

## Original Article

# Correlation between paternal serum hepatitis B virus DNA levels and vertical transmission from father to infant

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**Abstract:** This study aimed to investigate the relationship between paternal serum hepatitis B virus (HBV) DNA levels and father to infant vertical transmission. A total of 202 couples with mothers who tested positive for prenatal hepatitis B surface antibody (anti-HBs) were included in the observation group, while 196 couples with mothers who tested negative for prenatal anti-HBs were included in the control group. Fathers within the two groups were further divided into four groups (10e9-10e6 IU/ml, 10e6-10e4 IU/ml, 10e4-10e2 IU/ml, <10e2 IU/ml) based on their HBV DNA levels. HBV serologic markers (HBVM) and HBV DNA were detected by electrical chemiluminescence immunoassay and fluorogenic quantitative PCR, respectively. In the observation group, nine newborns (9/53) tested positive for HBV DNA as determined by testing the umbilical cord blood in the group where paternal serum HBV-DNA levels were 10e9-10e6 IU/ml. One infant (1/51) was positive for HBV DNA from the group where the paternal serum HBV load ranged from 10e6-10e4. The difference in rate of transmission between these two groups was statistically significant. There were no infants positive for HBV DNA in the other two groups where the fathers were HBV carriers with viral loads of less than 10e4 IU/ml. In the control group, eleven infants (11/52) were tested positive for HBV DNA using umbilical cord blood in the group where the paternal serum HBV DNA level was 10e9-10e6 IU/ml. Three neonates (3/50) were positive for HBV DNA in the group where the paternal serum HBV DNA load was in the range of 10e6-10e4 IU/ml. The difference in rate of transmission between these two groups was statistically significant. One case (1/52) tested positive for HBV DNA in the group where the paternal serum HBV DNA level was in the range of 10e4-10e2 IU/ml. This rate of transmission was not significantly different from that of the group with paternal serum HBV DNA levels in the range of 10e6-10e4 IU/ml. HBV DNA was not detected in newborns with paternal serum HBV DNA levels of less than 10e2 IU/ml. These results demonstrated that the HBV vertical transmission rate was positively correlated with the paternal serum HBV DNA level. Moreover, the father to fetus vertical transmission of HBV could not be blocked completely in the presence of high paternal serum HBV viral loads, even if the mother was positive for anti-HBs. The sample size of this study is small, and thus, more investigations on the stratification of prenatal anti-HBs status are required based on a larger sample size.

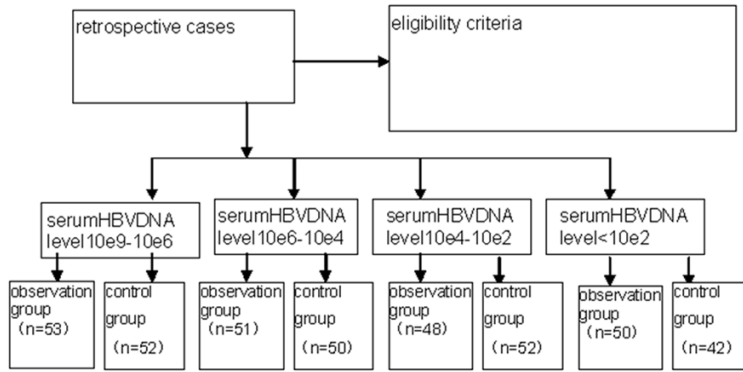
**Keywords:** Paternal serum HBV DNA, vertical transmission from father to infant, correlation

## Introduction

Modes of HBV transmission include vertical transmission from mother to infant and father to infant [1]. In recent years, some progress has been made on the HBV mother to child transmission route and prophylaxis, and gradually, there has been increasing attention towards paternal vertical transmission. In 2006, Ali et al reported that sperm-mediated HBV genes were

able to replicate and express in early embryonic cells, providing direct evidence for HBV transmission from father to infant [2]. A study by Wang et al confirmed the genotype consistency of HBV isolated from the father and his child. In addition, the high homology of the isolated HBV strains, to a great extent, precluded the possibility of mother to fetus transmission, and indicated that infant's HBV was transmitted from the father [3]. On the molecular level, this study

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**Figure 1.** A flow chart for patients enrollment.

