EARLY INVASIVE PRENATAL DIAGNOSIS IN HBsAg-POSITIVE WOMEN

P. M. GROSHEIDE*, H. W. P. QUARTERO†, S. W. SCHALM*, R. A. HEIJTINK‡ AND G. C. M. L. CHRISTIAENS§

Departments of Internal Medicine* and Obstetrics and Gynecology†, Academic Hospital Dijkzigt and Department of Virology‡, Erasmus University, Rotterdam; §Department of Obstetrics and Gynaecology, Academic Hospital Utrecht, Utrecht, The Netherlands

SUMMARY

From 1982 to 1989, pregnant women in two large city hospitals in The Netherlands had serum samples screened for hepatitis B surface antigen (HBsAg). Infants of mothers found to be HBsAg-positive received hepatitis B immune globulin immediately after birth and hepatitis B vaccine in the first year of life. Blood samples of infants were regularly tested for HBsAg and antibodies directed against HBsAg. A retrospective analysis of the pregnancy outcome in HBsAg-positive women who had invasive tests for prenatal diagnosis was carried out to determine whether amniocentesis and chorionic villus sampling (CVS) are risk factors for the intrauterine transmission of the hepatitis B virus. Amniocentesis was carried out in 17 HBsAg-positive women and CVS in one case. Only two women were HBsAg- and HBeAg-positive. Prenatal diagnosis led to the termination of pregnancy for fetal chromosome abnormality in three cases. The remaining 15 pregnancies were uneventful; all infants were negative for HBsAg and developed an active immune response to the vaccine. These data suggest that amniocentesis in HBsAg-positive women constitutes a low risk for the intrauterine transmission of the hepatitis B virus, but definite conclusions in HBeAg-positive women cannot be drawn.

KEY WORDS—Hepatitis B, amniocentesis, chorionic villus sampling, prenatal diagnosis, intrauterine infection, neonate.

INTRODUCTION

With increasing technical capabilities for more and earlier prenatal diagnosis, obstetricians should question the potential risks of invasive tests in hepatitis B surface antigen (HBsAg)-positive women.

Without prophylactic measures, the risk of perinatal infection among infants born to mothers infected with the hepatitis B virus (HBV) ranges from 10 to 90 per cent, depending on each mother's hepatitis B e antigen (HBeAg) status

(Beasley *et al.*, 1977; Stevens *et al.*, 1979). Up to 90 per cent of infants infected by their mothers at birth develop chronic HBV infection (Stevens *et al.*, 1975).

Most HBV infections occur perinatally; infants are exposed to the virus during labour (Stevens *et al.*, 1975; Gerety and Schweitzer, 1977; Lee *et al.*, 1978). Presumably mother-to-infant transmission occurs from haematological leaks across the placenta and/or birth canal exposure to maternal secretions or blood (Beasley *et al.*, 1982; Lin *et al.*, 1987).

Early administration of hepatitis B immune globulin (HBIg) to the newborn infant is therefore important to protect the infant from perinatal HBV infection. Passive–active immunization, consisting of HBIg and hepatitis B vaccine has been very effective (>90 per cent) in preventing HBV infection among infants born to HBsAg-positive

mothers (Stevens *et al.*, 1987; Schalm *et al.*, 1989). However, some HBeAg-positive mothers infect their infants before labour and delivery, i.e. during premature labour or threatened abortion (Ohto *et al.*, 1987). These infants will not be amenable to immunoprophylaxis and any immunization programme is likely to fail. Hepatitis B virus is considered not to pass the intact placenta. Besides placental leakage due to uterine contractions, disruption of the placental barriers may also result from invasive tests such as chorionic villus sampling (CVS) and amniocentesis for early prenatal diagnosis of fetal genetic disease. The question arises as to whether health-care professionals should offer an HBsAg-positive woman prenatal genetic testing where there is a chance of infecting the infant with HBV *in utero*.

Because data in the literature are scarce, we retrospectively investigated the impact of early CVS and amniocentesis on intrauterine transmissions of HBV from HBsAg-positive women to their offspring.

PATIENTS AND METHODS

In 1982 a multicentre trial was started in The Netherlands to detect HBsAg carrier mothers and to immunize their offpsring (Mazel *et al.*, 1984). Obstetricians in four treatment centres were asked to screen all pregnant women for HBsAg at the first antenatal visit. All confirmed HBsAg-positive samples were tested for HBeAg. The serum samples were assayed by radioimmunoassay (Abbott Laboratories, Chicago, IL).

Infants of HBsAg-positive mothers who agreed to passive–active immunization of their newborns received HBIg (100–150 IU anti-HBs/ml or 300 IU anti-HBs/ml, Central Laboratory of the Netherlands Red Cross Blood Transfusion Service) at birth and active immunization according to several dose and time schedules. Cord blood and blood samples from infants were regularly tested for HBsAg (Ausria II, Abbott) and anti-HBs (Ausab, Abbott) until at least 12 months of age.

