International Journal of Infectious Diseases 17 (2013) e54–e58

Contents lists available at SciVerse ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Is hepatitis B virus transmitted via the male germ line? A seroepidemiological study in fetuses☆

Qun-Xi Cai [a], Yi-Yang Zhu [b,*]

[a] Department of Obstetrics and Gynecology, Taizhou Hospital of Zhejiang Province, Wenzhou Medical College, Linhai City, China
[b] Center for Prenatal Diagnosis, Taizhou Hospital of Zhejiang Province, Wenzhou Medical College, Linhai City, 317000, China

ARTICLE INFO

Article history:
Received 22 June 2012
Received in revised form 18 August 2012
Accepted 3 September 2012

Corresponding Editor: Mark Holodniy, California, USA

Keywords:
Hepatitis B virus
Vertical transmission
Prenatal diagnosis
Seroepidemiology

SUMMARY

Objectives: To detect father-to-fetus transmission of hepatitis B virus (HBV) in utero.
Methods: We conducted a study at the prenatal diagnosis center of Taizhou City. Fetuses with one or both parents carrying the hepatitis B surface antigen (HBsAg) were identified before genetic testing during the period 2008–2010. Intrauterine samples were obtained by amniocentesis or cordocentesis and tested for serological markers and by quantitative DNA assays. All neonates received combined hepatitis B immunoprophylaxis after delivery, and serological follow-up tests were performed at 1 year of age.
Results: Of the 407 couples enrolled in the study, HBV was carried by fathers only in 164, and none of their fetuses were found to be HBV DNA-positive in utero. All fetal serological markers were found to be of maternal but not paternal origin. The response rate to postnatal vaccination was 98.6%, and none of the children who failed immunoprophylaxis were the offspring of the HBV carrier fathers.
Conclusions: The infection of fetuses with HBV from the spermatozoa of carrier fathers seems unlikely, especially in an area where pre-conception hepatitis B vaccination is routinely provided.

1. Introduction

Transmission of the hepatitis B virus (HBV) from mother to infant is the predominant route in most high prevalence areas such as China.[1] However, father-to-child transmission also plays an important role in the prevalence of hepatitis B.[2] The transmission of HBV infection to children from their carrier fathers could be either horizontal through intimate postnatal contact, or vertical via the male germ line.[3] The latter is considered an intrauterine infection. However, whether this really exists in humans remains to be determined.

In past decades, several studies have reported the presence of integrated HBV DNA in human spermatozoal chromosomes.[4,5] Studies on embryos hybridized with mammalian ova and human spermatozoa have also confirmed that sperm-integrated HBV DNA can replicate and express the HBV protein in two-cell hybrid embryos.[6–8] All of the above findings demonstrate that theoretically the father could transmit HBV to the fetus via their spermatozoa.

Since HBV is a blood-borne virus, unvaccinated pregnant women would be at risk of HBV exposure if their fetuses carried the virus from the fathers. On the other hand, maternal antibodies can pass through the placenta and enter the fetal circulation freely. If women are vaccinated for HBV prior to conception, and thus HBV-negative, it still needs to be determined what would happen to the maternal immune system if the fetus contracted HBV as a result of HBV-positive spermatozoa. Would the fetus be passively immunized due to hepatitis B immune globulin (HBIG) leaking from the maternal circulation? However, because the literature on the transmission of HBV by spermatozoa in vivo is scarce, the viral replicating status and fetal immune response in utero are unknown. Only one study has detected HBV DNA and serological makers in eight aborted fetuses with a suspected HBV transmission via spermatozoa,[9] but the sample size of the study was small and the maternal serological status was uncertain.

Specimens used for the evaluation of intrauterine infection include amniotic fluid, placental tissue,[10–13] and neonatal peripheral blood.[14–17] Because the postnatal sample is inevitably contaminated by the maternal blood during delivery, this would be of no use in the determination of the time when the infection occurred (before or during delivery). A better alternative is to detect fetal infection before delivery by prenatal diagnostic techniques. Compared to postnatal specimens, intrauterine specimens obtained from amniocentesis or cordocentesis can minimize the risk of contamination with maternal blood.[18] These techniques are also safe and the risk of nosocomial infection caused by invasive procedures is very low.[19–22]

☆ ClinicalTrials.gov ID: NCT01574521.

* Corresponding author. Tel.: +86 1381 9630569.
E-mail address: zuyy@tzhospital.com (Y.-Y. Zhu).

1201-9712/$36.00 – see front matter
http://dx.doi.org/10.1016/j.ijid.2012.09.002

The aim of our study was to investigate the fetal hepatitis B seroepidemiology by prenatal diagnostic technique and to seek evidence of HBV vertical transmission via spermatozoa.

2. Materials and methods

We conducted a prospective study at Taizhou Hospital of Zhejiang Province, a large prenatal diagnosis center with more than 2000 invasive procedures performed every year. The institutional review committees and ethics committees for reproductive medicine approved this study. The research involved pregnant women who received prenatal diagnosis during the period January 2008 to June 2010. Women and their husbands were screened for HBsAg before genetic testing. HBV carriers were identified and their fetuses were recruited to test for intrauterine infection. With regard to the women who were probably carrying an HBV carrier fetus, a supplemental informed consent form was signed following a full discussion on the risk of maternal HBV exposure due to the invasive procedure. The indications for amniocentesis are: screening when there is a high risk of Down syndrome, advanced maternal age, familial genetic disease, previous child with chromosome abnormalities, and where there are sonographic markers for chromosome abnormalities. The indications for cordocentesis are: rapid karyotyping, diagnosis of fetal thalassemia, and blood chemistry. Amniocenteses were performed between gestational weeks 16 and 23, and cordocenteses were performed between gestational weeks 22 and 32. Only women with an indication for amniocentesis or cordocentesis were recruited. Samples from first trimester invasive procedures, such as chorionic villous sampling (CVS), were not included because it was difficult to get enough specimens for analysis.

