | 92 (49.2) | 18.496 | <0.01 |
| HBeAg(+) (≥1.0 S/CO) | Not applicable | 170 (90.9) | – | – |
| HBV DNA (≥2 $\log_{10}$ IU/ml) | 30 (20.5) | 79 (42.2) | 17.531 | <0.01 |
| HBsAg(+) & HBV DNA(+) | 7 (4.8) | 54 (28.9) | 31.779 | <0.01 |

HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; HBeAg, hepatitis B e antigen.

higher than those in neonates born to HBeAg-negative mothers (all $P < 0.01$). The detectable proportion of HBeAg (≥1.0 S/CO) in cord blood of neonates of HBeAg-positive mothers was as high as 90.9%. Overall, the levels of HBV markers in neonatal cord blood were significantly lower than that in maternal blood samples, except for the HBV DNA levels in neonates of HBeAg-negative mothers (Table 2). Additionally, total anti-HBc was positive in all 333 neonates (four twin pairs) of 329 mothers, but none of the neonates was anti-HBc IgM positive in the cord blood.

### 3.3. HBV markers in infants at 7–14 months of age

Of the 290 infants who were followed up for HBV markers at 7–14 months of age, 6 (2.1%) were positive for HBsAg, HBeAg, anti-HBc, and HBV DNA (Fig. 1). All these 6 infected infants were among the 164 infants born to HBeAg-positive mothers, with the MTIT rate of 3.7%, while none (0%) of the 126 infants born to HBeAg-negative mothers was infected (Fisher's exact, $P = 0.038$). Thus, we compared the main relevant maternal and neonatal parameters between transmission and non-transmission just in infants born to

**Table 2**
Quantification of HBV markers in the neonatal cord and maternal sera.

| Items | Median level in cord serum | Median level in maternal serum just before or at delivery | $Z$ | P |
|---|---|---|---|---|
| HBeAg(−) mother | | | | |
| HBsAg(+) ($\log_{10}$ IU/ml), n = 38 | 0.21 (−1.22– 4.24) | 3.44 (0.30–5.13) | −5.272 | <0.01 |
| HBV DNA ≥ 2 ($\log_{10}$ IU/ml), n = 30 | 2.33 (2.00–3.74) | 2.59 (2.00–5.59) | −1.728 | 0.084 |
| HBeAg(+) mother | | | | |
| HBsAg(+) ($\log_{10}$ IU/ml), n = 92 | −0.66 (−1.22–4.27) | 4.48 (3.12–5.02) | −8.329 | <0.01 |
| HBeAg(+) (S/CO), n = 170 | 1.58 (0.04–3.05) | 3.05 (0.30–3.19) | −11.029 | <0.01 |
| HBV DNA ≥ 2 ($\log_{10}$ IU/ml), n = 79 | 2.52 (2.01–7.74) | 7.91 (2.00–9.48) | −7.573 | <0.01 |

Reference value: HBsAg(+) ≥0.05 IU/ml, HBeAg(+) ≥1.0 S/CO. HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; HBeAg, hepatitis B e antigen.

**Table 3**
Comparison of HBeAg-positive mothers and their neonates between transmission and non-transmission.

| Variables | Transmission | Non-transmission | Statistical analysis | P |
|---|---|---|---|---|
| Mother | n = 5 | n = 156 | | |
| Delivery age (years) | 26.6 ± 2.7 | 26.2 ± 3.3 | $t = 0.268$ | 0.789 |
| Twin (%) | 1 (20) | 2 (1.3) | – | 0.091 |
| HBV DNA ($\log_{10}$ IU/ml) | 7.43 ± 0.45 | 7.32 ± 1.60 | $T = 0.153$ | 0.879 |
| HBsAg ($\log_{10}$ IU/ml) | 4.30 ± 0.23 | 4.34 ± 0.45 | $T = 0.197$ | 0.844 |
| HBeAg(+) ($\log_{10}$ S/CO) | 2.94 ± 0.21 | 2.85 ± 0.63 | $T = 0.318$ | 0.751 |
| Neonate | n = 6 | n = 158 | | |
| Male (%) | 2 (33.3) | 94 (59.5) | $\chi^2 = 0.730$ | 0.393 |
| Cord blood | | | | |
| HBV DNA ≥ 2 $\log_{10}$ IU/ml (%) | 5 (83.3) | 70 (44.3) | $\chi^2 = 2.150$ | 0.143 |
| HBsAg(+) (%) | 4 (66.7) | 82 (51.9) | $\chi^2 = 0.087$ | 0.768 |
| Both (%) | 4 (66.7) | 46 (29.1) | $\chi^2 = 2.279$ | 0.131 |
| HBeAg(+) | 6 (100) | 145 (91.8) | – | 1.000 |

