Title page

Title: Optimal timing of hepatitis B virus DNA quantification and clinical predictors for higher viral load during pregnancy

Running title: HBV DNA quantification during pregnancy

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Disclosure

The authors have stated explicitly that there are no conflicts of interest in connection with this study

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Abbreviations

Hepatitis B virus, HBV; hepatitis B antigen status, HBsAg; hepatitis B e antigen, HBeAg; Receiver operating characteristic, ROC; Body mass index, BMI

Abstract

Introduction:

Authorities publish recommendations on the hepatitis B virus (HBV) viral load threshold to initiate antiviral treatment but the timing of quantification during pregnancy is not defined. HBV DNA level at 28-30 weeks predicts the risk of immunoprophylaxis failure. This study evaluated the correlation of HBV DNA <22 weeks with 28-30 weeks' level. Clinical predictive factors for HBV DNA > 6 log_{10} IU/ml, 7 log_{10} IU/ml and 8 log_{10} IU/ml were studied.

Material and Methods:

A retrospective analysis of HBV DNA <22 weeks and 28-30 weeks of gestation was carried out in 352 pregnant HBV carriers. HBV DNA was examined using the COBAS TaqMan HBV Monitor Test coupled with the COBAS Ampliprep extraction system (Both Roche Diagnostics, Branchburg, NJ).

Results:

A strong positive correlation was found between the viral load <22 weeks (mean 16.7 weeks) and 28-30 weeks of gestation, which was independent of the viral load level and gestational age of quantification ($r=0.942$, $p<0.001$). Univariate analysis showed positive hepatitis B e antigen (HBeAg), maternal age <35 years old and body mass index (BMI) $\leq 21 kg/m^2$ were associated with a higher mean viral load at 28-30 weeks of gestation ($p<0.05$). These factors

were also associated with a higher chance of viral load >6 log_{10} IU/ml, 7 log_{10} IU/ml and 8 log_{10} IU/ml at 28-30 weeks($p<0.05$). In multiple regression analysis, only viral load <22 weeks and positive HBeAg remained predictive of a higher mean viral load at 28-30 weeks of gestation ($p<0.05$). The receiver operating characteristic curve showed the HBV DNA <22 weeks was an excellent predictor for different viral load cut-offs at 28-30 weeks. The area under curve were 0.986, 0.998 and 0.994 for viral load 6 log_{10} IU/ml, 7 log_{10} IU/ml and 8 log_{10} IU/ml respectively.

Conclusions: HBV DNA quantification should be performed before 22 weeks of gestation and viral load cut-offs similar to that at 28 weeks to determine immunoprophylaxis failure could be used. Maternal positive HBeAg status was associated with a higher chance of viral load >6 log_{10} IU/ml,7 log_{10} IU/ml or 8 log_{10} IU/ml.

Keywords

Hepatitis B virus; Infectious Disease Transmission, Vertical; Pregnancy; Immunization; Viral load

Key messages:

1. The level of hepatitis B viral load before 22 weeks of gestation is similar to

that at 28-30 weeks of gestation.

2. Maternal positive HBeAg status is associated with a higher HBV viral load level during pregnancy.

INTRODUCTION

Hepatitis B virus (HBV) infection remains endemic with an estimated global prevalence of 3.5%.[1] In 2015, the estimated prevalence of chronic HBV infection was approximately 257 million and 887,000 people died from HBV.[2] Vertical transmission is a major source of HBV infection as more than 90% of perinatally infected infants would become chronic carriers.[3] Immunization to the infants of HBV carriers can effectively prevent vertical transmission,[4] but 1-4% of infants still suffer from persistent HBV infection after immunization.[5-9] The risk of immunoprophylaxis failure depends on the maternal HBV viral load during pregnancy and is greatly reduced by antiviral treatment during the third trimester.[10, 11] Various authorities have published recommendations on the viral load threshold to initiate antiviral treatment but the timing of viral load quantification during pregnancy is not defined.[12-14]

