Effect of hepatitis B vaccination in hepatitis B surface antibody-negative pregnant mothers on the vertical transmission of hepatitis B virus from father to infant

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Abstract. The aim of the present study was to investigate the effects of vaccination with the hepatitis B vaccine (HBVac) in HB surface antibody (HBsAb)-negative pregnant mothers on the vertical transmission of HB virus (HBV) from father to infant. All the fathers tested positive for the serum HBV DNA and HB surface antigen (HBsAg) markers. The pregnant females were divided into an observation group or a control group depending on whether their serum was HBsAb-negative or positive. A total of 93 healthy individuals without HBV infection were included in a blank group, while 96 females who were serum HBV marker-negative or HB core antibody (HBcAb)-positive/(HBsAb)-negative were included in the observation group. The control group comprised 89 females who all tested positive for serum HBsAb, HB envelope antibodies and HBcAb. In the observation group, the positive rate of HBV DNA in the newborns was 7.29% (7/96), the positive rate of HBsAg was 3.13% (3/96) and the positive rate of HBsAb was 81.3% (78/96). In the control group, the positive rates of HBV DNA, HBsAg and HBsAb in the newborns were 4.49% (4/89), 2.25% (2/89) and 89.9% (80/89), respectively. No statistically significant differences were observed between the two groups. Therefore, the results of the present study indicate that HBVac treatment for HBsAb-negative pregnant females may have a positive role in blocking the vertical transmission of HBV from father to infant, as long as the vaccination is able to induce the production of a sufficient quantity of HBsAb. The HBVac exhibited no difference compared with pre-pregnancy HBsAb in blocking the vertical transmission of HBV from father to infant.

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Introduction

Numerous studies have demonstrated the existence of vertical transmission of hepatitis B virus (HBV) from father to infant (1-5). In addition, previous studies have shown that when the father is positive for the HB surface antigen (HBsAg), the HB surface antibody (HBsAb) level in the mother prior to pregnancy may be significant in preventing the vertical transmission of HBV from father to infant (6-9). After 20 weeks of pregnancy, placental trophoblasts actively transfer the antibody of IgG from the mothers to the fetus (10). Previous studies have indicated that in mothers with HBsAb levels of >10 MIU/ml, the antibody may be transferred to the fetus via the placenta and confer passive immune protection (11). Zhang *et al* (12) observed that in 168 women prior to pregnancy, HBsAb levels were all >400 U/L, and their newborns exhibited no HBV infection. HBsAg was negative and HBsAb was positive in these newborns. If HBsAb levels in the mothers prior to pregnancy are sufficient, HBV infected factors from the fathers may be eliminated. Ayoola *et al* (13) inoculated 72 pregnant women without HBV infection with HBVac and observed that 59% of the newborns were positive for HBsAb, suggesting that the fetus obtained immunity *in utero*.

However, in a number of cases, females did not receive HBV testing prior to pregnancy, and as the fathers tested positive for HBsAg, they attended the liver outpatient clinic of the Qinhuangdao Third Hospital (Qinguangdao, China) for further consultation once they were pregnant and were subsequently tested. The present study aimed to investigate the effects of vaccination with HB vaccine (HBVac) in HBsAb-negative pregnant females on the vertical transmission of HBV from father to infant.

Materials and methods

Study participants. Since March 2006, any HBsAg-positive males in the outpatient department whose partners were

pregnant were asked to attend the liver clinic with their partners. Couples with good compliance, who underwent regular examinations and acceptable follow-up visits were selected and divided into an observation group and a control group.

Inclusion criteria. This study was approved by the ethical committee of Qinhuangdao Third Hospital and written informed consent was obtained from the couples. Couples were included in the study if their serum tested negative for hepatitis A-E and HIV, with no alcoholic or autoimmune liver diseases. In addition, the patients were required to have normal renal function. Markers for HBV DNA and HBsAg in the fathers were positive, with the exception of the healthy subjects, and their medical history spanned between 2 and 35 years.

In addition to the aforementioned inclusion criteria, there were specific inclusion criteria for the different groups. The participants in the observation group had to meet an additional criterion: The results of the HBV marker (HBVM) detection in 96 mothers were negative or marginally positive for HB core antibodies (anti-HBc). In the control group, the results of the HBVM tests, which included HBsAb, HB envelope (HBe) antibodies and anti-HBc detection, were positive in 89 mothers. In addition, a total of 93 couples were selected from pregnant women who received prenatal care in the Qinhuangdao Women and Children's Hospital (Qinhuangdao, China) since March 2006 for the blank group. The selected healthy parents were not HBV carriers. The couples provided written informed consent. Ultimately, there were 96 newborns in the observation group and 89 newborns in the control group, with no mortalities.

