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Perinatal factors influencing mother-to-child HBV transmission.

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Abstract

Background: Chronic hepatitis B virus (HBV) infection remains a key public health problem. In China, mother-to-child transmission is recognized as the major transmission pathway. The exact mechanism by which HBV enters the fetus through the maternal placenta is unclear, hampering the development of preventative interventions.
Objective: We aimed to identify perinatal factors influencing intrauterine transmission as determined by hepatitis B surface antigen (HBsAg) positivity 12 h after birth.
Study design: A total of 343 HBsAg(+) newborns with HBV(+) mothers were included in this study. The maternal marker expression pattern as well as associations of maternal HBsAg status with multiple perinatal risk factors for transmission (including sex, fetal position, mode of delivery, gestational age, number of complications, and infant HBV marker levels were analyzed.
Results: The majority of HBsAg(+) newborns were also HBeAg(+). The HBeAg and anti-HBe titers were significant higher in newborns from HBsAg(+) mothers than HBsAg(-) mothers (P=0.000). The frequency of ≥ 2 complications was significant higher in HBsAg(+) mothers than HBsAg(-) mothers (p=0.023). There was no significant difference in offspring in sex, mode of delivery, in utero position, or gestational age between HBsAg(+) and HBsAg(-) mothers (all P ≥ 0.05).
Conclusion: The HBsAg(+) newborns of HBsAg(+) mothers showed higher HBV viral replication rates than those of HBsAg(-) mothers. Maternal HBsAg-positivity and multiple pregnancy complications were significant risk factors for uterine mother-to-child HBV transmission. Offspring of HBV-infected mothers should be screened regularly for HBV markers to identify carriers.

Keywords: Hepatitis B virus, Mother-to-child transmission, Perinatal factor, Hepatitis B e antigen li.

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Introduction

Chronic infection by hepatitis B virus (HBV) is a global public health issue afflicting 240 million people worldwide [1]. According to previous studies in HBV-endemic areas, infection occurs mainly in infancy and early childhood, with mother-to-child transmission (MTCT), also called vertical transmission, accounting for more than 90% of chronic infection in newborns and up to 50% in children less than 3 years old [2]. In China, the rate of HBV infection among women of childbearing age is still high (7.18%) [3] and MTCT may occur prenatally, during delivery, or in the early postpartum period.

Hepatitis B virus vaccination has been adopted by national immunization programs in China and many other countries. The World Health Organization recommends administration of HBV active immunization with or without Hepatitis B immunoglobulins (HBIG) within 12 h after birth to newborns born to hepatitis B surface antigen (HBsAg)-positive mothers to reduce postpartum MTCT, and this is now applied as standard procedure for such infants [1]. The exact mechanism by which HBV enters the fetus through the maternal placenta remains unclear. The HBsAg is detectable in amniotic fluid, cord blood, breast milk, vaginal fluid, and infant gastric contents, suggesting multiple routes for MTCT [4,5].

To examine the factors associated with intrauterine infection, we measured marker expression at 12 h postnatal. The aim of this study is to analyze the perinatal factors that may influence MTCT, such as sex, fetal position, mode of delivery, gestational age (GA), number of complications, and infant HBV marker levels.

Materials and Methods

Study subjects

This study enrolled newborns HBsAg-positive [HBsAg(+)] as determined by peripheral blood testing within 12 h after birth and before HBIG injection at Songjiang Gynecology Infantile Health Hospital of Shanghai. A total 1113 neonates were screened from May 2013 to November 2015. Finally, serum

samples from 343 HBsAg(+) newborns and their mothers were analyzed for HBV markers. The study protocol was reviewed and approved by Songjiang Gynecology Infantile Health Hospital of Shanghai ethics committee (2012-III -21). Informed written consent was obtained from all parents.

Definitions of groups

For statistical analyses, maternal HBV status was divided according to the combination of antigen and antibody HBV markers (HBsAg, anti-HBs, HBeAg, anti-HBe, anti-HBc), mode of delivery into spontaneous and caesarean section, and maternal complications of pregnancy into none; one complication, and ≥ 2 complications.

