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Incidence and seroprevalence of hepatitis E virus infection in pregnant women infected with hepatitis B virus and antibody placental transfer in infants

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ABSTRACT

Background: Hepatitis E has poor outcomes in pregnant women. Superinfection of hepatitis E virus (HEV) in patients infected with hepatitis B virus (HBV) may worsen liver disease.
Objectives: To estimate the incidence and seroprevalence of HEV infection among HBV-infected pregnant women, to investigate the transplacental transfer of maternal anti-HEV IgG, and to compare the maternal and neonatal outcomes in anti-HEV positive and negative pregnant women.
Study design: Totally 391 HBV-infected pregnant women were recruited from April 2012 to October 2014. Paired mothers and infants were followed up at an average 9.8 months postpartum. Anti-HEV IgG and IgM were tested by ELISA.
Results: Of the pregnant women, none was anti-HEV IgM positive and 42 (10.7%) were IgG positive. At the follow-up, 3 seronegative women converted to anti-HEV IgG positive, with an estimated incidence of 17 per 1000 person-years. No significant differences of gestational age, preterm birth rate, Apgar score and birthweight were observed between newborns of anti-HEV IgG positive and negative mothers. Of the 42 neonates born to anti-HEV IgG positive mothers, 38 (90.5%) had anti-HEV IgG in their cord blood. The neonatal and maternal anti-HEV IgG levels were positively correlated ($r = 0.827$, $p < 0.05$). All infants were negative for both anti-HEV IgM and IgG at the follow-up.
Conclusions: HBV-infected pregnant women rarely have novel HEV infection during late pregnancy in Jiangsu, China. Maternal anti-HEV IgG efficiently transfers into the fetuses, and disappears in infants before 10 months old.

1. Background

Hepatitis E virus (HEV), the causal agent of hepatitis E, is initially considered to be endemic only in developing countries. However, an increasing number of sporadic cases occurred in industrialized countries over the last two decades [1], resulting in HEV infections in almost every population of the World. Although most of HEV infections are mild or subclinical, the course in pregnant women may be severe, especially in the third trimester of pregnancy, and may cause death in up to 20–25% pregnant women [2]. Many investigations have shown that the incidence of HEV infection in pregnancy is high and a significant proportion of infected pregnant women could suffer very severe hepatitis and may even

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lead to fulminant hepatic failure, spontaneous abortions and membrane rupture [3]. Maternal hepatitis E has also been reported to be associated with adverse perinatal outcomes, such as preterm birth, stillbirth and neonatal death [4]. Additionally, it is reported that superinfection of HEV in chronic hepatitis B patients may cause severe exacerbation of liver disease [5–7].

Anti-HEV IgM can be detected within 3–4 weeks after infection and anti-HEV IgG antibodies are detectable shortly after the appearance of IgM. The presence of anti-HEV IgM indicates recent infection and the co-existence of anti-HEV IgG is of supportive, whereas the specific IgG alone is indicative of previous infection or convalescent phase. Recent studies of anti-HEV IgG seroprevalence in European and African pregnant women have shown rates ranging from 3.6% to 29.3% and 11.6% to 84.3% respectively [8–10]. The positive rates of anti-HEV IgM in pregnant women from Spain and China were 0.67% [8] and 2.6% respectively [11]. However, there are limited data about the longitudinal cohort study of anti-HEV among pregnant women and postpartum, particularly in those infected with hepatitis B virus (HBV). Previously, we surveyed the incidence and prevalence of HEV infection in the general pregnant women and postpartum and found that low (0.6%) novel subclinical, but not clinical, infection rate occurred during pregnancy [12]. Considering the high prevalence of HBsAg in China and the harmful effect of superinfection of HEV, whether pregnant women infected with HBV are prone to HEV infection is unknown. Additionally, the transplacental transfer of maternal anti-HEV IgG and its natural decay in neonates are less studied.

2. Objectives

This study aimed to estimate the incidence and seroprevalence of HEV infection among pregnant women infected with HBV, to investigate the efficiency of transplacental transfer and the decay of maternal anti-HEV IgG in infants, and to observe the maternal and perinatal outcomes in HEV infected and non-infected individuals.

3. Study design

3.1. Study population

In a study to evaluate the efficacy of telbivudine used in the late pregnancy to prevent the mother-to-infant transmission of HBV, a total of 391 pregnant women with positive hepatitis B surface antigen (HBsAg) were initially recruited from the Department of Obstetrics and Gynecology of Nanjing Drum Tower Hospital, Taixing People's Hospital, Wuxi Maternal and Child Health Hospital, Zhenjiang Maternal and Child Health Hospital, and Kunshan First People's Hospital respectively, during April 2012 to October 2014. Maternal and cord blood samples were kept at −30 °C. Follow-up serum samples of mothers and their infants were collected at 7–14 months after delivery. Data on maternal age, gestational age, infant sex, Apgar scores and birthweight were collected.