Sample processing and observation index. The couples provided venous blood samples for the detection of liver function, HBVM and HBV DNA. In the female patients, the detection of liver function and HBVM was conducted dynamically following the injection of HBVac (at one-month intervals). In addition, venous blood samples were collected from the newborns immediately after birth to detect the presence of HBVM and HBV DNA. When collecting samples from the newborns, consideration was taken with regard to the umbilical cord blood containing residues from the placenta and umbilical cord when the fetus was born, or umbilical cord ligation and mutilation, which may have resulted in contamination of the blood samples. Furthermore, additional factors may have polluted the umbilical cord blood, such as the placenta, placental abruption or a cesarean sections.

Research methods. All the fathers tested positive for the serum markers, HBV DNA and HBsAg, while the pregnant females were divided into an observation and a control group according to whether their serum tested positive or negative for HBsAb. Healthy individuals without HBV infection were included in the blank group. The pregnant women in the observation group were separately injected with 20 μg HBVac (North China Pharmaceutical Jintan Biotechnology, Co., Ltd., Shijiazhuang, China) at weeks 28, 32 and 36 of pregnancy. Subjects in the control and blank groups did not receive any drug treatment.

Detection method. HBVM and HBV DNA were detected using the same sample from each patient. With regard to HBVM detection, electrochemiluminescence with an automatic analysis system for chemiluminescence immunoassays (cobas-e-411; Roche Diagnostics GmbH, Mannheim, Germany) was used at a speed of 86 tests/h, and the data were analyzed using cobas software (cobas-c-411; Roche Diagnostics GmbH). Reagents were provided by Roche Diagnostics GmbH. HBV DNA was detected using fluorogenic quantitative polymerase chain reaction (FQ-PCR), according to the manufacturer's instructions. A nucleic acid releasing agent was used to lyse and release HBV DNA from the serum samples. Using a pair of specific primers and a specific fluorescence probe targeting the conserved region of HBV gene, PCR and fluorescence quantitative PCR instrument, we realized the quantitative detection of HBV DNA via a fluorescence signal change. Reagents and the FQ-PCR instrument (SLAN-48P) were provided by Sansure Biotech (Changsha, China).

Statistical analysis. The data are presented as the mean ± standard deviation and were processed using SPSS medical statistical software (version 16.0; SPSS, Inc., Chicago, IL, USA). Measurement data were analyzed using the t-test, while the count data were analyzed using the χ^2 test, where $P<0.05$ was considered to indicate a statistically significant difference.

Results

Demographic data of the parents in the three groups. No statistically significant differences were observed with regard to the age, height and weight of the participants in the observation, control and blank groups when compared pairwise. In addition, the HBV DNA load of the fathers in the observation and control groups was not significantly different. In addition, no statistically significant difference was detected in the gravida of the mothers in the observation, control and blank groups, or in the prenatal HBsAb levels when comparing the observation and control groups (Tables I and II).

Complications of the mothers in the two groups during pregnancy. There were no statistically significant differences in the rate of hypertension, pre-eclampsia, diabetes and fever in the mothers during pregnancy in the observation, control and blank groups when compared pairwise (Table III).

General state of the newborns. No statistically significant differences were detected with regard to the gestational age, birth weight and length, gender, 1-min and 8-min Apgar scores, pathological jaundice, other internal medicine and surgery diseases, and delivery mode in the observation, control and blank groups when compared pairwise (Table IV).

HBVM and HBV DNA in the newborns. When compared with the control group, the positive rates of HBV DNA, HBsAg and HBsAb in the newborns in the observation group were not significantly different (Table V).

Discussion

The vertical transmission and propagation rate of HBV can be observed as follows: The pathways of the paternal fetal trans-

Table I. General conditions of the fathers in groups A, B and C.

Parameter	A	B	C	P-value			t-value		
				A vs. B	B vs. C	A vs. C	A vs. B	B vs. C	A vs. C
Cases (n)	96	89	93						
Age (years)	31.1±2.8	29.5±2.7	28.9±2.9	0.516	0.806	0.398	0.712	0.262	0.945
Height (cm)	179.1±5.7	176.2±6.5	174.9±5.9	0.592	0.810	0.425	0.581	0.257	0.887
Weight (kg)	68.5±5.5	64.4±4.3	65.1±4.8	0.367	0.860	0.465	1.017	-0.188	0.807
HBV DNA (IU/ml)[a]	$3.7\pm1.9\times10^6$	$2.9\pm1.9\times10^6$	- -	--	-	-	-		

Data are presented as the mean ± standard deviation. [a]P=0.633; t=0.516. A, observation group; B, control group; C, blank group; HBV, hepatitis B virus.

