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Amniocentesis in mothers who are hepatitis B virus carriers does not expose the infant to an increased risk of hepatitis B virus infection

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Abstract. Sixty-seven pairs of mothers with hepatitis B virus (HBV) surface antigen (HBsAg) and their infants were divided into two study groups to determine the effect of amniocentesis on intrauterine HBV infection. In the first study group (35 pairs), the infant's HBsAg status in cord blood was studied and the results were compared with those obtained in the cord blood from 65 infants born to HBsAg-positive women who did not have an amniocentesis. In the second study group (32 pairs), the HBV status of the infants was studied at the age of three months to five years and compared with the HBV status of 3,454 infants in the National HBV Prevention Program. In the first study group, one sample (2.9%) was weakly positive for HBsAg; while in the first control group, two (3.1%) were positive. In the second study group, three (10%) infants were positive for HBsAg. The failure rates of immunoprophylaxis in the second study and control groups were similar (9.4% vs 11% for HBsAg carrier mothers; 30% vs 14% for HBe antigen-positive carrier mothers). This suggested that genetic amniocentesis did not increase the risk of intrauterine HBV infection.

Key words: Hepatitis B virus – Genetic amniocentesis – Intrauterine infection

Introduction

It is estimated that more than 200 million people are carriers of hepatitis B surface antigen (HBsAg). Chronic carriage of HBsAg has been shown to be closely

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associated with liver cirrhosis, hepatocellular carcinoma and other chronic liver diseases (Chen and Sung 1978; Beasley et al. 1981). The earlier in life the infection occurs, the more likely is the chronic carrier state. In Taiwan, about 15 to 20 percent of pregnant women are carriers of HBsAg and perinatal mother-to-child infection occurs frequently. About 40 percent of the infants of HBsAg-carrier mothers becomes HBsAg-carriers in early life (Ko et al. 1986; Stevens et al. 1979).

To prevent perinatal transmission of the disease, a National Hepatitis B Virus (HBV) Prevention Program in Taiwan using HBV vaccine with or without hepatitis B immune globulin (HBIG) has been carried out and shown to be highly effective (Chen et al. 1987; Hsu et al. 1988). However, a small fraction of the infants receiving immunoprophylaxis become chronic carriers. Intrauterine infection with HBV has been proposed as an important cause of immunoprophylaxis failure (Beasley et al. 1983a and 1983b; Hsu et al. 1988; Lin et al. 1987).

Second-trimester genetic amniocentesis is widely used. The procedure may cause uterine or placental bleeding and may lead to blood exchange between the mother and her fetus (Mennuti et al. 1980). This study was intended to address the question of whether the fetus of an HBsAg-carrier has an increased risk of intrauterine infection with HBV.

Materials and methods

A total of 67 HBsAg-carrier women and their progeny were used for this study. All women had no antibody to HBsAg (anti-HBs). The infants were divided into two study groups. In the first study group, the infant's HBV status was evaluated on cord blood; in the second group (32 infants), the infant's HBV status was studied at the age of three months to five years. Nineteen (28.4%) women, nine in the first group and ten in the second group, were positive for hepatitis B e antigen (HBeAg). Two control groups were used. The first control group consisted of 65 cord blood samples from infants born to carriers who had not had an amniocentesis. The second control group comprised 3,454 infants from the National HBV Prevention Program.

Gestational age of amniocentesis

Gestational age was calculated from the last menstrual period and confirmed or corrected by ultrasound biometry. The mean gestational age $\pm$ SD at amniocentesis was 17.8 ± 2.5 (range 15–28) weeks in the first group and 17.3 ± 2.5 (range 14–27) weeks in the second group. Most of the amniocenteses were performed between 16–19 weeks, the predominant indication being maternal age.