HBV DNA level at 28-30 weeks can predict the risk of immunoprophylaxis failure.[9] However, maternal HBV DNA quantification and antiviral treatment in the third trimester raise the theoretical concern of inadequate viral load suppression. In particular, highly viremic carriers are at higher risk of preterm birth.[15] This will further shorten the duration of treatment and hence suboptimal viral load reduction. Preterm birth occurs before HBV DNA quantification also preclude these women from antiviral treatment. Therefore, earlier risk assessment of immunoprophylaxis failure and antiviral treatment may be

required in highly viremic HBV carriers. Previous studies evaluating the risk of immunoprophylaxis failure from maternal viral load did not specify the timing of viral load quantification or using postpartum samples.[5-8] Since pregnancy is an immunomodulated state to avoid rejection of the fetal allograft, the dynamic immune and hormonal changes throughout the gestation may affect the HBV replicating activity.[16-20] In this study, we aim to evaluate the correlation of HBV DNA level at early gestation (before 22 weeks) with 28-30 weeks' HBV DNA level. We also investigate the clinical predictive factors for high viral load (>6 $\log_{10}$ IU/ml) at 28-30 weeks.

MATERIAL AND METHODS

This was a secondary analysis of a prospective study carried out at the antenatal units in five public hospitals in Hong Kong.[9] HBV carriers were identified by positive hepatitis B surface antigen (HBsAg) status examined at booking visit. All women gave written informed consent. Hepatitis B e antigen status (HBeAg) and HBV DNA were then tested in HBsAg positive women after consent was obtained. HBV DNA was examined using the COBAS TaqMan HBV Monitor Test coupled with the COBAS Ampliprep extraction system (Both Roche Diagnostics, Branchburg, NJ), with a lower limit of detection of 100 copies/mL (~17.2 IU/ml) and upper limit of 990 000 000 copies/mL (~170 103 092 IU/ml) (1 IU = 5.82 copies). The inclusion criteria for this study was

confirmed HBV carrier by positive HBsAg status at booking, who had paired HBV DNA viral load quantification before 22 weeks and at 28-30 weeks of gestation. All women did not receive antiviral treatment throughout the pregnancy. All newborns received hepatitis B immunoglobulin and first dose of HBV vaccine within 12 hours of birth, followed by second and third dose of hepatitis B vaccine at one and six months of life. Immunoprophylaxis failure of infants was defined by HBsAg positivity at 9-12 months of age. Since the optimal viral load threshold to initiate antiviral treatment is still controversial, different viral load as cut-offs (> 6 log10, 7 log10, 8 log10IU/ml) were studied.

Statistical Analyses

As the HBA DNA value was highly skewed, a log transformation was performed. The interrelationships between the HBV DNA level, and time gap and the change of viral load before 22 weeks and 28-30 weeks of gestation were explored by Pearson's correlation. The differences of HBV viral load at 28-30 weeks of gestation on HBeAg status, maternal age, body mass index (BMI), parity, smoking and drinking (alcohol consumption) were investigated by t test. Multiple regression analysis was used to investigate the association of viral load at 28-30 weeks with those statistically significant factors in univariate analysis for a higher viral load. The clinical risk factors of high viral loads (> 6 log_{10}, 7 log_{10}, 8 log_{10} IU/ml) were explored by odds ratio (OR) with 95% confidential interval (95% CIs), Chi-square test was also used to investigate

the association between clinical risk factors and high viral load (> 6 $\log_{10}$, 7 $\log_{10}$, 8 $\log_{10}$ IU/ml). Receiver operating characteristic (ROC) curve was used to find out the best cut-off point of viral load before 22 weeks to predict the high viral load (6 $\log_{10}$, 7 $\log_{10}$, 8 $\log_{10}$ IU/ml) at 28-30 weeks of gestation. The best cut-off point was defined as the point with maximum Youden's index. A p-value <0.05 was considered as statistical significance. All data were analyzed with IBM Statistical Package for the Social Sciences, version 22 (Armonk, NY:IBM Corp.).

Ethical approval

This study received ethical approval from Institutional Review Board of the University of Hong Kong/Hospital Authority Hong Kong West Cluster on 30th August, 2018 (reference number: UW18-474).

RESULTS

325 pregnant HBV carriers with both HBV DNA viral load tested before 22 weeks and 28-30 weeks of gestation were analysed. Table 1 summarized the basic demographics and viral load levels of the women.