Reference value: HBsAg(+) ≥0.05 IU/ml, HBeAg(+) ≥1.0 S/CO. HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; HBeAg, hepatitis B e antigen.

HBeAg-positive mothers (Table 3). The results showed that the proportions of detectable HBV DNA or HBsAg or both in cord blood of infected infants were higher than those in cord blood of uninfected infants, yet the difference had no statistical significance.

Of 284 infants who were negative HBsAg at the follow-up, 281 (98.9%) had anti-HBs higher than 10 mIU/ml, and three others had 4.8, 8.2, and 9.45 mIU/ml anti-HBs respectively. None of 126 infants born to HBeAg-negative mothers was positive for anti-HBc, and 7 (4.4%) of 158 infants born to HBeAg-positive mothers were positive for both anti-HBs and anti-HBc, indicating the resolved transient infection. HBV DNA was not detected in these seven anti-HBc positive infants.

### *3.4. Outcomes of neonates with high titers of HBV markers in cord blood and characteristics of HBV infected infants*

To clarify whether high HBV DNA levels in cord blood may indicate the intrauterine infection, we paid particular attention to the seven neonates who had high titers of HBV DNA (≥5 $\log_{10}$ IU/ml) and HBsAg and HBeAg in their cord blood (infants 1–7 in Table 4). They were followed up at 8–13.5 months of age; three infants were infected, whereas four others were not infected (Table 4). In addition to three infected infants mentioned above, we also detected three other infants (infants 8–10) who were infected with HBV (Table 4). None of them was positive for anti-HBc IgM in cord blood. All these six infants, but one (infant 10), had normal ALT and were negative for anti-HBc IgM at follow-up. Infant 10 was conceived by in vitro fertilization. He was negative for HBsAg and HBV DNA in cord blood, but was positive for HBsAg, HBeAg, HBV DNA, and anti-HBc IgM and had ALT level of 231.7 U/L at seven months of age, demonstrating postnatal infection.

Sequencing data showed the identity of S gene between each mother-infant pair (Fig. 2), confirming the MTIT. None of the six infected infants had mutations in the *a* determinant of HBV S gene.

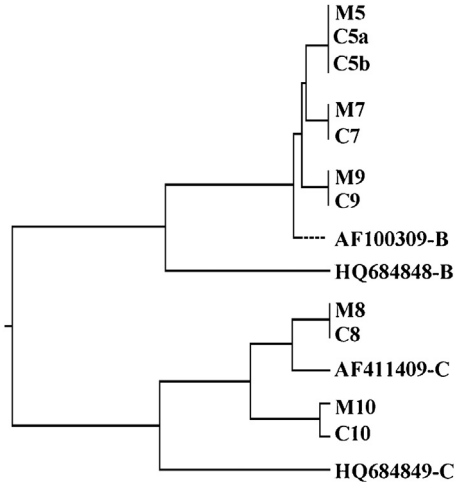


**Fig. 2.** Phylogenetic analyses of HBV S gene in HBV-infected child/mother pairs. The tree was constructed based on the S gene sequences from six HBV-infected children and their mothers. Reference sequences from GenBank included genotype B: AF100309 and HQ684848, and genotype C: AF411409 and HQ684849. HBV, hepatitis B virus; M, mother; C, child.

