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ORIGINAL ARTICLE

Chronic hepatitis B virus (HBV) infection in pregnancy

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Abstract
Background Hepatitis B is a considerable disease burden among Asians. Little is known about its disease behaviour in pregnant women.
Methods Clinical, laboratory and radiological data of pregnant and peri-partum females with chronic hepatitis B virus (HBV) infection who were seen between years 1999 and 2004 were studied. Their progress was documented up to 6 months post-partum. This was compared with the age-matched and HBe status-matched, non-pregnant, female patients with chronic HBV infection, who were consecutively selected from the department's registry as controls (ratio 1 mother: 4 non-pregnant controls), over the corresponding period.
Results A total of 35 mothers and 140 controls were studied. Mean age of patients was 30.7 ± 3.6 years. Majority of mothers (74.3%) presented during pregnancy itself. 1st:2nd:3rd trimester presentation = 20.0%:48.6%:5.7%. Majority (65.7%) were positive for HBe antigen (HBeAg) at the time of presentation. About 57.1% mothers had a clinical event in the form of alanine transferase (ALT) elevation and/or loss of HBeAg vs 28.8% among controls ($P = 0.002$). Among HBeAg-positive subjects, more mothers (14.3%) than controls (2.2%) had resultant HBeAg loss ($P = 0.02$). Among HBeAg negative subjects, more mothers than controls had serum ALT elevations in the post-partum period ($P = 0.007$). Overall, more mothers had elevated ALT levels than controls, regardless of their HBeAg status. Neither mothers nor control subjects decompensated clinically, neither required liver transplantation nor died during the study period.
Conclusions Pregnancy is associated with serum ALT elevation and HBeAg loss in patients with chronic HBV infection in the peri-partum period.

Keywords Hepatitis B · Seroconversion · Pregnancy · Maternal care · Acute exacerbation

Introduction

Despite the adoption of national hepatitis B immunisation programmes in some countries for almost two decades, chronic hepatitis B virus (HBV) infection still remains a considerable medical and financial burden, affecting young adults in many Asian countries. Most Asians tend to acquire chronic HBV infection peri-natally or during early childhood. Hence, most chronically infected females would, at some point, reach childbearing potential. As the evaluation and management of abnormal liver tests in the pregnant female is particularly challenging, the importance of understanding the natural history of chronic HBV infection in the peri-partum period is vital. This is especially so when antiviral agents can be deployed early in acute exacerbation of hepatitis B and could make a difference to maternal morbidity and mortality before hepatic decompensation. While much has been documented about peri-natal transmission of HBV to babies and the possible modes of prevention [1–4], there are minimal data in English literature on the effect of pregnancy on maternal HBV disease in the chronically infected mothers.

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Aim

The purpose of this study was to determine viral activity and clinical events associated with chronic HBV infection in pregnant and peri-partum females.

Method

Chronic HBV-infected females who were on our centre's regular surveillance follow-up for asymptomatic chronic HBV infection and became pregnant, or pregnant mothers who were incidentally found to be HBsAg positive on routine antenatal blood test and were referred to our centre between the years 1999 and 2004 were considered eligible for the study. All mothers' clinical progress, laboratory and radiological data obtained after they were known to be pregnant to minimum 3 months post-partum were reviewed. Mothers with clinical events were reviewed till 6 months post-partum or till resolution of event, whichever occurred earlier. Mothers without clinical events by third month post-partum were deemed to be event-free and data review was stopped by then. Mothers with obstetric complications (e.g. pre-eclampsia, acute fatty liver of pregnancy, intrahepatic cholestasis), concurrent use of hepatotoxic drugs (including those from traditional or herbal sources), with any concurrent chronic liver disease (e.g. hepatitis C or HIV), autoimmune disease or systemic diseases were excluded from the study.

Controls

Consecutive age-matched and HBe antigen (HBeAg)-status matched non-pregnant females were selected as controls from the centre's chronic HBV infection registry over the same study period. This registry was set up from the year 1991 to capture all consecutive chronic HBV patients on the centre's follow-up, regardless of disease activity. Controls were selected in the ratio of four controls for every one pregnant or peri-partum female studied. The same exclusion criteria for eligibility of mothers were applied to the selection of control subjects. In addition, none of the controls was on oral contraceptives. The same parameters of clinical progress, laboratory and radiological data were reviewed for control subjects for a total duration of 12 months (if pregnant or peri-partum patients had no clinically significant event) or 15 months (if pregnant or peri-partum patients had a clinical event), correspondingly.