A strong positive correlation was found between the viral loads measured before 22 weeks and 28-30 weeks of gestation (r= 0.942, p< 0.001). This correlation was independent from the gestational age of viral load

quantification but consistent in HBV carriers with different viral load levels (Figure 1). Although there was a wide range of timing of HBV DNA quantification before 22 weeks (10 – 22 weeks), it was not associated with the viral load level (r= 0.048, p= 0.385). The time gap between the two measurements was not associated with the change of viral load (viral load at 28-30 weeks - viral load before 22 weeks) (r= 0.028, p= 0.613).

The viral load level before 22 weeks and 28-30 weeks were studied, with respect to different clinical parameters (Table 2). Higher mean viral load before 22 weeks and 28-30 weeks of gestation were associated with positive HBeAg status, maternal age < 35 years old (at 28-30 weeks only) and BMI ≤ 21kg/m^2. The viral load level was not associated with parity, smoking or drinking. In the multiple regression analysis, only positive HBeAg status and viral load before 22 weeks correlated with a higher viral load at 28-30 weeks (R^2 = 0.904, $p < 0.05$) (Table 3). Every unit increase of viral load before 22 weeks was associated with an increase of 0.883 $\log_{10}$IU/ml viral load at 28-30 weeks. Positive HBeAg status was also significantly associated with a higher viral load of 0.561 $\log_{10}$IU/ml.

The relationship of clinical factors and different viral load levels as cut-offs at 28-30 weeks of gestation were evaluated. Positive HBeAg status, maternal age <35 years and BMI ≤21 kg/m^2 were associated with a higher chance of

viral load > 6 log_{10}, 7 log_{10} or 8 log_{10} IU/ml when tested by Chi-square test ($p<0.05$), odds ratios are shown in Table 4.

We examined the HBV DNA level before 22 weeks of gestation to predict different viral load levels at 28-30 weeks of gestation. The ROC curve showed the HBV DNA before 22 weeks of gestation was an excellent predictor for different viral load cut-offs at 28-30 weeks (Figure 2). The area under curve were 0.983, 1.000 and 0.997 for viral load 6 log_{10} IU/ml, 7 log_{10} IU/ml and 8 log_{10} IU/ml respectively. The best cut-off to predict 6 log_{10} IU/ml, 7 log_{10} IU/ml and 8 log_{10} IU/ml were 5.49 log_{10} IU/ml (sensitivity 0.904 and specificity 0.984), 6.80 log_{10} IU/ml (sensitivity 1.000 and specificity 1.000) and 7.66 log_{10} IU/ml (sensitivity 1.000 and specificity 0.982) respectively.

DISCUSSION

In this study, we showed the viral load before 22 weeks was highly correlated with that at 28-30 weeks of gestation. A greater proportion of women with viral load > 6 log_{10}, 7 log_{10}, 8 log_{10} IU/ml at 28-30 weeks may be identified by positive HBeAg status, maternal age <35 years or BMI ≤21 kg/m^2. Previous evidence on the change of HBV DNA level throughout the pregnancy was conflicting. Studies showed 5.2-9% of HBV carriers could have ≥ 2 log_{10} increase in DNA level during pregnancy.[17, 18] A mean 0.4 log_{10} increase in late pregnancy or early postpartum was observed.[16] However, significant change in

antenatal viral load was not demonstrated in other studies.[19, 20] Our finding was consistent with the latter that the viral load did not change significantly during pregnancy. Therefore, HBV DNA should be performed in early pregnancy (before 22 weeks) to determine the risk of immunoprophylaxis failure.

The optimal threshold and timing to start antiviral treatment to prevent immunoprophylaxis failure remains controversial. Our previous work showed the risks of immunoprophylaxis failure with maternal HBV DNA level of <7.2, 7.2–8.2, >8.2 $\log_{10}$ IU/ml were 0%, 8.6%, and 3.1%, respectively in 641 pregnancies.[9] The risk of immunoprophylaxis failure with borderline viral load of 5 $\log_{10}$ – 7 $\log_{10}$ IU/ml has also been reported.[5-8] Therefore, various cut-offs were evaluated and consistent findings were noted. Although effective reduction in immunoprophylaxis failure was shown in the third trimester use of antiviral treatment,[11] suboptimal viral load suppression at delivery raised the concern of failed protection from a shorter duration of antiviral treatment should a preterm birth occur.[15, 21] From the ROC analysis, the best HBV DNA level before 22 weeks to predict 28 weeks' is similar to the level at 28-30 weeks at various viral load cut-offs. Therefore, our date suggested similar viral load cut-off could be used in earlier gestation. Earlier HBV DNA quantification could allow time for HBV carriers to consider antiviral treatment, risk assessment for vertical transmission from prenatal invasive test (usually

performed in late first or early second trimester) and, more importantly possible earlier antiviral treatment in HBV carriers with extremely elevated viral loads or high risk of preterm birth, who have an increased chance of suboptimal viral load suppression at delivery and immunoprophylaxis failure.

