Effect of pregnancy on the activity and infectivity of hepatitis B virus in women with chronic hepatitis B infection

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KEY MESSAGES

1. One-half of hepatitis B surface antigen carrier mothers demonstrated hepatitis B virus (HBV) activity during pregnancy.
2. Hepatitis B e antigen (HBeAg) was the best maternal marker for HBV activity in pregnancy, but 59% of mothers with circulating HBV DNA were HBeAg negative.
3. In mothers with no detectable HBV DNA in the first trimester, viral activity still increased from 19.6% in the second trimester to 30.4% in the third trimester to 50% after delivery, but none of their infants had in-utero HBV infection.
4. In-utero HBV infection was evident in 8% of infants, and was related to maternal HBeAg status and high HBV DNA level during all three trimesters.

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Introduction

Chronic hepatitis B virus (HBV) infection, identified by a positive hepatitis B surface antigen (HBsAg) test, occurs in 10% of the obstetric population in Hong Kong.[1] The greatest concern is the high risk of vertical transmission,[2] because the suppression of the Th-1 and enhancement of the Th-2 immune systems during pregnancy may facilitate flare up of chronic HBV infection and increase infectivity. Antenatal screening with HBeAg detects only 10.5% of mothers with anti-HBe who have a viral load $>10^4$ IU/mL.[3] A detectable, and especially high level of, maternal serum HBV DNA is associated with a higher rate of intrauterine infection and consequent failure of passive-active immunoprophylaxis in the offspring.[4] Maternal antenatal antiviral treatment to prevent intrauterine infection can only reduce (not eliminate) the risk.[5]

Given the high prevalence of mothers with HBV infection in our community, routine assessment of every mother throughout pregnancy to monitor their viral DNA level enables selection of high-risk cases for treatment. Owing to resource and logistical concerns, the alternative is to screen and select high-risk women for antenatal treatment. Better understanding of the enhancement effect of pregnancy on HBV activity and infectivity, including the gestational effect, the magnitude of enhancement, and clinical and laboratory features of inflammation is necessary. We hypothesised that pregnancy is associated with increased viral activity in a significant proportion of women, some of whom will manifest features of inflammation that can be identified through antenatal monitoring. We aimed to (1) determine the effect of pregnancy on the activity and replication of HBV during the three trimesters, (2) identify the most sensitive clinically applicable markers of viral activity and maternal inflammation, and (3) examine the relationship of the effect of pregnancy and maternal HBV DNA level with cord blood HBV DNA positivity, taken as evidence of intrauterine infection.

Methods

This was a prospective longitudinal observational study conducted from October 2009 to March 2012 on asymptomatic pregnant women with positive antenatal HBsAg screening who had a singleton pregnancy and negative Down's screening.

As no data on HBV activity during pregnancy were available, we assumed this to be up to 20%. Assuming a type 1 error of 0.05 and a power of 80%, 65 to 174 women were required for the study. Although the study could tolerate a default rate of up to 15%, financial constraints limited the target number to 170 women screened positive for HBsAg.

Screening for maternal HBsAg was routinely performed at booking. Eligible subjects were recruited in the clinic and then invited to attend serial assessments at around 20-24 weeks gestation, 34-36 weeks gestation, and 6 weeks and 6 months after delivery. Blood samples were obtained for measurement of liver function, HBeAg status, HBV DNA level, complete blood count, and serum

ferritin and sensitive C-reactive protein (CRP). At delivery, umbilical cord blood was obtained for measurement of HBV DNA to determine whether in-utero transmission had occurred. HBV DNA measurements were performed using TaqMan real-time polymerase chain reaction (range of detection, 10^2 to 10^9 copies/mL; correlation coefficient of the standard curve, >0.990). The obstetricians were blinded to test results. Nonetheless, if the alanine transaminase (ALT) level was elevated, referral was made to the medical clinic for further assessment. Those who were on antiviral treatment before or during pregnancy were excluded. After delivery, newborns received passive (immunoglobulin) and active (vaccine) immunisation against hepatitis B as per protocol.

Not all recruited women attended all the antenatal or postnatal assessments, and a cord blood sample was not available in all cases, thus HBV DNA results at each time point varied. The final longitudinal study cohort comprised subjects who attended all three antenatal assessments, although not all had a complete set of cord blood or postnatal HBV DNA results.

