Original Article

Maternal viral load and hepatitis B virus mother-to-child transmission risk: A systematic review and meta-analysis

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Aim: The aim of this study was to assess the relationship between maternal viral load and mother-to-child transmission (MTCT) risk in hepatitis B envelope antigen (HBeAg)-positive mothers.

Methods: PubMed and Web of Science were systematically searched. We compared MTCT incidence between maternal hepatitis B virus (HBV)-DNA-positive and HBV-DNA-negative groups. We also examined the dose–response effect of this relationship.

Results: Twenty-one studies with 10,142 mother–child pairs were included in the studies. The mean MTCT incidence was 13.1% in the maternal HBV-DNA-positive group, compared with 4.2% in the negative group. The summary MTCT odds ratio of maternal HBV-DNA positive compared with negative was 9.895 (95% confidence interval [CI], 5.333 to 18.359; Z = 7.27, P < 0.00001) by random-effects model. In maternal HBV-DNA < 6 log10 copies/mL, 6–8 log10 copies/mL, and > 8 log10 copies/mL level stratifications, the pooled MTCT incidences were 2.754% (95% CI, 1.198–4.310%; Z = 3.47, P = 0.001), 9.932% (95% CI, 6.349–13.516%; Z = 5.43, P < 0.00001), and 14.445% (95% CI, 8.317–20.572%; Z = 4.62, P < 0.00001), respectively. A significant linear dose–response association was found between maternal viral load and MTCT risk, with the points estimate of increased MTCT risk 2.705 (95% CI, 1.808–4.047) at 6 log10 copies/mL compared with reference (3 log10 copies/mL), and 7.316 (95% CI, 3.268–16.378) at 9 log10 copies/mL. A significant non-linear dose–response association was also found between maternal viral load and HBV MTCT risk (model $\chi^2 = 23.43, P < 0.00001$).

Conclusion: Our meta-analysis indicated that maternal viral load was an important risk factor for MTCT in HBeAg-positive mothers, and maternal viral load was dose-dependent with HBV MTCT incidence.

Key words: hepatitis B virus, maternal viral load, mother-to-child transmission, risk factor

INTRODUCTION

HEPATITIS B VIRUS (HBV) infection is a major global health problem. According to World Health Organization estimates, 257 million people are chronically infected with hepatitis B. Hepatitis B virus infection not only causes acute or chronic hepatitis, but also leads to severe long-term, life-threatening complications, such as cirrhosis, liver failure, and hepatocellular carcinoma. More than 686,000 people die every year due to cirrhosis and liver cancer caused by HBV infection. Hepatitis B virus infection acquired by mother-to-child transmission (MTCT) is typically considered to be one of the major causes of chronic infection. According to a previous study, newborns have a 90% chance of becoming chronic carriers after infection with HBV; in children < 3 years old the chance is up to 50%. However, in adults the chance is only up to 5%. After appropriate postnatal hepatitis B immunoglobulin and a series of HBV vaccines, the MTCT rate is significantly reduced, but is still 5–10%. Therefore, effective prevention of MTCT of HBV from pregnant women to their newborns within the perinatal period is very important for interrupting chronic HBV infection.

Some studies have investigated the risk factors for MTCT in hepatitis B envelope antigen (HBeAg)-positive mothers.
Wong et al. found a linear association between duration of the first stage of labor and hepatitis B surface antigen (HBsAg) positivity in infants’ cord blood.5 Yang et al. reported elective cesarean section versus vaginal delivery could effectively reduce the rate of MTCT of HBV (elective cesarean section, 10.5%; vaginal delivery, 28.0%).6 Yi et al. reported amniocentesis carried out on HBsAg+ mothers significantly increased the frequency of MTCT, especially in mothers with HBV-DNA >7 log10 copies/mL.7 Some maternal and neonatal gene mutations or polymorphisms are related with MTCT, including Toll-like receptors 3 (c.1377C/T) and 9 (G2848A) gene polymorphisms,8 tumor necrosis factor-α 308G/A, and vitamin D receptor Apal and TaqI genotypes,9 HLA-DPA1 (rs3077) and HLA-DPB1 (rs9277535).10

Most importantly, HBV viral factors directly impact MTCT. Yin et al. reported that HBV pre-S/S gene mutations is a risk factor of vaccination failure and frequently causes HBV MTCT.11 Serum HBV-DNA level has been identified as the reliable marker of active HBV replication. Maternal HBV viral load is another important viral risk factor for MTCT. Many studies have shown increased risk of MTCT was related with higher maternal levels of HBV-DNA. Zhang et al. reported maternal-positive HBV-DNA was associated with higher MTCT incidence compared with negative, with odds ratio (OR) 11.362 (95% confidence interval [CI], 1.389–12.6%; P = 0.033).12 Wen et al. found that predictive MTCT incidence at maternal viral load levels of 7, 8, and 9 log10 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.13 Zou et al. reported that the incidence of immunophrophylaxis failure was 0%, 3.2% (3/95), 6.7% (19/282), and 7.6% (5/66) at mothers’ pre-delivery HBV-DNA levels of <6, 6–6.99, 7–7.99, and ≥8 log10 copies/mL, respectively (P < 0.001 for trend).14 However, these studies included small sample sizes. As far as we know, there is no systematic review that summarizes HBV load and the risk of MTCT.

