Development of occult hepatitis B viral infection in pregnancy: implications for antenatal screening in women from endemic areas

Philip Chang MBBS*, **Jeffrey Tu** MBBS*, **Antony Chesterman** MBBS*, **Robert Kim** MBBS*,
Peter Robertson BSc (Hons) PhD[†], **William D Rawlinson** PhD FRACP^{†§**} and **Stephen M Riordan** MD FRACP^{††}

*Gastrointestinal and Liver Unit; [†]Serology Division, South Eastern Area Laboratory Services; [‡]Virology Division, South Eastern Area Laboratory Services, The Prince of Wales Hospital; [§]School of Biotechnology and Biomolecular Sciences, Faculty of Science; ^{**}School of Medical Sciences, Faculty of Medicine; ^{††}Prince of Wales Clinical School, Faculty of Medicine, University of New South Wales, Sydney, Australia

Summary: Occult hepatitis B virus (HBV) infection, manifest clinically by the presence of HBV deoxyribonucleic acid (HBV DNA) in peripheral blood in individuals who test negative for the HBV surface antigen (HBsAg), may occur in various clinical contexts, including under the influence of pharmacological immunosuppression in patients from areas endemic for HBV and, hence, at risk of previous exposure. Pregnancy is a condition associated with immune suppression, but whether virus-specific immunity may be suppressed to an extent sufficient to allow occult HBV infection to develop is currently unknown. This is potentially relevant not only to the mother's health but also because vertical transmission has been reported in the occult HBV infection setting. We report a 30-year-old woman from a country endemic for HBV who, prior to pregnancy, was persistently HBsAg-negative with undetectable HBV DNA in peripheral blood, in whom HBV DNA became increasingly detectable during pregnancy, peaking in the third trimester, before returning to undetectable levels postpartum. HBsAg remained negative and liver function tests were normal throughout. Immunoglobulin M hepatitis B core antibody, a marker of the possibility of acquisition of a new HBV infection, was also negative. The baby received immunization against HBV infection from birth and has remained HBV negative at six months. This report documents for the first time that occult HBV infection may develop during pregnancy. Further data are required regarding the prevalence of this phenomenon, predisposing factors, impact on maternal health and risk of vertical transmission so that implications for current antenatal screening strategies that do not include measurement of HBV DNA in peripheral blood can be properly determined.

Keywords: hepatology, infection

INTRODUCTION

Chronic hepatitis B virus (HBV) infection remains an enormous global problem, with an estimated 350 million people affected. This is likely to be a substantial underestimation, as it takes no account of the recently recognized entity of occult HBV infection, manifest clinically by the presence of HBV deoxyribonucleic acid (HBV DNA) in peripheral blood in individuals who test negative for the HBV surface antigen (HBsAg). The absence of measurable HBsAg and the relatively low level of viral replication, as reflected by circulating HBV DNA concentrations, in occult HBV infection are mostly due to a vigorous host cellular immune response that limits replication activity and viral gene expression, rather than the result of infection with replication-defective or HBsAg-escape mutants¹ and instances of development of occult infection under the influence

of pharmacological immunosuppression have recently been reported in patients from areas endemic for HBV, and, hence, at risk of previous exposure.² Pregnancy is a condition associated with suppression of cellular immunity,^{3,4} but whether virus-specific immunity may be suppressed to an extent sufficient to allow occult HBV infection to develop is currently unknown. This is an issue of potential relevance not only to the mother's health but also because vertical transmission has been reported both clinically and in an animal model in the occult HBV infection setting.^{5,6}

CASE REPORT

A 30-year-old woman, born in China, a country endemic for HBV, presented for assessment on account of a family history of HBsAg-positive HBV infection involving at least two generations (mother, maternal aunt and two brothers), raising the possibility of vertical transmission. Family screening for HBV status had been undertaken after a brother, previously unaware of HBV infection, had recently been diagnosed with complicating hepatocellular carcinoma. She was asymptomatic and unaware of any previous clinical illness to suggest HBV

