Abnormal IL-10 levels were related to alanine aminotransferase abnormalities during postpartum in HBeAg positive women with chronic hepatitis B

Ming Wang, MD
Ying Hou, MD
Shi-Hui Meng, MD
Bo Yang, MD
Ping Yang, MD
Hua Zhang, MD
Yunxia Zhu, MD

Abstract
Alanine transaminase (ALT) abnormalities are common in chronic hepatitis B (CHB) carriers during postpartum period. Disturbances in cytokines are considered to be associated with hepatitis Flares. There are limited data on cytokines changes in HBeAg positive patients with ALT abnormalities.

This is an observational study. Pregnant patients with hepatitis B e-antigen (HBeAg) positive were enrolled from January 2014 to September 2018. Patients were assigned into three groups based on ALT levels in postpartum 6 to 8 weeks: ALT in normal range, ALT in 1 to 2-fold upper limits of normal (ULN) and ALT >2-fold ULN. Serum cytokines, ratios of regulatory T cells, and the concentration of cortisol were collected and compared among the three groups.

Of the 135 mothers enrolled, 80.7% (109/135) completed the postpartum 6-week study. 13.8% (15/109) patients had postpartum ALT higher than 2×ULN, 27.5% (30/109) patients had ALT in 1 to 2×ULN and 58.7% (64/109) patients had ALT in normal range. Compared to control group, patients with ALT >2 ULN had a higher IL-10 level (P < .05). No differences of IL-10 levels were found in the comparison of other inter comparison among three groups. No differences were found in the levels of other collected serum cytokines, cortisol, and regulatory T cells among three groups. On multivariate analysis, abnormal IL-10 level was independent risk factor for postpartum ALT elevating >2×ULN. At the same time, the incidence of postpartum ALT elevated >2×ULN were higher in patients with abnormal elevation IL-10 level than in patients with normal IL-10 level (14/68 vs 1/41, P = .008).

CHB patients with postpartum ALT abnormalities show higher IL-10 level and postpartum ALT abnormalities were mainly occurred in patients with abnormal IL-10 level. IL-10 may be an underlying predictor and treatment target of hepatitis B, and further studies are needed.

Abbreviations: ALT = alanine aminotransferase, CHB = chronic hepatitis B, G-CSF = granulocyte colony stimulating factor, HBeAg = hepatitis B e-antigen, HBsAg = hepatitis B surface antigen, HBV = hepatitis B virus, IL = interleukin, INF-γ = interferon-γ, LdT = telbivudine, MTCT = mother-to-child transmission, TDF = tenofovir disoproxil fumarate, TNF-α = tumor necrosis factor-α, ULN = upper limits normal.

Keywords: alanine aminotransferase, hepatitis B e-Antigen, interleukin-10, postpartum

1. Introduction
Chronic hepatitis B virus (HBV) infection is an epidemic that is associated with cirrhosis and liver cancer.[1] Without intervention, 80% to 90% of infants born of hepatitis B e-antigen (HBeAg) positive mothers may be infected with HBV.[2] After the widespread use of the HBV vaccine combined with hepatitis B immunoglobulin G. The mother to child transmission rate (MTCT) has been reduced from 90% to ~ 5% to 10%.[3,4]
Previous studies indicated that inhibiting HBV replication with lamivudine (LAM), telbivudine (LdT) or tenofovir disoproxil fumarate (TDF) during late pregnancy in HBsAg positive mothers with high viremia could further reduce the risk of MTCT.\(^7\)\(^8\) However, postpartum ALT level elevation is common in CHB patients, which ranged from 10% to 57%.\(^9\)\(^10\) In a study, 45 women with postpartum ALT level exceeding two times the upper limit of normal and HBV DNA level that had decreased more than 3 lgIU/mL or HBeAg titer that had decreased more than 50% were switched to peg-IFNs and adefovir (CPIA) for 96 weeks. 91.1% (41/45) of them achieved a virological response, 55.6% (25/45) achieved HBeAg clearance and 26.7% (12/45) achieved hepatitis B surface antigen (HBsAg) clearance or seroconversion. There were no cases of significant deterioration of liver function.\(^10\)

Although mild-to-moderate elevations of ALT levels during pregnancy have not been linked to pregnancy complications in mothers with CHB,\(^11\) a recent study reported that severe ALT flares can cause maternal death.\(^12\) Then, the postpartum ALT flares may mean a chance of better treatment or risk of liver deterioration.