In this study, we aim to: (i) compare the MTCT risk between HBV-DNA-positive mothers and HBV-DNA-negative mothers; (ii) summarize the MTCT incidence in different HBV-DNA level stratifications; and (iii) find the dose–response relationship between HBV-DNA level and MTCT risk. The PROSPERO CRD registration ID of this study is 42018087311.

METHODS

Search strategy

To identify eligible studies published before October 2017, we applied a systematic search strategy to PubMed and Web of Science databases. We used the following keywords and subject headings in combination to identify relevant articles in electronic databases: hepatitis B virus, HBV, HBV-DNA level, HBV load, mother–to-child transmission, mother–to-infant transmission, utero transmission, and vertical transmission. The search algorithm used in PubMed was: ("Hepatitis B virus" [MeSH Terms] OR hepatitis B virus [title] OR HBV [title]) AND (mother–to-child transmission [title] OR mother–to-infant transmission [title] OR intrauterine transmission [title] OR utero transmission [title] OR vertical transmission [title]). The search algorithm used in the Web of Science advanced search was: (TI = Hepatitis B virus OR TI = HBV) AND (TI = "mother–to-child transmission" OR TI = "intrauterine transmission" OR TI = "mother–to-infant transmission" OR TI = "utero transmission" OR TI = "vertical transmission"). We manually examined reference lists from retrieved articles and published reviews to identify additional manuscripts. The search was limited to English and Chinese articles.

Study eligibility

We defined studies as being eligible for inclusion in this analysis if they met the following criteria: (i) types of studies: observational studies (cohort studies and case–control studies were included); (ii) types of participants: HBsAg-positive mother and their children with documented HBV serology at birth and at least once between 6 months and 3 years of age; (iii) types of exposure: compared maternal HBV-DNA positive with negative, or compared different HBV-DNA level stratifications for the outcome; and (iv) types of outcome: studies included MTCT incidence. Mother-to-child transmission was defined by HBsAg or HBV-DNA positivity in the child. A mother with multiple births was recognized as a mother–child pair. We excluded publications when: (i) mother–child pairs were co-infected with hepatitis A, C, D, or E virus or HIV; (ii) the number of MTCT was <3; and (iii) the study did not provide crude numbers in contingency tables or maternal HBV-DNA level stratifications.

Study selection and data extraction

Study selection was initially carried out by review of titles and abstracts. When there was any possibility that it might be relevant, the full text of the article was downloaded and then reviewed for data retrieval. The following data were abstracted onto standardized forms: first author, publication year, country, study design, year of enrollment, number of mother–child pairs, maternal age at delivery, HBV-DNA detection method, definition of HBV-DNA positive, antiviral therapy, vaccine, infant follow-up, MTCT
incidence, crude numbers in contingency tables for HBV-DNA positive and MTCT risk, crude numbers of infant HBV infected and not infected in maternal HBV-DNA level stratifications, and the most fully adjusted ORs with 95% CIs for categories of HBV-DNA, or crude ORs. Data were extracted independently by two reviewers. Any differences of opinion were resolved by discussion and consensus reached by discussion with a third reviewer.

**Quality assessment**
The Newcastle–Ottawa Scale (NOS) was used to assess the quality of the included observational studies. The NOS contains eight items, categorized into three dimensions including Selection (4), Comparability (1), and Exposure (3). A high-quality study can be awarded a maximum of one star for each numbered item within the Selection and Exposure categories. A maximum of two stars can be given for Comparability. The NOS ranges from zero up to nine stars. Two reviewers independently assessed the quality of studies. Disagreements were resolved by a third reviewer.

**Statistical analyses**
First, we compared the MTCT incidence between HBV-DNA-positive mothers and -negative mothers. The meta-analysis model was chosen by heterogeneity between included studies. Heterogeneity was explored by the χ²-test and inconsistency (I²) statistics; P < 0.05 for the χ²-test or an I² value ≥25% represented heterogeneity. In the absence of significant heterogeneity, studies were pooled using a fixed-effect model. If heterogeneity was observed, a random-effects model was used. Overall effects of OR were determined using the Z test. Publication bias was evaluated using funnel graphs, Egger’s regression test, and Begg’s adjusted rank correlation test. We undertook subgroup meta-analyses by country (China and non-China), definition of HBV-DNA positive (>3 log₁₀ copies/mL and not >3 log₁₀ copies/mL), and MTCT diagnosis time.

![Flow diagram showing selection of studies assessing maternal viral load and hepatitis B virus (HBV) mother-to-child transmission (MTCT) risk](image_url)
Table 1 Characteristics of the included studies assessing maternal hepatitis B virus (HBV)-DNA and mother-to-child transmission (MTCT) risk