HBV serological assay

Peripheral blood samples (5 mL) were acquired from newborns within 12 h after delivery (prior to inoculations with HBIG and HBV vaccines) and processed within 12 h of collection. All newborns received the government-funded 3 doses of approved HBV vaccine (10 mg/dose). Administration of HBIG (100 IU) was successfully given within 24 hours after birth. Serum HBV markers were determined using reagents from Roche Diagnostics and a Cobas e601 analyzer (Roche Diagnostic GmbH, Mannheim, Germany).

Semi-quantification of serum HBV markers

Serum HBV markers were determined based on the competition principle by ELISA using reagents from Roche on a Cobas e601 analyzer. Samples were considered positive based on the following cutoff indexes: HBsAg ≥ 1; anti-HBs ≥ 10 mIU/mL; HBeAg ≥ 1; anti-HBe ≥ 1; anti-HBc ≥ 1.

Definition of newborn infants HBV infection and complications

HBsAg positivity in peripheral blood at 12 h after birth was considered evidence of mother-to-child intrauterine HBV transmission [6]. Definitions of complications were according to "Obstetrics and gynecology" [7].

Statistical analysis

The chi-square test and Student's t-test were applied to evaluate the differences between categorical variables and continuous variables, respectively. The Wilcoxon rank sum test was used to compare the differences in HBV marker titers among groups. All statistical tests were two-sided and performed using SPSS 18.0 for Windows (SPSS, Chicago, IL). A P value of <0.05 was considered statistically significant.

Results

Perinatal parameters of HBsAg(+) newborns

Average GA was 39.4 ± 1.25 w (range, 34.7-41.7 w). Average BW was 3336 ± 420 g (range, 1900-4350 w).The majority of HBsAg(+) infants (89.8%) were in the LOA position with only 2.92% in ROA and LSA positions secondly, respectively (Table 1). The most common maternal complication was anemia (29.4%), followed by diabetes (14.3%) (Table 2). The five most frequent combinations of ≥ 2 complications were anemia and hypothyroidism (2.3%), anemia and diabetes (2%), anemia and premature rupture of fetal membranes (2%), anemia plus abnormal placental position (2.0%), and anemia with fetal distress (1.5%).

Table 1. *Perinatal parameters of HBsAg(+) newborns.*

Perinatal factors	Frequency, n	%
Sex		
male	178	51.9
female	165	48.1
Position of fetus		
LOA (Left Occiput Anterior)	308	89.80
LOT (Left Occipito Transverse)	10	2.92
LOP (left Occipito Posterior)	10	2.92
ROA (Right Occiput Anterior)	6	1.75
ROT (Right Occipito Transverse)	4	1.17
ROP (Right Occiput Posterior)	2	0.58
LSA (left Sacro Anterior)	1	0.29
RSA (Right Sacro Anterior)	1	0.29
RST (Right Sacro Transverse)	1	0.29
Mode of the delivery		
spontaneous delivery	204	59.5
caesarean section	139	40.5
Maternal complications during pregnancy		
None	170	49.6
One complication	125	36.4
≥ 2 complications	40	11.7

Table 2. *Maternal complications during pregnancy.*

Complication	n*
Anemia	101
Diabetes	49
Fetal Distress	25
Hypothyroidism	21
Premature rupture of fetal membranes	15
Cholestasis	10
Abnormal placental position	9
Hypertension	6
Thrombocytopenia	5

Fibroid	3
Oligohydramnios	2
Hepatic dysfunction	2
Postpartum hemorrhage	1
Umbilical cord rupture	1
Intrauterine growth retardation	1
*out of 341 women	

Maternal HBV marker profile

There were 15 HBV marker combinations among mothers of the 343 HBsAg(+) newborns (Table 3), of which the most common was HBeAg(+)/anti-HBc(+) (43.7%), followed by anti-HBc(+), HBsAg(+)/HBeAg(+)/anti-HBc(+) (23.3%), and HBsAg(+)/anti-HBe(+)/anti-HBc(+) (17.5%).

