

RAPID COMMUNICATION

## A randomized controlled clinical trial: Interruption of intrauterine transmission of hepatitis B virus infection with HBIG

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### Abstract

**AIM:** To evaluate the efficacy of interruption of intrauterine infection of HBV with HBIG in pregnant women with positive HBeAg and HBsAg.

**METHODS:** A prospective randomized controlled trial was adopted. Sixty cases with positive HBeAg and HBsAg were coincident with the criteria of inclusion, and 8 cases were excluded. Fifty-two cases were analyzed (28 cases in trial group and 24 in control group). All cases in trial group received 200 IU HBIG intravenously every 4 wk for 3 times from the 28<sup>th</sup> wk. The cases of control group received placebo in the same way. All pregnant women were detected for HBeAg and HBV-DNA at the beginning of the trial and end of the trial (delivery). The cord blood of all newborns were collected for detecting HBeAg and HBV-DNA simultaneously.

**RESULTS:** For investigation of HBeAg of newborns in trial group, 6 of 28 cases of newborns had positive HBeAg, the HBeAg positive rate being 21.4%, the total rate of 95% CI being 8%-41%. In control group, 19 of 24 cases of newborns had positive HBeAg, HBeAg positive rate was 79.2%, the rate of 95%CI being 5%-93%. By statistical analysis,  $\chi^2 = 17.26$ ,  $P < 0.01$ , RR = 0.27, 95% CI (6.3 × 10<sup>-6</sup>, 8.6 × 10<sup>-5</sup>). For investigation of HBV-DNA of newborns in trial group, 7 of 28 cases of newborns had positive HBV-DNA, HBV-DNA positive rate being 25%, the total rate of 95% CI being 11%-45%. In control group, 20 of 24 cases of newborns had positive HBV-DNA, HBV-DNA positive rate was 83.3%, the total rate of 95% CI being 63%-95%. By statistical analysis,  $\chi^2 = 17.62$ ,  $P < 0.01$ , RR = 0.30, 95% CI (1.5 × 10<sup>-5</sup>, 1.7

× 10<sup>-4</sup>). The results indicated that there was significant difference in HBeAg positive rate and HBV-DNA positive rate of newborns between the two groups. In trial group, 7 of 28 newborns had HBV-DNA positive, but the HBV-DNA load of newborns was lower than that of their mothers. In control group, 20 of 24 newborns still had HBV-DNA positive, and the HBV-DNA load of newborns was close to those of their mothers. Statistical analysis indicated that there was no significant difference in HBV-DNA load between postnatal women without HBIG intervention and their filial generations ( $T = 81.5$ ,  $P > 0.1$ ).

**CONCLUSION:** It is effective and safe to prevent intrauterine infection of HBV with HBIG from the 28<sup>th</sup> wk in pregnant women with positive HBeAg and HBsAg. In clinical application, those pregnant women with negative HBeAg and positive HBV-DNA also need to be interrupted by HBIG.

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**Key words:** Interruption; Intrauterine; Transmission; Hepatitis B virus; HBIG

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### INTRODUCTION

Hepatitis B virus (HBV) is highly endemic in China. It has been estimated that there are more than 1.2 million of chronic carriers of HBV nationwide. Most of them are infected by mother-infant transmission. Mother-infant infection of HBV can result in chronic HBV carrier state, chronic hepatitis, cirrhosis, hepatocellular carcinoma and the cycling of mother-infant transmission. So to prevent mother-infant transmission is a key strategy to control hepatitis B prevalence in our country. For a long time, it has been successful to vaccinate the infants of HBV positive mother with hepatitis B vaccine or both with hepatitis B immune globulin (HBIG) after birth. However, 10%-20% of the infants have no response to vaccination<sup>[1]</sup>. The main reason of failing to immunization is intrauterine

infection of HBV. Therefore, it will be meaningful to interrupt the intrauterine infection of HBV. In this study, we try to explore the effects of interruption of intrauterine infection of HBV with HBIG in pregnant women with positive HBeAg. It will be reported as follows.