| First author, year | Country | Study design | Year of enrollment | Mother-infant pairs, n | Mother age, years | HBV-DNA detection method | HBV-DNA positive definition |
|-------------------|---------|--------------|--------------------|-----------------------|------------------|-------------------------|---------------------------|
| Zhang, 2016       | China   | Prospective cohort | 2008–2012          | 385                  | NR               | PCR-fluorescence         | ≥500 copies/mL          |
| Wen, 2016         | China   | Case-control  | 2011–2013          | 344                  | 27.1 ± 4.3       | PCR-fluorescence         | >3 log_{10} copies/mL   |
| Xu, 2015          | China   | Case-control  | 2008–2012          | 312                  | 26.8 ± 2.5       | PCR                     | >3 log_{10} copies/mL   |
| Lee, 2015         | Singapore | Prospective cohort | 2009–2013          | 154                  | 32.4 ± 4.7       | PCR                     | >2 × 10^5 IU/mL         |
| Zhang, 2012       | China   | Prospective cohort | 2004–2005          | 176                  | 28.5 ± 4.0       | PCR-fluorescence         | >3 log_{10} copies/mL   |
| Yi, 2014          | China   | Case-control  | 2008–2012          | 261                  | 29.4 ± 5.1       | PCR                     | ≥500 copies/mL          |
| Lu, 2014          | China   | NR            | 2010–2013          | 140                  | 21–40            | PCR                     | >10^6 IU/mL             |
| Yin, 2013         | China   | Prospective cohort | 2006–2010          | 1355                 | 28.83 ± 3.92     | PCR-fluorescence         | >3 log_{10} copies/mL   |
| Yin, 2013         | China   | NR            | 2006–2010          | 1360                 | 28.83 ± 3.92     | PCR                     | >3 log_{10} copies/mL   |
| Wen, 2013         | Taiwan  | Prospective cohort | 2007–2011          | 303                  | 33.1 ± 4.4       | PCR                     | >4 log_{10} copies/mL   |
| Pan, 2013         | China   | Retrospective | 2007–2011          | 1409                 | 27.2 ± 4.4       | PCR-fluorescence         | ≥500 copies/mL          |
| Guo, 2013         | China   | NR            | 2003–2009          | 1133                 | 26.6 ± 4.6       | PCR-fluorescence         | >3 log_{10} copies/mL   |
| Zou, 2012         | China   | Retrospective | 2007–2010          | 869                  | 27.8 ± 4.1       | PCR                     | ≥500 copies/mL          |
| Zhang, 2012       | China   | NR            | 2010–2011          | 46                   | NR               | PCR                     | >3 log_{10} copies/mL   |
| Li, 2012          | China   | NR            | 2008–2010          | 221                  | 27.31 ± 3.74     | PCR                     | >3 log_{10} copies/mL   |
| Singh, 2012       | Canada  | NR            | 2002–2007          | 64                   | 26.5–33.5        | PCR                     | >20 IU/mL               |
| Song, 2007        | Korea   | NR            | 1997–2002          | 144                  | 31.4 ± 4.0       | PCR                     | NR                      |
| Sha, 2007         | China   | Prospective   | 2002–2004          | 214                  | NR               | PCR                     | NR                      |
| Yin, 2006         | China   | NR            | 2003–2004          | 230                  | NR               | PCR                     | >3 log_{10} copies/mL   |

†In China, the immunoprophylaxis program is hepatitis B (HB) vaccine at 0, 1, and 6 months and HB immunoglobulin at 0, 3, and 6 months. NOS, Newcastle–Ottawa Scale; NR, not reported; PCR, polymerase chain reaction.

(24 h, 6–12 months, and >1 year). We also conducted meta-regression by MTCT incidence and publication year. Sensitivity analysis was carried out by only included prospective studies.

Second, we pooled the MTCT incidence in the maternal HBV-DNA level stratifications (<6 log_{10} copies/mL, 6–8 log_{10} copies/mL, and >8 log_{10} copies/mL) assuming random effects and binomial rate. Two-sided 95% CIs for the incidences with the Freeman–Tukey double arcsine variance-stabilizing transformation are also provided. The 95% CI excluding zero indicates a statistically significant overall result.

Finally, we examined the dose–response relationship of maternal HBV-DNA level and MTCT risk. The dose–response meta-analysis was carried out using the method proposed by Greenland and Longnecker and Orsini et al. We carried out linear dose–response analyses from the natural logarithm of the ORs across categories of HBV-DNA. This analysis is performed on the basis of the data for categories of HBV-DNA levels on median dose, number of cases and participants, and the adjusted OR with its 95% CI (or substituted by crude OR). We also assessed a potential non-linear dose–response relationship between HBV-DNA and MTCT risk using restricted cubic splines with three knots at 10%, 50%, and 90% of the distribution. A likelihood ratio test was used to assess the difference between the non-linear and linear models to test for non-linearity.

All statistical analyses were undertaken with Stata software, version 13.0 (Stata, College Station, TX, USA). Two-sided P < 0.050 was considered statistically significant. The following Stata commands were used: metan (HBV-DNA positive vs. negative, sensitivity analysis, and pooled MTCT incidence), metafunnel (funnel plot), metabias (Egger’s test and Begg’s test), metareg (meta-regression analyses), and glst (dose-response analyses).