**Table 1.** Characteristics of the fathers in the two groups

Group	Cases (n)	Age (years)	Height (cm)	Weight (kg)
Observation group	202	30±3.2	175±5.4	67±5.5
Control group	196	28±2.9	174±5.8	66±5.7
t-value		t=0.802	t=0.737	t=0.219
P-value		0.467	0.502	0.838

provides evidence for the vertical transmission of HBV from father to fetus. Meanwhile, HBV genes were detected in multiple organs of a deceased fetus whose father was positive for HBV while the mother was negative for HBV, identifying the vertical transmission of HBV from father to fetus [4]. In 2014, Zhang et al [5] explored the effects of paternal semen HBV-DNA levels on their offspring vertical transmission and pointed out a dose-response relationship for HBV-DNA levels between the paternal serum and semen. Their findings revealed that the risk factors for HBV transmission from father to fetus were paternal serum being positive for HBV-DNA, and hepatitis B virus antigen (HBeAg), and the semen being HBV-DNA positive. In addition, paternal serum HBV-DNA loads of >10e5 copies/ml and semen HBV-DNA loads of >10e3 copies/ml could increase the rate of father to fetus vertical transmission. In order to further explore the relationship between the paternal serum HBVDNA levels and father to fetus transmission, our retrospective study stratified statistics on the paternal serum HBVDNA levels. Our findings could provide evidence for a personalized approach to block the father to fetus vertical transmission of HBV.

## Materials and methods

### Patient selection

In this retrospective study, all the cases were from the Third Hospital of Qinhuangdao and the Women and Children's Hospital of Qinhuangdao from March 2006 to May 2013. Eligibility criteria for inclusion in this study were: (1) informed consent; (2) fathers testing HBsAg positive and HBVDNA positive or under the detection limit prior to conception;

(3) pregnant women testing negative for serum HBsAg and HBVDNA, and positive or negative for anti-HBs prenatal; (4) availability of test results from the newborns for HBVM and HBV-DNA. A total of 202 couples were included in the observation group in which pregnant mothers were positive for anti-HBs. An additional 196 couples were included in the control group with anti-HBs negative pregnant females.

The fathers were further stratified into four groups (10e9-10e6 IU/ml, 10e6-10e4 IU/ml, 10e4-10e2 IU/ml, <10e2 IU/ml) based on their HBV-DNA levels (**Figure 1**).

### Infant characteristics and outcomes (observed indicators)

Measurement of HBV-M and HBV-DNA in infants. General characteristics of infants including gestational age, weight, height, and internal and surgical diseases.

### 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 (cobase411; Roche Diagnostics GmbH, Mannheim, Germany) was used at a speed of 86 tests/h, and the data were analyzed using cobas software (cobase411; Roche Diagnostics GmbH). Reagents were provided by Roche Diagnostics GmbH. HBV DNA was detected using fluorogenic quantitative polymerase chain reaction (FQPCR), according to the manufacturers instructions. A

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**Table 2.** Characteristics of the mothers in the two groups

Group	Cases (n)	Age (years)	Height (cm)	Weight (kg)	Gravidity (n)	Quantitative anti-HBs prenatal (IU/l)
Observation group	202	27±2.1	163±4.9	56±5.6	2±1.0	405±25.3
Control group	196	25±2.6	159±5.9	54±5.8	2±1.0	0
t-value		t=1.036	t=0.903	t=0.430	t=0.00	t=27.727
P-value		0.359	0.417	0.690	1.00	0.000

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 FQPCR instrument (SLAN48P) were provided by Sansure Biotech (Changsha, China) [6].

### 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  $\chi^2$  test, where  $P < 0.05$  was considered to indicate a statistically significant difference. Binary Logistic regression analysis was performed using Wald Test with a significance level of 0.05 for independent variable and 0.1 for data rejection.

## Results

### Parent characteristics

Baseline characteristics of the parents in the two groups are shown in **Tables 1** and **2**.

