Relationship between HBV viremia level of pregnant women and intrauterine infection: neated PCR for detection of HBV DNA

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Abstract

AIM To determine the incidence of hepatitis B virus (HBV) in trauterine infection and to explore the relationship between HBV viremia level of pregnant women and HBV intrauterine infection.

METHODS Sixty-nine pregnant women were divided into three groups. Group A, 41 HBsAg positive patients, 14 of them were HBeAg positive (group A1), and 27 HBeAg negative (group A2); Group B, 12 HBsAg negative patients, but positive for anti-HBs and/or anti-HBe and/or anti-HBc; and Group C, 16 patients negative for all HBV markers. Blood samples of mothers were taken at delivery, samples of their infants were collected within 24 hours after birth (before injection of HBIG and HBV vaccine). All the serum samples were stored at - 20℃. HBV serum markers were tested by radioimmunoassay and HBV NDA were detected by nested polymerase chain reaction.

RESULTS In group C, all of 16 newborns were negative for HBsAg and HBV DNA. In group A, 7 infants were HBsAg positive (17.1%), and 17 (41.5%) were HBV DNA positive ($P < 0.05$). The incidence of intrauterine HBV infection was much higher in group A1 than that in group A2 (HBsAg 42.9% *vs* 3.7%, HBV DNA 92.9% *vs* 14.8%, $P < 0.05$). The incidence of HBV intrauterine infection was significantly different between high and low HBV viremia of mothers (93.3% *vs* 42.9%, $P<0.05$).

CONCLUSION The incidence of HBV intrauterine infection is high when HBV DNA in newborns detected with nested PCR is used as a marker of HBV infection. It is related to HBV viremia level of mothers.

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INTRODUCTION

The incidence of chronic HBV infection is high in China, more than 120 million people in China are carriers of HBV, 40% to 60% of them catch HBV infection from their mothers. So the key strategy for controlling HBV infection in China is to prevent HBV transmission from mother to infant. Transmission from mother to infant takes place in uterine, during delivery, and after birth. Vaccination after birth is of efficacy in preventing infant from HBV infection during delivery and after birth, but it can not interrupt HBV intrauterine infection. Previous studies showed that the HBV intrauterine infection rate was low (2.1% - 8.0%). However, recent investigations indicate that the rate is as high as 35% - 50%, indicating that intrauterine infection is the main route for HBV transmission from mother to infant[1-3]. We detected HBV DNA in the sera of newborns to determine HBV intrauterine infection rate and to explore its relation to HBV viremia level of mothers.

SUBJECTS AND METHODS

Subjects

Sixty-nine pregnant women and their newborns were investigated. All pregnant women were confirmed to be HBsAg positive by solid phase radioimmunoassay (spRIA), followed up and delivered at our hospital. They were divided into 3 groups. Group A, 41 cases positive for HBsAg, among them 14 were HBeAg positive (group A1) and 27 HBeAg negative (group A2). Group B, 12 cases negative for HBsAg but positive for anti-HBs and/or anti-HBe and/or anti-HBc (independent or combinant presence). Group C, 16 cases negative for all HBV markers.

None of the pregnant women had histories of hepatitis, symptoms and signs of hepatitis, threatened abortion, threatened premature delivery, and edema-hypertension-proteinuria syndrome. There was no significant difference in age, week of pregnancy at delivery, gravidity and parity among the three groups.

Methods

Blood samples of gravida were collected at delivery and the samples of newborns were taken within 24 hours after birth (before injection of hepatitis B vaccine and hepatitis B immunoglobulin). All serum

samples were stored at -20℃.

Serum HBV DNA was tested by nested polymerase chain reaction. Primers were designed according to the S region of HBV genome, synthesized by the Shanghai Institute of Cellular Biology. The sequences of primers are shown in Table 1.

Table 1 Sequences of primers

Number	Position	Sequence (5' - 3')
1	300-321	CATCTTCTTGTTGGTTCTTCTG
2	715-695	TTAGGGTTTAAATGTATACCC
3	421-441	TCTATGTTTCCCTCTTGTTGC
4	626-605	ACCACATCATCCATATATCTG

*1, 2 outer primers; 3, 4 inner primers

The product of first amplification was electrophoresed on 1.8% agarose gel, appearance of 416bp band was considered as strong positive for HBV DNA. If first amplification is negative, the product was used as plate for second amplification. The product of second PCR was electrophoresed on agarose gel, appearance of 206bp band was considered as weak positive for HBV DNA. Each sample was examined twice, there were positive, negative, and blank controls in each test.

Serum HBV markers were tested with solid phase radioimmunoassay (kits from 3V Company).

$X2$ test and direct calculation of probability on fourbolt data were used for statistical analysis.

RESULTS

Sixteen mothers in group C were negative for serum HBV DNA and their newborns were all negative for HBsAg and HBV DNA. The positive rates of HBsAg and HBV DNA in groups A and B are shown in Table 2.