RESULTS

Characteristics of included studies

We identified 346 unique papers after searching PubMed and Web of Science. Of these, 21 publications with 10 142 mother–child pairs were eligible for the evaluation of the relationship of maternal viral load and HBV MTCT risk. Figure 1 shows the stages in identifying studies for inclusion in the review. Most of the studies were published in the last decade and were carried out in the China. All identified studies followed an observational design, with six prospective cohorts and
Table 1 (Continued)

| First author, year | Antiviral therapy | Vaccination† | Time of MTCT diagnosis, months | MTCT incidence, % | Maternal HBV-DNA (+) | Maternal HBV-DNA (−) |
|--------------------|-------------------|--------------|--------------------------------|------------------|-----------------------|----------------------|
| Zhang, 2016        | NR                | NR           | 8–12                           | 4.4              | 17/204                | 0/164                |
| Wen, 2016          | NR                | NR           | 24 h                           | 12.2             | 27/143                | 15/159               |
| Xu, 2015           | NR                | Yes          | 24 h                           | 45.5             | 73/64                 | 69/106               |
| Lee, 2015          | NR                | Yes          | 6                              | 2.6              | 2/31                  | 2/119                |
| Zhang, 2014        | NR                | Yes          | 6                              | 5.1              | 8/59                  | 1/108                |
| Yi, 2014           | NR                | Yes          | 7–12                           | 3.4              | 9/132                 | 0/120                |
| Lu, 2014           | NR                | NR           | 1–3 year                       | 24.3             | 31/10                 | 3/96                 |
| Yin, 2013          | NR                | Yes          | 12                             | 10.7             | 121/549               | 24/661               |
| Yin, 2013          | NR                | Yes          | 12                             | 1.54             | 21/651                | 0/688                |
| Wen, 2013          | NR                | Yes          | 6                              | 3.3              | 10/99                 | 0/194                |
| Pan, 2013          | NR                | Yes          | 24 h                           | 2.8              | 40/828                | 0/541                |
| Guo, 2013          | NR                | Yes          | 24 h                           | 8.9              | 64/454                | 32/496               |
| Zou, 2012          | NR                | NR           | 7–12                           | 3.1              | 22/5                  | 47/795               |
| Zhang, 2012        | NR                | NR           | 24 h                           | 45.7             | 16/5                  | 6/19                 |
| Li, 2012           | NR                | Yes          | 24 h                           | 8.1              | 12/32                 | 6/171                |
| Singh, 2012        | NR                | Yes          | NR                             | 18.7             | 11/40                 | 1/12                 |
| Song, 2007         | NR                | NR           | 24 h                           | 11.8             | 17/47                 | 0/80                 |
| Shao, 2007         | NR                | NR           | 6                              | 4.7              | 7/33                  | 3/171                |
| Yin, 2006          | NR                | Yes          | 24 h                           | 9.6              | 18/101                | 4/107                |

Figure 2. Mother-to-child transmission (MTCT) incidence compared between the maternal hepatitis B virus (HBV)-DNA-positive group and the maternal HBV-DNA-negative group. The overall effects showed positive maternal HBV-DNA related with MTCT. [Color figure can be viewed at wileyonlinelibrary.com]
five case–control studies. All of the studies detected HBV-DNA by polymerase chain reaction, most of studies defined HBV-DNA positive as HBV-DNA $>3 \log_{10}$ copies/mL. The MTCT incidence ranged from 1.54% to 45.5%. The NOS for methodological quality assessment of the included studies scored at 7–8. A quality assessment scale showed the publications that met eligibility have acceptable quality to be included in our meta-analyses. Characteristics of the 21 articles included in the meta-analyses are shown in Table 1.

Incidence of MTCT compared between maternal HBV-DNA-positive and –negative groups

Nineteen publications\(^7,12,14,21–23,25,26,28–37\) with 9033 mother–child pairs met eligibility for this comparison. The mean MTCT incidence in the maternal HBV-DNA-positive group was 13.1% (range, 3.1–81.5%), compared with 4.2% (0.0–39.4%) in the maternal HBV-DNA-negative group. The MTCT incidence between the two groups is listed in Table 1.

There was significant heterogeneity between the included studies ($\chi^2_{(18)} = 106.61$, $P < 0.00001$; $I^2 = 81.3\%$). The summary MTCT OR of maternal HBV-DNA positive compared with maternal HBV-DNA negative was 9.895 (95% CI, 5.333–18.359; $Z = 7.27$, $P < 0.00001$) by random-effects model (Fig. 2).

The funnel plot showed asymmetry, which indicated evidence of publication bias (Fig. 3). Begg’s test ($z = -0.98$, $P = 0.327$) and Egger’s test ($t = -3.23$, $P = 0.005$) also suggested publication bias.

Subgroup meta-analysis by country showed the summary MTCT OR was 10.323 (95% CI, 5.315–20.052; $Z = 6.89$, $P < 0.00001$) in the China subgroup, and 7.387 (95% CI, 1.231–44.326; $Z = 2.19$, $P = 0.029$) in the non-China subgroup (Fig. 4a). Subgroup meta-analysis by HBV-DNA positive definition showed the summary MTCT OR was 4.891 (95% CI, 2.836–8.433; $Z = 5.70$, $P < 0.00001$) in the >3 log$_{10}$ copies/mL subgroup; in the not >3 log$_{10}$ copies/mL subgroup, the summary MTCT OR was 25.186 (95% CI, 9.411–67.404; $Z = 6.42$, $P < 0.00001$) (Fig. 4b). Subgroup meta-analysis by MTCT diagnosis time showed the summary MTCT OR was 4.292 (95% CI, 2.297–8.021; $Z = 4.57$, $P < 0.00001$) in the 24 h subgroup, 16.808 (95% CI, 6.708–42.118; $Z = 6.02$, $P < 0.00001$) in the 6–12 months subgroup, and 20.040 (95% CI, 0.673–59.149; $Z = 1.73$, $P = 0.083$) in the >1 year subgroup (Fig. 4c).