Definitions

'Mothers' was defined as any female subject who was included in the study who presented either during pregnancy (anytime from months 0 to 9 of conception) or in the peri-partum period (anytime from months 10 to 12 of conception; i.e. within 3 months of delivery). 'HBeAg loss in pregnancy' was defined as detectable HBeAg during pregnancy, which had become negative either during pregnancy or up to 6 months post-partum among mothers. 'HBeAg loss among control subjects' was defined as detectable HBeAg, which had become negative during the corresponding 15 months of review. 'HBeAg seroconversion' was defined as any HBeAg loss accompanied by the presence of detectable anti-HBe antibodies. A 'clinical event' was defined as an elevation in alanine transferase (ALT) levels (anytime during the pregnancy to 3 month post-partum), HBeAg-loss (anytime during the pregnancy to 6 months post-partum) or any clinical decompensation.

Data

Data were analysed with SPSS version 12.0. Categorical outcomes were obtained using Chi-square or Fisher's exact test where appropriate. Parametric variables were analysed by Student's *t*-test. Statistical significance was defined as P-value <0.05.

Results

From the period 1999 to 2004, 36 mothers were followed-up by the department. However, one mother was excluded from the study for the reason of pre-eclampsia.

Hence, a total of 35 mothers and 140 matched controls were included in the final analysis (Table 1).

Most mothers came for review either as part of their regular follow-up for their known chronic HBV infection (51.4%) or on advice by their primary physicians (45.7%), when the patient was found to be pregnant and HBsAg-positive, albeit well. Only one mother was referred to the department because of an abnormal liver function test. All mothers were asymptomatic prior to their pregnancy, and at time of presentation to our clinic when first found to be pregnant.

The mean age of mothers was 30.7 ± 3.6 years. Majority of mothers and controls were Chinese. Majority of mothers were included in the study during pregnancy (within months 0 to 9 of conception); and mostly in the second trimester (months 4 to 6 of conception). Majority of mothers in this study were in their second pregnancy (42.9%), followed by mothers who were in their first pregnancy (31.4%).

About 65.7% of mothers and matched controls were HBeAg positive.

Table 1 Characteristics of mothers and matched-controls

Characteristics	Mothers (%) $N = 35$	Controls (%) $N = 140$
Mean age (years)	30.7 ± 3.6	30.7 ± 3.6
HBeAg positive at presentation	23 (65.7)	92 (65.7)
HBeAg negative at presentation	12 (34.3)	48 (34.3)
Presented during pregnancy	26 (74.3)	NA
Trimester 1:2:3	7 (20.0):17 (48.6):2 (5.7)	NA
Presented in post-partum period	9 (25.7)	NA

The clinical events observed in this study included the elevation of sera ALT during pregnancy or post-partum, HBeAg loss or HBe-seroconversion (Table 2). Overall, more mothers had elevated sera ALT (s. ALT) levels than controls, regardless of their HBeAg status. HBeAg-positive subjects had more clinical events than HBeAg-negative subjects, among both mothers and controls. The median s. ALT level among mothers was 39 U/l, higher than that among controls (Table 3). By proportion, more mothers had ALT elevations above twice the upper limit of normal.

For those mothers ($n = 18$) who had been on regular follow-up by the Centre prior to the discovery of their pregnancies, we retrospectively reviewed their s. ALT levels that were measured 3 and/or 6 months prior to the discovery of their pregnancies during their previous routine follow-up visits. All 18 of them had s. ALT done 6 months prior, and 12 had additional s. ALT done 3 months prior to the visit when they reported pregnancy. It was found that their median s. ALT levels were within normal limits, measuring 24.5 and 27.5 U/l, 3 and 6 months earlier, respectively; 58.3 and 61.1% of them had s. ALT within the normal range in the previous 3 and 6 months prior to the discovery of their pregnancies.

Among HBeAg-positive subjects, more mothers than controls had HBeAg loss by the end of the study period. When these mothers were followed-up beyond the study period, all of them developed detectable HBe-antibodies within 0–12 months after e-antigen loss. All had sustained seroconversions up to the time of last follow-up. The minimum follow-up was at least 1-year post-partum and the longest follow-up for these mothers was 5 years (data not shown). All HBe-seroconversions among mothers were spontaneous and none required therapeutic intervention with antiviral agents.

None of the mothers or controls developed clinical decompensation or died within the study period.

Discussion

Our results show that pregnancy is significantly associated with ALT elevation and spontaneous HBe-seroconversion in HBeAg-positive mothers.

Pregnancy is an immunological juggle between maternal tolerance of paternal MHC antigens (and hence the foetus) and that of maintaining immunocompetence for defence against microbes and other antigens.