Results

A total of 278 subjects were recruited; 32 of them were excluded for a variety of reasons: ten became HBsAg negative in the index pregnancy, three had abortions, four were twin pregnancies, five received antiviral treatment, and ten were lost to follow-up. For the remaining 246 subjects, their mean age was 32.1±4.3 years and mean body mass index was 22.0±3.1 kg/m^2; 41.5% were nulliparous, and 241 (98.0%) delivered at the Prince of Wales Hospital. Mean gestation at delivery was 38.5±3.2 weeks and mean birth weight was 3121±557 g. Regarding HBV activity, 27% of mothers were HBeAg positive, 42% to 61% of mothers had HBV DNA detected during pregnancy and postpartum, and 8% of infants had in-utero infection (Table 1).

In 157 (64%) subjects who attended all three antenatal assessments, HBV DNA was detected in 111 and not detected in 46. The two groups were comparable in terms of maternal characteristics, gestation at delivery, birth weight, and the incidence of obstetric complications, but the former had an elevated serum ALT level at all three assessments (P=0.006 to P<0.001) and elevated ferritin level in the third trimester (P=0.022) [Table 2].

According to the first assessment findings, positive HBeAg status identified maternal HBV viral activity (odds ratio=31.2, 95% confidence interval=4.14-234.32), but 59.1% of HBeAg negative mothers also had circulating HBV DNA (Table 3). Most women with HBV DNA detected subsequently had HBV DNA detected at the first assessment, whereas 19.6% (9/46) and 30.4% (14/46) of those without HBV DNA detected initially also had HBV DNA detected in the second and third assessments, respectively. Nonetheless, in-utero infection was confined to those with HBV DNA detected initially. After delivery, HBV DNA detection was similar to that during the second and third trimester (all above 80%), but, alarmingly, 46.5 to 51.2% of women in whom HBV DNA was not detected initially had HBV DNA detected at the postnatal visits, compared with 19.6% and 30.4% at the second and third antenatal assessment, respectively.

For infants with in-utero infection (16/127 or 13%), the incidence of positive maternal HBeAg status was significantly higher (93.8% vs 19.8%, P<0.001), and maternal HBV DNA level was also higher at the first assessment (8.23±1.20 vs 4.40±1.93 $\log_{10}$ copies/mL, P<0.001), second assessment

TABLE 1. Maternal condition and hepatitis B virus (HBV) activity

Parameter	No. (%) of subjects
Nulliparous women	102/246 (42)
Caesarean delivery	61/246 (25)
Hepatitis B e antigen positive	67/235 (27)
Elevated alanine transaminase level (≥55 IU/L) at 1st assessment	13/192 (5)
Maternal HBV DNA detected at 1st assessment	117/168 (48)
Maternal HBV DNA detected at 2nd assessment	139/205 (57)
Maternal HBV DNA detected at 3rd assessment	146/210 (59)
Maternal HBV DNA detected at delivery	102/165 (42)
Placental HBV DNA detected	64/180 (26)
Cord blood HBV DNA detected	20/173 (8)
Maternal HBV DNA detected at 6 weeks postpartum	149/195 (61)
Maternal HBV DNA detected at 6 months postpartum	135/183 (55)

TABLE 2. Maternal characteristics and pregnancy outcome in subjects with or without hepatitis B virus (HBV) DNA detected at the first assessment

Parameter	Mean±SD or No. (%) of subjects		P value
	HBV DNA detected (n=111)	HBV DNA not detected (n=46)	
Maternal age (years)	32.1±4.3	32.8±4.0	0.368
Weight (kg)	55.2±8.6	56.5±10.8	0.440
Height (cm)	159.1±5.3	158.0±5.7	0.242
Body mass index (kg/m^2)	21.8±3.1	22.6±3.9	0.154
Born in Hong Kong	57/110 (51.8)	14/46 (30.4)	0.014
Gravidity=1	24 (21.6)	10 (21.7)	0.987
Parity=0	41 (36.9)	15 (32.6)	0.606
1st assessment			
Haemoglobin (g/dL)	11.8±1.0	11.7±1.4	0.683
White cell count (x10^9/L)	8.3±1.8	8.3±1.9	0.824
Alanine transaminase (IU/L)	25.8±18.3	20.1±14.6	0.006
Ferritin (pmol/L)	157.0±134.3	168.6±145.4	0.713
C-reactive protein (mg/L)	2.9±2.6	4.8±6.7	0.427
2nd assessment			
Haemoglobin (g/dL)	11.2±0.8	11.3±0.8	0.646
White cell count (x10^9/L)	8.8±1.9	9.1±2.4	0.428
Alanine transaminase level (IU/L)	35.3±124.5	16.1±5.7	<0.001
Ferritin (pmol/L)	81.9±120.3	73.7±81.5	0.753
C-reactive protein (mg/L)	2.9±3.0	4.4±5.2	0.423
3rd assessment			
Haemoglobin (g/dL)	11.4±1.1	11.2±1.1	0.197
White cell count (x10^9/L)	8.2±2.1	8.0±2.2	0.543
Alanine transaminase level (IU/L)	22.9±20.2	15.0±5.2	<0.001
Ferritin (pmol/L)	43.2±31.5	36.5±42.1	0.022
C-reactive protein (mg/L)	3.2±3.6	3.5±3.4	0.565
Gestation at delivery (weeks)	39.0±1.3	39.2±1.2	0.315
Birthweight (g)	3162±386	3138±419	0.739