Meta-regression analyses showed that MTCT incidence ($t = -0.62$, $P = 0.543$) and year of publication ($t = -0.20$, $P = 0.847$) were not related with MTCT risk (Fig. 5).

A sensitivity analysis including only prospective studies showed the summary MTCT OR was 6.990 (95% CI, 4.666–10.472; $Z = 9.43$, $P < 0.00001$) (Fig. 6).

Incidence of MTCT in different maternal HBV-DNA level stratifications

Ten publications\(^{12,14,21,23,24,26,28,31,37}\) with 3258 mother–child pairs met eligibility for the MTCT incidence for $<6 \log_{10}$ copies/mL of maternal HBV viral load. The MTCT incidence ranged from 0.0% to 6.7%. The pooled MTCT incidence was 2.754% (95% CI, 1.198–4.310%; $Z = 3.47$, $P = 0.001$) by random-effects model (Fig. 7a).

Seven publications\(^{12,14,21,24,31,37}\) with 1019 mother–child pairs met eligibility for the MTCT incidence for 6–8 $\log_{10}$ copies/mL of maternal HBV viral load. The MTCT incidence ranged from 5.3% to 19.6%. The pooled MTCT incidence was 9.932% (95% CI, 6.349–13.516%; $Z = 5.43$, $P < 0.00001$) by random-effects model (Fig. 7b).

Seven publications\(^{12,14,21,24,31,37}\) with 232 mother–child pairs met eligibility for the MTCT incidence for $>8 \log_{10}$ copies/mL of maternal HBV viral load. The MTCT incidence ranged from 7.6% to 33.3%. The pooled MTCT incidence was 14.445% (95% CI, 8.317–20.572%; $Z = 4.62$, $P < 0.00001$) by random-effects model (Fig. 7c).

The MTCT incidence in different maternal HBV-DNA level stratifications are listed in Table 2.

Dose–response relationship between HBV-DNA and MTCT risk

Fourteen publications\(^{12–14,21–24,26–29,31,32,37}\) with 8851 mother–child pairs met eligibility for the evaluation of
Figure 4 Subgroup meta-analysis of studies assessing maternal viral load and hepatitis B virus (HBV) mother-to-child transmission (MTCT) risk by (a) country subgroup, (b) HBV-DNA positive definition subgroup, and (c) time of MTCT diagnosis after birth. CI, confidence interval; OR, odds ratio. [Color figure can be viewed at wileyonlinelibrary.com]
the dose–response relationship of maternal viral load and HBV MTCT risk. Linear dose–response showed significant and evolutionary risk of HBV MTCT risk along with maternal viral load increasing, with OR 1.393 (95% CI, 1.218–1.594) for each log_{10} copy/mL increase. Several representative points value enhanced the association: the point estimate of maternal viral load at 6 log_{10} copies/mL had an increased OR of MTCT 2.705 (95% CI, 1.808–4.047) compared with reference (3 log_{10} copies/mL), whereas maternal viral load at 9 log_{10} copies/mL had an increased OR of MTCT 7.316 (95% CI, 3.268–16.378) compared with the reference. A significant non-linear dose–response association was also found between maternal viral load and HBV MTCT risk (model χ^2 = 23.43, P < 0.00001). Linear dose–response and non-linear dose–response are listed in Figure 8.

**DISCUSSION**

**In our meta-analysis,** we found the mean MTCT incidence was higher in the maternal HBV-DNA-positive group compared with the -negative group (13.1% vs. 4.2%), with the pooled OR 9.895 (95% CI, 5.333–18.359; Z = 7.27, P < 0.00001). A sensitivity analysis that included only prospective studies showed the results were robust. In the linear dose–response model, the MTCT risk increased along with maternal viral load, with OR 1.393 (95% CI, 1.218–1.594) for each log_{10} copy/mL increase. Yin et al. reported the adjusted OR of MTCT increased by 1.57 (95% CI, 1.12–2.21) for each log_{10} IU/mL increase, adjusted by pregnancy-induced hypertension syndrome, HBeAg, HBeAb, and other risk factors by the logistic model. Liu et al. reported the adjusted OR of MTCT increased by 1.57 (95% CI, 1.12–2.21) for each log_{10} IU/mL increase, adjusted by the delivery mode, also by logistic regression. These two adjusted ORs were similar to our pooled ORs from the linear dose–response model. The placenta represents an efficient barrier for HBeAg transfer and that the HBeAg does not tolerize cytotoxic T cells. However, when the HBV load is increased, HBsAg is expressed in cells of the ovarian follicle or placental capillary endothelium, and HBsAg can pass through the placenta by cellular transfer, possibly contributing to MTCT. Our non-linear dose–response model showed that the MTCT
risk increased rapidly when HBV-DNA was $< 7 \log_{10}$ copy/mL, but MTCT was maintained at a higher risk when HBV-DNA was $\geq 7 \log_{10}$ copy/mL (Fig. 8).