Table II. General conditions of the mothers in groups A, B and C.

Parameter	A	B	C	P-value			t-value		
				A vs. B	B vs. C	A vs. C	A vs. B	B vs. C	A vs. C
Cases (n)	96	89	93						
Age (years)	25±2.1	25.6±2.2	26.1±1.90	0.750	0.781	0.538	-0.342	-0.298	-0.673
Height (cm)	165±5.2	159±4.6	158±5.1	0.209	0.813	0.171	1.497	0.252	1.665
Weight (kg)	61.2±5.6	54.6±4.7	50.9±4.9	0.193	0.399	6.075	1.564	0.944	2.398
Gravidity	2±1.0	2±1.0	2±1.0	1.000	1.000	1.000	0.000	0.000	0.000
Prenatal HBsAb (IU/ml)[a]	495±39.8	487±41.8	-	-	-	-	-	-	-

Data are presented as mean ± standard deviation. [a]P=0.822; t=0.240. A, observation group; B, control group; C, blank group; HBsAb, hepatitis B surface antibody.

Table III. Common complications of the mothers during pregnancy.

				P-value			χ^2		
Parameter (n)	A	B	C	A vs. B	B vs. C	A vs. C	A vs. B	B vs. C	A vs. C
Cases	96	89	93	-	-	-	-	-	-
Hypertension	11	8	12	0.580	0.399	0.761	0.306	0.712	0.092
Pre-eclampsia	0	0	0	-	-	-	-	-	-
Diabetes	0	0	0	-	-	-	-	-	-
Fever	0	0	0	-	-	-	-	-	-

A, observation group; B, control group; C, blank group.

Table IV. Birth conditions of the newborns in the three groups.

				P-value			t-value		
Parameters	A	B	C	A vs. B	B vs. C	A vs. C	A vs. B	B vs. C	A vs. C
Pregnancy weeks	38.95±1.21	9.21±1.34	39.19±1.22	0.815	0.986	0.821	-0.249	0.019	-0.242
Birth weight (kg)	3.33±0.32	3.34±0.31	3.30±0.41	0.971	0.899	0.925	-0.039	0.135	0.100
Birth length (cm)	48.73±1.59	48.60±1.53	49.70±1.61	0.924	0.439	0.499	0.102	-0.858	-0.742
Gender, male/female (n)	52/44	49/40	50/43	0.908	0.260	0.233	0.124	0.031	0.003
Apgar score 1 min	9.94±0.50	9.89±0.49	9.25±0.69	0.857	0.816	0.688	0.192	-1.310	1.403
Apgar score 8 min	9.93±0.58	9.84±0.57	9.72±0.61	0.903	0.861	0.956	0.025	0.249	0.432
Pathological jaundice (n)	5	3	6	0.539	0.338	0.715	0.377	0.918	0.133
Other diseases (n)	0	0	0	-	-	-	-	-	-
Delivery manner, caesarean/head (n)	51/45	45/44	51/42	0.727	0.563	0.813	0.122	0.334	0.056

A, observation group; B, control group; C, blank group.

Table V. Serum HBV markers in the newborns in groups A and B.

Groups	Cases (n)	HBV DNA (+)	HBsAg (+)	HBsAb (+)
A (n)	96	7	3	78
B (n)	89	4	2	80
χ^2		0.646	0.135	2.764
P-value		0.421	0.713	0.096

A, observation group; B, control group; HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; HBsAb, hepatitis B surface antibody.