Three hundred and two HBsAg-positive women were identified and delivered over a 7-year period in the two university hospitals participating in the vaccination trial. Both hospitals act as centres for prenatal diagnosis. Amniocentesis or CVS is offered to women of advanced maternal age (≥ 36 years) or with a history of fetal chromosomal anomaly or neural tube defects.

At the start of the vaccination programme, the potential risks of transmitting the hepatitis B virus through transplacental leakage caused by invasive diagnostic procedures were unknown. Since no records were kept on invasive testing for prenatal diagnosis in HBsAg-positive pregnant women, we performed a retrospective study of the files of HBsAg-positive women who had their newborn infants immunized within the trial. If prenatal genetic testing had been performed, this was followed by data collection on the sampling procedure and pregnancy outcome. In addition, the HBV DNA levels of the HBsAg carrier mothers who had prenatal diagnosis performed were assayed quantitatively by HBV DNA assay (Abbott), since at present it is thought that the HBV DNA levels of the HBsAg carrier mothers are a better marker for infectivity than HBeAg (Lee *et al.*, 1986). Intrauterine HBV infection was defined as the occurrence of HBsAg in cord blood and in the subsequent blood samples of infants.

RESULTS

Invasive prenatal diagnosis was carried out in 18 of the 302 women found to be HBsAg-positive. Of 40 HBsAg-positive women aged 36 years or older, only six (15 per cent) had undergone prenatal diagnosis. In another two women aged over 36 years, prenatal diagnosis was not carried out because they were HBsAg-positive. In the other 12 women, prenatal diagnosis was performed for reasons other than maternal age. Of the 18 HBsAg-positive women, two were also HBeAg-positive. One HBeAg-positive woman had high levels of HBV DNA (61 pg/ml). The other HBeAg-positive woman had an acute HBV infection at the time that she underwent prenatal diagnosis but her blood sample was not available for additional HBV DNA testing. All other women were HBV DNA-negative.

Amniocentesis was performed in 17 cases and CVS in the remaining case. Indications for prenatal diagnosis, HBeAg status of the women, maternal and gestational age, and pregnancy outcome are presented in Table I. Amniocentesis was performed at 15·4–18 weeks' gestation; one woman, however, underwent amniocentesis at 30 weeks' gestation because multiple malformations were identified by ultrasound. CVS was performed at 10 weeks' gestation. No transplacental amniocentesis was performed and the liquor samples showed no

Table I—Characteristics of the HBsAg-positive women and the prenatal procedures carried out

	Amniocentesis*, $n=17$ (%)	Chorionic villus sampling, $n=1$
Indication		
Maternal age	6 (35·3)	—
Other	11 (64·7)	1
HBeAg status		
HBeAg-positive	2 (11·8)	—
HBeAg-negative	13 (76·4)	1
Unknown	2 (11·8)	—
Duration of gestation		
First trimester	—	1
Second trimester	16	—
Third trimester	1	—
Pregnancy outcome		
Termination	3 (17·6)	—
Liveborn	14 (82·4)	1

*Not transplacental.

signs of blood staining. The woman who had an acute hepatitis B infection and was both HBsAg- and HBeAg-positive at the time of amniocentesis displayed a low degree HBsAg-positive liquor (van Os *et al.*, 1991). In all other cases, amniotic fluid was not tested for HBsAg.

The pregnancy was terminated in three cases because of anencephaly (14·6 weeks) or multiple malformations (18·4 and 31 weeks). These women were all HBsAg-positive but HBeAg-negative. The remaining 15 pregnancies were uneventful and resulted in the delivery of 15 healthy infants. The mean gestational age for livebirths was 39·1 ± 2·3 weeks (range 32–42 weeks). The median birth weight was 3558 ± 722·6 g (range 1660–4920 g). None of the neonatal blood samples was HBsAg-positive (0/15; 95 per cent confidence interval 0–21·8). All infants were HBsAg-negative at the age of 3 months and thereafter. At the age of 12 months, they had developed protective levels of anti-HBs following active immunization.

DISCUSSION

We have presented the outcome of invasive prenatal diagnosis in 15 women who were HBsAg-positive at the time of the procedure. All women, including those highly infective during invasive testing, gave birth to a healthy infant without signs of intrauterine HBV infection. HBsAg positivity within the first months of life despite immuno-prophylaxis at birth may be due to infection acquired *in utero* (Ohto *et al.*, 1987). The patient population, however, was selected and numbers may be too small to declare invasive prenatal diagnosis in HBsAg-positive women completely safe. The upper unit of the 95 per cent confidence limit on a stroke rate of 0 per cent among 15 patients is 22 per cent.

