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• *BRIEF REPORTS* •

Transformation of hepatitis B serologic markers in babies born to hepatitis B surface antigen positive mothers

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Abstract

AIM: To better understand the clinical significance of hepatitis B serologic markers in babies born to hepatitis B surface antigen (HBsAg) positive mothers, the incidence of maternal serologic markers of hepatitis B via placenta and its transformation in these babies were investigated.

METHODS: Mothers with positive HBsAg were selected in the third trimester of pregnancy. Their babies received immunoprophylaxis with hepatitis B immunoglobulin and hepatitis B vaccine after birth, and were consecutively followed up for hepatitis B serologic markers and HBV DNA at birth, mo 1, 4, 7, 12, and 24.

RESULTS: Forty-two babies entered the study, including 16 born to hepatitis B e antigen (HBeAg)-positive HBsAg carrier mothers and 26 to HBeAg-negative HBsAg carrier mothers. Apart from four babies born to HBeAg-positive carrier mothers and demonstrated persistent positive HBeAg eventually became HBV carriers, all other babies developed anti-HBs before 12 mo of age. Among the other 12 babies born to HBeAg-positive carrier mothers, HBeAg was detected in 7 at birth, in 4 at mo 1, and in none of them thereafter. No antibody response to the transplacental HBeAg was detected. Among the babies born to HBeAg-negative carrier mothers, anti-HBe was detected 100% at birth and mo 1, in 88.5% at mo 4, in 46.2% at mo 7, in 4.2% at mo 12 and none in mo 24. Among all the immunoprophylaxis-protected babies born to either HBeAg-positive or HBeAg-negative carrier mothers, anti-HBc was detected in 100% at birth, mo 1 and mo 4, in 78.9% at mo 7, in 36.1% at mo 12 and in none at mo 24.

CONCLUSION: HBeAg can pass through human placenta from mother to fetus and become undetectable before 4 mo of age, but no antibodies response to the transplacental HBeAg can be detected till mo 24 in the immunoprophylaxis-protected babies. The sole existence of anti-HBe before 1 year of age or anti-HBc before 2 years of age in babies born to HBsAg carrier mothers may simply represent the transplacental maternal antibodies, instead of indicators of HBV infection status.

Key words: Hepatitis B e antigen; Hepatitis B e antibody; Hepatitis B; Chronic; Maternal-infantile transmission; Hepatitis B surface antigen; Children

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INTRODUCTION

Hepatitis B virus (HBV) infection is of major public health importance worldwide. Globally, there are more than 350 million chronic carriers of HBV who are at high risk of developing severe sequelae, such as end-stage cirrhosis and hepatocellular carcinoma[1]. In highly endemic areas, such as China, mother-to-child transmission of HBV plays an important role in keeping the high prevalence of the carrier status[2]. The vast majority of untreated infants born to hepatitis B e antigen (HBeAg) positive mothers become infected and leads to chronicity. However, infants born to HBeAg-negative hepatitis B surface antigen (HBsAg)-positive carrier mothers are likely to develop acute hepatitis but less frequently progress to chronicity[3]. Since the introduction of hepatitis B vaccine in the early 1980s, passive-active immunoprophylaxis with hepatitis B immunoglobulin (HBIG) and hepatitis B vaccine has been proved to be highly effective in preventing perinatal transmission of HBV infection. Nevertheless, a small proportion of the children born to HBV carrier mothers, especially that with HBeAg positive, still become HBsAg carriers despite receiving passive-active immunoprophylaxis[4-6].

Detection of hepatitis B serologic markers is fundamental to the judgment of HBV infection status and prognosis of an individual[7]. It has long been known that the antibodies to the variety HBV antigens can pass through placenta from

HBV infected mother to babies. It is also suggested that HBeAg, because of its small size, may transverse the placenta and elicit HBe/HBcAg-specific T helper cell tolerance *in utero*[8]. Although the ability of HBeAg to cross the murine placenta recently has been questioned, significant evidence has documented that HBeAg could infect the fetus via human placenta[9,10]. However, little is known about how long the transplacental HBV markers could persist in the babies, and whether the transplacental HBeAg could induce an antibody response in human infants has never been investigated[10,11]. To answer these questions, a consecutive follow-up observation was done in babies who were born to HBsAg carrier mothers.

MATERIALS AND METHODS

Subjects

From January 2000 to December 2001, all pregnant women who received regular antenatal examinations at Zhongshan Hospital (affiliated teaching hospital of Fudan University) or Shanghai No. 9 People's Hospital were screened for HBsAg in the third trimester of pregnancy. If the test was positive, HBsAg was redetected and other HBV markers including HBeAg, and serum alanine transaminase (ALT) level were examined simultaneously before delivery. Their babies received two doses of 200 IU of HBIG (Shanghai Institute of Biological Products, Shanghai, China) intramuscularly within 24 h after birth and at d 15. Then these babies were inoculated with three doses of 10 μg of recombinant yeast-derived hepatitis B vaccine (manufactured by SmithKline Beecham, packaged by Shanghai Institute of Biological Products, Shanghai, China) at mo 1, 2, and 7. Those babies were followed up in a specific clinic in our hospital. Consecutive serum samples from these babies were collected by inguinal vein puncture at birth, and at mo 1, 4, 7, 12, and 24 and were kept at -40 ℃ until determination. The research protocol was approved by the Ethical Committee of our hospital and informed consents were obtained from the parents of all babies before delivery.