Correspondence to: Professor Stephen Riordan,
Gastrointestinal and Liver Unit, The Prince of Wales Hospital,
Barker Street, Randwick 2031, New South Wales,
Sydney, Australia
Email: sriordan@ozemail.com.au

infection. Physical examination was unremarkable. Liver function tests were normal. Results of HBV serology were as follows: HBsAg negative, as measured by an highly sensitive chemiluminescent microparticle immunoassay (CMIA) with additionally high capability to detect HBsAg-escape mutants (Abbott Architect HBsAg qualitative CMIA; Abbott Laboratories, IL, USA); HBV early antigen (HBeAg) negative (Abbott Architect HBeAg qualitative CMIA); HBV early antibody (HBeAb) negative (Abbott Architect anti-HBe CMIA); total HBV core antibody (HBcAb) (immunoglobulin [Ig]G and IgM) positive (Abbott Architect anti-HBc II assay); IgM HBcAb (Abbott Architect anti-HBc IgM assay) negative and HBV surface antibody (HBsAb) negative (Abbott Architect anti-HBs assay). This pattern of serology is indicative of previous, apparently resolved exposure to HBV. In order to assess for the possibility of occult HBV infection, HBV DNA was sought in peripheral blood and found to be undetectable using a sensitive technique (Cobas AmpliPrep/Cobas Taqman 48 real-time polymerase chain reaction assay; Roche Molecular Systems Inc, Pleasanton, CA, USA; sensitivity 54.5 IU/mL). All assays were performed according to the manufacturers' instructions. Liver ultrasonography was normal.

The patient was concerned regarding the finding of her previous HBV exposure and, in particular, at the possibility of any future reactivation and complication and, at her request, returned for review 12 months later. She had remained asymptomatic and there were no abnormal signs evident on physical examination. Liver function tests were normal. HBV serology was unchanged, with the HBsAg assay remaining negative, the HBeAg assay negative, the HBeAb assay negative, the total HBcAb assay positive and the HBsAb assay negative. HBV DNA remained undetectable in peripheral blood. Liver

ultrasonography remained normal. Given instances of occult HBV infection becoming evident for the first time in patients from endemic areas undergoing immunosuppressive therapy² and because both pregnancy is a condition associated with immune suppression^{3,4} and vertical transmission from mother to baby may occur in the occult HBV infection setting,^{5,6} it was decided to reassess for the possibility of the development of occult HBV infection should the patient become pregnant.

The patient thus returned five months later when she was seven weeks pregnant. She described no symptoms and there were no liver-related signs on physical examination. Liver function tests remained normal. HBV serology was unchanged. However, HBV DNA was now detectable at low concentration in peripheral blood. IgM HBcAb, measured as a marker of the possibility of acquisition of a new HBV infection to account for the increased HBV DNA level, was negative. At week 36 of pregnancy, the circulating HBV DNA level had increased further. Notably, the previously positive total HBcAb titre had become only equivocal by this time. HBsAg, HBeAg, HBeAb and HBsAb assays remained negative (Figure 1). The patient had received no immunosuppressive medication and there had been no change in her general health during pregnancy. Her liver function tests remained normal.

A live infant was born by vaginal delivery at 38 weeks and received HBV immune globulin and vaccination from birth. By six weeks postpartum, the mother's HBV DNA level in peripheral blood had again become undetectable, although the total HBcAb assay remained equivocal, returning to its prepregnancy positive result by six months. HBsAg, HBeAg, HBeAb and HBsAb assays remained negative throughout postpartum monitoring (Figure 1). The mother remained well with

Figure 1 Serial hepatitis B virus deoxyribonucleic acid (HBV DNA) levels in peripheral blood in relation to hepatitis B serology prepregnancy, during pregnancy and postpregnancy. HBsAg = hepatitis B virus surface antigen; HBeAg = hepatitis B e antigen; HBeAb = hepatitis B virus e antibody; HBcAb = total hepatitis B virus core antibody; HBsAb = hepatitis B virus surface antibody

normal liver function tests during this time. The baby remains HBsAg and HBV DNA negative at six months.

The mother was subsequently vaccinated (Engerix-B; GlaxoSmithKline, Boronia, Australia; 20 µg by intramuscular injection, thrice over 6 months), resulting in a protective HBsAb level of 7300 IU/L, with the aim of limiting the possibility of recurrent occult HBV infection, or even overt HBsAg-positive HBV infection,⁷ developing in subsequent pregnancies.

DISCUSSION

Suspected of existing since the 1980s, the entity of occult HBV infection has become identified and better characterized in recent years, as a result of the availability of highly sensitive molecular biology techniques.¹ Occult HBV infection is significantly associated with the presence of anti-HBV antibodies, most often IgG HBcAb, reflecting previous HBV exposure, but more than 20% of cases may occur in subjects negative for all antibody markers,⁸ meaning that their absence cannot be taken to exclude the possibility of occult infection. This seronegativity is presumably the result of a progressive disappearance of antibody markers over time following resolution of acute infection, but may, conceivably, reflect the absence of an antibody response from the time of the infection.¹

