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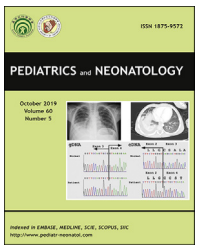

Original Article

Maternal HBeAg positivity and viremia associated with umbilical cord blood hepatitis B viremia

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Key Words
cord blood hepatitis B viral (HBV) DNA;
hepatitis B core antibody;
in-utero exposure to HBV;
maternal hepatitis B viral (HBV) DNA

Background: Hepatitis B (HBV) transmission may result from in utero transmission. We aimed to determine the correlation between maternal serum and umbilical cord blood HBV DNA levels in infants delivered by chronic HBV-infected mothers and to describe the effect of cord blood viremia on vertical transmission.
Methods: A prospective cohort of 92 chronic HBV-infected mother-and-child pairs recruited over three years was analyzed. Maternal and cord blood were tested for HBV DNA by real-time PCR. Standard immunoprophylaxis with both active and passive immunization was administered to all infants. Serological testing was performed on all infants at 9 months of age.
Results: Moderate positive correlation of the maternal HBV DNA with cord blood HBV DNA was demonstrated ($r^2 = 0.521$, $p = <0.001$). HBeAg +ve mothers were younger with higher HBV and cord viremia. At 9 months of age, one infant was infected. Infants delivered by HBeAg positive mothers and mothers with high HBV DNA of more than 6 LOG IU/mL (1×10^6 IU/mL) have increased relative risk of cord blood viremia.

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Conclusions: Maternal HBV DNA and presence of HBeAg were positively correlated to cord blood HBV DNA in infants delivered by chronic HBV-infected mothers. Our data suggest that reducing maternal viremia during the antenatal period may help to reduce cord blood viremia.

1. Introduction

Neonates infected during the perinatal period have a 90% risk of chronic infection themselves and may transmit hepatitis B to non-infected children via blood and bodily-fluid exposure.[1] Despite the combination of early active hepatitis B vaccination and passive immunization with hepatitis B immunoglobulin (HBIG), failure of immunoprophylaxis occurs in up to 1–10% of infants.[2–6] The mechanism behind this remains unclear and is speculated to be due to high maternal hepatitis B viral load, transplacental viral transmission resulting in intrauterine infection, or vaccine failure in recipients unable to mount adequate response due to possible HLA sub-type.[7] Several studies have described the effect of a high maternal viral load and the presence of hepatitis B markers (HBV DNA and HBV antibodies) in neonatal specimens on immunoprophylaxis failure.[5,8,9] Although the correlation between umbilical cord blood HBV DNA and immunoprophylaxis failure is known, the relationship between cord blood HBV DNA and maternal HBV DNA is less certain.[5,8,9] Both the EASL (European Association For The Study Of The Liver)[10] and AASLD guidelines[11] advocated the use of antivirals in pregnant women with high HBV DNA load to reduce immunoprophylaxis failure, although the treatment endpoints were not clearly defined. Low-level persistence of HBV viraemia is reported in 31% of affected infants who received standard immunoprophylaxis.[12] The mechanisms leading to immunoprophylaxis failure in these infants are uncertain, and intrauterine transmission together with low-level peripartum fetal viraemia may play a contributory role. Thus, there is a need to identify the potential risk factors of perinatal viral exposure, and detection of HBV markers remains essential in identifying the risk associated with perinatal viral exposure.

Our study aims to determine the correlation between maternal serum and umbilical cord blood HBV DNA levels in infants delivered by chronic HBV infected mothers and to describe the effect of cord blood viremia on vertical transmission.

2. Methods

This prospective cohort study was approved by the National Healthcare Group Domain Specific Ethics Review Board (IRB B/09/100). All pregnant women positive for hepatitis B surface antigen (HBsAg) who delivered at the National University Hospital (NUH), Singapore, from 1 June 2009 to 1 June 2012 were invited to participate in the study. Informed consent was obtained from all the study participants.

2.1. Inclusion and exclusion criteria

Participating mothers allowed their infants to receive passive immunization at birth and the standard three-dose hepatitis B vaccination schedule used in Singapore. Mothers with co-existing human immunodeficiency virus (HIV) or a family history of hereditary immunodeficiency diseases were excluded from the study.