Amniocenteses and cordocenteses were performed using 22-gauge spinal needles, free-hand manipulation, and continuous ultrasound guidance. To obtain a pure fetal specimen, the first syringe of amniotic fluid was discarded to avoid a 'blood-tip' and transplacental puncture was avoided if possible. Fetal blood samples were collected from the floating cord and verified by hemoglobin electrophoresis, which can identify the maternal hemoglobin (HbA2) within the fetal sample.

After having collected enough specimens for genetic analysis, a further 0.5–1 ml of sample was extracted by final syringe for hepatitis B serological and HBV DNA tests. Specimens for HBV DNA analysis were stored at −20 °C until assay, and serological tests were performed immediately after the puncture. If an intrauterine infection was detected, post-exposure prophylaxis combined with HBIG and hepatitis B vaccine was administered immediately for

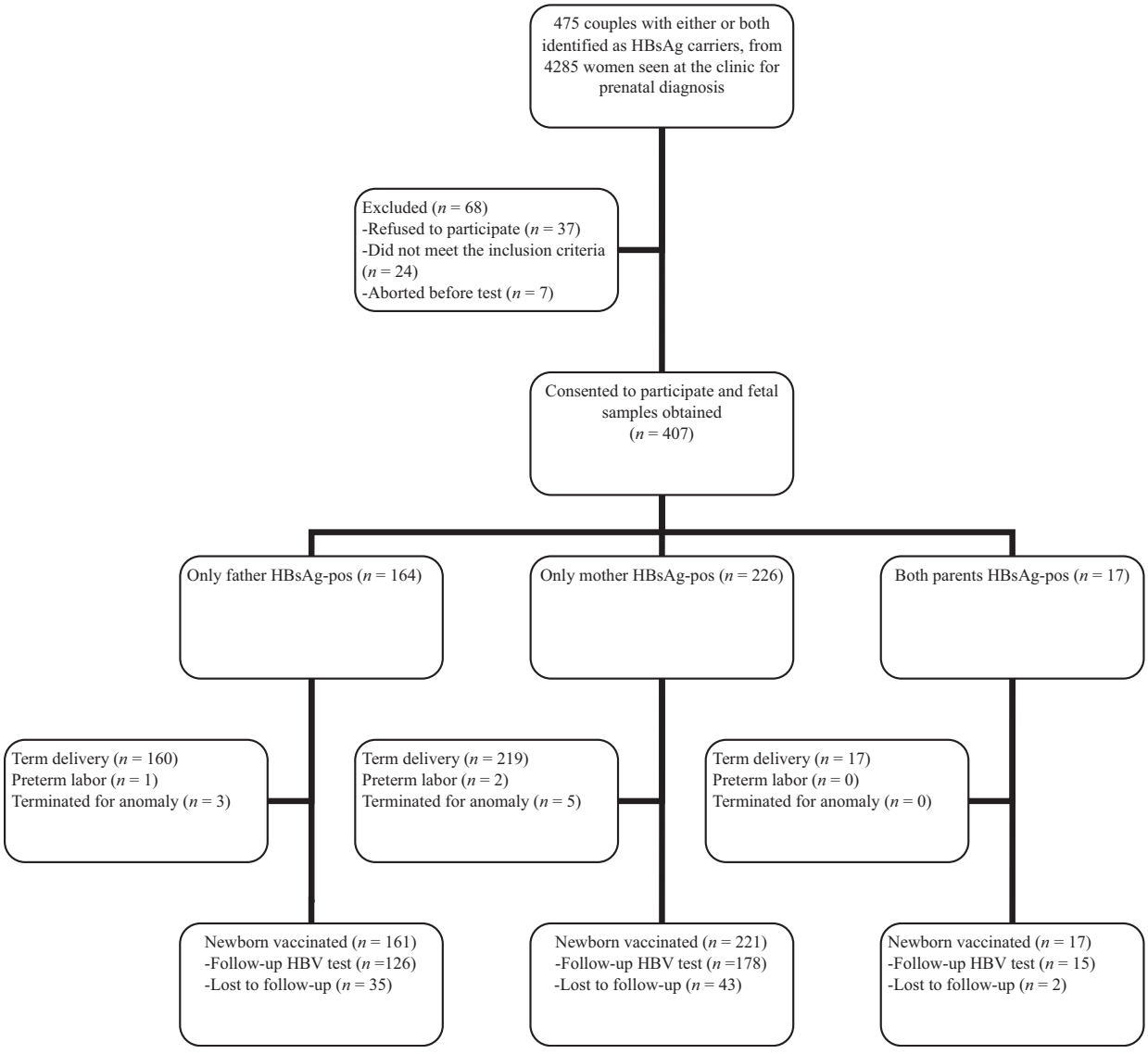

Figure 1. Flow chart of study participants.