The prevalence of occult HBV infection from country to country reflects the general prevalence of HBV infection in various geographical locations. This entity is a rare occurrence in most western countries, whereas prevalence rates in the order of 2–15% have been reported in apparently healthy subjects in Asia and parts of Europe and Africa,^{1,9,10} The prevalence may be even higher when liver tissue, rather than peripheral blood, is analysed.¹ In the only analysis of the prevalence of occult HBV infection in pregnant women reported so far, a positivity rate of HBV DNA in peripheral blood of 11.4% was recently documented in a cross-sectional study in Korea, a figure in keeping with the general prevalence of occult hepatitis B in that country.¹¹

The infectivity of occult HBV infection is increasingly recognized, with reports of inadvertent transmission of infection by both blood transfusion and organ transplantation.¹ Risk of vertical transmission has also been reported both clinically and in an animal model.^{5,6} Experience in HBsAg-positive pregnant women suggests that most instances of vertical transmission probably occur at or near the time of birth¹² and immunization of the infant from birth using both HBV immune globulin and vaccination to produce antibodies to HBsAg is highly effective in preventing vertical transmission, especially at lower levels of maternal viraemia, as reflected by the circulating HBV DNA concentration.¹² This approach is likely to be especially effective in protecting against vertical transmission in babies born to mothers with occult HBV infection, since most cases are not the result of infection with an HBsAg-escape mutant strain¹ and, as in our patient, circulating HBV DNA levels are generally only modestly elevated in the occult infection setting.¹

Pregnancy is associated with disturbed cellular immunity. In particular, the maternal Th1 immune response may be suppressed during normal pregnancy to avoid rejection of the fetus, resulting in a predominant Th2 phenotype which allows the pregnancy to continue.^{3,4} While this Th2-dominant phenotype at the maternal–fetal interface allows fetal survival, the presence of anti-inflammatory cytokines such as interleukin

(IL)-4 and IL-10 may predispose to increased risk of infection, including reactivation of latent viruses, such as HBV.^{3,4}

The unique longitudinal nature of our report has allowed us to document for the first time that, in a previously exposed patient from an endemic area, but with persistently undetectable HBV DNA in peripheral blood prior to pregnancy, occult HBV infection may become evident during pregnancy, with the magnitude of the HBV DNA level in peripheral blood, and thus possible risk of vertical transmission,¹² increasing in the third trimester. The change in total HBcAb status from positive to only equivocal and the absence of a measurable IgM HBcAb response coincident with the detection of HBV DNA in peripheral blood together point to an important role played by reduction of virus-specific immunity, rather than the possibility of new HBV exposure, in the development of the occult infection in our patient, in whom the peak HBV DNA level in pregnancy was comparable to that recently reported in patients from an endemic area who developed occult HBV infection in the context of pharmacological immunosuppression.² The risk of occult HBV infection developing in this latter circumstance was 6% in the only reported analysis to date.² The prevalence of occult HBV infection developing in pregnancy is currently unknown. Data regarding this, along with assessment of predisposing factors for this phenomenon, its possible impact on maternal health and the risk of vertical transmission, are required so that implications for current antenatal screening strategies that do not include measurement of HBV DNA in peripheral blood can be properly determined.

The natural history of HBsAg-positive HBV infection in pregnancy is variable but generally favourable.^{13,14} In a recently reported retrospective cohort study of 17 HBsAg-positive pregnant Asian-American women who were managed at a community gastroenterology practice and a university hepatology clinic in the United States over an eight-year period,¹³ and for whom baseline liver function tests prior to pregnancy were available, the ratio of maximum serum alanine aminotransferase (ALT) level during pregnancy to baseline ALT level was found to increase in 10/17 (58.8%) patients, decrease in 6/17 (35.3%) patients and remain unchanged in 1/17 (5.9%) patients. Two patients (11.8%) developed an at least three-fold increase in ALT level during pregnancy, with ALT values peaking at 295 and 1540 U/L, respectively (normal generally less than 45 U/L). Marked hepatocellular injury, as reflected by peak ALT levels of 586 and 2522 U/L, respectively, also occurred during pregnancy in 2/11 (18.2%) additional patients for whom no baseline ALT level was available. Three of these four patients with biochemical evidence of marked hepatocellular injury during pregnancy decompensated clinically, with the development of jaundice and hepatic encephalopathy and the necessity of emergency liver transplantation in one case.

