

# Motor development delay in offspring is associated with prenatal telbivudine exposure

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## Abstract

Telbivudine is an orally nucleoside analog with potent and specific antihepatitis B virus (HBV) activity, and it has been reported to block mother-to-infant transmission. However, few studies have focused on the safety of prenatal exposure for offspring development.

This is a prospective noninterventional study. Participants were enrolled during delivery through the Women's Hospital of Zhejiang University School of Medicine between January 2012 and September 2013. Neonate's umbilical cord arterial blood (UCAB) was collected after delivery. Hepatitis B virus DNA copy, HBV serology, alanine aminotransferase (ALT), creatine kinase (CK), creatinine (CRE), and blood urea nitrogen (BUN) were measured. The development of the offspring was evaluated by the Chinese Revision of Bayley Scales of Child Development (BSCD-CR) at 12 to 24 months old.

Around 30 and 31 chronic hepatitis B mothers were recruited in untreated group (non-LdT group) and telbivudine-treatment group (LdT group), respectively, and 2 children (one in non-LdT group and 1 in LdT group) were lost in follow-up. Sixty-one normal women and their children were recruited as a normal control (control group). Compared with non-LdT group, telbivudine treatment effectively blocks HBV transmission from mother to infant. However, CK in UCAB was significantly increased in the LdT group. Moreover, children with prenatal telbivudine exposure showed lower level of serum creatinine than non-LdT group, reduction of psychomotor developmental index and increased risk of motor development delay.

Prenatal telbivudine exposure is correlated with motor development delay in offspring.

**Abbreviations:** ALT = alanine aminotransferase, BMI = body mass index, BSCD-CR = Chinese Revision of Bayley Scales of Child Development, BUN = blood urea nitrogen, CHB = chronic hepatitis B, CK = creatine kinase, Cre = creatinine, HBIG = hepatitis B immunoglobulin G, HBsAg = hepatitis B virus surface antigen, HBV = hepatitis B virus, LdT = telbivudine, MDI = mental development index, MTCT = mother to child transmission, NA = nucleoside analog, PDI = psychomotor development index, UCAB = umbilical cord arterial blood.

**Keywords:** motor development, offspring, telbivudine

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CZ and YY contributed equally to this work.

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## 1. Introduction

Chronic hepatitis B (CHB) virus infection is an epidemic disease infecting more than 350 million people worldwide, and a leading cause of cirrhosis and liver cancer-associated mortality.<sup>[1,2]</sup> In China, ~11% of fertile women are positive for hepatitis B virus (HBV) surface antigen (HBsAg),<sup>[3]</sup> and the leading mode of CHB transmission is from mother to child (MTCT). Without intervention, a pregnant woman with CHB has a high risk of vertical transmission of HBV to her fetus, and 80% to 90% of infants of CHB mother develop into chronic infection.<sup>[4]</sup> Passive-active immune-prophylaxis, including administration of HBV vaccine and immunoglobulin G (HBIG), can reduce the incidence of CHB-MTCT to ~5% to 10%.<sup>[5]</sup> But with high maternal viral load (>10<sup>6</sup> copies/mL), the rate of vertical transmission of HBV to infants is still nearly 30%, even with immune-prophylaxis intervention.<sup>[6–8]</sup>

Therefore, antiviral treatment during pregnancy is necessary for CHB mothers to prevent MTCT. Telbivudine (β-L-2'-deoxythymidine, LdT) is an oral nucleoside analog (NA) used to reduce HBV viral replication by inhibiting HBV DNA polymerase. LdT was assigned to pregnancy category B by the US Food and Drug Administration in 2006; therefore, it can be used during pregnancy. LdT treatment shows effectiveness of blocking MTCT even with high viral loads, starts either in late pregnancy only or throughout the entire pregnancy.<sup>[9–12]</sup> Moreover, some CHB women can become pregnant during the

antiviral treatment. Hence, in these pregnancies, fetuses experience intrauterine exposure to antiviral drug, which may affect offspring development. Preclinical study has shown LdT crossing the placenta in animal.<sup>[15]</sup> At present, safety assessment of LdT mainly focuses on adverse events during pregnancy, delivery or of newborns, including pregnancy complications, stillbirth, preterm birth, neonatal death, abnormal birth weight or apgar score.<sup>[10,13,14]</sup> However, limited data are available on the growth and development of children prenatally exposed to LdT. In this study, we aim to evaluate the growth and development of offspring prenatally exposed to LdT to investigate the safety of LdT prenatal exposure.