Apart from locally acting mechanisms that specifically delete maternal allo-reactive cells, a variety of autoimmune diseases have been found to improve during gestation in mice and humans, with a higher risk of relapse after delivery [5, 6]. Recently, it has become clear that

Table 2 HBV-related events in the peri-partum period

HBV-related events	Mothers (%)	Controls (%)	*P*-value
Overall ($N = 175$)	$N = 35$	$N = 140$	
All clinical events	20 (57.1)	40 (28.8)	0.002
Raised ALT in pregnancy	9 (34.6)	34 (24.6)	NS
Raised ALT post-partum	15 (50.0)	14 (10.5)	<0.001
HBeAg-positive (at presentation)	$N = 23$	$N = 92$	
All clinical events	16 (69.6)	37 (40.7)	0.01
HBeAg loss	3 (14.3)	2 (2.2)	0.02
Raised ALT in pregnancy (or corresponding months 0–9)	8 (50.0)	31 (34.1)	NS
Raised ALT post-partum (or corresponding months 10–12)	12 (66.7)	14 (16.3)	0.01
HBeAg-negative (at presentation)	$N = 12$	$N = 48$	
All clinical events	4 (33.3)	3 (6.3)	0.009
Raised ALT in pregnancy (or corresponding months 0–9)	1 (10.0)	3 (6.4)	NS
Raised ALT post-partum (or corresponding months 10–12)	3 (25.0)	0 (0.0)	0.007

Table 3 Magnitude of ALT elevation among mothers and controls

	ALT median (U/l)	ALT normal (%)	ALT > 1–2 × ULN[a] (%)	ALT > 2–5 × ULN[a] (%)	ALT 5–10 × ULN[a] (%)	ALT ≥ 10 × ULN[a] (%)
Mothers	39.0	42.4	27.3	15.2	6.1	9.1
Controls	26.0	69.4	14.9	11.2	2.9	1.5

[a] Magnitude of ALT elevation expressed as multiples of the upper limit of normal

mechanisms that promote T cell suppression are central to the induction of peripheral immune tolerance [7]. A significant proportion of the T lymphocyte population consists of regulatory T cells that are capable of suppressing the activation of 'effector' CD4 and CD8 lymphocytes [8]. The establishment of chronic HBV infection is associated with a progressive decline of the adaptive immunity with a lower number of both circulating and intrahepatic virus-specific CD8 and CD4 T cells and low production of virus-specific antibodies [9]. Although sustained T-cell hyporesponsiveness is found in patients with chronic HBV infection, such hyporesponsiveness is known to be reversible. Acute exacerbation of hepatitis B can be preceded by recovery of CD4-mediated T cell reactivity to HBV nucleocapsid antigens [10, 11] that have been suggested to be triggered by the increasing concentrations of HBeAg and HBcAg [12]. Acute exacerbation of hepatitis B can also be followed by a significant rise in IL-12 and Th1 cytokine production that can precede or occur spontaneously with HBeAg seroconversion [10].

Although not well documented for chronic HBV infection in the peri-partum period, the increased rate of seroconversion could be related to a post-partum immune rebound. It has been documented that the level of endogenous cortisol levels rise in the second trimester and reach their peak in the third trimester (up to thrice the normal levels in a non-pregnant female), before falling rapidly to pre-pregnant levels after delivery [13, 14]. In association with this, it is interesting to note that Soderstrom et al. [15] reported an increase in HBV DNA level by mean 0.4 log copies late in pregnancy and the early post-partum period, in association with ALT elevation in 33 subjects (55 pregnancies). Although to a differing degree, this phenomenon was seen in both HBeAg-positive and HBeAg-negative mothers. Similar findings were also reported in two Asian populations. In 1989, a study by Lin et al. [16] on chronic HBV-infected mothers who were HBeAg positive reported that 5 out of 30 mothers seroconverted in their first 3 months post-partum. In contrast, none of the patients in their control group had HBe seroconversion. Also, overt liver dysfunction was observed in 43% of mothers who were HBeAg positive within the first post-partum month in a Japanese study [17] that involved 269 pregnant women with chronic HBV infection.

Lin et al. [18] further studied 40 HBeAg-positive, chronic HBV-infected mothers prospectively and recently reported that low HBeAg titres and low HBV DNA viral loads were associated with HBeAg loss. This was based on virologic studies of serum taken from these infected mothers at the time of delivery, 3–4 months and 1-year post-partum. However, by multiple logistic regression analysis, only low HBeAg titres were found to be significantly associated with HBeAg loss. There was no particular HBV genotype (genotypes B and C were included in the study) significantly associated with the loss of HBeAg among the mothers.