Amniocentesis

Before amniocentesis, 3–5 ml of maternal blood was drawn by venepuncture for study of HBV status. Amniocentesis was done with a No. 22 needle under continuous ultrasound guidance. Care was taken to avoid puncturing the placenta. If it was the anterior, the thinnest portion was chosen for tapping. In all cases, only one tap was sufficient to obtain 20–40 ml of amniotic fluid. Bleeding into the amniotic cavity from the uterine wall or placenta could be seen after the tap in some but always stopped spontaneously within two to three minutes. In the first study group, the placenta was punctured in 11 (31.4%) cases and post-tap bleeding was noted in three; in the second study group, the placenta was punctured in nine (28.1%) cases and post-tap bleeding was noted in four.

Immunoprophylaxis

Within 24 hours of delivery, the infants born to HBeAg-positive carrier women received an intramuscular injection of 0.5 ml of HBIG (HyperHep, Cutter Laboratories, CA) and then an intramuscular or subcutaneous injection of 5 μg of plasma-derived hepatitis B vaccine (Hevac B, Institut Pasteur, France) at the age of one, five and nine weeks, with a booster at 12 months. For the infants born to HBeAg-negative carrier women, HBIG injection was omitted and only four doses of vaccine were given (Chen et al. 1987; Hsu et al. 1988).

Collection of cord, neonatal and infant blood

In the first study and control groups, 3–5 ml of cord blood was drawn for assay of HBV status. To avoid maternal blood contamination, the umbilical cord was first rinsed with normal saline, wiped clean with a piece of sterile gauze and then carefully sampled using a disposable syringe and needle. In ten study group infants, blood was collected by femoral or jugular vein puncture within four days of birth.

In the second study group, postnatal HBV status was studied at the age of three months to five years – 20 (62.5%) at three months, four (12.5%) at six months, and eight (25%) at 18 months to five years. In seven infants, neonatal blood specimens were also available for study.

Assay for HBV status

HBsAg, HBeAg and anti-HBs were measured with radioimmunoassay kits (Ausria-II, Abbott-HBe and Ausab respectively, Abbott Laboratories, North Chicago, IL.).

Results

In the first group, 34 (97.1%) of the 35 samples of cord blood and all of the ten samples of neonatal blood were negative for HBsAg; in the first control group, 63 (97.0%) of the 65 samples were negative for HBsAg. The difference in the HBsAg-positive rates between the two groups was statistically not significant. In the study group, one infant born to an HBeAg-positive carrier woman had weakly positive HBsAg in his cord blood. No neonatal blood of this infant was available for study, however, the infant developed anti-HBs after immunoprophylaxis.

In the second study group, 29 (90.6%) infants (7 born to HBeAg-positive women and 22 to HBeAg-negative carrier women), were HBsAg-negative during follow-up. The other three infants (9.4% of the whole group, or 30% of the HBeAg-positive carrier women), all born to HBeAg-positive mothers, were persistently positive for HBsAg and HBeAg at the age of 26 months, 30 months and 36 months respectively (Table 1). In two cases, the placenta was not punctured and post-tap bleeding was not seen; while in one case, the placenta was punctured but no post-tap bleeding was noted. Neonatal blood was available from two infants and both samples were positive for HBsAg. In the second control group from the National HBV Prevention Program, 11% (for the whole group) and 14% (for those born to HBeAg-positive women) of the infants failed to be protected by immunoprophylaxis. The differences in the immunoprophylaxis failure rate between the study and control groups (9.4% vs 11% for HBsAg-positive mothers; 30% vs 14% for HBeAg-positive carrier mothers [Fisher's exact probability ~ 2.5]) did not have statistical significance.