## 2. Materials and methods

### 2.1. Participants

Pregnancy mothers with singleton neonates, aging between 20 and 40 years, were enrolled during delivery in the Women's Hospital of Zhejiang University School of Medicine from January 2012 to September 2013. Mothers diagnosed with CHB and positive for HBsAg, HBeAg before pregnancy were defined as CHB mothers and were eligible for the study group. Neonates whose CHB mothers had been treated with only LdT of 600 mg once daily (Novartis, Inc, Basel, Switzerland) were the exposed group (LdT group), and neonates whose CHB mothers were unwilling to receive any antiviral therapy were the nonexposed group (non-LdT group). We created a 1:1 matched control group comprising normal women (without HBV infection) and their children. The matching factors were gestational age (within 1 week), birth weight (within 150 g), and age of children (within 1 month).

All mothers underwent routine pregnancy examinations. All infants were given the HBV vaccine 10 µg (Dalian Hissen BioPharm Inc., Liaoning, China) at 0, 1, and 6 months of age. All infants born from HBV mothers were also given the hepatitis B immunoglobulin G (HBIG) 100 IU (Shandong Taibang Biological, Shandong, China) immediately after birth. Patients co-infection with other hepatitis viruses (hepatitis A, C, D, and E), syphilis or HIV, evidence of hepatocellular carcinoma or a husband infected with HBV were excluded as well as those who experienced severe pregnancy complications or delivery complications including gestational diabetes, gestational hypertension and intrahepatic cholestasis of pregnancy, intrauterine asphyxia and asphyxia neonatorum. CHB mothers treated with other antiviral drugs, such as lamivudine, adefovir and entecavir were excluded. Parents with genetic diseases or showing elevated creatine kinase (CK) were excluded. The exclusion criteria listed were referred to Williams Obstetrics, 23rd edition.

The study protocol was approved by the Ethical Committee of Women's Hospital, School of Medicine, Zhejiang University. All authors have access to the study data and reviewed and approved the manuscript. Children were included after informed written consent was obtained from their parents or guardians. This investigation was registered in the Chinese Clinical Trial Registry (ChiCTR-OPC-16009018).

### 2.2. Mother and infant data collection

Gestational data, including maternal age, gestational age at delivery, birth weight, fetal gender and delivery mode, perinatal complication, medication use and other clinical characteristics, including mother's alanine aminotransferase

(ALT), CK, creatinine (Cre), blood urea nitrogen (BUN), and HBV serology, including HBV DNA copy and markers of HBV infection at delivery were collected from the clinical database of Women's Hospital.

Umbilical cord arterial blood (UCAB) of infants was collected immediately after delivery to detect ALT, CK, Cre, BUN, and HBV DNA copy. HBV serology was examined using blood of femoral vein, which was obtained after the infants were washed, within 24 hours of birth.

### 2.3. Offspring follow-up assessments

Children were brought back to the hospital at the age of 12 to 24 months. Their height and weight were measured and peripheral blood was collected to detect ALT, CK, Cre, BUN, and HBV DNA copy (except children of control group). The Chinese Revision of the Bayley Scales of Child Development (BSCD-CR) was used to evaluate motor and mental development. The test is a revision of Bayley Scales of Child Development-Second Edition (BSCD-II) and provides 2 subscales, a motor scale providing a psychomotor developmental index (PDI) and a mental scale yielding a mental developmental index (MDI). The mean score ( $\pm$  standard deviation) for both indexes for a normal population is  $100 \pm 15$ , and a score of less than 85 indicates developmental delay.<sup>[16]</sup> The assessment was conducted by a well-trained test professional who was blind to the groups to maximize reliability of results while minimize potential bias and both inter- and intraexaminer variability.