2.2. Laboratory investigation

Antenatal screening for maternal HBsAg, hepatitis B e antigen (HBeAg) and HIV antibodies (anti-HIV) was performed routinely for all participating mothers. Maternal antenatal HBV DNA is not standard obstetric care; it was measured as part of the study, in their last trimester, close to their delivery date using *artus*® HBV PCR kit (QIAGEN GmbH, Hilden, Germany). Similarly, measurement of cord blood HBV DNA is also part of the study protocol and not analyzed routinely in clinical practice. The lower limit for detection of HBV DNA is 1.13 LOG IU/mL. HBsAg, anti-HBs and anti-HBc antibodies were measured by Architect HBsAg Qualitative II, Architect Anti HBs and Anti-HBc II, (Abbott Laboratories, Singapore) respectively, as per manufacturer's protocol. Post neonatal testing at 9 months of life is recommended as a routine practice and the measurement of anti-HBc was part of the research study protocol. A semi-quantitative analysis was performed for the anti-HBc antibodies using the signal over cut off ratio (S/CO) values obtained. S/CO is defined as sample/cut off ratio = sample relative light units (RLU)/Cut off RLU. Cut off RLU is the calibrator 1 mean RLU times 1.0. The results are interpreted as S/CO $<$ 1.0 are non-reactive and $>$1.0 are reactive. Anti-HIV was measured by Architect HIV Ag/Ab Combo, (Abbott Laboratories, Singapore) according to manufacturer's instructions.

2.3. Clinical management

None of the participants received antiviral therapy during pregnancy as the study was carried out before the EASL guidelines were published.[10] Umbilical cord blood (UCB) was collected for routine post-natal newborn screening tests, including glucose-6-phosphate dehydrogenase deficiency, congenital hypothyroidism, and blood group typing, following the standard management at the third stage of labor. Leftover blood samples from the UCB of infants were collected if parental consent was given. The UCB was obtained directly from the umbilical vessels after clamping and cutting the umbilical cord. This UCB collection

Table 1 Comparison of characteristics of HBeAg positive and HBeAg negative mothers.

Characteristics	HBeAg +ve mothers (n = 27)	HBeAg −ve mothers (n = 65)	P value
Mean maternal age (SD), yrs	29.6 (4.2)	32.6 (5.0)	0.008
Mean maternal HBV viral load LOG IU/mL (SD)	5.98 (2.30)	1.26 (1.60)	<0.001
Mean cord HBV viremia LOG IU/mL (SD)	2.64 (1.76)	1.41 (1.34)	<0.001
Mean gestational age at birth of infants (SD), weeks	38.9 (1.1)	38.8 (1.2)	0.77
Mean birthweight of infants (SD), kg	3.05 (0.38)	3.13 (0.38)	0.35
Anti-HBc positivity %	70.4	45.3	0.029
Mean Anti-HBs titers (SD) IU/L	514 (382)	562 (399)	0.60
Semi-quantitative HBc	4.35 (3.86)	2.51 (3.29)	0.035
Vertical transmission (%)	3.7	0	–

Anti-HBs = Hepatitis B surface antibody; HBV = Hepatitis B virus; HBeAg = hepatitis B e antigen; UCB = Umbilical cord blood.

technique is validated for neonatal screening tests at our center, and hence the risk of maternal blood contamination was very low.[13]

2.4. HBV vaccination

At birth, all infants received 110 international units (IU) of hepatitis B immunoglobulin (HyperGAM, Grifols Therapeutics Inc, USA) and a 10 mcg dose of hepatitis B vaccine (Engerix, Glaxo-Smith-Kline, Belgium). A second dose of hepatitis B vaccine was administered at 4–6 weeks of birth, with a third dose given at 6 months of age.

2.5. Data collection

Baseline demographics, maternal and antenatal history, as well as neonatal data, including birth weight, gestational age, and gender, were collected. Infants were assessed at 0, 1, 6 and 9 months for evidence of jaundice, breast-feeding, and compliance to follow-up vaccines. At nine months of age, blood was taken for serological testing (HBsAg, anti-HBs and anti-HBc). Infants with positive HBsAg were further evaluated for the presence of HBV DNA level and HBV surface gene sequencing as part of the study. Vertical transmission was defined as HBsAg positivity and anti-HBs antibody less than 10IU/L at the 9th month of age.

2.6. Statistical analysis

Statistical analysis was performed with SPSS software version 20. The characteristics of infants with positive cord blood HBV DNA were compared with those without using Student *T*-tests for continuous variables and Mann-Whitney test for variables that were not in normal distribution. Chi-squares tests were used to compare the proportions of HBV serology tests in the study subjects. Linear correlation was analyzed using Pearson's method for maternal and cord blood HBV DNA. A *P* value of less than 0.05 was considered statistically significant.

3. Results

The cohort of 120 mother-child pairs was recruited over a 3-year period. Maternal HBV and serological data were available for all mothers. Cord blood HBV DNA was not available for 22 infants due to insufficient remaining UCB following routine neonatal screening (n = 12), and failure to collect additional UCB due to precipitous or after-hours delivery (n = 10). In an additional six cases, the infants were not brought for serological testing at nine months of age. Thus, complete sets of maternal-infant HBV DNA and serological data were available for 92 mother-child pairs.