## 4. Discussion

In the present study, we found that none of the infants born to HBV carrier mothers with negative HBeAg was infected with HBV and 3.7% infants of HBeAg-positive mothers were infected, demonstrating the high protective efficacy of combined use of HBIG and hepatitis B vaccine. Also, an absolute majority (98 of 103, 95.1%) of infants, positive for HBV DNA and/or HBsAg in cord blood, were not infected with HBV, indicating that the presence of HBV markers in cord blood just means exposure to, but not infection with HBV. The present study reinforces that it is almost impossible to diagnose intrauterine HBV infection based on detection of HBV markers in cord blood.

The presence of HBsAg and HBeAg in cord blood (Table 1) is predictable, because maternal HBsAg and HBeAg may pass through placenta [20,21]. The detection of HBV DNA in cord blood of 103 neonates (Fig. 1) demonstrated the presence of HBV in these neonates; however, absolute majority (95.1%) of them did not become chronically infected (Fig. 1), indicating that the virions were derived from their mothers during birth, but not resulted from the intrauterine infection. Earlier studies also showed that the presence of HBsAg in cord blood was not the marker of neonatal infection [22]. However, many scholars still considered that the presence of HBsAg and/or HBV DNA in cord blood was the evidence of infection, leading to the transmission rate as high as 30–60% [3–6]. The exaggerated transmission rate may cause parents' overwhelming fears of babies being infected with HBV, and may have both physicians and parents to take unreasonable strategies to prevent the transmission [23], such as maternal HBIG administration in late pregnancy, elective cesarean section, and giving up breast feeding, all of which have been proved to be futile [24–26].

Interestingly, of seven neonates with high HBV DNA levels (>$10^5$ IU/ml) and high titers of HBsAg and HBeAg in cord blood, three became chronically infected, but four others were not infected (Table 4). Undoubtedly, the virus in the four uninfected infants was derived from their mothers but the immunoprophylaxis blocked the infection. It is possible that the virus inside the three neonates who became chronically infected was also derived

**Table 4**
Serum testing results in infants with HBV DNA ≥ 5 log IU/ml or HBV infection and their mothers.

| No. | Mother (just before or at delivery) | | | | Neonate (cord blood) | | | Immunoprophylaxis after birth[a] | | | | Infant at 7–14 months old | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | HBV genotype | HBV DNA (log IU/ml) | HBsAg (log IU/ml) | HBeAg (log S/CO) | HBV DNA (log IU/ml) | HBsAg (log IU/ml) | HBeAg (log S/CO) | HBIG (min) | Hepatitis B vaccine | | | HBV genotype | HBV DNA (log IU/ml) | HBsAg | HBeAg | Total anti-HBc | anti-HBc IgM | anti-HBs (mIU/ml) |
| | | | | | | | | | 1st (min) | 2nd (m) | 3rd (m) | | | | | | | |
| 1 | C | 8.14 | 4.64 | 3.02 | 7.74 | 4.27 | 2.69 | 30 | 10 | 1 | 6 | n/a | (−) | (−) | (−) | (−) | n/a | 35 |
| 2 | B | 6.84 | 4.24 | 3.09 | 7.21 | 4.22 | 3.05 | 20 | 20 | 1 | 6 | n/a | (−) | (−) | (−) | (−) | n/a | >1000 |
| 3 | C | 7.61 | 4.29 | 2.93 | 7.00 | 2.73 | 2.10 | 10 | 10 | 1 | 6 | n/a | (−) | (−) | (−) | (−) | n/a | >1000 |
| 4 | B | 7.69 | 4.53 | 3.02 | 5.01 | 2.19 | 1.51 | 120 | 2 | 1 | 6 | n/a | (−) | (−) | (−) | (−) | n/a | >1000 |
| 5[b] | B | 7.64 | 4.48 | 3.11 | 7.69 | 3.78 | 2.51 | 30 | 10 | 1 | 6 | B | 9.18 | (+) | (+) | (+) | (−) | (−) |
| 5[c] | | | | | 7.42 | 3.70 | 2.49 | 30 | 10 | 1 | 6 | B | 8.94 | (+) | (+) | (+) | (−) | (−) |
| 7 | B | 6.98 | 4.52 | 3.11 | 5.25 | 1.72 | 2.11 | 40 | 40 | 1 | 6.5 | B | 8.18 | (+) | (+) | (+) | (−) | (−) |
| 8 | C | 6.94 | 3.93 | 2.78 | 3.96 | 0.30 | 1.92 | 290 | 290 | 1 | 6 | C | 8.12 | (+) | (+) | (+) | (−) | (−) |
| 9 | B | 7.62 | 4.31 | 2.67 | 2.03 | (−) | 1.46 | 30 | 10 | 1 | 6 | B | 8.87 | (+) | (+) | (+) | (−) | (−) |
| 10 | C | 7.95 | 4.27 | 3.05 | (−) | (−) | 2.45 | 30 | 10 | 1 | 6 | C | 7.19 | (+) | (+) | (+) | (+) | (−) |

