

Mechanism of intrauterine infection of hepatitis B virus

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Abstract

AIM: To explore the possible mechanism of intrauterine infection of hepatitis B virus (HBV).

METHODS: HBV DNA was detected in vaginal secretion and amniotic fluid from 59 HBsAg-positive mothers and in venous blood of their newborns by PCR. HBsAg and HbCag in placenta were determined by ABC immunohistochemistry.

RESULTS: The rate of HBV intrauterine infection was 40.1% (24/59). HBV DNA was detected in 47.5% of amniotic fluid samples and 52.5% of vaginal secretion samples respectively. HBsAg and HbCag were detected in placentas from HBsAg-positive mothers. The concentration of the two antigens decreased from the mother's side to the fetus's side, in the following order: maternal decidual cells > trophoblastic cells > villous mesenchymal cells > villous capillary endothelial cells. However, in 4 placentas the distribution was in the reverse order. HBsAg and HbCag were detected in amniotic epithelial cells from 32 mothers.

CONCLUSION: The main route of HBV transmission from mother to fetus is transplacental, from the mother side of placenta to the fetus side. However, HBV intrauterine infection may take place through other routes.

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INTRODUCTION

Hepatitis B virus infection is a worldwide health problem. China is one of the high prevalent areas, with a positive rate of HBsAg in population more than 10%^[1]. Recent data indicate that the rate of intrauterine infection of HBV is 10%-44.4%^[2,3]. Intrauterine infection is one of important routes of HBV transmission, and the main cause of HBV chronic infection. To explore the possible mechanism of intrauterine infection, we detected HBV DNA in the vaginal secretion and amniotic fluid from HBsAg-positive mothers and venous blood from the neonates by PCR, and also detected the

distribution of HBsAg and HbCag in the placenta by ABC immunohistochemical method.

MATERIALS AND METHODS

Patients

Pregnant women who gave birth in the Hospital of Women's and Children's Health Care in Zhaoqing, Guangdong Province, China and their full-term newborns were recruited into this study. All the mothers received a regular prenatal examination in the clinic during pregnancy, and were detected for HBV serum markers (HBVM) by ELISA. Fifty-nine HBsAg-positive mothers and their newborns were studied, 10 HBsAg-negative mothers and their newborns served as control. All the mothers had no threatened abortion or related history, no pregnancy related complications. There was no difference in age, pregnant frequency of mother and gestational age of fetus between the two groups. Sixty-nine mothers gave birth to sixty-nine newborns.

HBV DNA detection

Vaginal secretion of mothers was taken before amnion rupture. After entering labor of the mother, at a proper time or just after amnion rupture, amniotic fluid was taken, and strictly prevented from blood contamination. After birth, 3 ml of neonatal venous blood was taken and separated for serum. All specimens were stored at -20 °C and HBV DNAs were detected simultaneously. PCR test kits were purchased from Hua Mei Biological Engineering Company. The tests were performed strictly according to the manufacturer's instructions.

Determination of HBsAg and HbCag in placenta

Placental tissues of 1 cm×1 cm×2 cm, were taken from the fetal side and the maternal side respectively, fixed in 10% formalin, embedded with paraffin according to routine procedure and sliced in 5 μm thickness. Rabbit McAb against HbCag and mouse McAb against HBsAg were used for immunohistochemical test and DAB staining kits were purchased from Wuhan BoShide Biological Engineering Ltd Company. HBsAg and HbCag positive livers from autopsy were used as positive control, and placentas from HBVM negative mothers served as negative control. At the same time, we used PBS instead of the first antibody as blank control. Dark brown yellow in cytoplasm or nucleus was regarded as strongly positive, brown yellow as positive, and light brown yellow as weakly positive.

Diagnosis of intrauterine infection

The presence of HBV DNA in neonatal venous blood was regarded as intrauterine infection.