Associations of maternal HBsAg status with infant HBV marker status and perinatal factors

The 343 maternal peripheral blood samples were divided into two groups: HBsAg(+) and HBsAg(-). 82 (24%) infants were born to HBsAg(+) mothers. The rate of ≥ 2 maternal complications was significantly higher in HBsAg(+) mothers than HBsAg(-) mothers (p=0.023), whereas there was no significant difference in offspring sex, mode of delivery, uterine position, GA or BW between the two groups (P ≥ 0.05) (Table 4). The titers of HBeAg and anti-HBe were significant higher in newborns of the 82 HBsAg(+) mothers than in infants of the HBsAg(-) mothers (P=0.000), while HBsAg titer was higher in newborns from HBsAg(-) mothers. In total, 70.3% of infants were HBsAg(+)/HBeAg(+) as defined by ≥ 1 for both.

Table 3. HBV marker status of mothers (n=341).

Status of HBV makers	Frequency, n	%
HBeAg(+)/anti-HBc(+)	150	43.7
anti-HBc(+)	80	23.3
HBsAg(+)/HBeAg(+)/anti-HBc(+)	60	17.5
HBsAg(+)/anti-HBe(+)/anti-HBc(+)	16	4.7
anti-HBs(+)/anti-HBe(+)/anti-HBc(+)	7	2.0
HBeAg(+)/anti-HBe(+)/anti-HBc(+)	5	1.5
anti-HBs(+)	4	1.2
anti-HBs(+)/anti-HBc(+)	4	1.2
anti-HBe(+)/anti-HBc(+)	4	1.2
HBsAg(+)/anti-HBc(+)	3	0.9
HBsAg(+)/anti-HBs(+)/anti-HBe(+)/anti-HBc(+)	3	0.9
anti-HBs(+)/HBeAg(+)/anti-HBc(+)	3	0.9
HBsAg(+)	2	0.6
anti-HBe(+)	2	0.6
Total	343	100

HBsAg(+): hepatitis B surface antigen positivity

anti-HBs(+): anti- hepatitis B surface antibody positivity

HBeAg(+): hepatitis B e antigen positivity

anti-HBe(+): anti- hepatitis B e antibody positivity

anti-HBc(+): anti- hepatitis B core antibody positivity

Discussion

Chronic hepatitis B virus (HBV) infection remains a critical public health problem in China, and MTCT during the perinatal period is considered the most important reason for the failure of passive-active immunoprophylaxis [8-10]. If the fetus cannot remove the hepatitis B virus from the mother, the infant with carry the virus chronically, and 9% will develop chronic HBV infection in adulthood [1]. The mechanisms by which HBV enters the fetus through the human placenta are still unclear. In the mid 1980's, immunological pathology and molecular biology techniques detected hepatitis B virus in the liver cells of stillbirths from HBV-positive mothers, confirming intrauterine infection. It is generally accepted that neonatal blood HBsAg positivity or HBV-DNA positivity [6,11,12] is indicative of intrauterine infection. Here we show that neonatal mother-to-child infection is strongly associated with maternal viral status and pregnancy complications.

In this study, the titers of HBeAg and anti-HBe were related to the mothers' HBsAg status. Vertical transmission of HBV can occur by three routes: intrauterine transmission through the placenta, intrapartum transmission, and postnatal transmission through breast milk [13]. The latter two modes of transmission can be blocked by HBIG and HBV vaccines, but there is currently no effective preventive measure for intrauterine transmission by placenta during pregnancy [14]. Intrapartum transmission means a small amount of maternal blood into the fetal blood circulation caused by leakage the postpartum horizontal transmission due to placental villous vascular contraction of the uterus rupture when delivery. Studies on HBV-related intrauterine transmission have found that infection is related to transmission of maternal HBsAg, HBeAg titer, and HBV-DNA content, which is similar to this study.

Table 4. Associations of maternal HBsAg status with perinatal factors and newborn HBV marker titers.