Babies satisfying the following criteria were enrolled in this study. Firstly, their mothers were HBsAg positive at two occasions, with a normal serum level of ALT and without any symptoms or signs of hepatitis. Secondly, the newborn's gestational week was greater than 37 and less than 42. The babies with an obvious abnormality, or birth weight less than 2 500 g, or the Apgar scores less than 8 at 1 or 5 min after birth were excluded.

Laboratory methods

Hepatitis B serologic markers (HBsAg, anti-HBs, HBeAg, anti-HBe and IgG anti-HBc) were determined by using commercial AxSYM system (AxSYM HBSAG 2.0, AUSAB 2.0, HBE 2.0, ANTI-HBE 2.0, CORE, Abbott Laboratories, Chicago, IL, USA) according to the manufacturer's instructions. HBV-DNA was determined by semi-nested PCR[12]. In brief, 5 μL of DNA extracted from the serum was added to the following amplification mixture: 5 μL of Taq polymerase buffer, 1 μL of 10 mmol/L deoxyribonucleotide triphosphate, 1.5 units of Taq (SABC, China), 10 pmoL of sense (HBMF1: 5'-YCCTGCTGGTGGCTCCAGTTC-3') and antisense primers (HBMR2: 5'-AAGCCANACARTGGGGGAAAGC-3') in a 50 μL reaction volume. The amplification profile was 6 min at 96 ℃, followed by 25 cycles at 94 ℃ for 45 s (denaturation), 45 s at 60 ℃ (annealing) and 45 s at 72 ℃ (extension), and then extended for 5 min. The reaction was performed in a 60-well cycler (PTC150, MJ Research, MA, USA). Five microliters of the first-round PCR product was then added to a second-round PCR mixture with the same composition but with a different inner sense primer (HBMF2: 5'-GTCTAGACTCGTGGTGGACTTCTCTC-3'). Ten microliters of the second-round PCR product was then analyzed by electrophoresis in 2% agarose gels, stained with ethidium bromide, and visualized under ultraviolet light. A band at 485 bp was judged as HBV-DNA positive.

RESULTS

Basic information

Forty-two HBsAg carrier mothers, including 16 who were HBeAg and anti-HBc positive and 26 who were HBeAg negative, but anti-HBe and anti-HBc positive were enrolled with their babies (male 20, female 22). Forty babies were followed up to 12 mo of age, and 37 were followed up to 24 mo of age. The reasons for dropout are mainly as follows: fear of puncture, long distance from hospital or move abroad. Four babies became HBsAg carriers despite the passive-active immunoprophylaxis. All other babies developed anti-HBs response till 12 mo of age. All four carrier babies were born to HBeAg-positive carrier mothers, none of the babies born to HBeAg-negative carrier mothers were found HBsAg or HBV-DNA positive during the follow-up period, yielding an immunoprophylaxis failure rate of 25% in babies born to HBeAg-positive carrier mothers comparing to zero in babies born to HBeAg-negative carrier mothers.

Transformation of HBV markers in immunoprophylaxis failure babies

Two of the immunoprophylaxis failure babies were found HBsAg and HBV-DNA positive at birth, which may indicate an *in utero* infection. Other two immunoprophylaxis failure babies were HBsAg and HBV-DNA negative at birth, but one of them was found HBsAg and HBV-DNA positive since mo 1, another was found HBsAg and HBV-DNA positive since mo 12 and subsequently. All the four immunoprophylaxis failure babies were HBeAg and anti-HBc positive, anti-HBs and anti-HBe negative persistently at birth and thereafter.

Transformation of HBV markers in immunoprophylaxis protected babies

HBeAg positivity Among the 12 babies born to HBeAg-positive carrier mothers and who had been successfully immunized, HBeAg was detected in 7 at birth. Four of them remained positive at mo 1, but none of them detected positive thereafter. It is different from the four babies who became carriers, in whom the HBeAg was positive throughout the follow-up period. No HBeAg had been detected in the 26 babies born to HBeAg-negative carrier

mothers.

Anti-HBe positivity Anti-HBe was detected in 100% (26/26) of the babies born to HBeAg-negative and anti-HBe positive carrier mothers at birth and mo 1, in 88.5% (23/26) at mo 4, in 46.2% (12/26) at mo 7, in 4.2% (1/24) at mo 12, and none in mo 24. It was detected in none of the 16 babies born to HBeAg-positive carrier mothers in the whole follow-up period.

Anti-HBc positivity The anti-HBc is persistently positive since birth in the four babies who became HBsAg carriers. In other 38 babies, anti-HBc was detected in 100% at birth, mo 1 and mo 4, in 78.9% (30/38) babies at mo 7, in 36.1% (13/36) babies at mo 12, and the anti-HBc become undetectable in all of them at mo 24.