The mechanism of ALT flares in these patients has not been fully understood. ALT flares or exacerbations are mainly observed in mothers with elevated ALT or HBV DNA levels ≥5 log10 IU/mL at delivery.\(^13\) In pregnancy, immune alterations such as regulatory T (Treg) cells expansion and distinct regulation of Th1, Th2, and Th17 cytokines occur to prevent fetus rejection.\(^13\)\(^14\) Maternal immune responses and cytokines are mediators of inflammation, indicate immune activation, and play a role in viral clearance.\(^15\) Previous reports suggested that immunological changes during pregnancy may stimulate HBV clearance in highly viremic mothers.\(^8\) It is hypothesized that postpartum hepatic flares in HBV or autoimmune hepatitis are due to immune system reactivation after parturition.\(^16\)\(^17\) We selected patients who received antiviral treatment during pregnancy and compared their cytokines in different postpartum ALT groups. We aimed to study the relationship between postpartum ALT abnormalities and cytokines during postpartum non-treatment patients with high viremia.

2. Study methods

2.1. Design and patient selection

This is an observational case-control study in which patients were recruited from a tertiary care hospital, Beijing Youan Hospital in China between January 2014 and September 2018. The trial was approved by the institutional ethics review committee (approval number: Jing-you-ke-lun-zi [2016]15) and has been registered on the Chinese Clinical Trial Registration (no. ChiCTR-OIC-17010869). Pregnant women fulfilling the inclusion and exclusion criteria were offered participation in the study. All CHB pregnant carriers were assessed in the second trimester and followed until 6 to 8 weeks postpartum. Patients were assigned into three groups based on the ALT in postpartum 6 to 8 weeks: control (normal postpartum ALT levels), 1 to 2 ULN postpartum ALT levels and >2 fold ULN postpartum ALT levels. Serum quantitative hepatitis B virus (HBV) markers, HBV-DNA, ALT, cytokines, ratios of regulatory T cells and the concentration of cortisol were collected and compared among the three groups.

Beijing Youan Hospital is the appointed hospital by the department of health in Beijing to evaluate mothers with hepatitis B. Pregnant mothers were screened for the following criteria of eligibility: age between 20 and 45 years, HBsAg and HBeAg positivity, and HBV DNA levels above 5 log10IU/mL; gestational age 24 to 28 weeks, Key exclusion criteria included ALT ≥80 U/ mL (ULN = 40 U/mL); co-infection with hepatitis C, D, E, or HIV; evidence of hepatocellular carcinoma or cirrhosis; concurrent treatment with immune modulators, cytotoxic drugs, or steroids; evidence of fetal deformity by ultrasound examination.

2.2. Study procedures and data collections

Chronic Hepatitis B (CHB) was diagnosed according to the national guidelines in China for the timing of initiating on preventing mother to child transmission (MTCT). Patients were followed at 4-week intervals before the due date and the 6 to 8 weeks after delivery.

Clinical data recorded included age, ALT, HBV-DNA, HBV-markers, cortisol, ratio of regulatory T cells, and cytokines. A significant ALT flare was defined as >2-fold upper limits normal. The cytokines were detected with Bio Plex cytokine assay. According to the assay manufacturers, the sensitivity (expressed as the minimum detectable dose) and the upper limit of detection, the cytokines were detected with Bio Plex cytokine assay. According to the assay manufacturers, the sensitivity (expressed as the minimum detectable dose) and the upper limit of detection, respectively, for the various assays are: regulator T cells (5–10%), IL-2 (0 pg/mL, 99.44 pg/mL), IL-4 (0 pg/mL, 3.25 pg/mL), IL-6 (0 pg/mL, 8.53 pg/mL), IL-8 (0 pg/mL, 116.07 pg/mL), IL-10 (0 pg/mL, 1.53 pg/mL), INF-γ (0 pg/mL, 124.08 pg/mL), TNF-α (0 pg/mL, 60 pg/mL), and GM-CSF (0 pg/mL, 122.19 pg/mL).

