Hepatitis B virus serosurvey and awareness of mother-to-child transmission among pregnant women in Shenyang, China

An observational study

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Abstract
Preventing hepatitis B virus (HBV) mother-to-child transmission (MTCT) is the key to controlling the prevalence of chronic HBV infection. Adequate awareness of hepatitis B in hepatitis B s antigen (HBsAg) positive pregnant women may be helpful to reduce HBV MTCT.

The aim of this study was to explore HBV seroprevalence among pregnant women and investigate the level of hepatitis B awareness among HBsAg positive pregnant women.

HBV serum biomarkers were tested among pregnant women visiting Shengjing Hospital of China Medical University. HBsAg-positive pregnant women received a HBV DNA test and completed a questionnaire. The different HBV DNA loads were interpreted as follows: $2 \times 10^3$ to $<2 \times 10^6$ IU/mL was low viral load, $2 \times 10^6$ to $<2 \times 10^9$ IU/mL was intermediate viral load and $\geq 2 \times 10^9$ IU/mL was high viral load. The pregnant women with high viral load were treated with telbivudine (LdT). HBV DNA at different times was tested. The rate of HBV MTCT was confirmed at 28 weeks postpartum.

HBsAg prevalence among pregnant women was 3.1% (441/14314). There was significant difference in comparing HBsAg prevalence in different age groups ($\chi^2 = 13.86$, $P < .01$). Among 441 HBsAg-positive pregnant women, 151 (34.2%) were hepatitis B e antigen (HBeAg) positive and 112 (25.4%) had high viral load. After 4 weeks of treatment, the average HBV DNA load of 66 cases with high viral load was $(5.0 \pm 0.8)\log_{10}$IU/mL. The average HBV DNA load at 4 weeks postpartum rebounded to $(7.9 \pm 1.0)\log_{10}$IU/mL, which was not significantly different from that at baseline ($t = 1.23$, $P = .22$). At 28 weeks postpartum, the rate of HBV MTCT in the treatment group was significantly lower than that in the observation group (0% vs 12.2%; $P = .02$). Only 23.4% of pregnant women knew their HBV status before gestation and 17.7% of pregnant women knew the HBV status before delivery. However, only 21.3% of pregnant women realized to need antiviral treatment to prevent MTCT.

The pregnant women in Shenyang had a low HBsAg prevalence. Antiviral treatment for pregnant women with high viral load can effectively reduce the rate of HBV MTCT. HBV screening and education among HBsAg-positive pregnant women should be strengthened.

Abbreviations: ALT = alanine aminotransferase, anti-HBc = hepatitis B c antibody, anti-HBe = hepatitis B e antibody, HBeAg = hepatitis B e antigen, HBIG = hepatitis B immunoglobulin, HBsAg = hepatitis B s antigen, HBV = hepatitis B virus, LdT = telbivudine, MTCT = mother-to-child transmission, TDF= tenofovir disoproxil fumarate.

Keywords: hepatitis B virus, mother-to-child transmission, pregnancy, questionnaire, telbivudine

1. Introduction
In China, mother-to-child transmission (MTCT) is the main transmission route of hepatitis B virus (HBV). Preventing HBV MTCT is the key to controlling prevalence of chronic HBV infection. Despite immunoprophylaxis with hepatitis B vaccine and hepatitis B immunoglobulin (HBIG), the rate of HBV MTCT is still 10% to 15%, which involves in the women with high viral
load and hepatitis B e antigen (HBeAg) positive.\textsuperscript{[2]} Antiviral treatment during middle and late gestation in pregnant women with high viral load can effectively reduce HBV MTCT.\textsuperscript{[3,4]} Aging women, hepatic flare, HBV mutation, and quasispecies may lead to a seroprevalence change in pregnant women with chronic HBV infection.\textsuperscript{[5–8]} Measures such as improving hepatitis B awareness in hepatitis B s antigen (HBsAg) positive childbearing-age women, HBV screening and education before gestation, follow-up, and timely intervention during gestation can reduce HBV MTCT in HBsAg-positive women.\textsuperscript{[9,10]}

2. Objectives

In this study, we investigated HBV seroprevalence among pregnant women in Shenyang, China. We also tested the dynamic change of HBV DNA load for pregnant women with high viral load during antiviral treatment. We further investigated the level of hepatitis B awareness among HBsAg-positive pregnant women by a questionnaire survey.