Maternal and infant demographics and characteristics of the infants born to HBeAg +ve and HBeAg −ve are

Table 2 Risks factors for cord blood viremia.

Risk factor	No. with cord viremia	No. without cord viremia	Viremia rate	RR (95%CI)
Maternal HBeAg −ve	36	29	0.55	1
Maternal HBeAg +ve	22	5	0.81	1.48 (1.11–1.95)
Maternal HBV DNA < 6 LOG IU/mL (1×10^6 IU/mL)	40	32	0.56	1
Maternal HBV DNA > 6 LOG IU/mL (1×10^6 IU/mL)	18	2	0.90	1.62 (1.25–2.09)

HBV = Hepatitis B virus; HBeAg = hepatitis B e antigen; UCB = Umbilical cord blood.

summarized in Table 1. Maternal age, gestational age and, birth weight were similar in both groups. Four mothers had amniocentesis prior to delivery, six mothers underwent preterm labor but delivered at a median age of 36 weeks, and similarly 4 mothers had pregnancy-induced hypertension but none had pre-eclampsia. The majority of the mothers delivered vaginally; 28 women underwent Caesarean deliveries for obstetric indications. Infants delivered by HBeAg +ve women were younger and had significantly higher maternal HBV viremia and UCB viremia. The relative risk for cord blood HBV viremia was 1.48 (95% CI, 1.11−1.95, $p < 0.05$) for HBeAg positive mothers and was further elevated to 1.62 (95% CI, 1.25−2.09, $p < 0.001$) for those mothers with HBV viremia exceeding 6 LOG IU/mL (1×10^6 IU/mL) of blood (Table 2). The presence of cord blood viremia did not vary based on the obstetric risk factors of mothers (data not shown).

A moderate positive correlation was present between detectable maternal serum and cord blood HBV DNA ($r^2 = 0.521$, $p < 0.001$) (Fig. 1). The higher the antenatal maternal HBV DNA, the higher the UCB viral load detected at birth.

3.1. Vertical transmission

The vertical transmission rate was 1/27 in the group with HBeAg +ve mothers and none (0/65) in the HBeAg −ve group. Anti-HBs antibody titers were similar in both groups. Despite having had detectable HBV DNA in their cord blood, almost all infants (n = 57/58) were able to mount an immune response and achieve anti-HBs antibody levels greater than 10IU/L. However, those delivered by HBeAg +ve mothers were more likely to be positive for HBc antibody and the semi-quantitative analyses were also higher.

The one child with vertical transmission was found to have positive anti-HBc, HBsAg and anti-HBs < 10IU/L at nine months of age. She also had HBV DNA detected by PCR in her serum at nine months. Her mother's pregnancy and delivery were uncomplicated. There was no premature breach of amniotic membranes. Both maternal and umbilical cord HBV DNA exceeded 1×10^5 IU/mL. Cord blood HBsAg was not measured. The child was not breastfed by her mother's personal choice.

4. Discussion

This study showed that the levels of umbilical cord HBV DNA increased with maternal HBV DNA in the third trimester and correlated to the maternal HBeAg positivity.

Zhang et al.[14] showed that UCB is a reliable mode of blood testing in infants; however, they did not analyze the correlation of cord or fetal blood viremia with maternal serum. The modes of HBV transmission from mother to child are germline transmission via infected oocytes,[15] transplacental intra-amniotic transmission and perinatal transmission during birth. Germline transmission[16] as a mechanism is difficult to demonstrate conclusively despite the supportive data from animal studies.[16–18] These germline infections are impossible to prevent.

Vertical transmission of HBV is thought to occur perinatally, hence the significant fall in infection rates with early