## MATERIALS AND METHODS

### Design of clinical trial

A prospective randomized controlled trial was adopted. Trial group and control group were determined by randomized number table. The study (from January, 1997 to May, 2002) were undertaken by 4 sub-centres, including the First Affiliated Hospital, Xinjiang Medical University, Urumqi Military General Hospital; Maternal and Children's hospital; Xinjiang Jian Gong Hospital. According to the estimation of sample number, 30 cases were included in each group. With regard to the missing of follow-up, we started the clinical trial based on 40 cases in each group.

### Subjects

The criteria for inclusion and exclusion were determined before starting the trial. The criteria for inclusion: the pregnant women given pre-labor checkups at obstetric clinic of above hospitals, with positive HBeAg and better general condition, without threatened abortion or threatened premature labor, and hypertension, with normal liver function, and to deliver at the same hospital. The criteria for exclusion: to stop pregnancy for some reasons, to deliver at other hospitals and lose follow-up, and to be administered HBIG not according the regulation.

Sixty cases of pregnant women were coincident with the criteria for inclusion, but 8 cases were excluded, including , 3 cases who stopped pregnancy; 2 cases who transferred to other hospitals; 2 cases who lost to follow-up for unknown reasons and 1 case who used HBIG not according the regulation. So at last, 52 cases were analyzed, including 28 cases in trial group and 24 cases in control group. There was no significant difference about pregnant age, parity, fetal age, delivery mode and median of HBV-DNA (respectively  $4.3 \times 10^8$  and  $3.98 \times 10^8$  copy/mL) between those two groups. There was no history of fetal distress in all newborns of the two groups. Fifty-two pregnant women with positive HBeAg labored 52 newborns. No side effects were observed in trial group.

### Methods of treatment

All cases in trial group received 200 IU HBIG intravenously every 4 wk for 3 times (28 wk, 32 wk, 36 wk). And side effects were observed. The cases of control group were followed up for checkups, without any treatment.

### HBeAg and HBV-DNA test

All pregnant women were detected for HBeAg and HBV-DNA at the beginning of the trial and the labor day. The cord blood of all newborns were collected, separated for serum and stored at -20°C for detecting HBeAg and HBV-DNA simultaneously. HBeAg was tested by ELISA (commercial kit from Huamei Biotechnology Company, No

**Table 1 Comparison of cord blood: HBeAg and HBV-DNA positive rate**

Groups	cases <i>n</i>	HBeAg(+)		HBV- DNA(+)	
		<i>n</i>	%	<i>n</i>	%
Trial	28	6	21.4	7	25
Control	24	19	79.2	20	83.3

**Table 2 HBV-DNA levels in 7 mother-infant pairs in trial group (gene copy/mL)**

Pair No.	Pregnant women	Filial generation
1	$1.16 \times 10^8$	$1.88 \times 10^5$
2	$4.45 \times 10^8$	$2.84 \times 10^4$
3	$2.74 \times 10^8$	$1.83 \times 10^5$
4	$4.78 \times 10^7$	$3.56 \times 10^5$
5	$1.53 \times 10^8$	$2.66 \times 10^5$
6	$3.94 \times 10^8$	$2.92 \times 10^4$
7	$2.28 \times 10^8$	$1.68 \times 10^5$

20020605), HBV-DNA was tested by FQ-PCR (commercial kit from Da'an Biotechnology Company, No.s19990003). The procedures of detection were performed according to the instruction of the manufacturers.

### Statistical analysis

Relative risk (RR) and 95% CI were calculated,  $\chi^2$  test was used to compare the difference of qualitative data, Wilcoxon matched rank test was used to compare the difference of HBV-DNA load.