### Infant characteristics in observation and control groups (**Table 3**)

Relationship between HBV-DNA levels in paternal serum and infant umbilical cord blood. In the observation group, a total of 202 fathers were divided into four groups based on serum HBVDNA levels. Nine infants (9/53) tested positive for HBVDNA using umbilical cord blood in the group where the paternal serum HBV DNA level was  $10^9$ - $10^6$  IU/ml. Only one infant (1/51) tested positive for HBV-DNA in the sec-

ond group where the paternal serum HBV DNA loads ranged from  $10^6$ - $10^4$  IU/ml, which is significantly different from the first group ( $\chi^2=6.747$ ,  $P=0.009$ ). There were no infants positive for HBVDNA in other two groups with lower HBVDNA levels. These results indicated that the HBV vertical transmission rate from father to fetus decreases with lower HBVDNA levels in the paternal serum. Moreover, we found that the father to fetus vertical transmission of HBV could not be completely blocked, but rather increased with higher HBV viral loads in the paternal serum, even if the mother was positive for anti-HBs (**Table 4**).

In the control group, 196 fathers were divided into four groups based on serum HBV viral loads. Eleven infants (11/52) tested positive for HBVDNA using umbilical cord blood in the first group where paternal serum HBV DNA levels were  $10^9$ - $10^6$  IU/ml. Three neonates (3/50) were positive for HBVDNA in the second group where the paternal serum HBVDNA loads ranged from  $10^6$ - $10^4$  IU/ml. One case (1/52) was found to be HBVDNA positive in the third group where the paternal serum HBVDNA levels ranged from  $10^4$ - $10^2$  IU/ml. HBVDNA was not detected in any infants in the fourth group where the paternal serum had HBVDNA levels less than  $10^2$  IU/ml. These results demonstrated that the rates of the neonatal umbilical cord blood that tested positive for HBVDNA were decreased with reduced HBV viral loads in paternal serum (**Table 4**).

### Risk factor for HBV father to infant transmission

To exclude the confounding factor, binary logistic regression was performed with the HBV infection of infants served as dependent variables and paternal HBVDNA and maternal HBsAb used as independent variables. Results showed that classification of equation was 81.2% when the maternal HBsAb was selected

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**Table 3.** General situation of the neonates at birth

	Pregnancy week	Weight (kg)	Height (cm)	Gender, M/F (n)	1 min apgar score	8 min apgar score	Jaundice	Other internal and surgical diseases (n)	Delivery mode, caesarean/head (n)
Observation group (n=202)	39.31±1.31	3.41±0.32	48.92±1.53	105/97	9.76±0.52	9.87±0.66	11	0	103/99
Control group (n=196)	39.25±1.33	3.35±0.34	49.45±1.52	106/90	9.89±0.46	9.79±0.59	9	0	104/92
$\chi^2$ (t)	t=0.056	t=0.223	t=-0.426	$\chi^2=0.675$	t=-0.324	t=0.157	$\chi^2=0.697$		$\chi^2=0.171$
P-values	0.958	0.835	0.692	0.176	0.762	0.883	0.152		0.679

M, Male; F, Female.

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**Table 4.** Paternal serum HBVDNA loads and newborns vertical transmission rate in two groups

		Cases	Newborns umbilical cord blood HBVDNA positive (IU/L cases)	Newborns HBVDNA positive rate (%)	$\chi^2$	P-value
10e9-10e6	Observation group (A)	53	9	16.98	A vs. B: 6.747	0.009
	Control group (C)	52	11	21.12	C vs. D: 4.943	0.026
10e6-10e4	Observation group (B)	51	1	1.96	D vs. E: 1.124	0.289
	Control group (D)	50	3	6		
10e4-10e2	Observation group	48	0	0	C vs. E: 9.420	0.002
	Control group (E)	52	1	1.92		
<10e2	Observation group	50	0	0		
	Control group	42	0	0		

at the first step. And the classification of equation reached 92.7% when the paternal HBVDNA was selected. The maternal HBsAb and paternal HBVDNA had statistical significance on neonates HBV; the regression coefficient  $B < 0$  for the maternal HBsAb and  $> 0$  for paternal HBVDNA, indicating positive maternal HBsAb was a protective factor and elevated paternal HBVDNA was a risk factor for the HBV infection of neonates. The Wald value of paternal HBVDNA  $>$  Wald value of maternal HBsAb, suggesting that paternal HBVDNA high loads had a greater influence on neonates HBV infection.