Lowering the level of maternal HBV-DNA during pregnancy is essential for the prevention of MTCT in mothers with high viremia. The European Association for the Study of the Liver and the Asian Pacific Association for the Study of the Liver suggest that, in addition to neonatal immunoprophylaxis, treatment with antiviral agents such as tenofovir or telbivudine during pregnancy, beginning at 28–32 weeks of gestation, may be safe and effective in preventing MTCT. However, there is still no consensus on the optimal cut-off value of maternal viral load for antiviral treatment. Some randomized controlled trials use different cut-off values for antiviral treatment, which included 6 log_{10} copies/mL, 7 log_{10} copies/mL, and 1000 MEq/mL (approximately 9 log_{10} copies/mL). However, most studies regarded viral load of HBV-DNA >6 log_{10} copies/mL (approximately 5.3 log_{10} IU/mL) as the main risk of MTCT. Yi et al. recommended HBV-DNA >6 log_{10} copies/mL as the cut-off for antiviral therapy in pregnant women. In our included studies, some reported no MTCT in the <6 log_{10} copies/mL stratified level. However, our meta-analysis showed in the maternal HBV-DNA <6 log_{10} copies/mL stratified level that the pooled MTCT incidence was 2.754% (95% CI, 1.198–4.310%). The result indicated that newborns are still at risk when HBV-DNA <6 log_{10} copies/mL. Therefore, we think that antiviral treatment should be given when mothers are HBV-DNA-positive, rather than at >6 log_{10} copies/mL. However, this inference should be confirmed by other randomized controlled trials. Wakano et al. also reported that, if a first child

Figure 5 Meta-regression analysis of studies assessing maternal viral load and hepatitis B virus mother-to-child transmission (MTCT) risk by (a) MTCT incidence and (b) year of publication. Each circle represents a study, the size of the circle represents the sample size of the study, and the shading represents the 95% confidence interval. [Color figure can be viewed at wileyonlinelibrary.com]

![Figure 5](image_url)

Figure 6 Sensitivity analysis of studies assessing maternal viral load and hepatitis B virus mother-to-child transmission risk by only included prospective studies. CI, confidence interval; OR, odds ratio. [Color figure can be viewed at wileyonlinelibrary.com]

![Figure 6](image_url)
became an HBV carrier despite immunoprophylaxis, antiviral therapy is recommended in the second pregnancy. Our meta-analysis also found the pooled MTCT incidence was 9.932% (95% CI, 6.349–13.516%) in the $6 \log_{10}$ copies/mL stratified level, and 14.445% (95% CI, 8.317–20.572%) in the $>8 \log_{10}$ copies/mL stratified level. The results also showed the MTCT incidence increased along with maternal HBV viral load.

There are some limitations in this meta-analysis. First, some of the included studies were retrospective case-control studies. The most important drawback in case-control studies relates to the difficulty of obtaining reliable information about individuals’ exposure status over time. Case-control studies are therefore placed low in the hierarchy of evidence. Second, only a few studies reported adjusted ORs of stratified HBV-DNA levels. We used crude

Figure 7 Mother-to-child transmission (MTCT) incidence in different maternal hepatitis B virus (HBV)-DNA level stratifications in studies assessing maternal viral load and HBV MTCT risk: (a) $<6 \log_{10}$ copies/mL; (b) $6-8 \log_{10}$ copies/mL; (c) $>8 \log_{10}$ copies/mL. [Color figure can be viewed at wileyonlinelibrary.com]
Table 2  Characteristics of the included studies for dose–response meta-analysis for maternal hepatitis B virus (HBV)-DNA and mother-to-child transmission risk

| Study ID | HBV-DNA category, log10 copies/mL | Infant HBV infected (+) | Infant HBV infected (−) | Infection rate, % | Odds ratio (95% CI) |
|----------|----------------------------------|-------------------------|------------------------|------------------|-------------------|
| Zhang, 2016 | <2 | 0 | 164 | 0.0 | NA |
| | 2–5.99 | 0 | 53 | 0.0 | NA |
| | 6–6.99 | 6 | 56 | 10.7 | 1.007 (0.308–3.960) |
| | 7–7.99 | 8 | 70 | 11.4 | 1.120 (0.304–3.960) |
| | ≥8 | 3 | 25 | 12.0 | 1.120 (0.168–5.757) |
| Wen, 2016 | <3 | 15 | 159 | 8.6 | 1.000 (ref.) |
| | 3–5 | 8 | 50 | 13.8 | 2.827 (0.945–7.832) |
| | 5–7 | 3 | 11 | 21.4 | 2.891 (0.464–12.626) |
| | >7 | 16 | 82 | 16.3 | 2.068 (0.905–4.728) |
| Xu, 2015 | <3 | 17 | 158 | 9.7 | 1.000 (ref.) |
| | 3–6 | 9 | 32 | 22.0 | 2.61 (1.07–6.38) |
| | 6–9 | 35 | 61 | 36.5 | 5.33 (2.78–10.22) |
| Liu, 2015 | <6 | 0 | 127 | 0.0 | NA |
| | 6–6.99 | 3 | 42 | 7.1 | 1.000 (ref.) |
| | 7–7.99 | 6 | 64 | 9.4 | 1.313 (0.262–8.531) |
| | ≥8 | 1 | 13 | 7.7 | 1.077 (0.019–14.775) |
| Zhang, 2014 | <3 | 1 | 98 | 1.0 | NA |
| | 3–4 | 0 | 22 | 0.0 | NA |
| | 4–5 | 0 | 12 | 0.0 | NA |
| | 5–6 | 1 | 7 | 12.5 | 1.000 (ref.) |
| | 6–7 | 1 | 8 | 11.1 | 0.875 (0.010–78.385) |
| | 7–8 | 2 | 10 | 16.7 | 1.400 (0.060–94.228) |