3. Study design

All pregnant women visiting Shengjing Hospital of China Medical University between January and December 2016 were enrolled. HBV serum biomarkers were tested for all pregnant women. HBsAg-positive pregnant women also received a HBV DNA test and completed a questionnaire.

HBV serum biomarkers were tested with chemiluminescence microparticle immunoassay (Architect i2000; Abbott, IL). Negative was defined as HBsAg < 0.05 IU/mL, HBeAg < 1.0 s/co, hepatitis B e antibody (anti-HBe) > 1.0 s/co, and hepatitis B c antibody (anti-HBc) < 1.0 s/co, respectively. HBV DNA was tested with real-time quantitative PCR (COBAS AmpliPrep/TaqMan 48 analyzer; Roche, CA). The different HBV DNA loads were interpreted as follows: < 200IU/mL as negative, 20 to < 2 x 10\(^3\)IU/mL was low viral load, 2 x 10\(^3\) to < 2 x 10\(^4\)IU/mL was intermediate viral load, and ≥ 2 x 10\(^4\)IU/mL was high viral load. The pregnant women with high viral load signed informed documents and were divided into the treatment group and observation group according to their own preference. Telbivudine (LdT; 600mg, once daily; Beijing Novartis Pharmaceutical Co., Ltd., Beijing, China) was orally administered from 24 to 28 weeks gestation and immediately discontinued after delivery in the treatment group. HBV DNA was tested at baseline, after 4 weeks of treatment, before delivery, and at 4 weeks postpartum in the treatment group. HBV DNA was tested at baseline and 4 weeks postpartum in the observation group. All these infants received 100IU HBIG (Hualan Bioengineering Co., Ltd., Xinxing, China) and 10μg hepatitis B vaccine (Kangtai Biological Products Co., Ltd., Shenzhen, China) intramuscularly within 12 hours after birth. The other 2 doses of 10μg hepatitis B vaccines were scheduled at 1 and 6 months of age. The rate of HBV MTCT was defined as the proportion of infants with HBV DNA and HBsAg-positive in the infants born to HBsAg-positive mothers at 28 weeks of age.

The HBsAg positive pregnant women were requested to complete a written questionnaire survey anonymously. They were taken into a separate room to allow privacy during the survey. The questionnaire was jointly developed by the study team. Content was intensely discussed among the study team. The questionnaire consisted of 10 items, divided into 3 parts: the time when HBsAg status was known (4 items: Q1, Q2, Q3, Q4), their knowledge of HBV MTCT (3 items: Q5, Q6, Q7), and strategies of preventing HBV MTCT (3 items: Q8, Q9, Q10). For each item there were 2 response options: “yes” or “no.” Data generated were analyzed with SPSS 22.0 software (IBM, Armonk, NY). Baseline characteristics and laboratory results were summarized by mean values including percentage and mean±standard deviation. The t test or Mann–Whitney U test was employed in the comparison of continuous data and the Chi-square test was used for the comparison of count data. P < .05 (bilateral test) was statistically significant.

4. Results

4.1. HB seroprevalence among pregnant women

4.1.1. HBsAg prevalence among pregnant women. There were 14,314 pregnant women visiting Shengjing Hospital of China Medical University from January to December 2016. The age range of these pregnant women was 19 to 45 years and the average age was (31.1 ± 4.5) years. The HBsAg prevalence among these pregnant women was 3.1% (441/14314). The HBsAg prevalence among pregnant women in different age groups is summarized in Table 1. The HBsAg prevalence in the group 19 to 25 years of age was lowest (1.2%). Whereas, the HBsAg prevalence in the group 26–35 and 36–45 years of age were 2.8 and 2.9 times than that in the youngest group. There was significant difference in comparing HBsAg prevalence in different age groups (χ\(^2\) = 13.86; P < .01).