	Maternal HBsAg(+)	Maternal HBsAg(-)	χ^2 or t value	P
Sex (male:female)	44:38	134:127	0.134	0.714
GA	39.52 ± 1.21	39.38 ± 1.26	0.881	0.379

BW	3271 ± 440	3356 ± 412	-1.607	0.109
Position of fetus (LOA)	72 (87.8%)	236 (90.4%)	9.039	0.339
Mode of the delivery			1.190	0.275
Spontaneous delivery	53 (64.6%)	151 (57.9%)		
Caesarean section	29 (35.4%)	110 (42.1%)		
Maternal complications during pregnancy			7.577	0.023*
None	37 (45.1%)	133 (51%)		
One complication	26 (31.7%)	99 (37.9%)		
≥ 2 complications	19 (23.2%)	29 (1.1%)		
Semi-quantification of newborn HBV markers				
HBsAg	2367.59 ± 205	3236.75 ± 161.90	-2.795	0.005*
anti-HBs (mIU/mL)	5.23 ± 2.21	12.67 ± 5.22	-0.792	0.429
HBeAg	1045.25 ± 67.33	661.74 ± 40.87	4.670	0.000*
anti-HBe	6.17 ± 0.40	4.26 ± 0.22	4.272	0.000*
anti-HBc	0.02 ± 0.01	0.04 ± 0.01	-0.933	0.352
Data presented as number (n) or mean ± SD.				

Interesting, we found HBsAg(+) mother is high risk of more complications during pregnancy. Most of the complications are easy to injure the placental barrier. Fetal and maternal circulatory systems are separated but can exchange substances through the placental barrier. Reports claimed that it is difficult for HBsAg to pass through the normal placental barrier, while HBeAg can pass through easily [15,16]. HBeAg is a major component of the virus nucleocapsid polypeptide and circulates in blood during active viral replication (i.e., when the host is infectious) [17]. It can exist in both a free state or in a IgG complex in the blood, and the complex can traverse the placenta and enter the fetal blood circulation. The immature fetal immune system can produce tolerance to HBeAg, so HBV accumulates mutations that minimize the expression of epitopes recognized by CD8+ T cells, particularly in the enhancer II/basal core promoter/precore (EnhII/BCP/preC) region and the preS/S regions, which may impair cytotoxic T cell function necessary to remove HBV from infected liver cells [18,19]. Therefore, HBeAg can enter the fetal circulation and interfere with immune cell function and even affect the specificity of antibodies, resulting in fetal infection [20-22]. In the study, infants of HBsAg(+) mothers exhibited higher HBeAg and HBeAb titers than infants of HBsAg(-) mothers. Thus, the risk of infants' chronic hepatitis B is higher in HBsAg(+) mothers.

In this study, we found that HBsAg(+) mothers were significantly more prone to complications than HBsAg(-) mothers, including fetal distress (6/19 cases), abnormal placental position (5 cases), premature rupture of fetal membranes (3 cases), and postpartum hemorrhage (1 case). It appears that placental leakage greatly increases the probability of intrauterine MTCT, as the virus does not easily pass through the intact barrier. In late pregnancy, women produce large amounts of estrogen, which is metabolized by the liver. Estrogen inactivation is reduced when liver cells are injured by HBV infection, which causes the incidence of postpartum hemorrhage. Maternal HBV infection can also markedly increase the incidence of fetal distress as infected mothers may develop placental chorionic vascular disease, which impairs placental circulation. During delivery, a small amount of maternal blood leaks into the fetal circulation during contraction of the uterus, and abnormal placenta position was recognized as a risk factor for maternal blood leakage. Therefore, HBsAg(+) women with multiple pregnancy complications are at highest risk for MTCT of HBV infection, and so maybe receive active intervention [23,24].

Of all newborns positive for HBsAg(+), those from HBsAg(+) mothers showed significantly higher HBV viral replication than those from HBsAg(-) mothers. Mothers with HBsAg(+) status and multiple pregnancy complications were at highest risk of mother-to-child HBV transmission. Thus, this population should be targeted for preventative therapy. Newborns of infected mothers should be screened regularly for HBV markers to detect chronic carriers.

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