(8.13±1.16 vs 4.45±2.18 $\log_{10}$ copies/mL, P<0.001), and third assessment (7.93±1.30 vs 2.91±2.60 $\log_{10}$ copies/mL, P<0.001). There was no significant difference in infant characteristics, Apgar score, or cord blood pH and base deficit level.

Discussion

This study longitudinally examined HBV viral activity during pregnancy in asymptomatic HBsAg positive mothers. Although only 5% of women had an elevated ALT level at their initial assessment, HBV DNA was detected in half, and this proportion remained consistent across the three trimesters. Furthermore, one in 12 infants showed evidence of intrauterine HBV infection. A similar proportion of these mothers also showed evidence of HBV viral activity for up to 6 months postpartum. These women had persistent viral activity during and after pregnancy despite being asymptomatic.

Mothers with initial HBV DNA detection had a significantly higher incidence of HBeAg positivity (odds ratio=31.15) and elevated ALT level throughout pregnancy. Nonetheless, HBeAg status was unreliable in the identification of those with HBV activity: 59.1% of mothers with circulating HBV DNA were HBeAg negative. Similarly, the markedly overlapping ALT level, ferritin, CRP, and blood count were unhelpful. Therefore, HBV DNA testing for every HBsAg carrier mother is suggested if feasible. If not, at least HBeAg testing and serial ALT measurements should be performed.

The initial absence of HBV DNA could not exclude increased viral activity later on, as some of these subjects had HBV DNA detected in the second (19.6%) and third (30.4%) trimesters. The incidence

TABLE 3. Subsequent maternal hepatitis B virus (HBV) activity in relation to HBV DNA status at first assessment

Parameter	No. (%) of subjects		P value
	HBV DNA detected (n=111)	HBV DNA not detected (n=46)	
HBeAg positive status	45/110 (40.9)	1/46 (2.2)	<0.001
2nd assessment			<0.001
HBV DNA detected (n=106)	97/111 (87.4)	9/46 (19.6)	
HBV DNA not detected (n=51)	14/111 (12.6)	37/46 (80.4)	
3rd assessment			<0.001
HBV DNA detected (n=107)	93/111 (83.8)	14/46 (30.4)	
HBV DNA not detected (n=50)	18/111 (16.2)	32/46 (69.6)	
Cord blood HBV DNA status			0.006
Positive (n=16)	16/92 (17.4)	0/35 (0)	
Negative (n=111)	76/92 (82.6)	35/35 (100)	
6 weeks postnatal assessment			<0.001
HBV DNA detected (n=115)	93/104 (89.4)	22/43 (51.2)	
HBV DNA not detected (n=32)	11/104 (10.6)	21/43 (48.8)	
6 months postnatal assessment			<0.001
HBV DNA detected (n=104)	84/96 (87.5)	20/43 (46.5)	
HBV DNA not detected (n=35)	12/96 (12.5)	23/43 (53.5)	

increased to 51.2% at 6 weeks and 46.5% at 6 months postpartum. Although no in-utero infection was found in this subgroup, these subjects might have a higher maternal risk than those with HBV DNA detected initially. Further studies are required to elucidate the long-term maternal implications.

In-utero infection was associated with a maternal positive HBeAg status and higher HBV DNA load throughout pregnancy, but infant characteristics were comparable. This suggests maternal tolerance to chronic HBV infection with no consequent adverse perinatal outcome in most cases.

Conclusion

Half of the asymptomatic maternal carriers of HBsAg showed evidence of HBV activity, and subjects with undetectable HBV DNA in the first trimester had increased viral activity in the second (19.6%) and third (30.4%) trimesters and within 6 months of delivery (around 50%). No in-utero infection was found in mothers with initial undetectable viral activity. HBeAg was the best maternal parameter to identify subjects with viral activity; HBV DNA level should be measured for every HBsAg carrier mother, as 59% of HBeAg negative mothers also have circulating HBV DNA.

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