The exact mode of intrauterine transmission of HBV from the mother to the infant is unknown. In cases of invasive testing involving the chorion or the placenta, HBV may be directly transmitted from maternal to fetal blood. Another possible route of HBV transmission may be the swallowing of HBsAg-contaminated amniotic fluid by the fetus. So, on a theoretical basis it seems safe to avoid transplacental procedures and/or CVS in HBsAg carrier pregnant women and certainly in the more infectious subgroup which is also HBeAg-positive or has high levels of HBV DNA. Any vascular area in the uterine wall should be avoided so as to limit the contamination of the amniotic cavity with maternal blood.

Several important factors associated with maternal–fetal transmissions of HBV need further discussion. A likely explanation for the absence of HBV infections in our study is that direct blood to blood contact did not take place. By comparison, HBV transmission by needle-stick has been shown to vary between 5 and 11 per cent (Grady *et al.*,

1978; Seeff et al., 1978). The risk of infection depends on the level of infectivity of the injected material (Alter et al., 1976). After a percutaneous exposure to HBeAg-positive blood, the risk of transmission increases to approximately 30 per cent (Grady et al., 1978; Seeff et al., 1978). Since only two women may be considered as highly infective, the risk for the development of HBV infection through microleakage of maternal blood after invasive prenatal diagnostic techniques may have been negligible. The only woman who underwent CVS was HBeAg-negative, so no conclusions can be drawn on the safety of this procedure in HBeAg-positive women.

Wong et al. (1980) performed third-trimester amniocentesis in 61 HBsAg-positive women and found HBsAg in 16 (26 per cent) liquor samples. Amniocentesis in HBsAg-positive women did not affect the incidence of HBsAg in 1-month-old babies (14·3 per cent), in comparison with a control group (5 per cent) who did not have amniocentesis ($P>0·1$). However, a positive correlation ($P=0·04$) was reported in HBeAg-positive mothers between the presence of HBsAg in amniotic fluid and the finding of antigen in newborns at the age of 1 month. The authors concluded that amniocentesis should be discouraged if mothers are HBsAg- and HBeAg-positive.

The risk of infection also depends on the quantity of injected material. Introduction of an amount as small as 10^{-8} ml of HBeAg-positive blood is sufficient for infection (Shikata et al., 1977). The incidence of maternal–fetal transfusion in the literature varies from 2 to over 50 per cent, depending on complications during pregnancy and the methods of testing used (Schröder, 1975). The overall risk of fetal red cells entering the maternal circulation following amniocentesis was 10 per cent according to Sebring and Polesky (1990), and considerable fetal–maternal haemorrhage after CVS was described by Mantingh (1988), who found increased concentrations of maternal serum alpha-fetoprotein in 60 per cent of the blood samples. The degree of maternal–fetal transfusion and disruption of placental barriers as a result of invasive testing for prenatal diagnosis, however, is not known.

Transmission of HBV by the oral route has been documented in an experimental setting but appeared far less effective than the parenteral route (Krugman and Giles, 1970). In the natural situation, the risk of infection is considered to be very low. This finding is partly based on experience in endoscopy; HBV infection through endoscopy appeared low or non-existent (Villa et al., 1984). As 20 per cent of placental perforations are associated with bloody taps (Kappel et al., 1987), ingestion of HBsAg-contaminated amniotic fluid by the fetus is therefore not likely to be an important mode of transmission. However, transplacental procedures were not performed in our HBsAg-positive women. In the one woman who had weakly HBsAg-positive amniotic fluid, contamination with maternal blood is the most likely explanation.

Finally, it is suggested that the fetal organs only become susceptible to HBV during the third trimester of pregnancy (Gerety and Schweitzer, 1977). The risk of the hepatitis B virus being transmitted from a freshly infected mother to her fetus was 70 per cent during the third trimester, but appeared to be virtually non-existent in women who were infected at an earlier stage (Schweitzer, 1975). Failure to transmit HBV from the mother to the fetus before the third trimester may be due to failure of the HBV to pass the placenta or failure of the fetus to support the HBV. For instance, no integration of HBV DNA was detected in fetal liver cells from 48 terminated pregnancies after 20–32 weeks of gestation (Li et al., 1986). Also, conception and development of the pre-embryo in an HBsAg-positive woman occur in a potentially infectious medium, and even the accidental HBV exposure of pre-embryos to a contaminated culture medium during in vitro fertilization procedures did not result in intrauterine infections (van Os et al., 1991).

Although data are scarce, the available information may support the idea that HBV will not harm the fetal liver early in pregnancy. The present uncertainties, however, point to the need for a more careful and considered approach to the widespread use of invasive prenatal diagnostic techniques in HBsAg-positive women. For highly infectious women (HBeAg-positive or HBV DNA-positive), a firm conclusion on the safety of invasive prenatal diagnosis, especially CVS, cannot be drawn. Perhaps, extensive studies in countries with a high prevalence of HBV may solve some of these issues.

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