HBV DNA testing is not available in many resource-limited settings, rendering difficulty in predicting immunoprophylaxis failure of infants in countries with high HBV prevalence. Pregnant HBV carriers at risk of higher viral load, using various cut-offs of 6 $\log_{10}$, 7 $\log_{10}$, 8 $\log_{10}$ IU/ml, can be identified by maternal age >35 years old, BMI $\leq$21 kg/m^2 or positive HBeAg status. Similar observation was found in non-pregnant HBV carriers.[22, 23] However, the association of lower BMI and younger age with higher viral load was not observed in the multivariate analysis. As younger HBV carriers are more likely to have both lower BMI and be HBeAg positive, age and weight could be confounders rather than true predictors. Only HBeAg remains a significant factor. Although HBeAg appears to be a better predictor than age and BMI, the latter require no additional cost and remain a viable option for targeted HBV DNA quantification in countries with limited resources. Previous studies did not study the effect of age and BMI on HBV DNA during pregnancy; therefore our findings merit further evaluation.

The strength of this study is that the precise timing of HBV DNA testing using a

sensitive assay permits accurate and standardized viral load assessment throughout pregnancy. The wide range of HBV DNA quantification before 22 weeks allows flexibility in clinical practice. By using different viral load cut-offs for analysis, our data are still applicable if there is a future change in the viral load cut-offs to start antiviral treatment. Our study has some limitations. Firstly, a small number of samples before 13 weeks of gestation may limit our conclusion to testing before this gestation. Secondly, dilution for further HBV DNA quantification was not performed for HBV DNA exceeded the upper limit of assay. However, this would not affect the clinical application of our results as the threshold to consider antiviral treatment is below the upper limit of the assay. Thirdly, all women were Chinese and not put on antiviral treatment during pregnancy. Liver function test and HBV genotype were not available which could affect the generalizability of our result. Finally, although we showed risk assessment of immunoprophylaxis failure feasible in early pregnancy, a randomized trial would be required to test whether HBV carriers with extremely high viral load need earlier treatment before 28-30 weeks to prevent immunoprophylaxis failure.

CONCLUSION

HBV DNA quantification should be performed before 22 weeks of gestation and viral load cut-offs similar to that at 28 weeks to determine immunoprophylaxis failure could be used. Maternal positive HBeAg status was

associated with a higher chance of viral load >6 $\log_{10}$ IU/ml,7 $\log_{10}$ IU/ml or 8 $\log_{10}$ IU/ml. Maternal age <35 years or BMI $\leq$21 kg/m^2 may be used for targeted HBV DNA testing in resource limited setting.

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Table 1. Basic demographics and maternal viral load level	
	Median (IQR), Mean (SD) or n (%)
Age (years), mean (SD)	32.6 (4.6)
Body mass index (kg/m^2), mean (SD)	22.2 (3.1)
Gravida, median (IQR)	2.0 (2.0)
Parity, median (IQR)	0.0 (1.0)
Nulligravida, n(%)	116 (35.7%)
Nulliparity, n (%)	174 (53.5%)
Smoking, n (%)	21 (6.5%)
Drinking, n (%)	6 (1.8%)
Chinese, n (%)	325 (100%)
Maternal HBsAg positive, n (%)	325 (100%)
Maternal HBeAg positive, n (%)	81 (24.9%)
Viral load before 22 weeks (log_{10} IU/ml), mean (SD)	3.6 (2.4)
- Viral load > 6 log_{10} IU/ml, n(%)	66 (20.3%)
- Viral load > 7 log_{10} IU/ml, n(%)	57 (17.5%)
- Viral load > 8 log_{10} IU/ml, n(%)	47 (14.5%)
Viral load at 28-30 weeks (log_{10} IU/ml), mean (SD)	3.7 (2.5)
- Viral load > 6 log_{10} IU/ml, n(%)	73 (22.5%)
- Viral load > 7 log_{10} IU/ml, n(%)	58 (17.8%)
- Viral load > 8 log_{10} IU/ml, n(%)	47 (14.5%)
Gestational age at recruitment (weeks), mean (SD)	16.7 (2.6)
<13 weeks, n (%)	10 (3.1%)
13-14^{+6} weeks, n (%)	68 (20.9%)
15-16^{+6} weeks, n (%)	138 (42.5%)
17-18^{+6} weeks, n (%)	45 (13.8%)
19-20^{+6} weeks, n (%)	27 (8.3%)
21-22^{+6} weeks, n (%)	37 (11.4%)