2.3. Outcome measurements and endpoints

This study aimed to compare the concentrations of various cytokines, the ratio of regulator T cell, and cortisol in plasma samples in different postpartum ALT levels in patients with high viremic on-treatment.

2.4. Statistical analysis

In our trial, the mean value of IL-10 was 11.5 ± 4.8 pg/mL in CHB patients and 4.8 ± 0.4 pg/mL in immune tolerant patients. Based on the data, 15 patients with ALT abnormal and 64 patients in immune tolerant phase could achieve more than 90% power to a significance level of 0.05 both one-tailed and two-tailed. Considering the incidence of Alanine aminotransferase flares was observed 17.1% to 45% in LdT or TDF treated mothers\(^6\) and 20% drop-out rates, 126 cases were a reasonable least sample size.

Baseline characteristics and laboratory results were summarized for the three groups utilizing descriptive statistics, including percentage, mean ± standard deviation (SD). Statistical analysis was performed using analyzed by SPSS 23.0 (SPSS, Inc., NYC, IBM) and GraphPad Prism Version 7.0 (GraphPad, La Jolla, CA). For the quantitative variable, the one-way ANOVA was used to compare three group differences. For categorical variables, the
Kruskal–Wallis rank sum test was used for three group comparisons. Student’s t test was used to assess continuous variables of two groups. Multivariate classification logistic regression was used to adjust IL-10 on predicting ALT elevation. Abnormal cytokine values were replaced with 1 and normal cytokine values were replaced with 0 during regression. The significance level was set at P < .05.

3. Results

3.1. Study population

Our study screened 159 CHB patients with HBeAg positive during the second trimester, 6 patients had ALT higher than 2 ULN at baseline and 153 patients were in the immune tolerant phase (ALT < 2 ULN). Of the 153 patients in immune tolerant phase, 135 patients started antiviral treatment after baseline research blood sample collection and 18 patients were not administered antiviral drugs. 109 of 153 patients completed the study (Fig. 1). At the time of postpartum follow-up, the ALT of 15 patients were increased more than 2 ULN (177.81 ± 166.53 IU/L), 30 patients from I ULN to 2 ULN (55.82 ± 10.92 IU/L), and 64 patients in the normal range (26.27 ± 7.04 IU/L). Patients disposition is shown in Figure 1. The mean (SD) gestational weeks of delivery were 38.47 ± 1.41, 39.16 ± 1.05, 39.45 ± 1.53 weeks (P = .036) in three groups respectively, and the time of postpartum follow-up were 6.33 ± 1.23, 6.74 ± 1.15, 6.93 ± 2.32 weeks (P = .524) after delivery. Patients with postpartum ALT elevation had a higher HBV level in the baseline and lower HBV level in the postpartum, indicating more patients stepped into immune clearance phase (Table 1). There were no differences in the levels of HBsAg, HBeAg, and ALT, except the higher HBV-DNA in the patients with postpartum ALT elevated more than 2 ULN.

3.2. Serum IL-10 flares are frequent in CHB carriers with postpartum ALT flares

We tested Th1 (IL-2, IFN-γ, TNF-α), Th2 (IL-4, IL-6, IL-10), Treg (IL-10) and mononuclear macrophage-related (IL-8, GM-CSF) serum cytokines previously reported to be associated with CHB flares. There was no significant difference in the levels of IL-2, IL-4, IL-8, IL-10, IFN-γ, HBeAg, GM-CSF, Treg, and cortisol (P > .05). However, IL-10 level was higher in patients with ALT elevating than in patients with normal ALT (P < .05) (Fig. 2A). To better understand whether IL-10 values can predict postpartum ALT elevation, we performed multivariate classification logistic regression analysis to compare the cytokine values of mothers with postpartum ALT higher than 80 IU/L to those of mothers who were in tolerant phase. After adjusting for the other cytokine variables listed in Supplementary Table S2, abnormal IL-10 was still the independent predictors for abnormal IL-10 was still the independent predictors for postpartum ALT elevation (Wald= 3.995, Exp [B] = 11.887, 95% CI[1.049, 134.653], P = .034) (see Table. Supplemental Table S1. Supplemental Table S1, http://links.lww.com/MD/D372. Multiple variable logistic analysis on the postpartum abnormal ALT (>2ULN) with postpartum cytokines). After adjusting for the age, genotype, postpartum HBsAg, postpartum HBeAg, and postpartum HBV DNA levels, abnormal IL-10 was still the independent predictors for postpartum ALT elevation (Wald= 4.450, Exp [B] = 10.894, 95% CI[1.184, 100.209], P = .035) (see Table. Supplemental Table S2, http://links.lww.com/MD/D373. Multiple variable logistic analysis on the postpartum abnormal ALT (>2ULN) with postpartum cytokines). After adjusting for the age, genotype, postpartum HBsAg, postpartum HBeAg, and postpartum HBV DNA levels, abnormal IL-10 was still the independent predictors for postpartum ALT elevation (Wald= 4.450, Exp [B] = 10.894, 95% CI[1.184, 100.209], P = .035).