RESULTS

HBV DNA status in amniotic fluid, vaginal secretion and neonatal venous blood

The positive rates of HBV DNA in amniotic fluid and vaginal secretions were 47.5% (28/59) and 55.9% (31/59) respectively. No HBV DNA was detected in amniotic fluid and vaginal secretion in control group. Of the 59 newborns born to mothers with HBsAg-positive, 24 were HBV DNA positive in neonatal venous blood, the rate of intrauterine infection was 40.1%

(24/59). No HBV DNA was detected in neonatal venous blood from newborns whose mothers were HBsAg-negative.

HBsAg and HBcAg in placenta

The positive rates of HBsAg and HBcAg in placentas from 59 mothers with HBsAg- positive were 81.4% (48/59) and 61.1% (36/59) respectively. HBsAg in placenta appeared as inhomogeneous dark brown yellow granules in plasma of all kinds of cells. It was 76.27% (45/59) in maternal decidual cells, 72.88% (43/59) in trophoblastic cells, 62.71% (37/59) in villous mesenchymal cells, 52.54% (31/59) in villous capillary endothelial cells, 54.24% (32/59) in amniotic epidermic cells. The positive coloration of HBsAg was seen in part of the villous interstices. The distribution of positive cells was patchy or conglomerate. The stained HBcAg in placentas was homogeneous granule, existing in nuclei of the positive cells which were distributed in focus or dispersion, mainly including 59.32% (35/59) of maternal decidual cells, 55.93% (33/59) of trophoblastic cells, 50.85% (30/59) of villous mesenchymal cells, 44.07% (26/59) of villous capillary endothelial cells, 49.15% (29/59) of amniotic epidermic cells. No HBsAg or HBcAg was detected in placentas of the control. The number of positive cells of HBsAg or HBcAg and the degree of staining were gradually decreased from maternal decidual cells to villous capillary endothelial cells.

On the contrary, the distribution of HBsAg- and HBcAg- stained cells in 4 placentas was gradually decreased from villous capillary endothelial cells to maternal decidual cells. The staining degree of HBsAg and HBcAg was also decreased in the same order.

DISCUSSION

Most researchers hold that the mechanism of HBV intrauterine infection is transplacental infection. In 1987, Lin detected 32 placentas of HBsAg and HBcAg positive mothers using PAP immunohistochemistry, and did not find HBsAg^[4]. Tang detected HBV DNA in placentas of induced labor from HBsAg- positive mothers using dot blot hybridization, and found HBV DNA in 2 cases^[5]. Lucifora detected 12 placentas of HBsAg carriers with no symptoms by immunohistochemistry and found HBsAg and HBcAg in villous capillary endothelial cells^[6,7]. Xu^[8] and Yan^[9] detected placentas from HBsAg- positive mothers by ABC immunohistochemistry and *in situ* hybridization and found HBsAg, HBcAg and HBV DNA in all kinds of placental cells. Wang *et al* detected 24 placentas of HBsAg and HBcAg positive mothers using *in situ* hybridization and found HBV DNA was mainly distributed in maternal decidual cells, while no HBV DNA- positive cells were in the villi^[10]. The results above were different obviously. In the present study, by using PCR for the determination of intrauterine HBV infection and ABC immunohistochemistry for the detection of the presence of HBsAg and HBcAg in placenta, we detected 59 placentas of HBsAg- positive mothers and found the positive rates of HBsAg and HBcAg were 81.4% (48/59) and 61.1% (36/59) respectively. The detection rate of HBsAg and HBcAg, the proportion of positive cells and the degree of staining were gradually decreased from the maternal side to the fetus side of placenta (decidual cells > trophoblastic cells > villous mesenchymal cells > villous capillary endothelial cells). The villous capillary endothelial cells were infected by HBV in 31 mothers, from whom 22 newborns had HBV intrauterine infection. These results indicated that HBV could infect all kinds of cells in placenta, which was the possible mechanism of intrauterine infection that HBV infected cells from maternal decidua to villous capillary endothelia or that HBV infected trophoblastic cells directly, then to villous mesenchymal cells

and villous capillary endothelial cells resulting in fetus infection.