HBV-DNA positivity

HBV-DNA was only detected in the four immunoprophylaxis failure babies. Two of them were positive since birth, one since mo 1, and another since mo 12. It was at the same time when the positive HBsAg was detected. HBV-DNA was negative in all immunoprophylaxis protected babies.

DISCUSSION

HBV infection in early life often results in chronicity[13]. The infection can be persistent even life-long. It has been estimated that 25% of them will die from HBV-related hepatocellular carcinoma or end-stage cirrhosis in future[1]. Hepatitis B vaccine is a hallmark in preventing the transmission of HBV. It has been demonstrated that universal vaccination also had decreased the incidence of children hepatocellular carcinoma[14,15]. Unfortunately, there are still a small proportion of the babies born to HBsAg carrier mothers become infected despite receiving passive-active immunoprophylaxis[4-6]. In the present study, with passive-active immunoprophylaxis, 24 babies born to HBeAg negative HBsAg positive mothers were protected from HBV infection. However, 4 of 16 babies born to HBeAg-positive HBsAg carrier mothers still became persistently infected with HBV. Two of them had positive HBsAg and HBV-DNA since birth, indicating that the babies were infected *in utero* (antenatal transmission). This result coordinates with our previous publications and others that intrauterine infection is the main cause of the failure of immunoprophylaxis to interrupt the mother-to-babies transmission of HBV[6,16]. No vaccination strategy (active alone, or passive-active) until now could prevent this kind of transmission. Some of the fetuses that have contacted HBV antigens early in embryonic development become immunologically tolerant to HBV antigens. Hence, HBV cannot be effectively eliminated, leading to chronic HBV infection.

The mechanism of immunological tolerance may be influenced by different HBV markers. It has been well documented that babies born to mothers who are carriers of HBV and who express HBeAg are more likely to become persistently infected than babies born to HBeAg-negative carriers[3,4,8]. In the present study, all the four persistently infected babies were born to HBeAg-positive hepatitis B carriers. HBsAg does not usually cross the placenta. However, there have been suggestions that maternal HBeAg could pass through placenta from mother to fetus and induce T-cell tolerance *in utero*[3,8]. Although one group[17,18] had reported that HBeAg cannot pass the murine placenta efficiently in H-2b mice, others and us[9-11] had proved that HBeAg can indeed cross the human placenta from mother to fetus. Our present study further showed that the transplacental HBeAg can still be detected at 1 mo of age at about 33.3% of babies (4/12) born to HBeAg-positive carrier mothers, but it would disappear before 4 mo of age in uninfected babies. The babies with HBeAg positive persistently over 4 mo of age always were accompanied by HBV infection breakthrough. Because HBeAg and HBcAg are highly cross-reactive in terms of T-helper cell recognition, the exposure to HBeAg in uterus may lead to fetal immunotolerance not only to HBeAg but also to HBcAg[3,19]. The HBV markers should be further followed up in babies who are HBeAg positive beyond 4 mo of age, because of the possibility of HBV infection breakthrough.

Anti-HBe and anti-HBc are also important markers to judge HBV infection status. Although it has been well known that these antibodies can transverse human placenta from mother to fetus, little knowledge is available about how long these antibodies persist and if antibody response to the transplacental HBeAg can be detected in the successfully immunized babies. In the present study, we demonstrated that transplacental anti-HBe disappeared in nearly all babies (95.8%) before 12 mo of age, and no antibody response to transplacental HBeAg was detected in the immunoprophylaxis protected babies born to HBeAg-positive HBsAg carrier mothers. Positive transplacental anti-HBc can last longer time than anti-HBe in the babies born to HBsAg carrier mothers. It can still be detected in about one-third of the babies at 12 mo of age, but disappeared before 24 mo of age. Therefore, the sole existence of anti-HBe before 1 year of age and/or anti-HBc before 2 year of age not along with positive HBsAg in babies born to hepatitis B carrier mothers may simply represent the transplacental maternal antibodies to the virus, and may not be indicators that babies has experienced an infection of HBV actively or previously. However, if sole anti-HBc is detected in babies over 2 years of age, it could be an indicator of past infection. Other researchers reported that the existence or high level of transplacental maternal anti-HBc may correlate significantly with the outcome of *in utero* HBV infection[11,20]. In the present study, transplacental anti-HBc can be detected in all babies born to HBsAg carrier mothers before 4 mo of age. Therefore, no significant correlation can be discovered between the absence of maternal anti-HBc and immunoprophylaxis failure.

Above all, our present study suggests that the maternal HBeAg can transverse the human placenta from mother to fetus, but it will disappear before 4 mo of age in the babies born to HBeAg-positive carrier mothers. The sole existence of anti-HBe before 1 year of age or anti-HBc before 2 year of age in babies born to HBsAg carrier mothers may simply represent the transplacental maternal antibodies, instead of indicators of HBV infection status. Exposure of the immature immune system in uterus and early life to transplacental HBeAg might have induced immunotolerance, so that no antibodies response to HBeAg could be detected.

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