Being a retrospective study, unfortunately the detailed virologic profile of our study population was not well defined and no T cell study corresponding to the clinical event was carried out. However, the fact that the clinical outcome of our pregnant mothers was consistent with that found in other earlier studies would substantiate the interplay of immunologic and virologic events underlying the clinical manifestation in mothers with chronic HBV infection, as suggested by the preceding findings of our colleagues. With a relatively immunocompromised state in the second and third trimesters of pregnancy facilitating viral replication, hence the increase in viral load, it is conceivable that the lowering maternal cortisol levels coinciding with the end of pregnancy leads to restoration of maternal immunocompetence, thus the orchestration of an immunoclearance of HBV. Whether such peri-partal phenomenon will necessarily lead to successful HBe antigen loss or seroconversion will, understandably, be dependent on the viral load or HBeAg titre, as suggested by Lin et al. [18], in addition to the scale of the immunologic activities mounted, at the start of such an event. This phenomenon is analogous to that following the withdrawal of iatrogenic corticosteroids in non-pregnant patients with chronic HBV infection.

In order to be sure that our mothers were not already in the immunoclearance phase of their chronic HBV infection that may coincidentally account for our findings, we looked back at their s. ALT of the recent past, prior to the discovery of their pregnancies. We found that their earlier s. ALT levels were not significantly different from that of our controls and a larger proportion of them had s. ALT within normal limits just before they were pregnant. Hence,

suggesting the fact that there were more mothers than controls in our study having a higher degree of ALT elevation, as reflected in the median ALT levels and peak elevation, is likely to be related to their pregnancies, rather than prior predisposition.

Nevertheless, we would like to acknowledge several limitations in our study. Firstly, this is a retrospective cross-sectional study that makes it difficult to extrapolate the findings to the actual natural history of this condition in pregnancy. Nevertheless, our findings do suggest that chronic HBV infection is not necessarily quiescent when a female patient goes through pregnancy. Secondly, we have tried as best as possible to match a similar cohort of non-pregnant patients as controls, by their gender, age and HBeAg status and shown that when these confounders are controlled, more chornic HBV infected mothers have clinical events as compared to controls. Although our controls were not matched for viral genotype, it is notable that an earlier-mentioned study did not find this to be a significant determinant for HBeAg loss [18]. Just like the Taiwanese population studied, we have previously found that our HBV-infected patients were also predominantly infected by HBV genotypes B and C [19]. We are also aware that we did not include non-HBV infected pregnant females as separate controls. This was because previous study [20] has already shown that s. ALT in pregnancy, albeit higher in the second trimester of pregnancy in normal female subjects when compared with non-pregnant controls, remained well within the normal limits. Thus pregnancy per se could not have accounted for the elevated s. ALT in our pregnant, HBV-infected patients. Thirdly, as this was a retrospective study involving asymptomatic mothers, they tend to report about their pregnancy only as and when they return for their originally scheduled routine follow-up for HBV infection at our centre, this explains why all mother's data or sera were not drawn at the same time points of their pregnancy. Hence, while some mothers were identified and monitored from the first trimester of pregnancy, others were included in the study from a later part of their pregnancy. Also, a proportion of patients were referred by their primary care doctors who had been following their pregnancies till a screening test for HBV infection was positive. Although these patients were largely asymptomatic (except for one who was referred for an elevated ALT level), they tended to be referred to our centre later in pregnancy, usually at second or third trimester.

Finally, it is important to appreciate the variability in the clinical manifestation of acute exacerbation of chronic hepatitis B during the peri-pertum period. The clinical events in our study were limited to elevated ALT levels, HBeAg loss or seroconversions, and mothers with clinical events had only mild disease followed by spontaneous resolution. It was fortunate that none of our patients had a fatal clinical event. However, this is not always the case. In a retrospective study conducted by Wong et al. [21] in Singapore, the medical records of obstetric patients with abnormal liver function tests were reviewed to determine the common causes. In this study, a total of 48 patients were studied, of which the cause in three patients was attributed to hepatitis B 'flare'. Two of these patients required urgent referrals to a liver transplant centre. Unfortunately, both deteriorated rapidly and demised before they could be transplanted. One deteriorated while in the third trimester while the other deteriorated in the second week post-partum. At the time of decompensation, one patient had HBeAg-positive viraemia, while the other had HBeAg-negative viraemia. All three patients presented while in their third trimester of pregnancy. It is not known if any of these patients were on herbal or immunomodulatory drugs at the time of disease activity.

Conclusions

The findings of our cohort study, consistent with those in some of the earlier reports, suggest that pregnancy is likely to be associated with serum ALT elevation and HBeAg loss in patients with chronic HBV infection in the peri-partum period. While a large scale, prospective study of HBV patients going through pregnancy would be necessary to help shed more light to this condition, it would be prudent for now to keep a closer follow-up on chronic HBV-infected, pregnant females in order to render them early treatment, whenever indicated, to avoid potentially serious maternal morbidity and mortality that may be associated with acute exacerbations of chronic hepatitis B.

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