Table 1

Comparison of pregnant and postpartum clinical information in patients with different postpartum ALT levels (n = 109).

| Median (SD) | Control (ALT ≤40 IU/L) (n = 64) | 1–2 ULN (40 IU/L ≤ ALT ≤ 80 IU/L) (n = 30) | >2 ULN (ALT > 80 IU/L) (n = 15) | F, P |
|------------|----------------------------------|---------------------------------|-------------------------------|----|
| Age of pregnant (yrs) | 28.19 ± 3.57 | 29.47 ± 3.57 | 28.40 ± 2.41 | 5.23, .007 |
| Gestational weeks of delivery (weeks) | 39.16 ± 1.05 | 39.45 ± 1.33 | 38.47 ± 1.41 | 3.42, .036 |
| Time of postpartum follow-up (weeks) | 6.05 ± 2.32 | 6.74 ± 1.15 | 6.33 ± 1.23 | 0.65, .52 |
| ALT at baseline (IU/L) | 22.78 ± 16.09 | 21.09 ± 7.97 | 23.14 ± 14.50 | 0.18, .84 |
| ALT at delivery (IU/L) | 19.00 ± 17.24 | 22.07 ± 11.65 | 23.47 ± 14.73 | 0.73, .48 |
| ALT at postpartum (IU/L) | 26.27 ± 7.04 | 58.52 ± 10.92 | 177.81 ± 166.53 | 34.77, .001 |
| HBV DNA at baseline (×10^3 IU/mL) | 11.29 ± 6.89 | 7.05 ± 6.68 | 13.29 ± 8.49 | 5.23, .007 |
| HBV DNA at delivery (×10^3 IU/mL) | 6.62 ± 1.13 | 6.86 ± 1.07 | 4.87 ± 7.07 | 0.18, .83 |
| HBV DNA at postpartum (×10^3 IU/mL) | 6.55 ± 7.70 | 2.97 ± 5.32 | 2.52 ± 5.97 | 6.92, .002 |
| HBsAg at baseline (IU/mL) | 29.332 ± 28.975.57 | 21.746.72 ± 18.061.39 | 27.906.57 ± 18.532.09 | 0.93, .40 |
| HBsAg at delivery (IU/mL) | 26.592.69 ± 18.252.08 | 25.264.85 ± 18.198.07 | 29.164.47 ± 18.394.05 | 0.23, .80 |
| HBsAg at postpartum (IU/mL) | 32.614.02 ± 20.065.52 | 26.283.75 ± 19.743.59 | 27.012.53 ± 20.240.06 | 1.30, .28 |
| HBeAg at baseline (COI/mL) | 1593.72 ± 696.78 | 1717.30 ± 2051.47 | 1597.02 ± 3075.51 | 0.13, .88 |
| HBeAg at delivery (COI/mL) | 1554.48 ± 784.06 | 1476.09 ± 716.94 | 1623.58 ± 392.17 | 0.22, .80 |
| HBeAg at postpartum (COI/mL) | 1434.08 ± 797.35 | 1312.82 ± 782.65 | 1270.73 ± 512.38 | 0.43, .65 |

ALT = alanine aminotransferase, HBsAg = hepatitis B e-antigen, HBsAb = hepatitis B immune globulin, HBeAb = hepatitis B surface antigen, HBV = hepatitis B virus, ULN = upper limits normal.
logistic analysis on the postpartum abnormal ALT (≥2ULN) with postpartum IL-10.