In our study, the number of HBsAg- and HBcAg- positive cells was gradually decreased from villous capillary endothelial cells to maternal decidual cells in 4 placentas. The degree of staining was decreased in the same order from the fetus side to the mother side of placenta. HBV DNA was positive in 2 of the venous blood samples. This indicated that HBV infected the fetus first, and then infected cells in different layers of placenta. In these 4 cases HBsAg and HBcAg were detected in amniotic epidermic cells, HBV DNA in amniotic fluid and vaginal secretion was also detected, suggesting that the ascending infection from vagina might exist, that is to say, HBV in vaginal secretion infected fetal membrane, amniotic fluid, fetus and cells of different layers in placenta or HBV infected fetal membrane first then infected cells in different layers of placenta from the fetus side to the mother's side.

From the 1980's, researchers all over the world have proved that HBV DNA was existent in all generations of spermatogenic cells and sperms in HBV- infected males. Researchers studied male HBV carriers whose wives were not infected with HBV, and their fetuses. The results of HBV DNA sequencing showed that the homology between the father and his son or daughter was 98%-100%. Some researchers found HBsAg in follicular fluid of HBsAg- positive women by immunohistochemistry. Still others found HBV DNA in ovary from a woman who died of severe hepatitis using *in situ* hybridization. HBV DNA was mainly in plasma of ovum and interstitial cells. Now that human oocytes can be infected by HBV, the possibility of HBV transmission through oocytes may exist. In our study, although the fetus HBV infection through oocyte has not been proved in the 2 cases, we could not exclude the possibility.

In conclusion, intrauterine HBV infection is mainly transmitted through the placenta from the maternal blood to the fetus. HBV infection through vagina or oocytes may exist.

REFERENCES

- 1 **Xu DZ**. The epidemic state of hepatitis B virus in present. *Zhonghua Linchuang Yisheng* 2002; **30**: 2-3
- 2 **Ruff TA**, Gertig DM, Otto BF, Gust ID, Sutanto A, Soewarso TI, Kandun N, Marschner IC, Maynard JE. Lombok Hepatitis B Model Immunization Project: toward universal infant hepatitis B immunization in Indonesia. *J Infect Dis* 1995; **171**: 290-296
- 3 **Zhang SL**, Han XB, Yue YF. Relationship Between HBV viremia level of pregnant women and intrauterine infection: nested PCR for detection of HBV DNA. *World J Gastroenterol* 1998; **4**: 61-63
- 4 **Lin HH**, Lee TY, Chen DS, Sung JL, Ohto H, Etoh T, Kawana T, Mizuno M. Transplacental leakage of HBeAg- positive maternal blood as the most likely route in causing intrauterine infection with hepatitis B virus. *J Pediatr* 1987; **111**(6 Pt 1): 877-881
- 5 **Tang SX**, Yu GL, Cheng SY. Study on the mechanism and effected factors of HBV intrauterine infection. *Zhonghua Liuxingbingxue Zazhi* 1991; **12**: 325-327
- 6 **Lucifora G**, Martinez F, Calabro S, Carroccio G, Brigandi A, de Pasquale R. HBcAg identification in the placental cytotypes of symptom-free HBsAg-carrier mother: a study with the immunoperoxidase method. *Am J Obstet Gynecol* 1990; **163**(1 Pt 1): 235-239
- 7 **Lucifora G**, Calabro S, Carroccio G, Brigandi A. Immunocytochemical HBsAg evidence in placentas of asymptomatic carrier mothers. *Am J Obstet Gynecol* 1988; **159**: 839-842
- 8 **Xu DZ**, Yan YP, Zou S, Choi BC, Wang S, Liu P, Bai G, Wang X. Role of placental tissues in the intrauterine transmission of hepatitis B virus. *Am J Obstet Gynecol* 2001; **185**: 981-987
- 9 **Yan YP**, Xu DZ, Wang WL, Liu B, Liu ZH, Men K, Zhang JX, Xu JQ. The HBV infection of HBsAg positive women with different pregnant periods placentas. *Zhonghua Yixue Zazhi* 1998; **78**: 76-77
- 10 **Wang FS**, Li ZL, Zhang Y, Xu DZ, Wang CJ. The detection and significance of HBV DNA in PBM and placental tissue of HBVM- positive mothers. *Zhongguo Yishi Zazhi* 1999; **1**: 31-32