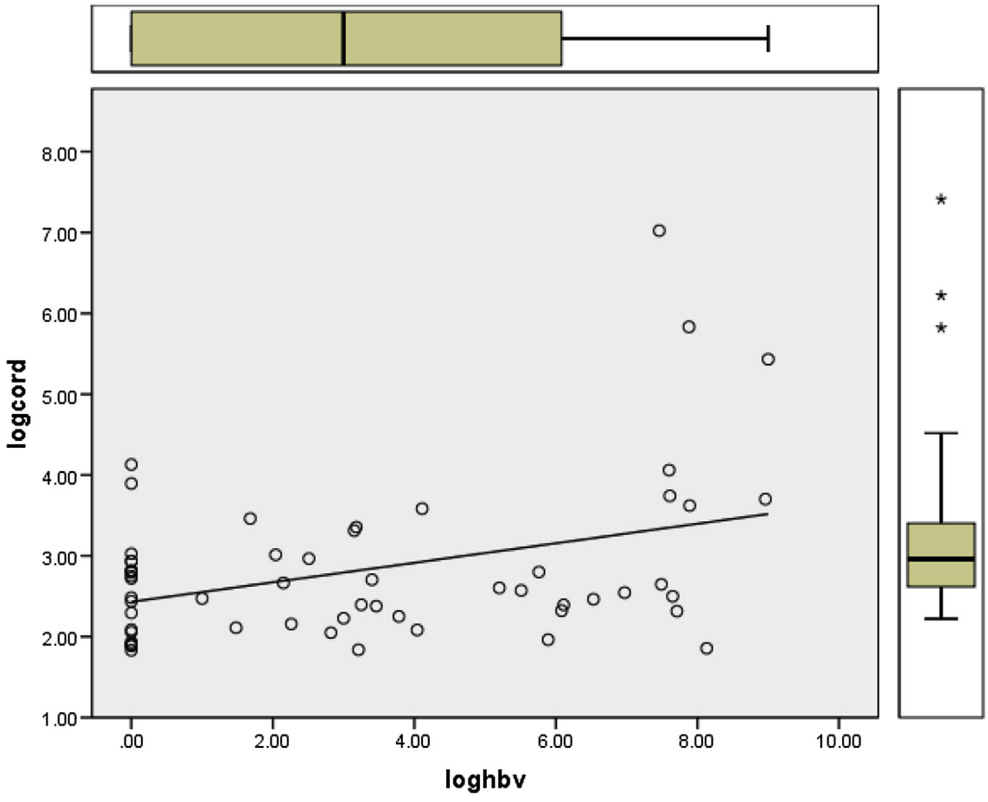

Figure 1 Correlation graph of maternal viral DNA (log IU/mL) vs cord blood viral DNA (log IU/mL).

combined active and passive immunization. Immunoprophylaxis failure is almost always associated with very high maternal HBV loads,[19] which is likely due to either high placental or high UCB viral concentrations. In these cases, the use of antiviral therapy during the third trimester may aid in reducing perinatal transmission.[20]

Shao and colleagues reported an intrauterine HBV infection rate of 43.3% by examining aborted second trimester fetuses from HBsAg positive mothers.[21] This was attributed to transplacental transfer of HBV via peripheral blood mononuclear cells. In contrast, the incidence of intrauterine HBV infection in amniotic fluid was 10%.[8] Similarly, in our study, a high proportion of infants (63%) demonstrated the presence of UCB HBV. Interestingly, the HBV DNA levels in UCB were not as high as those of the maternal HBV DNA supporting the findings by Zou et al.[5]

Intrapartum HBV transmission demonstrates a positive association with the sequestration of the virus within placental villous capillary endothelial cells.[15,22] Viral quantities decreased from the maternal to the fetal surface of the placenta, while the risk of transplacental transmission is higher with the concentration of virus on the fetal surface of the placenta.[23–25] Events that disrupt maternal blood vessels and allow intra-amniotic "leakage" of viral particles increase the risk of intrapartum infection, such as preterm labor. Transplacental transmission of HBeAg in early pregnancy may induce fetal immune tolerance to the antigen and contribute to the inability of the child to mount an appropriate response to the deactivated vaccine.[8] The presence of HBsAg immune-activated cells which was recently demonstrated in neonatal cord blood plasma specimens obtained from HBV positive mothers further supports in utero exposure.[26]

Our study shows a positive but weak correlation of the maternal HBV DNA with the umbilical cord DNA, supporting earlier studies[5,9] and guidelines that advocate antenatal treatment with antiviral agents during pregnancy to reduce maternal HBV viral load to less than 6 LOG IU/mL (1×10^6 IU/mL) and hence minimize exposure risk for infants.

The major limitation of this study is the small sample size. We were not able to demonstrate definite correlation of UCB viremia and vertical transmission. This is possibly related to the low vertical transmission rates in the study population and to the small number of the mothers ($n = 15$) with high viral loads of more than 6 LOG IU/mL (1×10^6 IU/mL). The incidence of vertical transmission in our study is similar to Zou's[5] although our patients received only 1 dose of HBIG at birth despite the presence of cord blood viremia. This finding is similar to the results of Elefsiniotis et al.[27] and Zhu et al.,[8] who also did not find any increased risk of immune-prophylaxis failure with cord viremia.[5,27] Future studies involving mothers with high viral load of more than 6 LOG IU/mL (1×10^6 IU/mL) and high cord viremia may be able to test this hypothesis further.

We conclude that the cord blood HBV DNA correlates positively with maternal HBV DNA and maternal HBeAg positivity. However, the overall vertical transmission rate is not increased in our study population even in the presence of UCB viremia with the current standard HBV immunoprophylaxis regimen.

Declarations of interest

None.

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