### 3.3. More ALT abnormalities in patients with Serum IL-10 abnormalities

To further explore the relationship of postpartum IL-10 with postpartum ALT flare, we compared the ratio of postpartum ALT flares between patients with IL-10 in the normal range and patients with IL-10 elevation higher than the normal range. In patients with normal IL-10 level, ALT in 1 patient was ALT elevating (>2ULN), 11 were mildly to moderately elevated (40–80 U/L), 29 were within the normal range (≤40 U/mL). In patients with elevated IL-10 level, ALT in 14 patients were ALT elevating (>2ULN), 20 were mildly to moderately elevated (40–80 U/L), 35 were within the normal range (≤40 U/mL). Statistically significant differences were seen in elevated postpartum IL-10 patients compared to normal postpartum IL-10 patients (p < .05) (Fig. 2b; Table 2). To further validate the relationship of IL-10 and ALT flare, we compared the ratio of ALT flares before delivery between patients with IL-10 in the normal range and patients with abnormal IL-10 elevation. Compared to patients with normal IL-10, more patients had abnormal ALT in abnormal IL-10 group. No difference of HBV-DNA levels between two groups of different IL-10 levels (see table. Supplemental Table S3, http://links.lww.com/MD/D374. Frequency of ALT abnormalities in pregnant patients with abnormal IL-10 levels before delivery (n = 109)). In patients with IL-10 elevation before delivery, 2 of 66 patients had ALT elevating more than 2 ULN, 5 of 66 patients had ALT elevating to 1–2ULN, 39 of 66 patients had ALT in normal range. However, in 43 patients with postpartum IL-10 before delivery, only 1 patient had ALT elevating to 40.7 IU/L and the remaining 42 patients had ALT in normal range.

### 4. Discussion

In previously published studies, 10% to 57% treated mothers experienced ALT flares[17,18] and 28.27% untreated CHB mothers were observed with abnormal ALT levels[19,20] after delivery. However, factors that can predict ALT flares during the postpartum period were still unknown. Cytokines are a complex and pleiotropic group of cell-signaling proteins that involve proliferation, maturation, migration, differentiation, activation, chemotaxis of immune cells, and predominantly responses to viral infections.[18] The production of pro-inflammatory cytokines (e.g., IL-2, IFN-γ, TNF-α) are associated with Th1 cells and responsible for cell-mediated immunity, such as cytotoxic damage. By contrary, anti-inflammatory cytokines (e.g., IL-4, IL-5, IL-6, IL-9, IL-10) are usually produced by Th2 cells and promote humoral immunity.[19] No studies have reported on the relationships of postpartum ALT elevation and cytokine abnormalities in off-treatment postpartum populations.[20,21] Our research showed the serum levels of IL-10 were higher in postpartum patients with ALT abnormalities.

In the immune tolerance phase, some HBV infected patients remain HBeAg positive with high levels of serum HBV DNA but with few or no symptoms, normal ALT levels, and minimal histological activity in the liver.[22] When the tolerogenic effect is lost during the immune tolerance phase, immune mediated lysis of infected hepatocytes occurs, along with elevations in ALT levels. However, recent immunological studies challenge the concept of both immune tolerance and lack of disease activity based on clinical markers.[23,24] It has been suggested that rapid

### Table 2

Frequency of ALT abnormalities in patients with abnormal postpartum IL-10 levels (n = 109).

| n (mean ± SD) | IL-10 elevation (n = 68) | IL-10 normal (n = 41) | P      |
|--------------|-------------------------|----------------------|--------|
| ALT levels   |                         |                      |        |
| >2ULN       | 14 (183.89 ± 171.08)    | 1 (92.70)            | Z = 2.453, .014 |
| 1–2ULN      | 20 (55.92 ± 10.41)      | 11 (56.76 ± 12.41)   |       |
| ≤1 ULN      | 35 (27.55 ± 7.32)       | 29 (24.72 ± 6.47)    |       |
| HBV-DNA     | 5.18 ± 7.08             | 4.53 ± 6.86          | t = 0.463, .645 |