In group A, the intrauterine infection rate was 17.1% (7/41) when HBsAg was used as a marker of intrauterine infection, the rate was high up to 41.5% (17/41) when HBV DNA was used, the difference was significant, $P < 0.05$. The HBV intrauterine infection was closely related to the mothers' HBeAg status (Table 2).

Table 2 Detection rate of HBsAg and HBV DNA

Group	Cases	Mother	Newborn	
		HBV DNA+ (%)	HBV DNA+ (%)	HBsAg+ (%)
A1	14	14 (100.0)	13 (92.9)	6 (42.9)
A2	27	7(25.9)	4 (14.8)	1 (3.7)
B	12	1 (8.3)	0	0

HBV intrauterine infection was related to mothers' status and levels of serum HBV DNA (Tables 3 and 4).

Table 3 Relationship between HBV intrauterine infection and mothers' HBV DNA status

Mothers' HBV DNA	No. of neonates	HBV DNA+	Intrauterine infection rate (%)
+	22	17	77.2
-	31	0	0

[b]$P<0.01$ *vs* mothers' HBV DNA positive

Table 4 Relationship between HBV intrauterine infection and mothers' serum HBV DNA levels

Mothers' HBV DNA level	No. of neonates	HBV DNA+	Intrauterine infection rate (%)
Strong positive	15	14	93.3
Weak positive	7	3	42.9

[a]$P<0.05$ *vs* mother HBV DNA weak positive.

DISCUSSION

Nested polymerase chain reaction (n-PCR) for HBV DNA detection is a sensitive and specific method for determining HBV intrauterine infection. The incidence of HBV intrauterine infection reported by different researchers is greatly discrepant, ranging from 2.1% to 50%, due to different sensitivities of methods used[3]. Yi *et al* detected HBV antigen in the liver of artificially aborted fetus with immunohistochemical assay and immuno-electromicroscopy, 43.75% of fetus from HBsAg positive pregnant women were infected with HBV[4]. Tang *et al* found that the HBV intrauterine infection rate was 44.4% (12/27) when HBV DAN in liver of fetus from HBsAg positive mothers was detected with Southern Blot, but the rate was only 18.5% when HBV antigen in fetal liver was detected by immunohistochemical method[5]. In this study, the HBV intrauterine infection rate was 17.1% when HBsAg in newborn serum detected by spRIA was used as a marker for diagnosis of intrauterine infection, but the rate was 41.5% when HBV DNA in newborn serum detected with n-PCR was used as a marker for diagnosis ($P < 0.05$). The results indicated that n-PCR for detecting HBV DNA in serum of newborn was a sensitive and specific method for diagnosis of HBV intrauterine infection. The sensitivity of spRIA for HBsAg was at ng level, and tissues and cells of fetus were not mature, resulting in low expression level of HBV antigen. So detection of HBsAg in newborn serum underestimated the incidence of HBV intrauterine infection[5]. The sensitivity of PCR for HBV DNA detection ranged from 10 fg/ml to l ag/ml. Nested PCR prevented the "plateau" of one-time amplification, and increased the sensitivity and specificity by changing the primers and plates[6]. Nested PCR for detection of serum HBV DNA in newborn had a more practical value than the methods used by Yi[4] and Tang[5], it can be used in clinical research into the mechanism of HBV intrauterine infection and in evaluation of the effect of methods for interrupting HBV intrauterine infection.

There is a closely positive correlation between the level of HBV viremia in mother and HBV intrauterine infection. HBeAg is a serum marker indicating HBV active replication. It has been reported that the HBV infection risk of infants from HBeAg positive mothers is 80% - 90%[1,7]. In our study all 14 HBeAg positive mothers had HBV DNA in serum, the HBV DNA detection rate of their newborns was as high as 92.9% (13/14). However, only 25.9% of HBeAg negative mothers were positive for HBV DNA, 14.8% (4/27) of their newborns were positive for HBV DNA in serum. These results show that the risk of HBV intrauterine infection is much higher in HBeAg positive mothers than that in negative ones ($P<0.05$).

The presence of HBV DNA is a direct marker of HBV active replication. The incidence of HBV intrauterine infection was much higher in newborns of mothers with HBV DNA than that of mothers without HBV DNA (77.3% *vs* 0, $P < 0.01$), and that the incidence was higher in newborns of mothers with high level of serum HBV DNA (strong positive for HBV DNA) than that of mothers with low level (weak positive for HBV DNA) of HBV DNA (93.3% *vs* 42.9%, $P<0.05$). These results confirmed that HBV intrauterine infection was positively related to the level of HBV replication in the mothers.

In conclusion, the incidence of HBV intrauterine infection is high, and it is positively related to the level of HBV replication in mothers. In order to control the epidemic of hepatitis B in China, it is important to explore the mechanism of HBV intrauterine infection and to develop effective methods for interrupting intrauterine infection. We have finished a prospective control trial, confirming that multiple injections of hepatitis B immunoglobulin during pregnancy can prevent fetus from HBV infection effectively (unpublished data).

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