4.1.2. HBeAg status and HBV DNA level among HBsAg-positive pregnant women. HBeAg status and HBV DNA load among 441 HBsAg-positive pregnant women are summarized in Table 2. Among 441 HBsAg-positive pregnant women, 151 (34.2%) were HBeAg positive, and 207 (46.9%) were HBV DNA

| Table 1 | HBsAg prevalence among pregnant women in different age groups. |
|---------|--------------------------------------------------|
| Age, y  | N | HBsAg positive (%) | HBsAg negative (%) | OR | 95% CI |
| 19–25 | 1035 | 12 (1.2%) | 1023 (98.8%) | 1.0 |
| 26–35 | 8862 | 284 (3.2%) | 8578 (96.8%) | 2.8 | 1.58–5.05 |
| 36–45 | 4417 | 145 (3.3%) | 4272 (96.7%) | 2.9 | 1.60–5.23 |

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| Table 2 | HBeAg status and HBV DNA load among HBsAg-positive pregnant women. |
|---------|-------------------------------------------------------|
| HBsAg DNA | Total | HBeAg positive | HBeAg negative |
| n = 441 | n = 151 | n = 290 |
| Negative | 234 (53.1%) | 9 (5.9%) | 225 (77.6%) |
| Low viral load | 44 (9.9%) | 6 (4.0%) | 38 (13.1%) |
| Intermediate viral load | 51 (11.6%) | 29 (19.2%) | 22 (7.6%) |
| High viral load | 112 (25.4%) | 107 (70.9%) | 5 (1.7%) |

Among 441 HBsAg-positive pregnant women, 151 (34.2%) were HBeAg positive, and 207 (46.9%) were HBV DNA positive. There were 112 (25.4%) pregnant women with high viral load. Among 112 pregnant women with high viral load, 107 cases were HBeAg positive and the remaining 5 cases were HBeAg negative.
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### 4.1.3. Dynamic change of HBV DNA after antiviral treatment among the pregnant women with high viral load.

One hundred twelve pregnant women with high viral load were divided into the treatment group (n = 66) and observation group (n = 46). All pregnant women in the treatment group were treated for more than 8 weeks and no subject was prematurely delivered before 28 weeks gestation. The baseline conditions in the 2 groups are described in Table 3. There was no significant difference in age, alanine aminotransferase (ALT), and HBV DNA load between the 2 groups. The change of HBV DNA load at different times in the treatment group is described in Fig. 1. After 4 weeks of treatment, the average HBV DNA load was (5.0 ± 0.8) log_{10} IU/mL, which decreased by 3.1 log_{10} IU/mL compared with that at baseline (t = 29.78, P < .01). The average HBV DNA load continued to decrease an extra 0.9 log_{10} IU/mL compared with that at baseline (t = 1.01, P = .315).

### Table 3

| Items                          | Treatment group (n = 66) | Observation group (n = 46) | t  | P   |
|-------------------------------|-------------------------|---------------------------|----|-----|
| Age                           | 31.3 ± 4.4              | 30.4 ± 4.2                | 1.01 | 0.315 |
| Alanine aminotransferase, U/L | 27.4 ± 11.0             | 25.1 ± 12.3               | 1.05 | 0.295 |
| HBV DNA, log_{10} IU/mL       | 8.1 ± 0.4               | 7.9 ± 0.5                 | 1.89 | 0.061 |

There was no significant difference in age, alanine aminotransferase, and HBV DNA load between the 2 groups.

