Title page

Title: Immunoprophylaxis failure of infants born to Hepatitis B carrier mothers following routine vaccination

Short title: Hepatitis B vaccination failure of infants

Ka Wang Cheung [1] MRCOG

Mimi Tin Yan Seto [1] MRCOG

Anita Sik Yau Kan [1] MRCOG

Daniel Wong [2] MRCOG

Thomas Kam On Kou [3] MRCOG

Po Lam So [4] FHKAM (O&G)

Wai Lam Lau [5] FRCOG

Khair Jalal [6] FHKAM (Paed)

Yuet Yee Chee [6] FHKAM (Paed)

Rosanna Ming Sum Wong [6] FHKAM (Paedi)

Chin Peng Lee [1] FRCOG

Ernest Hung Yu Ng [1] FRCOG (UK)

1: Department of Obstetrics and Gynaecology, Queen Mary Hospital, the University of Hong Kong, Hong Kong SAR, China.

2: Department of Obstetrics & Gynaecology, Pamela Youde Nethersole Eastern Hospital, Hong Kong SAR, China.

3: Department of Obstetrics & Gynaecology, Queen Elizabeth Hospital, Hong Kong SAR, China.

4: Department of Obstetrics & Gynaecology, Tuen Mun Hospital, Hong Kong SAR, China.

5: Department of Obstetrics & Gynaecology, Kwong Wah Hospital, Hong Kong SAR, China.

6: Department of Paediatrics & Adolescent Medicine, the University of Hong Kong, Hong Kong SAR, China.

Grant support

This research was funded by a grant from Health and Medical Research Fund, Hong Kong (Reference number 11121661)

Abbreviation

Immunoprophylaxis failure (IF), Hepatitis B e antigen (HBeAg), Hepatitis B immunoglobulin (HBIG), Hepatitis B surface antigen (HBsAg), Hepatitis B virus (HBV)

Corresponding Author:

Ka Wang Cheung

Address: 6/F, Professorial Block, Queen Mary Hospital, 102 Pokfulam Road, Hong Kong

Telephone: (852) 22554517

Fax number: (852) 28550947

Email: kelvincheung82@hotmail.com

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

Writing assistance

None

Author Contributors

KWC, MTYS, ASYK, DW, TKOK, PLS, WLL, KJ, YYC, RMSW, CPL and EHYN contributed to the study design. KWC, MTYS, DW, TKOK, PLS and WLL contributed with subjects recruitment. KWC, MTYS, DW, TKOK, PLS, WLL, KJ, YYC and RMSW contributed to the data collection. KWC wrote the first draft of the manuscript. All authors contributed to final data interpretation and contributed to and approved the final draft of the manuscript.

Introduction

Hepatitis B virus (HBV) infection remains the commonest form of chronic hepatitis worldwide. The risk of vertical transmission leading to chronic infection is dramatically reduced by giving hepatitis B immunoglobulin (HBIG) to newborns at birth together with a complete course of HBV vaccination (1). A high maternal HBV DNA level during pregnancy is the strongest risk factor leading to immunoprophylaxis failure (IF) (2). Due to the retrospective nature (3), heterogeneity of the studied population (4), and different or unknown timing of HBV DNA quantification (3-5), the optimal HBV DNA level to categorize the high-risk HBV mothers and subsequent use of antiviral treatment remains not well defined. We aimed to evaluate the chance of IF in relation to the maternal HBV DNA level at 28 to 30 weeks of gestation, and with other maternal, obstetric and neonatal factors.

Methods

A prospective multicenter study was conducted from January 2014 to December 2016 at five hospitals in Hong Kong. All pregnant women in Hong Kong were tested for hepatitis B surface antigen (HBsAg) during their first antenatal visit. Women with a positive HBsAg status were recruited. Women receiving antiviral treatment during pregnancy were excluded. All gave a written informed consent and were enrolled under protocols approved by the Institutional Review Board of each hospital.

Maternal hepatitis B e antigen (HBeAg) was tested once upon recruitment and the HBV DNA was quantified at 28-30 weeks 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). All newborns received both 10μg HBV vaccines (Engerix^{TM}-B, GlaxoSmithKline, Belgium)

and 110IU HBIG (HyperHEP® B, Grifols, USA) intramuscularly at a different site within 12 hours of birth, followed by hepatitis B vaccine of same dosage at one and six months of life. HBsAg of infants was examined at 9-12 months. IF of infants (either infant in case of twin pregnancy) was defined as HBsAg positive at 9-12 months.

The sample size was calculated based on comparing the proportions of IF in infants between high and low maternal pre-delivery HBV DNA levels. A total of 624 subjects were required for the study with a power of 80% and a type I error of 5%. The Student's t-test or Wilcoxon Rank Sum test was used to compare quantitative variables, and the Chi-square test or Fisher's exact test were used to compare qualitative variables, respectively. P values < 0.05 were considered statistical significant. All data were analyzed with SAS software, version 9.2 (SAS Institute Inc. Cary, NC).