### 2.4. Laboratory testing

Quantitation of HBV DNA was detected by diagnostic kit (#DA-D051, DAAN Gene). Approximately 2 mL Blood sample was collected by vacuum blood collection tube, and serum was segregated for detection by centrifuge (1500 rpm, 5 minutes). Serum DNA was extracted by diagnostic kit (#DA-D051, DAAN Gene) according to the manufacture's instruction. DNA solutions of 20 µL mixed with 30 µL PCR amplification system (27 µL buffer, 3 µL Taq polymerase, #DA-D051, DAAN Gene) were detected by ABI 7500 real-time PCR system (Applied Biosystems). Reaction conditions were: 93°C, 2minutes; 93°C, 45seconds; 55°C, 60seconds; 10 cycles; (c) 93°C, 30seconds; and 55°C, 45 seconds; 30 cycles. The detection range is 100 IU/mL to  $1.0 \times 10^8$  IU/mL. HBV DNA levels lower than 100 UI/mL is undetectable. Serum ALT, CK, Cre, and BUN were detected with the Abbott Architect C16000 clinical chemistry diagnostic system (Abbot Diagnostics). HBV markers, including HBsAg, hepatitis B surface antibody (HBsAb), hepatitis B e antigen (HBeAg), hepatitis B e antibody (HBeAb), and hepatitis B core antibody (HBcAb), were measured by chemiluminescent microparticle immunoassays in the Abbott Architect i2000 system (Abbot Diagnostics).

### 2.5. Statistical analysis

Data were analyzed using the SAS software 9.2 (SAS Institute, Inc.). For continuous variables, one-way ANOVA with Tukey's multiple comparisons test or student *t* tests were used for comparison of 3 or 2 groups and expressed as mean  $\pm$  standard deviation. Categorical variables were compared by Fisher's exact test where necessary. Relative risk (RR) and its 95% confidence interval (CI) were calculated to evaluate the risk association between prenatal exposure to LdT and mental and motor development using the Cochran-Mantel-Haenszel  $\chi^2$  test. All

reported *P* values are two-sided and *P* < .05 was considered statistical significance. We calculated the sample size with online tool (<http://powerandsamplesize.com>). Considering score difference of 10 in BSCD-CR between non-LdT and LdT subjects (SD=10; power N 0.9; α=5%; sampling ratio=1), 21 cases in each group were required.

### 3. Results

#### 3.1. Study population

There are totally 84 CHB mother collected from January 2012 to September 2013. Forty-six CHB mothers receiving antiviral treatment were recruited as potential subjects of exposed group, and 41 subjects who did not receive antiviral treatment were absorbed into non-LdT group. After retrieving the clinical database, 3 mothers were excluded due to receiving other antiviral drug and 23 mothers (11 in non-LdT group and 12 in LdT group) were excluded due to severe pregnancy complications or delivery complications. Finally, 30 CHB mothers of non-LdT group and 31 CHB mothers of LdT group were enrolled in this study, and 2 children (1 in non-LdT group and 1 in LdT group) were lost in follow-up (Fig. 1). Among the 30 mothers in the LdT group, 21 took LdT orally during their entire pregnancy, and the rest 9 began in the second or third trimester. Another 61 normal women and their children matched by gestational age, birth weight and age of children were recruited as control group.