### Discussion

#### Neonatal characteristics

No significant differences were observed between the infants in the observation and control groups in terms of gestational age, weight, height, Apgar scores at one and eight minutes after birth, pathologic jaundice, and delivery methods. There were no malformed or dead fetuses in either group. The aforementioned indexes were also not influenced by the vertical transmission of HBV to the fetus from HBsAg positive fathers (Table 3). Other investigations have found that HBV carriers exhibit higher risk of sperm chromosomal aberrations, sterility, miscarriage, stillbirth, perinatal infant mortality, and fetal malformation compared with healthy controls [7-10].

#### Detection of HBV in neonates

Previous studies have shown that the neonates had higher HBV infection rates when the fathers were positive for HBsAg [11-13]. However, these studies did not reach a consistent con-

clusion in terms of the HBV transmission rates [14, 15]. Such inconsistencies are possibly due to inconsistent diagnostic criteria and discrepancies in the objectives of the studies. In the observation group, our investigation revealed that vertical transmission of HBV from fathers was not observed in fetuses with anti-HBs positive mothers and fathers who were HBVDNA under the detection limit or had viral loads of less than 10e4 IU/ml, which is consistent with a previous report [16]. Moreover, higher viral loads of HBV could not completely block the father to fetus transmission, even if the mother was positive for anti-HBs. The vertical transmission to fetuses when the paternal serum HBVDNA levels were 10e9-10e6 IU/ml was significantly different from the transmission when the paternal serum HBVDNA levels were between 10e6-10e4 IU/ml HBVDNA. It has been reported that the use of antiviral therapy by fathers before pregnancy could reduce their HBV viral loads, and thus, effectively block the father to fetus vertical transmission [16]. In the control group, the rate of HBV DNA positive neonates with anti-HBs negative pregnant mothers and HBV carrier fathers with viral loads 10e9-10e6 IU/ml was significantly different than that HBV carrier fathers with viral loads ranging between 10e6-10e4 IU/ml. No significant difference between the rates of HBV positive infants was observed between the groups with paternal serum HBVDNA levels greater than 10e6-10e4 IU/ml and those with 10e4-10e2 IU/ml. HBVDNA was not detected in newborns in the group where the paternal serum HBV DNA level was less than 10e2 IU/ml. These results indicated that the father to fetus HBV vertical transmission rate was positively correlated with the paternal se-



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rum HBVDNA levels. HBsAb positive status (> 400 IU/L) in females prior to pregnancy, to a certain extent, is reported to be able to block the father to fetus vertical transmission of HBV [17]. In addition, If they are anti-HBs negative females prior to pregnancy, it is beneficial to block the father to child vertical transmission by vaccinating hepatitis B vaccine (HBVac) at week 28, 32 and 36 of gestation (they were anti-HBs positive prenatal) [18]. This is because the placenta begins to actively transmit immunoglobulin G (IgG) to the fetus starting at week 20 of gestation, and active transmission of antibody reaches a peak in late pregnancy (4 to 6 weeks before birth) [19].

### *Risk factor for HBV father to infant transmission*

Previous study showed that [20] serum HBV-DNA high loads were a risk factor for HBV father to infant transmission. In our study, logistic regression analysis suggested that paternal serum HBV-DNA high loads was a risk factor for the vertical transmission of HBV. In addition, positive HBsAb prior to conception was helpful to block the HBV infection in neonates, suggesting that serum HBV-DNA negative fathers (at least with a lower HBV-DNA loads) and HBsAb positive mothers before conception may reduce the father to infant transmission of HBV.

This study used a stratified analysis of the paternal serum HBVDNA levels. In the future, we will continue to investigate the stratification of anti-HBs status in pregnant women with a larger sample size.

### **Disclosure of conflict of interest**

None.

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