Table 1. The outcome for children whose mothers were HBsAg-positive and had genetic amniocentesis during pregnancy

	Maternal HBeAg	No. of children	No. with positive HBsAg (%)
Group 1	Positive	9	0[a] (0)
	Negative	26	0 (0)
	Subtotal	35	0[a] (0)
Group 2	Positive	10	3 (30)
	Negative	22	0 (0)
	Subtotal	32	3 (9.4)

[a] One child with weakly positive HBsAg was protected by immunoprophylaxis

Discussion

HBV is highly infectious. Susceptible subjects may be infected by exposure to the virus through accidental needle stick or ingestion (Bancroft et al. 1977; Beasley and Hwang 1983; Wong et al. 1980). With amniocentesis on HBsAg-positive mothers, the fetus may be exposed to HBV by two routes: (1) maternal-fetal blood exchange, (2) ingestion of HBV-contaminated amniotic fluid. Placental puncture inevitably injures some chorionic villi and results in admixture of maternal and fetal blood. Uterine wall puncture also causes fetal-maternal bleeding through capillaries in the fetal membranes. Such a fetal-maternal bleeding has been demonstrated (Mennuti et al. 1980). In addition to entering the fetal circulation, HBV may also contaminate the amniotic fluid which is ingested by the fetus.

It is generally accepted that fetuses infected in utero by HBV will have HBsAg in cord blood or become refractory to postnatal immunoprophylaxis against HBV (Beasley et al. 1983a and 1983b; Lin et al. 1987). However, HBsAg in cord blood may be due to maternal blood contamination and immunoprophylaxis failure may be caused by many factors. In this series, the HBsAg-positive rates in the study and control cord blood samples were similar. Only one cord blood sample in the study group was weakly positive for HBsAg. On follow-up, this infant was protected by immunoprophylaxis. The low level of HBsAg in this sample was therefore most likely due to maternal blood contamination during blood sampling.

Most of our amniocenteses were performed about four to five months before delivery. If intrauterine HBV infection had occurred at amniocentesis, the cord blood should have contained HBsAg.

The results in the second study group could be used to assess the value of immunoprophylaxis after possible intrauterine HBV infection. Three infants were not protected by immunoprophylaxis, two were delivered vaginally after an uneventful pregnancy and labor and the third was delivered by elective cesarean section. The mechanism of HBV infection in these infants was unclear. However, intrauterine HBV infection could occur in a natural setting and has been proposed as a major factor in immunoprophylaxis failure (Beasley et al. 1983a and 1983b; Lin et al. 1987). In unimmunized infants born to HBsAg-carrier mothers, as many as 85 to 90 percent of those from HBeAg-positive mothers and 10% from

HBeAg-negative carrier mothers will develop HBsAg by three months of age (Ko et al. 1986; Beasley et al. 1983 a). With immunoprophylaxis, most of these infants would be protected. Since we have already started the National HBV Prevention Program, it was unethical to have a control group with no immunoprophylaxis. Cases from the National HBV Prevention Program were therefore used as controls. The difference in the immunoprophylaxis failure rates between the two groups did not reach statistical significance. If genetic amniocentesis had increased the risk of intrauterine HBV infection, the failure rate in the second study group should have been higher than that in its control group.

The absence of increased intrauterine HBV infection after amniocentesis may be explained in part by the presumed hepatotropism of HBV. Recent research has demonstrated the existence of a receptor for polymerized human serum albumin (pHSA) on HBV particles. The receptor was found to be species-specific for human and chimpanzee albumins. There is also some evidence to show that similar albumin binding sites may also be present on the surface of hepatocytes (Imai et al. 1979; Neurath et al. 1986; Okamoto et al. 1986; Pontisso et al. 1983). Early in the second-trimester when most amniocenteses were done, the albumin binding sites of fetal hepatocytes may not exist or be mature enough; in addition, the concentration of serum pHSA may be very low because the abundant alpha-fetoprotein (an alpha 1 globulin) replaces some albumins (Cowchock 1976) and the existing albumins may not be old enough to form polymers. Under these conditions, the hepatotropism and the infectivity of HBV may be reduced. In a recent communication, London and O'Connell (1986) also proposed that maturation of the fetal liver is required before viral replication can take place. Further studies are necessary to test these hypotheses.

In summary, we have found that second-trimester genetic amniocentesis did not increase the risk of intrauterine fetal infection with HBV. Therefore maternal HBsAg carriage would not appear to be a contraindication to genetic amniocentesis.

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