#### 3.2. Clinical characteristics of the mothers and neonates

Table 1 presents the clinical characteristics of the mothers and infants in this study. There were no statistical differences in maternal age, delivery mode, and gender of infants between three groups. Maternal BUN of LdT group was higher than non-LdT and control group, whereas no statistical differences were observed in maternal ALT, CK, and Cre. Due to LdT therapy, the percentage of maternal HBV DNA copy exceeding 100 UI/mL and the number of HBV DNA copy was significantly lower in the LdT group than non-LdT group. Moreover, the number of HBsAg-positive infants in the LdT group was significantly reduced compared with the non-LdT group (3.23% vs 30.0%), showing LdT effectiveness of blocking HBV MTCT. In UCAB, the CK levels of the LdT group were significantly higher than the control and non-LdT group, but no differences were observed in ALT, Cre, and BUN (Fig. 2).

#### 3.3. Reduced level of serum creatinine and PDI in the LdT group

As can be seen in Table 2, the height, weight, and body mass index value of the children were similar between 3 groups. ALT, CK, and BUN in children peripheral blood between non-LdT and LdT group showed no significant difference (Fig. 3). However, Cre in LdT group ( $23.8 \pm 4.86$ ) is statistically lower than non-LdT group ( $31.055 \pm 10.52 \mu\text{M}$ ) (Fig. 3C). The percentage of