Table 2. Clinical parameters and viral load level at < 22 weeks and 28-30 weeks				
	< 22 weeks		28-30 weeks	
	Viral load (log_{10}IU/ml)		Viral load (log_{10}IU/ml)	
	Mean (SD)	P value	Mean (SD)	P value
Maternal age < 35 years old				
Yes	3.8 (2.6)	0.060	3.9 (2.7)	0.049
No	3.2 (2.0)		3.3 (2.1)	
Body mass index ≤21kg/m^2				
Yes	4.2 (2.6)	<0.001	4.3 (2.7)	<0.001
No	3.2 (2.2)		3.3 (2.3)	
HBeAg positive				
Yes	7.1 (1.9)	<0.001	7.2 (1.9)	<0.001
No	2.4 (1.1)		2.5 (1.3)	
Nulliparity				
Yes	3.7 (2.4)	0.546	3.8 (2.5)	0.456
No	3.5 (2.4)		3.5 (2.5)	
Smoking				
Yes	3.9 (2.6)	0.530	4.1 (2.7)	0.463
No	3.6 (2.4)		3.6 (2.5)	
Drinking				
Yes	1.8 (0.6)	0.071	1.8 (0.6)	0.072
No	3.6 (2.4)		3.7 (2.5)	

†: p value was calculated by student's t test.

Table 3. Multiple regression on the predictors of HBV viral load (log_{10}IU/ml) at 28-30 weeks (n=325)			
	Coefficient or Odds ratio	95% CIs	p-value
Viral load < 22 weeks of gestation (log_{10}IU/ml)	0.883	0.81, 0.95	<0.001
Maternal age < 35 years old	0.016	-0.18, 0.21	0.874
Body mass index ≤21 kg/m2	0.064	-0.13, 0.26	0.516
Positive HBeAg	0.561	0.17, 0.95	0.005

Table 4. Clinical factors associated with higher viral load using different HBV DNA cut-off values									
	≤6 $\log_{10}$ IU/ml (n=252)	>6 $\log_{10}$ IU/ml (n=73)	OR (95% confidence interval)	≤7 $\log_{10}$ IU/ml (n=267)	>7 $\log_{10}$ IU/ml (n=58)	OR (95% confidence interval)	≤8 $\log_{10}$ IU/ml (n=278)	>8 $\log_{10}$ IU/ml (n=47)	OR (95% confidence interval)
Maternal age < 35 years old	157 (62.3%)	60 (82.2%)	2.79 (1.46, 5.36)	169 (63.3%)	48 (82.8%)	2.78 (1.35, 5.75)	177 (63.7%)	40 (85.1%)	3.26 (1.41, 7.55)
Body mass index ≤21 kg/m^2	87 (34.5%)	37 (50.7%)	1.93 (1.14, 3.26)	91 (34.1%)	33 (56.9%)	2.52 (1.42, 4.50)	94 (33.8%)	30 (63.8%)	3.42 (1.79, 6.51)
Positive HBeAg	13 (5.2%)	68 (93.2%)	250.0 (86.1, 726.0)	23 (8.6%)	58 (100%)	NA	34 (12.2%)	47 (100%)	NA

Figure 1. The association between HBV viral load < 22 weeks and 28-30 weeks of gestation

Figure 2. The receiver operating characteristic curve of viral load < 22 weeks of gestation and DNA level a) >6log10, b) >7 log10 and c) >8log10 IU/ml at 28-30 weeks of gestation