ALT = alanine alanine aminotransferase, IL = interleukin, ULN = upper limits normal.
changes in the levels of maternal corticosteroids during postpartum period lead to active immunological responses to HBV.[13] But no differences were found in the levels of cortisol among three groups in our study. We found IL-10 levels were higher in patients with ALT elevation than in patients with normal ALT (P < .05). Within the liver, IL-10 comes from many cells, such as Toll-like receptor activated Kupffer cells, LSEC primed CD4+ T cells, CD4+CD25+Foxp3+ and antigen-induced CD8+ T cells.[25,26] Increasing evidence has demonstrated that virus-specific T cells (i.e., CD8+CTL and CD4+Th cells) play a central role in the clearance of HBV.[27-29] After adjusting for the other cytokine variables, abnormal IL-10 was an independent predictor for postpartum ALT elevation. A previous study[30] showed that IL-10 levels were significantly positively correlated to HBV DNA load and ALT levels in the HBeAg-negative patients. After adjusting for the age, genotype of HBV, postpartum HBsAg, postpartum HBeAg, and postpartum HBV DNA levels, abnormal IL-10 was still the independent predictors for postpartum ALT elevation.

A successful pregnancy needs maternal immune suppression and fetal tolerance resulted from a shift from Th1-mediated cytotoxic attacks to Th2-mediated anti-inflammatory responses to foreign fetal cells.[31] IL-10 is a negative regulator of inflammation which tends to be raised in normal pregnancies[32] and reduced in preterm delivery pregnancies. Serum cytokine levels are dynamic and involve continuous modulation of cytokine balance by both exogenous (e.g., pregnancy-induced hypertension or gestational diabetes) and endogenous (e.g., progestosterone) factors. The Th2-biased response resolves to pre-pregnancy ratios by 4 weeks postpartum.[13] Besides, increased IL-10 seems to offer some degree of protection in normal pregnancy. The reduced IL-10 levels seen in preterm delivery and high-risk patients could be permissive of an excessively inflammatory, destructive local Environment.[34] Compared to placebo in the third trimester, IL-10 levels were increased in women who received progesterone.[35] The time of postpartum follow-up was 6.33 ± 1.23, 6.74 ± 1.15, 6.95 ± 2.32 weeks after delivery in our study when the influence of progesterone on cytokine attenuated. However, similar outcomes of more ALT abnormalities occurred in patients with elevated IL-10 than patients with IL-10 in normal ranges before delivery in our study.

However, mechanisms underlying the association of IL-10 and the cytotoxic effects on liver cells are not fully understood. Th2 cytokines (IL-4, IL-6, and IL-10) can attenuate inflammatory responses which include the suppression of other cytokine production, the alteration of professional antigen presenting cells’ function and proliferation of CD4 and CD8 T cells.[36] Abnormal ALT is preceded by the change of immunological status suitable for active viral replication. In previous study, the parallel increase of serum IL-10 and HBV DNA levels suggested that IL-10 may trigger the surge of viral load by both suppressing antiviral inflammatory activity and enhancing viral replication. Elevated IL-10 has been repeatedly reported in patients with CHB and has been correlated with increased disease severity and viral load.[37] However, both higher IL-10 levels and lower HBV-DNA levels were found in our patients with ALT >2ULN than in immune tolerant patients. More patients had abnormal ALT in patients with abnormal IL-10 than in patients with normal IL-10 levels. Previous studies showed that increased expression of IL-10 is the primary factor that causes chronic HBV infection[30] and blockage of the IL-10 receptors could recover HBV specific CD8+ T cell response, accelerate virus elimination, and suppress the chronicity of viral infection.[37,38] IL-10 can induce the activation and proliferation of B cells and NK cells and enhance the cytotoxicity of CD8+ T cells, while inhibiting the production of IFN-γ selectively without changing the cytotoxicity of NK cells.