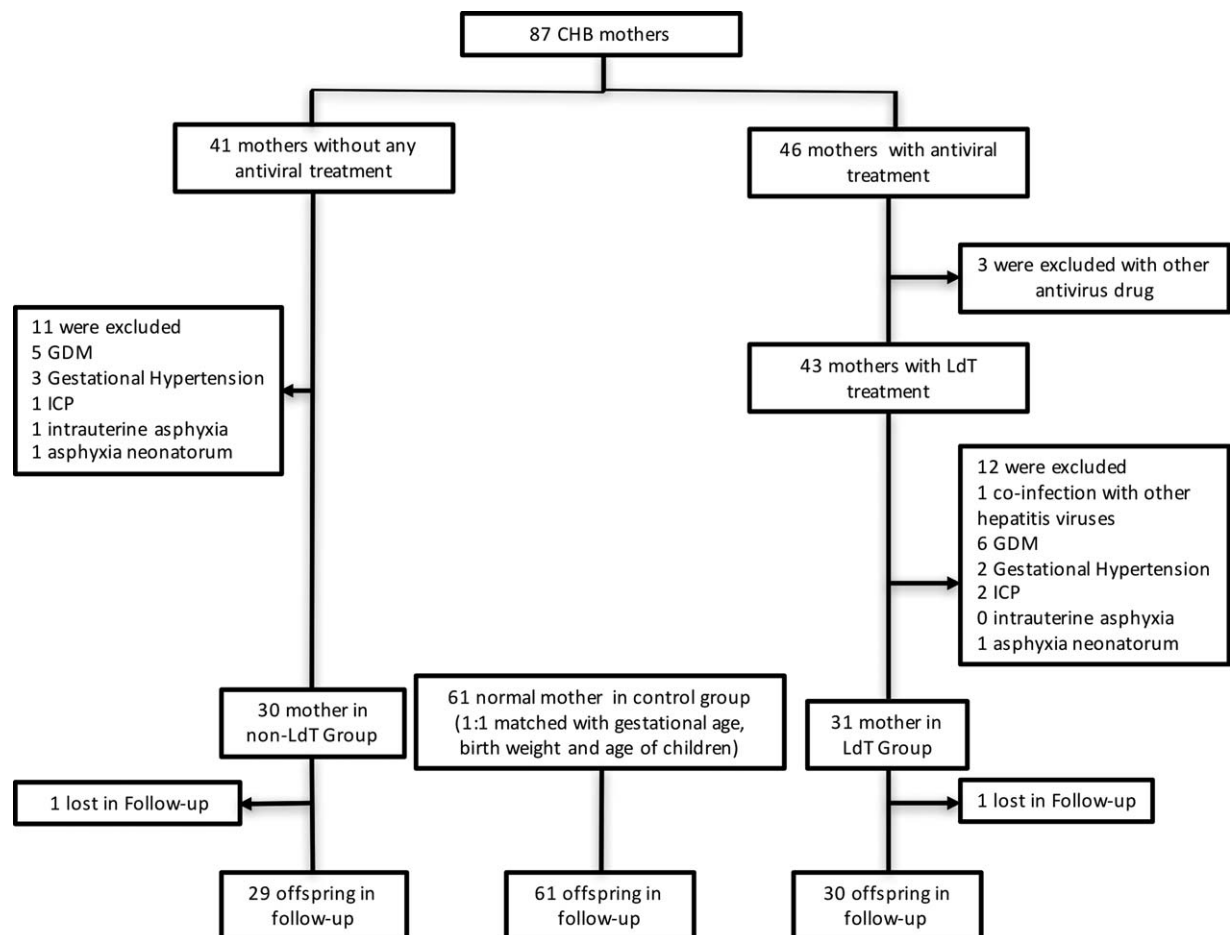


Figure 1. Study design and recruitment.

**Table 1**

**Clinical characteristics of pregnant women and offspring.**

Characteristics*	Control (n=61)	Non-LdT (n=30)	LdT (n=31)	P value† (control vs non-LdT)	P value (control vs LdT)	P value (non-LdT vs LdT)
<b>Mothers</b>						
Age, years	29.22±3.20	29.00±2.85	29.8±2.48	.935	.618	.509
HBV DNA>100 UI/mL, n (%)	–	30 (100)	12 (38.7)	–	–	<.001
HBV DNA Level, log <sub>10</sub> UI/mL‡	–	6.15±1.11	2.55±0.90	–	–	<.001
ALT, U/L	15.1±9.53	24.70±23.19	21.5±12.91	.011	.121	0.679
CK, U/L	75.0±59.8	73.07±34.90	104.2±85.49	.990	.092	.133
Cre, μmol/L	63.2±8.53	65.29±9.22	64.82±10.42	.581	.720	.978
BUN, mmol/L	2.93±0.92	2.95±0.77	3.87±0.99	.993	<.001	<.001
<b>Children</b>						
Gestational age, weeks	39.2±1.22	39.17±1.34	39.06±1.32	.973	.827	.947
Gender, male (%)	31 (50.8)	15 (50)	17 (54.8)	>.999	.826	.800
Birth weight, g	3474±441	3408±331	3344±427	.862	.325	.713
Delivery mode, cesarean section (%)	33 (54.1)	16 (53.3)	20 (64.5)	>.999	.379	0.440
Apgar scores (5 minutes)	9.85±0.48	9.867±0.43	9.96±0.03	.987	.414	.603
HBsAg+, n (%)	–	9 (30.0)	1 (3.23)	–	–	.005
HBsAb+, n (%)	–	13 (43.3)	16 (51.6)	–	–	.611
HBeAg+, n (%)	–	22 (73.3)	12 (38.7)	–	–	<0.001
HBeAb+, n (%)	–	1 (3.33)	11 (35.5)	–	–	0.003
HbCAb+, n (%)	–	25 (83.3)	26 (83.9)	–	–	1.000
HBV DNA>100 UI/mL, n (%)	–	6 (20.0)	0 (0)	–	–	.011
HBV DNA level, log <sub>10</sub> UI/mL‡	–	2.49±1.17	2.00±0.00	–	–	.03
ALT, U/L	10.37±5.04	9.97±4.79	13.10±7.03	.941	.072	.075
CK, U/L	232.5±80.7	292.7±189.8	545.5±467.7	.552	<.001	.001
Cre, μmol/L	65.0±10.4	68.89±9.90	63.88±13.1	.259	.888	.183
BUN, mmol/L	3.30±1.04	3.56±0.93	3.66±1.09	.499	.263	.927

ALT = alanine aminotransferase, BUN = blood urea nitrogen, CK = creatine kinase, Cre = creatinine, HBV = hepatitis B virus, HBsAg = hepatitis B surface antigen, HBsAb = hepatitis B surface antibody, HBeAg = hepatitis B e antigen, HBeAb = hepatitis B e antibody, HbCAb = hepatitis B core antibody, LdT = CHB mother with LdT therapy; LdT = telbivudine, Non-LdT = CHB mother without any antiviral therapy, SD = standard deviation.