## RESULTS

In trial group, 6 of 28 cases of newborns had positive HBeAg (Table 1). HBeAg positive rate was 21.4%, the total rate of 95% CI being 8% and 41%. In control group, 19 of 24 cases of newborns with HBeAg positive (Table 1), HBeAg positive rate was 79.2%, the rate of 95%CI being 5% and 93%. By statistical analysis,  $\chi^2 = 17.26$ ,  $P < 0.01$ , RR = 0.27, 95% CI:  $6.3 \times 10^{-6}$  and  $8.6 \times 10^{-5}$ . The results indicated that there was significant difference in HBeAg positive rate of newborns between the two groups. The risk of intrauterine transmission could be reduced by immunoprophylaxis with HBIG. In trial group, 7 of 28 cases of newborns had positive HBV-DNA (Table 1), HBV-DNA positive rate was 25%, the total rate of 95% CI being (11%, 45%), In control group, 20 of 24 cases of newborns had positive HBV-DNA, (Table 1), HBV-DNA positive rate was 83.3%, the total rate of 95% CI being 63% and 95%. By statistical analysis,  $\chi^2 = 17.62$ ,  $P < 0.01$ , RR = 0.30, 95% CI  $1.5 \times 10^{-5}$  and  $1.7 \times 10^{-4}$ . It indicated that there was significant difference in HBV-DNA positive rate of newborns between the two groups. It was effective to decrease the HBV-DNA load of pregnant women with HBV-DNA positive by application of HBIG.

In 28 cases of postnatal women with HBIG intervention, 21 cases of newborns had negative HBV-DNA, 7 cases had HBV-DNA positive, but the HBV-DNA

load was lower than that of their mothers (Table 2). By Wilcoxon matched rank test ( $T = 28$ ,  $P = 0.02$ ), it indicated that the HBV-DNA levels of filial generation decreased after intervention with HBIG. In control group, only 4 of 24 cases with negative HBV-DNA, the others were still HBV-DNA positive, and the HBV-DNA load was close to those of their mothers. By Wilcoxon matched rank test ( $T = 81.5$ ,  $P > 0.1$ ), it indicated that there was no significant difference in HBV-DNA load between postnatal women without HBIG intervention and their filial generations.

## DISCUSSION

The prevention of HBV infection by vaccinating newborns has been previously demonstrated to be extremely efficacious. However, some newborns fail to respond to immunization<sup>[2]</sup>. Intrauterine infection is regarded as the main reason of failure. It has been reported that the rate of intrauterine infection is up to 10%-44%<sup>[3]</sup>. It shows that intrauterine infection plays an important role in mother-infant transmission of HBV. The intrauterine infection may occur as early as on the 19<sup>th</sup> wk of pregnancy, but the main time is possibly in the third-trimester of pregnancy<sup>[4]</sup>. Recently more and more researchers are trying to interrupt the intrauterine infection by intervention. HBIG is a common method to prevent HBV transmission from mother to baby<sup>[5,6]</sup>.

The mechanism of intrauterine infection of HBV has been studied by some researchers<sup>[7,8]</sup>, the findings proved that the main route of HBV transmission from mother to fetus is transplacental. HBIG is an antibody of IgG type. The nourish cells with Fc receptor in placenta can transmit the IgG antibody from mother to infant since the 20 wk during pregnancy. In one study<sup>[9]</sup>, HBIG was injected frequently to pregnant women with positive HBeAg starting from the 20<sup>th</sup> wk during pregnancy, the positive rate of anti-HBs of newborns was up to 91.42% and no side effect and birth defect were observed in both mothers and newborns. The result showed that anti-HBs can get into the body of infants via placenta after using HBIG during pregnancy. That is to say the mechanism of preventing intrauterine infection of HBV by HBIG is production of fetal passive immunity<sup>[9]</sup>.

This study is a randomized controlled trial to interrupt intrauterine transmission of HBV by HBIG in pregnant women with positive HBeAg. The results showed that it was effective to waken the effectiveness of mother-infant transmission of HBV after using HBIG frequently. In trial group, a few newborns were still HBeAg positive, but the HBV-DNA load had decreased obviously. It was likely the result of application of HBIG before labor. We presume that HBV-DNA load may decrease further if we keep on using HBIG and hepatitis B vaccine to those newborns with positive HBeAg. This deserves to study in the future. No side effect was found in both mothers and babies during the clinical trial.