In other studies, elevated serum IL-10 (Th2 cytokine) was correlated with early spontaneous seroconversion and degree of liver inflammation.[39] The expression of IL-10 is affected by the polymorphisms in the regulatory regions.[40] Haplotypes carrying the homozygous ATA had low level of IL-10 while haplotype carrying the homozygous GCC had high level of IL-10. Meanwhile, the ACC (−1082, −819, and −592) haplotype could improve individuals’ anti-hepatitis B surface antigen (anti-HBs) antibody production to almost twice of individuals without this haplotype.[41] The mutation from −592G into −592A could enhance HBV susceptibility, while mutation from −1082A into −1082G could reduce HBV susceptibility. Mutation into −1082G allele was the protective factor against HBV infection, and the SNPs of IL-10 at −592 and −1082 sites were associated with HBV infection.[42,43] Wu et al[44] found that the polymorphisms of IL-10−1082 G/G genotype have correlation with lower HBV viral load and earlier HBeAg seroconversion. Different polymorphisms might explain the conflicting results between different results. In patients with abnormal IL-10 level, it is unclear whether the IL-10 elevation was the reflection of T-cell responses or IL-10 elevation lead to inflammatory activity and ALT elevation. This finding favors the close relationship between IL-10 levels and T cell cytotoxicity on HBV-infected liver. IL-10 could be a likely predictive marker for postpartum ALT elevation in patients with CHB virus. Besides, IL-10 may also be an underlying target for the control of HBV infection.

There are several limitations in our study. First, the sample size of this study was relatively small. Based on the data of our trial, 15 patients with ALT abnormal and 64 patients in immune tolerant status suitable for active viral replication. In previous study, the parallel increase of serum IL-10 and HBV DNA levels suggested that IL-10 may trigger the surge of viral load by both suppressing antiviral inflammatory activity and enhancing viral replication. Elevated IL-10 has been repeatedly reported in patients with CHB and has been correlated with increased disease severity and viral load.[37] However, both higher IL-10 levels and lower HBV-DNA levels were found in our patients with ALT >2ULN than in immune tolerant patients. More patients had abnormal ALT in patients with abnormal IL-10 than in patients with normal IL-10 levels. Previous studies showed that increased expression of IL-10 is the primary factor that causes chronic HBV infection[30] and blockage of the IL-10 receptors could recover HBV specific CD8+ T cell response, accelerate virus elimination, and suppress the
Author contributions

Conceptualization: Ming Wang, Yun-xia Zhu.
Data curation: Ming Wang, Yun-xia Zhu.
Formal analysis: Ming Wang.
Funding acquisition: Ming Wang, Hua Zhang, Yun-xia Zhu.
Investigation: Ming Wang, Ying Hou, Bo Yang, Ping Yang, Yun-xia Zhu.
Methodology: Ming Wang.
Resources: Hua Zhang, Yun-xia Zhu.
Supervision: Yun-xia Zhu.
Writing – original draft: Ming Wang, Shi-hui Meng.
Writing – review & editing: Ming Wang, Shi-hui Meng, Yun-xia Zhu.