\* Data are shown as mean ± standard deviation or number (percentage).

† P values were calculated by one-way ANOVA with Tukey's multiple comparisons test or Chi-square test (Fisher's exact test).

‡ HBV DNA level less than 100 UI/mL was counted as 100 UI/mL.

children HBV DNA copy exceeding 100 UI/mL and the number of HBV DNA copy in non-LdT group was still significantly higher than LdT group.

Motor and cognitive development of the offspring was evaluated by the BSCD-CR. Children of 3 groups showed comparable MDI scores, suggesting similar intellectual development status. Nevertheless, the mean PDI score for the LdT group was 89.47±18.96, which was significantly lower than the control group and non-LdT group (Fig. 3).

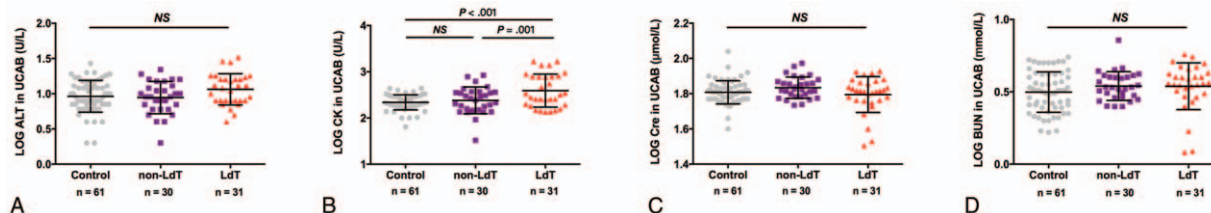
**3.4. Increased risk of motor development delay in offspring with prenatal LdT exposure**

We further investigated the incidence of development delay reflected by low PDI and MDI scores (<85) in 3 groups (Table 3).

The LdT group showed elevated incidence of low PDI score compared with the control group and non-LdT group, but there was no significant difference in MDI scores between 3 groups. Furthermore, risk ratio (RR) analysis showed that compared with control group (RR=6.01, 95% CI: 2.147–17.33) and non-LdT group (RR=2.90, 95% CI: 1.056–7.962), abnormal PDI score was significantly associated with prenatal LdT exposure.

**4. Discussion**

This is a prospective noninterventional study. We explored potential effects of prenatal LdT exposure on the development of neonates. Our results showed that prenatal LdT exposure group showed increased CK in fetal UCAB, reduced serum Cre, PDI score and thus appears to increase the risk of delayed motor development.



**Figure 2.** Elevation of creatine kinase in umbilical cord arterial blood in the LdT group. UCAB was collected immediately after delivery, and levels of ALT (A), CK (B), Cre (C), and BUN (D) were measured and compared. Horizontal lines and bars represent the mean and standard deviation, respectively. ALT=alanine aminotransferase, BUN=blood urea nitrogen, CK=creatine kinase, Cre=creatinine, NS=not significant, UCAB=umbilical cord arterial blood.



**Table 2**  
Follow-up evaluation.

Characteristic*	Control (n=61)	Non-LdT (n=29)	LdT (n=30)	P value† (control vs non-LdT)	P value (control vs LdT)	P value (non-LdT vs LdT)
Age, months	16.8±2.99	17.7±3.11	16.5±3.16	.335	.855	.215
Height, cm	79.0±3.65	80.7±4.24	78.9±4.78	.169	.990	.214
Weight, kg	11.3±1.54	11.28±1.46	10.81±1.41	.999	.306	0.444
BMI, kg/m <sup>2</sup>	18.1±2.08	17.32±1.65	17.36±1.60	.169	.199	.995
HBV DNA>100 UI/mL, n (%)	–	7 (24.1)	0 (0)	–	–	.005
HBV DNA Level, log <sub>10</sub> UI/mL‡	–	3.12±2.26	2.00±0.00	–	–	.012
ALT, U/L	–	25.72±11.94	22.13±5.90	–	–	.146
CK, U/L	–	133.1±49.89	150.8±81.90	–	–	.323
Cre, μM	–	31.0±10.52	23.8±4.86	–	–	.001
BUN, mM	–	3.44±0.97	3.44±0.81	–	–	.969
MDI	111.9±14.51	104.8±18.21	111.6±17.03	.126	.997	.236
PDI	110.2±15.97	112.8±20.86	89.47±18.96	.774	<.001	<.001