Why were there still 7 cases of newborns with HBeAg positive in trial group, in our opinion, one of reasons was that cord serum used for HBeAg test was not better than vein serum, because cord serum is easy to be contaminated

by the blood of mother with positive HBeAg. Another reason may be the high load of HBV-DNA in serum of these 7 mothers.

How to improve the effects of interruption of intrauterine transmission, other methods can be taken into consideration. Yue *et al*<sup>[10]</sup> used HBIG and together with hepatitis B vaccine to interrupt 30 pregnant women every 4 week from the 20<sup>th</sup> wk until delivery. The results showed that 29 newborn were HBsAg negative, 27 HBV-DNA negative and 10 were anti-HBs positive. In another clinical trial<sup>[11]</sup>, 56 cases were given 200 IU HBIG intramuscularly every 4 wk from the 28<sup>th</sup> wk, while 43 cases received 100 mg lamivudine orally every day, 52 cases in the control group received no special treatment. The results demonstrated that the risk of HBV intrauterine infection effectively reduced in the 3rd trimester of HBsAg positive pregnant women, and there was no significant difference between HBIG and lamivudine groups. In present study, Su *et al*<sup>[12]</sup> has reported that 38 cases of pregnant women were given lamivudine before pregnancy, during pregnancy, until after labor. The result showed that none of 38 newborns was chronic carrier of HBsAg. In infant animals, lamivudine does not cause dysplasia however evidence of safety in human infants is limited. So some researchers prefer to use lamivudine from the 16<sup>th</sup> wk or 24<sup>th</sup> wk to those pregnant women with positive HBeAg for the safety of infants. We are waiting for the outcomes.

HBeAg is a marker which indicates active duplication of HBV, high concentration of HBV and strong possibility of infection. So we chose HBeAg positive pregnant women as subjects in this study. However it is unsuitable for clinical application to prevent from intrauterine transmission of HBV because the mutation of pre-C region of HBV can result in negative HBeAg, but they still had high load of HBV-DNA<sup>[13]</sup>. In clinical application, those pregnant women with negative HBeAg and positive HBV-DNA also need to be interrupted by HBIG. It has been reported that there is closed relationship between HBV-DNA load of serum of pregnant women and intrauterine infection<sup>[14,15]</sup>. Therefore it will be better to guide the application of prevention of mother-infant transmission of HBV by HBV-DNA load test quantitatively.

It is clear that intrauterine infection is the main route of HBV transmission. According to present study, there is still a route of paternal-infants transmission of HBV<sup>[16]</sup>. Zhang *et al*<sup>[17]</sup> detected HBV markers of sperm of male patients with hepatitis B, HBV markers of peripheral blood of their partners and their newborns, the outcome was: HBV markers of mothers were negative, HBV markers of newborns were positive, situations were same as those of their fathers. It indicated that there was possibility of HBV transmission from farther to infant. Zhao *et al*<sup>[18]</sup> have detected HBV-DNA of sperma in male patients with hepatitis B, then a capture test of normal sperma was performed. The samples of infants from induced labor were tested for HBsAg and HBV-DNA, the result showed that HBV can cause vertical transmission via sperma. So in order to control intrauterine infection of HBV, we need to consider both mother and father. For noninfected pregnant women, while their partners are HBV carriers, application of pro-

phylaxis also should be considered in these fathers.

The limited number of HbsAG positive women and excluded cases may have produced a bias in our results warranting further study.