In another recent report of mostly Chinese women attending a hospital clinic in Singapore,¹⁴ the clinical course of 35 HBsAg-positive pregnant women with chronic HBV infection was monitored during pregnancy and for three months postpartum and compared with that of 140 non-pregnant controls matched for age and HBeAg status. No significant difference in the incidence of an increase in ALT value was apparent during pregnancy compared with that in control patients (34.6% versus 24.6%, respectively). However, a flare in hepatitis, as reflected by an increase in ALT level, was significantly more common in the first three months following pregnancy than in controls (50.0% versus 10.5%, respectively; $P < 0.001$). Notably no patient decompensated clinically in this series. Risk of flare

of HBV-related hepatitis during the early postpartum period has also been documented in separate studies from Sweden¹⁵ and the Netherlands,¹⁶ a phenomenon attributed to immune reconstitution following pregnancy.^{12,14} Restoration of immune responsiveness was presumably responsible for HBV DNA again becoming undetectable in peripheral blood and the total HBcAb again becoming positive in the postpartum period in our patient, in whom no biochemical evidence of associated liver injury was evident.

The role of vaccination in patients with occult HBV infection is currently a matter for conjecture. We decided to offer vaccination to our patient in this unique clinical context prior to any future pregnancies, in an attempt to limit her chance of developing recurrent occult HBV infection, or even progressing to overt HBsAg-positive HBV infection, given a recent report in Sicilian patients with occult HBV infection at baseline that demonstrated that 28% developed HBsAg-positive infection during pharmacological immunosuppression and that all such patients subsequently experienced severe hepatitis, with ALT levels in peripheral blood peaking in excess of 10 times normal.

REFERENCES

- 1 Raimondo G, Pollicino T, Cacciola I, Squadrito G. Occult hepatitis B virus infection. *J Hepatol* 2007;**46**:160–70
- 2 Georgiadou SP, Zachou K, Liaskos C, Gabeta S, Rigopoulou EI, Dalekos GN. Occult hepatitis B virus infection in patients with autoimmune liver diseases. *Liver Int* 2009;**29**:434–42
- 3 Mitchell MD, Trautman MS, Dudley DJ. Cytokine networking in the placenta. *Placenta* 1993;**14**:249–75
- 4 Wegmann TG, Lin H, Guilbert L, Mosmann TR. Bidirectional cytokine interactions in the maternal–fetal relationship: is successful pregnancy a TH2 phenomenon? *Immunol Today* 1993;**14**:353–6
- 5 Saito T, Shinzawa H, Uchida T, *et al.* Quantitative DNA analysis of low-level hepatitis B viremia in two patients with serologically negative chronic hepatitis. *B J Med Virol* 1999;**58**:325–31
- 6 Coffin CS, Michalak TI. Persistence of infectious hepadnavirus in the offspring of woodchuck mothers recovered from viral hepatitis. *J Clin Invest* 1999;**104**:203–12
- 7 Ferraro D, Pizzillo P, Di Marco V, *et al.* Evaluating the risk of hepatitis B reactivation in patients with haematological malignancies: is the serum hepatitis B virus profile reliable? *Liver Int* 2009;**29**:1171–7
- 8 Torbenson M, Thomas DL. Occult hepatitis B. *Lancet Infect Dis* 2002;**2**:479–86
- 9 Kim SM, Lee KS, Park CJ, *et al.* Prevalence of occult HBV infection among subjects with normal serum ALT levels in Korea. *J Infect* 2007;**54**:185–91
- 10 Allain JP. Occult hepatitis B virus infection. *Transfus Clin Biol* 2004;**11**:18–25
- 11 Kwon CI, Hwang SG, Shin SJ, *et al.* Occult hepatitis B virus infection in pregnant women and its clinical implication. *Liver Int* 2008;**28**:667–74
- 12 Jonas MM. Hepatitis B and pregnancy: an underestimated issue. *Liver Int* 2009;**29**(Suppl. 1):133–9
- 13 Nguyen G, Garcia RT, Nguyen N, Trinh H, Keefes EB, Nguyen MH. Clinical course of hepatitis B virus infection during pregnancy. *Aliment Pharmacol Ther* 2009;**29**:755–64
- 14 Tan HH, Lui HF, Chow WC. Chronic hepatitis B virus (HBV) infection in pregnancy. *Hepatol Int* 2008;**2**:370–5
- 15 Soderstrom A, Norkrans G, Lindh M. Hepatitis B virus DNA during pregnancy and post partum. Aspects on vertical transmission. *Scand J Infect Dis* 2003;**35**:814–19
- 16 ter Borg MJ, Leemans WF, de Man RA, *et al.* Exacerbation of chronic hepatitis B infection after delivery. *J Viral Hepatol* 2008;**15**:37–41

(Accepted 3 June 2010)