(Continues)
ORs for the dose–response meta-analysis, which was calculated from raw data provided by included studies. Some other risk factors will affect the MTCT, such as prolonged labor, vaginal delivery, amniocentesis, maternal gene mutations or polymorphisms, or others. Crude ORs could lead to bias. Third, significant heterogeneity was found between studies. We carried out subgroup analyses by country, HBV-DNA positive definition, and MTCT diagnosis time. We also evaluated meta-regression analysis by the MTCT incidence and the year of publication. However, we still did not find the potential source of heterogeneity. Finally, there was publication bias in the meta-analysis. These limitations need to be considered when evaluating the conclusion.
In conclusion, our meta-analysis indicated that maternal HBV viral load was an important risk factor for MTCT in HBeAg-positive mothers, and maternal viral load was dose-dependent with HBV MTCT incidence.

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REFERENCES

1. World Health Organization. Hepatitis B (updated July 2016). 2016. http://www.who.int/mediacentre/factsheets/fs204/en/
2. Naghavi M, Wang HD, Lozano R et al. Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet 2015; 385: 117-71.
3. Yi P, Chen R, Huang Y, Zhou RR, Fan XG. Management of mother-to-child transmission of hepatitis B virus: propositions and challenges. J Clin Virol 2016; 77: 32-9.
4. Pan CQ, Duan ZP, Bhamidimarri KR et al. An algorithm for risk assessment and intervention of mother to child transmission of hepatitis B virus. Clin Gastroenterol Hepatol 2012; 10: 452-9.
5. Wong VC, Lee AK, Ip HM. Transmission of hepatitis B antigens from symptom free carrier mothers to the fetus and the infant. Br J Obstet Gynaecol 1980; 87: 958-65.
6. Yang J, Zeng XM, Men YL, Zhao LS. Elective caesarean section versus vaginal delivery for preventing mother to child transmission of hepatitis B virus – a systematic review. Virol J 2008: 28: 5.
7. Yi W, Pan CQ, Hao J et al. Risk of vertical transmission of hepatitis B after amniocentesis in HBs antigen-positive mothers. J Hepatol 2014; 60: 523-9.
8. Gao Y, Cuo J, Zhang F et al. Evaluation of neonatal Toll-like receptors 3 (c.1377C/T) and 9 (G2848A) gene polymorphisms in HBV intrauterine transmission susceptibility. Epidem Infect 2015: 143: 1868-75.
9. Chatzidakis V, Choumerianou D, Dimitriou H, Kouroumalis E, Galanakis E. Genetic variants associated with susceptibility to mother-to-child transmission of hepatitis B virus. Eur J Gastroenterol Hepatol 2012; 24: 1185-90.
10. Lau KC, Lam CW, Law CY et al. Non-invasive screening of HLA-DPA1 and HLA-DPB1 alleles for persistent hepatitis B virus infection: susceptibility for vertical transmission and toward a personalized approach for vaccination and treatment. Clin Chim Acta 2011: 412: 952-7.
11. Yin Y, Zhang P, Tan Z, Zhou J, Wu L, Hou H. The association of pre-S/S gene mutations and hepatitis b virus vertical transmission. Hepat Mon 2016; 16: e32160.
12. Zhang Z, Li A, Xiao X. Risk factors for intrauterine infection with hepatitis B virus. Int J Gynaecol Obstet 2014; 125: 158-61.
13. Wen WH, Chang MH, Zhao LL et al. Mother-to-infant transmission of hepatitis B virus infection: significance of maternal viral load and strategies for intervention. J Hepatol 2013; 59: 24-30.
14. Zou H, Chen Y, Duan Z, Zhang H, Pan C. Virologic factors associated with failure to passive-active immunoprophylaxis in infants born to HBsAg-positive mothers. J Viral Hepat 2012; 19: e18-e25.
15. Wells GA, Shea BJ, O’Connell D et al. The Newcastle–Ottawa Scale (NOS) for assessing the quality of non-randomized studies in meta-analysis. 2000. http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp
16. Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. Stat Med 2002; 21: 1539-58.
17. Freeman MF, Tukey JW. Transformations related to the angular and the square root. Ann Math Statist 1950; 21: 607-11.
18. Greenland S, Longnecker MP. Methods for trend estimation from summarized dose-response data, with applications to meta-analysis. Am J Epidemiol 1992; 135: 1301-9.
19. Orsini N, Bellocco R, Greenland S. Generalized least squares for trend estimation of summarized dose-response data. Stat Methods Med Res 2006; 6: 40-57.
20. Orsini N, Li RF, Wolk A, Khudyakov P, Spiegelman D. Meta-analysis for linear and nonlinear dose–response relations: examples, an evaluation of approximations, and software. Am J Epidemiol 2012; 175: 66-73.
21. Zhang L, Gui XE, Wang B et al. Serological positive markers of hepatitis B virus in femoral venous blood or umbilical cord blood should not be evidence of in-utero infection among neonates. BMC Infect Dis 2016; 16: 408.
22. Wen HK, Zhang F, Wang T et al. Effects related to HBsAg status and mode of delivery as well as the interactions on
intrauterine transmission among HBsAg-positive mothers]. Zhonghua Liu Xing Bing Xue Za Zhi 2016; 37: 791–5. (In Chinese.)