[a]The numbers in columns denote the time points (min, minute; m, month) of injecting HBIG or hepatitis B vaccine after birth. Infants 5[b] and 5[c] were twins. n/a, not applicable. Reference value: HBsAg ≥ 0.05 IU/ml, HBeAg ≥1.0 S/CO, HBV DNA ≥100 IU/ml.
HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; HBeAg, hepatitis B e antigen; anti-HBs, antibody against hepatitis B surface antigen; anti-HBc, antibody against hepatitis B core antigen; HBIG, hepatitis B immunoglobulin.

from mothers during birth, but the postnatal immunoprophylaxis did not prevent the infection. On the other hand, there appears to be a gradient of cord blood HBV DNA levels and neonatal infection risk, as 1 (1.1%) of 89 neonates with level $<10^2$ IU/ml, 2 (2.9%) of 68 with level $\geq 10^2$–$<10^5$, and 3 (42.9%) of 7 with level $\geq 10^5$ were infected at the follow-up (Fig. 1), indicating that exposure to higher levels of HBV during birth appears to increase the likelihood of becoming chronic infection.

The data in the present study, together with the documented literatures, may help clarify whether intrauterine HBV infection is frequent or rare. Since the administration of passive-active immunoprophylaxis against hepatitis B in neonates, MTIT of HBV has reduced from 10 to 30% to nearly 0% and from 70 to 90% to 4–12% in infants born to HBeAg-negative and −positive mothers respectively [2,7–11,27]. In the present study, none of 126 infants born to HBeAg-negative mothers and 6 (3.7%) of 164 infants born to HBeAg-positive mothers were infected, although 28 and 75 infants had detectable HBV DNA in their cord blood respectively (Fig. 1). Recent reports showed that, when HBIG and hepatitis B vaccine were administered immediately after birth, MTIT of HBV was reduced to as low as 1.28–2.0% in infants born to HBeAg-positive mothers [15,16]. Additionally, antiviral therapy from gestation 28–32 weeks, together with the postnatal immunoprophylaxis, almost completely blocked MTIT of HBV in pregnant women with high viral loads [28–30]. Had in utero infection of HBV occurred, either postnatal immunoprophylaxis in neonates or combined with antiviral therapy in mothers would not have completely blocked MTIT of HBV. Therefore, these results indicate that intrauterine HBV infection is extremely rare.

The main limitation in this study is that the infants were followed up only one time (at 7–14 months of age), making it impossible to observe the dynamic changes of HBV DNA levels and to detect anti-HBc IgM in a timely manner, which would be helpful to determine the infection time (in utero, intrapartum, or postpartum). Anyhow, a definite intrauterine infection should be based on the detection of HBV DNA in the liver of fetuses, which is practically infeasible. Another limitation is that the number of HBV infected infants was small. Thus, a much larger group of mother-infant pairs are required in the future study.

In conclusion, the present study demonstrates that the presence of HBV DNA and/or viral antigens, even at high levels, in cord blood just indicates having exposed to, but not being infected with HBV. The findings that total absence of anti-HBc IgM in cord blood, no infection in infants of HBeAg-negative mothers and very low infection rate in infants of HBeAg-positive mothers suggest that intrauterine HBV infection is rare.

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## Ethics approval and consent to participate

The study, including the informed consent form, was approved by the institutional ethics review committee of Nanjing Drum Tower Hospital, Taixing People's Hospital, and Zhenjiang Fourth People's Hospital, respectively, and was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. Written informed consent was obtained from each participant woman.

## Conflict of interest

Non declared.

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