4.2. Questionnaire survey among HBsAg-positive pregnant women

Four hundred forty-one questionnaires completed by HBsAg-positive pregnant women were collected with the results described in Table 4. Only 23.4% (103/441) of HBsAg-positive pregnant women knew their HBV status before gestation and 17.7% (78/441) of HBsAg-positive pregnant women knew their HBV status before delivery. There were 66.2% (292/441) of HBsAg-positive pregnant women who knew that a HBsAg-positive mother could transmit HBV to her children; however, only 21.52% (94/441) of HBsAg-positive pregnant women knew that pregnant women with high viral load should need antiviral treatment to prevent MTCT. About 69.2% (305/441) of HBsAg-positive pregnant women were aware of the need for the inoculation of hepatitis B vaccine and HBIG in neonates.

4.3. The rate of HBV MTCT

All these infants received immunoprophylaxis with hepatitis B vaccine and HBIG and visited follow-up. At 28 weeks postpartum, the rate of HBV MTCT among the infants born to mothers in the treatment group was significantly lower than that in the observation group (Fig. 2). The rate of HBV MTCT in the treatment group was 0% (0 of 66 infants) versus 12.2% (5 of 46 infants) in the observation group (χ² = 5.18, P = .02).

5. Discussion

According to the data reported by the Chinese Disease Control and Prevention Center in 2007, HBsAg prevalence among
A consensus is to treat pregnant women with high viral load in middle or late gestation. Therefore, it is of clinical significance to understand HBV DNA load among pregnant women.\textsuperscript{[3,26]} In our study, the proportion of pregnant women with HBV DNA positive in 2016 was 46.9\%, which was lower than that in 2010 (81.9\%). The reason may be that some pregnant women received antiviral treatment before or during gestation because of hepatic flare. The proportion of pregnant women with high viral load was 25.4\%. High viral load can occur in both HBsAg-positive and negative pregnant women (although in the minority).

In this study, 66 pregnant women with high viral load were treated orally with LdT. After 4 weeks of treatment, HBV DNA load significantly decreased. HBV DNA load at 4 weeks postpartum approximately rebounded to that at baseline. At 28 weeks postpartum, the rate of HBV MTCT in the treatment group (0\%) was lower than that in the observation group (12.2\%). After antiviral treatment, the dynamic change of HBV DNA in pregnant women and the rate of HBV MTCT were consistent with previous reports.\textsuperscript{[17]} However, the latest study by Jourdain et al\textsuperscript{[28]} in Thailand failed to obtain a positive result on their issue. Their result was generalized to pregnant women in the area with higher MTCT rate (MTCT rate in Thailand is 2\%). It should not be generalized to pregnant women in the area with lower MTCT rate (MTCT rate in China is 10–15\%).

It is necessary for HBsAg-positive pregnant women to have adequate awareness of hepatitis B, which has important influence on the screening and follow-up during gestation. Our survey was administered among HBsAg-positive pregnant women to determine the level of hepatitis B awareness. We found that relatively a few (23.4\%) of HBsAg-positive pregnant women knew their HBV status before gestation. Only 21.3\% of pregnant women knew that pregnant women with high viral load need antiviral treatment to prevent HBV MTCT. HBV screening and education among HBsAg-positive pregnant women still need to be strengthened. In China, the neonates born to HBsAg-positive mothers are provided free inoculation of hepatitis B vaccine and HBIG within 12 hours after delivery, which significantly decreases HBV MTCT. Over 60\% of HBsAg-positive pregnant women knew about the HBV-free immunization policy.

The limitation of this study included potential long-term side effects of antiviral treatment on mothers and children. This aspect will require further investigation. These pregnant women treated with LdT were self-selected, and the questionnaire survey was not administered among HBsAg-negative pregnant women, which may be potential selective bias. It was still a limitation for the lack of the validation about our survey tool.

In 2015, China Foundation for Hepatitis Prevention and Control launched “Zero HBV MTCT Project” and standardized clinical management for preventing HBV MTCT. In 2016, the World Health Assembly endorsed World Health Organization global health sector strategies on hepatitis. These strategies call for the elimination of HBV MTCT by 2030.\textsuperscript{[29]} Furthermore, HBV screening, education, as well as antiviral treatment in pregnant women are initiated. “Zero HBV MTCT ” is expected to realize in China and all over the world.

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