mission of HBV can be divided into horizontal propagation and vertical transmission (14). Since neonatal immunity is weak, a father with HBV infection can transmit the virus to the newborn through daily contact; this transmission method is termed horizontal transmission. By contrast, vertical transmission is a pattern of transmission that propagates the virus through germ cells. Horizontal transmission by the combined injection of HB immunoglobulin (HBIG) and HBVac has been demonstrated to have a better efficacy at preventing horizontal propagation. However, there are no effective measures to prevent vertical transmission due to the manner of the transmission. Vertical transmission is the second most important method of spreading HBV (15), particularly in fathers who are HBsAg-, HBeAg- and anti-HBc-positive. The presence of HBsAb in females prior to pregnancy is widely accepted to play a positive role in blocking vertical transmission from father to infant; however, in married couples with an HBsAg-positive father, it is common for pregnancy-associated testing to identify no HBsAb, for

reasons including lack of testing prior to marriage and patients providing misinformation (16). The remedial measures require further study. The results of the present study demonstrated that in the observation group, the positive rate of HBV DNA in the newborns was 7.29% (7/96), the positive rate of HBsAg was 3.13% (3/96) and the positive rate of HBsAb was 81.3% (78/96). In the control group, the positive rates of HBV DNA, HBsAg and HBsAb in the newborns were 4.49% (4/89), 2.25% (2/89) and 89.9% (80/89), respectively, with no statistically significant differences identified between the two groups (Table V). These results indicate that even if a woman does not have HBsAb prior to pregnancy, as long as she receives timely injections of HBVac and produces HBsAb throughout the pregnancy, the power to block the vertical transmission of HBV from father to infant is significant. This result was consistent with the study by Li (17).

The general conditions of the newborns were measured using a variety of parameters, including weeks of gestation, birth weight and length, 1-min and 8-min Apgar scores, pathological jaundice or other diseases, and delivery manner. When compared with the blank group, there were no statistically significant differences in the observation and control groups with regard to the birth data. In addition, there were no fetal malformations or stillbirths in either group. The vertical transmission of HBV from the HBsAg-positive fathers to the infants also had no significant effect on the aforementioned newborn indicators (Table IV). Previous studies have shown that an HBV-infected male, whose sperm chromosome aberration rate is significantly increased compared with healthy controls, may experience an increased risk of infertility, abortion, stillbirth, perinatal mortality and fetal malformations (18-21). The present results are not consistent with these findings.

The positive rate of HBV DNA in the newborns was higher than the positive rate of HBsAg, and the presence of HBVM and HBV DNA does not occur simultaneously. A study by Wang *et al* (22) that included 16 newborns infected with HBV from their fathers revealed that the serum detection ratios of HBV DNA were all positive, however, there were no serological markers for HBV. In addition, He *et al* (23) found that the detection rate of HBV DNA in the serum was higher compared with that of HBsAg in newborns infected with HBV from their fathers, and the difference was statistically significant. This phenomenon is generally explained by the imperfect immune function of a fetus during the embryonic period, which is also affected by immune pressures from the mother (16). Therefore, a newborn does not show signs of serological HBV, only HBV DNA in the serum, liver and white blood cells. With the improvement of immune function following birth, the majority of infected offspring can remove pathogens; however, a small number of individuals are likely to become stable and sustained carriers, exhibiting viral replication and serological markers.

From week 20 of gestation, the placenta has a positive role in transporting IgG antibodies to the fetus, particularly during the late stages of pregnancy (4-6 weeks prenatal) (10). An injection of HBVac at weeks 28, 32 and 36 may be meaningful to block the vertical transmission of HBV from the father to the infant, as long as the mother produces a sufficient amount of HBsAb for transmission to the fetus. The results of the present study further demonstrated this hypothesis. Furthermore, several studies have previously demonstrated that administration of HBVac injection during pregnancy is safe, with numerous observations of the HBVac injection without any adverse reactions over a number of years (10,23,24). The adverse fetal and neonatal rate, including abortion, malformation, stillbirth, premature birth and neonatal asphyxia, also exhibited no growth, and there was no increase in the rates of fever and allergic reactions.

Zhang *et al* (12) reported that when the level of HBsAb in pregnant mothers was ≥400 IU/l, even if the sperm carried HBV, newborns may be protected from HBV infection. An injection of HBIG in HBsAb-negative females during the late stage of pregnancy has been suggested to be effective at providing protection, although achieving an effective concentration remains a challenge (14). However, additional studies have demonstrated that the application of HBIG during pregnancy has no effect on preventing the vertical transmission of HBV from father to infant (25-27).

In conclusion, the present study adopted remedial measures of HBVac injection in pregnancy, and quantities of HBsAb in prenatal maternal serum are shown in Table II. The results demonstrated that injections of HBVac in HBsAb-negative pregnant females may have a positive role in blocking the vertical transmission of HBV from father to infant, providing that the mother can produce a sufficient amount of HBsAb. However, the present study was limited due to the small sample size; thus, further investigations with a greater sample size are required to further confirm the conclusions reached.

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