Result:

Data from 641 women and 654 infants (13 pairs of twins) were available for final analysis. All infants had received the HBIG within 12 hours of birth and completed the whole course of hepatitis B vaccine on schedule. 24.2% women were HBeAg positive. There were 7 cases of IF (1.1%, 7/641), all born to women with positive HBeAg status and HBV DNA >8$\log_{10}$ copies/mL (~17,000,000 IU/mL). The risks of IF with HBV DNA level of <8, 8-8.99, >9 $\log_{10}$ copies/mL were 0%, 8.6% and 3.1% respectively (Table 1). Positive HBeAg and HBV DNA ≥8$\log_{10}$ copies/mL (~17,000,000 IU/mL) at 28-30 weeks were significant predictive factors for IF (4.5% (95% CI 1.83-9.08%) versus 0% (95% CI 0-0.76%), and 5.8% (95% CI 2.36-11.56%) versus 0% (95% CI 0-0.71%), respectively, *p*-value <0.0001). No other maternal, obstetrical and neonatal factors were significant for IF.

Discussion:

Various authorities suggested the use of antiviral therapy in women with high viral load to reduce the risk of IF (6, 7). Although a cut-off of 200,000IU/ml (~6$\log_{10}$ copies/mL) was recommended (6, 7), the optimal cut-off of viral load to initiate antiviral treatment remained debatable (6). The cut-off was mainly from a retrospective observational study carried out in China (3) and a randomized controlled trial (8). In our series, the lowest HBV DNA of IF was > 8$\log_{10}$ copies/mL (~17,000,000 IU/mL) which echoed with another prospective study (16), after excluding one case with invasive prenatal test and delay first HBV vaccination. Therefore, viral load of 8$\log_{10}$ copies/mL could be the optimal HBV DNA cut off at 28-30 weeks to predict IF and to start antiviral treatment. The use of antiviral treatment in women with low viral load <6$\log_{10}$copies/mL seems not justifiable.

Acknowledgements

We thank Chi Tao Ng, Clinical Trials Centre, The University of Hong Kong, for the statistical analysis.

Reference:

1. Lee C, Gong Y, Brok J, Boxall EH, Gluud C. Effect of hepatitis B immunisation in newborn infants of mothers positive for hepatitis B surface antigen: systematic review and meta-analysis. BMJ 2006;332:328–36.
2. Cheung KW, Seto MT, Wong SF. Towards complete eradication of hepatitis B infection from perinatal transmission: review of the mechanisms of in utero infection and the use of antiviral treatment during pregnancy. Eur J Obstet Gynecol Reprod Biol. 2013;169:17-23.
3. Zou H, Chen Y, Duan Z, Zhang H, Pan C. Virologic factors associated with failure to passive-active immunoprophylaxis in infants born to HBsAg-positive mothers. J Viral Hepat. 2012;19:e18-25.
4. Wen WH, Chang MH, Zhao LL, et al. Mother-to-infant transmission of hepatitis B virus infection: significance of maternal viral load and strategies for intervention. J Hepatol. 2013;59:24-30.
5. Wiseman E, Fraser MA, Holden S, et al. Perinatal transmission of hepatitis B virus: an Australian experience. Med J Aust. 2009;190:489-92.
6. Terrault NA, Bzowej NH, Chang KM, Hwang JP, Jonas MM, Murad MH; American Association for the Study of Liver Diseases. AASLD guidelines for treatment of chronic hepatitis B. Hepatology. 2016;63:261-83.
7. European Association for the Study of the Liver. EASL 2017 Clinical Practice Guidelines on the management of hepatitis B virus infection. J Hepatol. 2017:18.
8. Pan CQ, Duan Z, Dai E, et al. Tenofovir to prevent Hepatitis B transmission in mothers with high viral load. N Engl J Med.

Table 1. Immunoprophylaxis failure rate of different hepatitis B e antigen status and HBV DNA levels				
		Immunoprophylaxis failure		
HBV DNA at 28-30 weeks (log10 copies/ml)	N	n	%	95% CI
<6 (~ 171,821 IU/mL)	474	0	0	0-0.8
6-6.99	24	0	0	0-14.3
7-7.99	22	0	0	0-15.4
8-8.99	58	5	8.6	2.9-19.0
≥9 (~ 170,000,000 IU/mL)	63	2	3.2	0.4-11.0
≥ 8 (~ 17,000,000 IU/mL)	121	7	5.8	2.4-11.6
HBeAg status				
Negative	486	0	0	0-0.8
Positive	155	7	4.5	1.8-9.1

HBeAg hepatitis e antigen