23 Xu YY, Liu HH, Zhong YW et al. Peripheral blood mononuclear cell traffic plays a crucial role in mother-to-infant transmission of hepatitis B virus. Int J Biol Sci 2015; 11: 266–73.

24 Liu CP, Zeng YL, Zhou M et al. Factors associated with mother-to-child transmission of hepatitis B virus despite immunoprophylaxis. Internal Med 2015; 54: 711–16.

25 Lee YK, Aw M, Rauff M, Loh KS, Lim SG, Lee GH. Hepatitis B immunoprophylaxis failure and the presence of hepatitis B surface gene mutants in the affected children. J Med Virol 2015; 87: 1344–50.

26 Lu LL, Chen BX, Wang J et al. Maternal transmission risk and antibody levels against hepatitis B virus e antigen in pregnant women. Int J Infect Dis 2014; 28: 41–4.

27 Kang W, Ding Z, Shen L et al. Risk factors associated with immunoprophylaxis failure against mother to child transmission of hepatitis B virus and hepatitis B vaccination status in Yunnan province. China Vaccine 2014; 32: 3362–6.

28 Yin YZ, Zhou J, Zhang PZ, Hou HY. [Identification of risk factors related to the failure of immunization to interrupt hepatitis B virus perinatal transmission]. Zhonghua Gan Zang Bing Za Zhi 2013; 21: 105–10. (In Chinese.)

29 Yin Y, Wu L, Zhang J, Zhou J, Zhang P, Hou H. Identification of risk factors associated with immunoprophylaxis failure to prevent the vertical transmission of hepatitis B virus. J Infect 2013; 66: 447–52.

30 Pan CQ, Zou HB, Chen Y et al. Cesarean section reduces perinatal transmission of hepatitis B virus infection from hepatitis B surface antigen-positive women to their infants. Clin Gastroenterol Hepatol 2013; 11: 1349–55.

31 Guo Z, Shi XH, Feng YL et al. Risk factors of HBV intrauterine transmission among HBsAg-positive pregnant women. J Viral Hepat 2013; 20: 317–21.

32 Zhang RL, Wang MY, Chen QY, Xiu XY, Ren KH, Qiu IY, Huang X. [Study on the risk factors related to vertical transmission of HBV positive couples to their infant.] Zhonghua Liu Xing Bing Xue Za Zhi 2012; 33: 1283–7. (In Chinese.)

33 Li F, Wang QX, Zhang L et al. The risk factors of transmission after the implementation of the routine immunization among children exposed to HBV infected mothers in a developing area in northwest China. Vaccine 2012; 30: 7118–22.

34 Singh AE, Plot SS, Osiowy C et al. Factors associated with vaccine failure and vertical transmission of hepatitis B among a cohort of Canadian mothers and infants. J Viral Hepat 2011; 18: 468–73.

35 Song YM, Sung I, Yang S, Choe YH, Chang YS, Park WS. Factors associated with immunoprophylaxis failure against vertical transmission of hepatitis B virus. Eur J Pediatr 2007; 166: 813–18.

36 Shao ZJ, Xu DZ, Xu JQ et al. Maternal hepatitis B virus (HBV) DNA positivity and sexual intercourse are associated with HBV intrauterine transmission in China: a prospective case–control study. J Gastroenterol Hepatol 2007; 22: 165–70.

37 Yin YZ, Chen XW, Li XM, Hou HY, Shi ZL. [Intrauterine HBV infection: risk factors and impact of HBV DNA]. Nan Fang Yi Ke Da Xue Xue Bao. 2006; 26: 1452–4. (In Chinese.)

38 Reifenberg K, Deutschle T, Wild J et al. The hepatitis B virus e antigen cannot pass the murine placenta efficiently and does not induce CTL immune tolerance in H-2b mice in utero. Virology 1998; 243: 45–53.

39 Yu M, Jiang Q, Gu X et al. Correlation between vertical transmission of hepatitis B virus and the expression of HBsAg in ovarian follicles and placenta. PLoS One 2013; 8: e54246.

40 Wei JN, Xue SL, Zhang JF, Wang SP, Wang B. Study of the relationship in pregnant women between hepatitis B markers and a placenta positive for hepatitis B surface antigen. J Perinat Med 2015; 43: 191–9.

41 Pan CQ, Han GR, Jiang HX et al. Telbivudine prevents vertical transmission from HBsAg-positive women with chronic hepatitis B. Clinical Gastroenterol Hepatol 2012; 10: 520–6.

42 Han GR, Cao MK, Zhao W et al. A prospective and open-label study for the efficacy and safety of telbivudine in pregnancy for the prevention of perinatal transmission of hepatitis B virus infection. J Hepatol 2011; 55: 1215–21.

43 Xu WM, Cui YT, Wang L et al. Lamivudine in late pregnancy to prevent perinatal transmission of hepatitis B virus infection: a multicentre, randomized, double-blind, placebo-controlled study. J Viral Hepat 2009; 16: 94–103.

44 Bzowej NH. Optimal management of the hepatitis B patient who desires pregnancy or is pregnant. Curr Hepat Rep 2012; 11: 82–9.

45 Petersen J. HBV treatment and pregnancy. J Hepatol 2011; 55: 1171–3.

46 Wakano Y, Sugïura T, Endo T et al. Antiviral therapy for hepatitis B virus during second pregnancies. J Obstet Gynaecol Res 2018; 44: 566–9.