## REFERENCES

- 1 **Yue YF**, Yang XJ, Zhang SL, Han XB. Clinical study on the prevention of HBV transmission from mother to infant by injection of HBIG in pregnant women with HbsAg. *Zhongguo Shiyong Fuchanke Zazhi* 1999; **15**: 547-548
- 2 **Towers CV**, Asrat T, Rumney P. The presence of hepatitis B surface antigen and deoxyribonucleic acid in amniotic fluid and cord blood. *Am J Obstet Gynecol* 2001; **184**: 1514-1518
- 3 **Zhu QR**, Yu GJ, Lu Q, Ya H, Gu XH, Zhang XZ, Dong ZQ. A study of cotrolled clinical trial on interruption of HBV intrauterine transmission. *Zhongguo Erke Zazhi* 2002; **40**: 478-480
- 4 **Yan Y**, Xu D, Wang W. [The role of placenta in hepatitis B virus intrauterine transmission]. *Zhonghua Fuchanke Zazhi* 1999; **34**: 392-395
- 5 **Li XM**, Shi MF, Yang YB, Shi ZJ, Hou HY, Shen HM, Teng BQ. Effect of hepatitis B immunoglobulin on interruption of HBV intrauterine infection. *World J Gastroenterol* 2004; **10**: 3215-3217
- 6 **Zhu Q**, Yu G, Yu H, Lu Q, Gu X, Dong Z, Zhang X. A randomized control trial on interruption of HBV transmission in uterus. *Chin Med J (Engl)* 2003; **116**: 685-687
- 7 **Yue YF**, Jiang H, Shi L, Li LF, Xi BS, Yu YL, Chen GF. [Study on the mechanism of intrauterine infection of hepatitis B virus]. *Zhonghua Fuchanke Zazhi* 2004; **39**: 224-226
- 8 **Zhang SL**, Yue YF, Bai GQ, Shi L, Jiang H. Mechanism of intrauterine infection of hepatitis B virus. *World J Gastroenterol* 2004; **10**: 437-438
- 9 **Yue Y**, Yang X, Zhang S. Prevention of intrauterine infection by hepatitis B virus with hepatitis B immune globulin: efficacy and mechanism. *Chin Med J (Engl)* 1999; **112**: 37-39
- 10 **Yue YF**, Li YJ, Bai GQ, Zhang RJ. The study on Interrupting intrauterine infection by HBIG and hepatitis B vaccine. *Xi'an Jiaotong Daxue Yuebao Yixueban* 2004; **25**: F003-F004
- 11 **Li XM**, Yang YB, Hou HY, Shi ZJ, Shen HM, Teng BQ, Li AM, Shi MF, Zou L. Interruption of HBV intrauterine transmission: a clinical study. *World J Gastroenterol* 2003; **9**: 1501-1503
- 12 **Su GG**, Zhao NF, Fang SH. The observation of efficacy and safety of Lamivudine on pregnant women with chronic hepatitis B. *Hepatology* 2002; **7**: 84-85
- 13 **Zhang Q**, Tian GS. The advanced study and problems about intrauterine transmission of HBV. *Zhonghua Shiyan He Linchuangbingduxue Zazhi* 2003; **17**: 385-387
- 14 **Li XM**, Shi XF, Yang YB. The study of interruption of intrauterine infection in pregnant women by HBIG. *Zhongguo Yousheng Yu Yichuan Zazhi* 2002; **10**: 63-66
- 15 **Liu Z**, Xu D, Yan Y. The relationship of serum hepatitis B virus DNA load in HBsAg positive pregnant women to the intrauterine infection of newborns. *Zhonghua Fuchanke Zazhi* 1999; **34**: 133-134
- 16 **Wang SS**, Li MM, Peng GF, Li WL, Jin HL, Xiao H, Zeng NH, Wang ZB, Huang JL, Su JX. [HBV C gene mutation in the transmission from father to infant]. *Zhonghua Erke Zazhi* 2003; **41**: 845-848
- 17 **Zhang LY**, Fang ZS, Zhen Z, Liu XY, Gong XP, Wu YX. The study of transmission route of HBV from father to infant. *Zhonghua Chuanranbing Zazhi* 1994; **12**: 199-201
- 18 **Zhao LS**, Liu XS, Zhang ZX, Wang JR, Liu L, Lei BJ. Study on HBV vertical transmission via infected spermatozoa. *J Infect Dis* 1998; **16**: 154-157

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