Since the project on the antiviral therapy during the pregnancy was approved by the institutional review board (IRB) of each of above five hospitals, the serum samples of pregnant women and infants were used in this study via an exemption approved by the IRB of each hospital.

3.2. Laboratory tests

Serum samples were tested for anti-HEV IgM and IgG by an in-house ELISA. The unique in-house ELISA is based on combination of high reactivity of the recombinant immunodominant polypeptide 459–607 and poor reactivity of truncated polypeptide 472–607 [13]. The polypeptide 459–607, covering amino acids (aa) 459–607 on the open reading frame 2 (ORF2), and the polypeptide 472–607, covering aa 472–607, were both derived from ORF2 of HEV genotype 4 [13]. Importantly, the polypeptide 459–607 contains the most immunogenic site on ORF2, and the performance of ELISA based on this immunodominant polypeptide is comparable with that of the ELISA based on the insect-cell expressed ORF2 protein (aa 112–607) developed in the National Institutes of Health [14,15], which has been widely used in the investigation of seroprevalence and incidence of HEV infection [16–20]. The protocol of the in-house ELISA was as previously described [13]. Briefly, ELISA microplates were separately coated with purified recombinant ORF2 polypeptides 459–607 and 472–607 (100 μl/well) with 1 μg/ml in carbonate-bicarbonate buffer (pH 9.6) at 4 °C overnight. After washed three times with 0.05% Tween-20 in phosphate-buffered saline (PBS-T), the wells were blocked with 300 μl/well 5% skimmed milk (dissolved in PBS-T) at 37 °C for 1 h. Then, 100 μl diluted serum at a dilution of 1:100 in 5% skimmed milk was added to each well and incubated at 37 °C for 1 h. After five washes, horseradish peroxidase (HRP)-conjugated anti-human IgM or IgG (Sigma, MO, USA) diluted 3000-fold in PBS-T was added into the microplates (100 μl/well). Following incubation at 37 °C for another 0.5 h, 100 μl of tetramethylbenzidine solution (Sigma) was added to the wells. Finally, after incubation for 15 min at room temperature, 50 μl/well of 2 M H_2SO_4 was added to stop the color development. The optical density (OD_{450}) was read at 450 nm using a microplate reader (Bio-Rad, CA). The OD_{450} value of the immunodominant polypeptide (aa 459–607) was denoted as $OD_{459–607}$, and the truncated polypeptide (aa 472–607) was as $OD_{472–607}$. Samples with $OD_{459–607} \geq 0.5$ and $OD_{459–607}$: $OD_{472-607} > 2$ or $OD_{459-607} – OD_{472–607} > 0.5$, were considered to be positive for anti-HEV IgM or IgG.

3.3. Data analysis

Descriptive statistics were shown as mean ± SD to evaluate main characteristics of the pregnant women and neonates. The prevalence of anti-HEV IgM and IgG in pregnant women and the rate of transplacental transfer of anti-HEV IgG and their 95% CI were estimated. The correlation between the maternal and paired cord anti-HEV IgG levels (based on $OD_{459–607}$ values) was calculated and presented in a scatter plot. All statistical analyses were conducted using SPSS 13.0 (SPSS, Inc., Chicago, IL, USA). $P < 0.05$ was considered as statistically significant.

4. Results

4.1. Positive rates of anti-HEV in the pregnant women at and after delivery

Totally 391 pregnant women with positive HBsAg were recruited during the study period. Of them, 328 were also positive for hepatitis B e antigen (HBeAg); the high proportion of HBeAg positivity was a result of selection effect. The mean maternal age was 26.4 ± 4.1 years and mean gestational age was 38.8 ± 1.6 weeks. Twenty-eight women (2 HBeAg negative and 26 HBeAg positive) presented abnormal serum alanine aminostransferase (ALT, >40 U/L). The proportion of the term deliveries (gestation 37 and 41 weeks) was 92.5%. Of the 391 HBsAg-positive pregnant women, none was anti-HEV IgM positive, indicating no recent novel infection, and 42 (10.7%, 95% CI: 7.9–14.3%) were positive for anti-HEV IgG. In 328 pregnant women with positive HBeAg, 38 (11.6%, 95% CI: 8.4–15.7%) were positive for anti-HEV IgG, while that in 63 pregnant women with negative HBeAg, 4 (6.3%, 95% CI: 2.0–16.2%) were positive for anti-HEV IgG; there was no significant difference between HBeAg positive and HBeAg negative individuals