References

[1] Tong MJ, Pan CQ, Han SB, et al. An expert consensus for the management of chronic hepatitis B in Asian Americans. Aliment Pharmacol Ther 2018;47:1181–200.
[2] Park JS, Pan CQ. Viral factors for HBV mother-to-child transmission. J Hepatol 2017;67:476–80.
[3] Centers for Disease Control and Prevention (CDC). Assessing completeness of perinatal hepatitis B virus infection reporting through comparison of immunization program and surveillance data – United States. MMWR Morb Mortal Wkly Rep 2011;60:410–3.
[4] Zou HB, Chen Y, Zang H, et al. Prevention of hepatitis B virus vertical transmission: current situation and challenges. Zhonghua Gan Za Zhi 2010;18:556–8.
[5] Pan CQ, Duan Z, Dai F, et al. Tenofovir to prevent hepatitis B transmission in mothers with high viral load. N Engl J Med 2016;374:2324–34.
[6] Zhang H, Pan CQ, Pang Q, et al. Telbivudine or lamivudine use in late pregnancy: regulatory T cells control maternal immune tolerance toward the fetus. Immunol Lett 2014;162(1 Pt A):41–43.
[7] Wang M, Bao Q, Zuo Y, et al. Real-world study of tenofovir disoproxil fumarate to prevent hepatitis B transmission in mothers with high viral load. Aliment Pharmacol Ther 2019;49:211–7.
[8] Chang CY, Aziz N, Poongkunran M, et al. Serum alanine aminotransferase and hepatitis B DNA flares in pregnant and postpartum women with chronic hepatitis B. Am J Gastroenterol 2016;111:1410–5.
[9] Tan HH, Liu HF, Chow WC. Chronic hepatitis B virus (HBV) infection in pregnancy. Hepatol Int 2008;2:370–5.
[10] Lu JF, Liu Y, Ma LN, et al. Efficacy of combination antiviral therapy following childbirth in pregnant HBV carriers receiving telbivudine for hepatitis B virus infection after delivery. J Viral Hepat 2008;15:37–41.
[11] Yang G, Liu J, Han S, et al. Association between hepatitis B virus infection and outcome in Han population. Eur J Med Res 2016;21:23.
[12] Vorwerk I, Ruzas R, et al. Th1/Th2 cytokine profile in preterm delivery. Am J Reprod Immunol 2010;64:398–406.
[13] Chung JJ, Thompson AJ, Visvanathan K, et al. The phenomenon of hepatitis B virus-specific T cells differ in the liver and blood in chronic hepatitis B virus infection. Hepatology 2007;46:1332–40.
[14] Bonn C, Fascico P, Valdatta C, et al. Characterization of hepatitis B virus (HBV)-specific T-cell dysfunction in chronic HBV infection. J Virol 2007;81:4215–25.
[15] Rehermann B, Nasciembni M. Immunology of hepatitis B virus and hepatitis C virus infection. Nat Rev Immunol 2005;5:215–29.
[16] Alatrukchi N, Kozel MJ. Antiviral T-cell responses and therapy in chronic hepatitis B. J Hepatol 2003;39:631–4.
[17] Das A, Ellis G, Pellant C, et al. IL-10-producing regulatory B cells in the pathogenesis of chronic hepatitis B virus infection. J Immunol 2012;189:3925–35.
[18] Sverremark-Ekstrom E. Division of cytokines into Th1/Th2: a word of caution. Reprod Sci 2010;17:318–9.
[19] Denney JM, Nelson EL, Wadhwa PD, et al. Longitudinal modulation of immune system cytokine profile during pregnancy. Cytokine 2011;53:170–7.
[20] Sykes L, Macintyre DA, Yap XJ, et al. The Th1/Th2 dichotomy of pregnancy and preterm labour. Mediat Inflamm 2012;2012:1–2.
[21] Malikseem M, Raghupathy R, El-Shazly S, et al. Pro-inflammatory maternal cytokine profile in preterm delivery. Am J Reprod Immunol 2003;49:308–18.
[22] Norman JE, Yuan M, Anderson L, et al. Effect of prolonged in vivo administration of progesterone in pregnancy on myometrial gene expression, peripheral blood leukocyte activation, and circulating steroid hormone levels. Reprod Sci 2011;18:435–46.
[23] Blackburn SD, Wherry EJ. IL-10, T cell exhaustion and viral persistence. Trends Microbiol 2007;15:143–6.
[24] Brooks DG, Trifilo MJ, Edelmann KH, et al. Interleukin-10 determines viral clearance or persistence in vivo. Nat Med 2006;12:1301–9.
[25] Ermases M, Filippini CM, Martina MM, et al. Resolution of a chronic viral infection after interleukin-10 receptor blockade. J Exp Med 2006;203:2461–72.
[26] Yang G, Liu J, Han S, et al. Association between hepatitis B virus infection and HLA-DRB1 genotyping in Shaanxi Han patients in northwestern China. Tissue Antigens 2007;69:170–5.
[27] Höfler T, Reuss E, Freitag CM, et al. A functional polymorphism in the IL-10 promoter influences the response after vaccination with HBsAg and hepatitis A. Hepatology 2005;42:72–6.
[28] Gao L, Chen X, Zhang L, et al. IL-10 polymorphisms with hepatitis B virus infection and outcome in Han population. Eur J Med Res 2016;21:23.
[29] Miyazoe S, Hamsaki K, Nakata K, et al. Influence of interleukin-10 gene promoter polymorphisms on disease progression in patients chronically infected with hepatitis B virus. Am J Gastroenterol 2002;97:2086–92.
[30] Yang GC, Niu YH, Lin YT, et al. Human interleukin-10 genotypes are associated with different precore/core gene mutation patterns in children with chronic hepatitis B virus infection. J Pediatr 2011;158:808–13.
[31] Li MH, Lu Y, Zhang L, et al. Association of cytokines with alamine aminotransferase, hepatitis B virus surface antigen and hepatitis B envelope antigen levels in chronic hepatitis B. Chin Med J 2018;131:1813–8.