LdT=CHB mother with LdT therapy, MDI=mental developmental index, Non-LdT=CHB mother without any antiviral therapy, PDI=psychomotor developmental index.

\* Data are shown as mean±standard deviation.

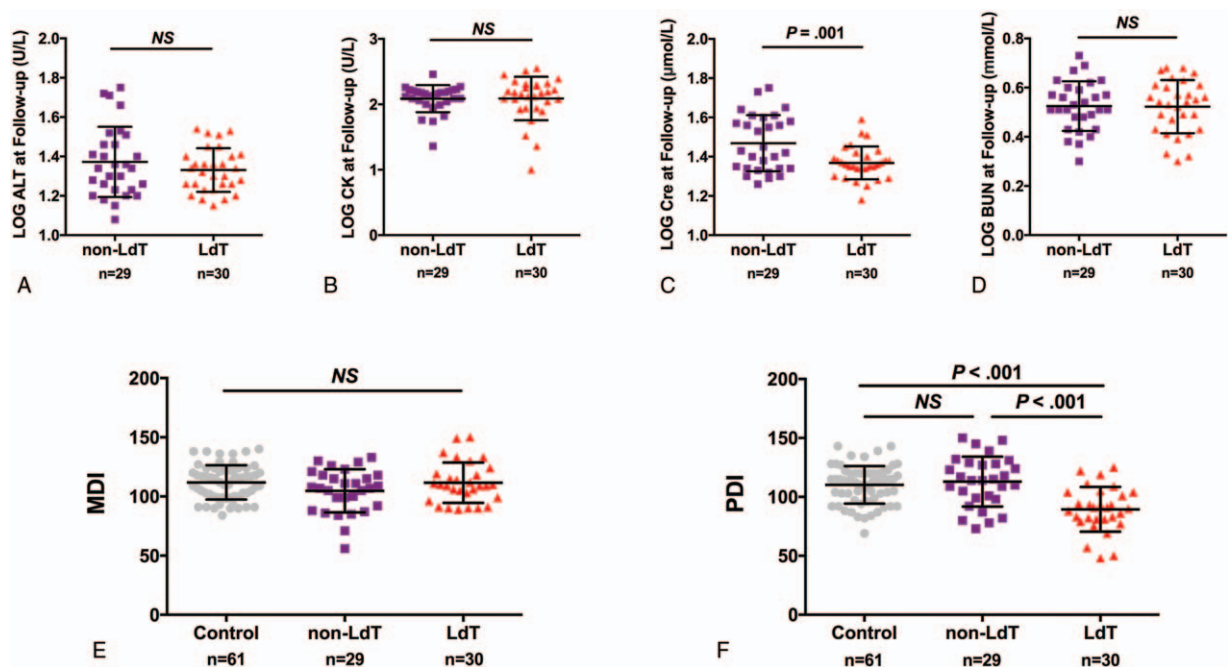
† P values were calculated using one-way ANOVA with Tukey's multiple comparisons test, Student's t test or Chi-square test (Fisher's exact test).

‡ HBV DNA level less than 100 UI/mL was counted as 100 UI/mL.

LdT has demonstrated superior efficacy in the treatment of CHB patients to other nucleoside analogs such as lamivudine.<sup>[17]</sup> In a 2-year GLOBE trial, LdT was associated with a higher rate of serum HBV DNA negativity, ALT normalization, and HBeAg loss and seroconversion than was Lamivudine at 1 and 2 years in HBeAg-positive patients.<sup>[18]</sup> In our study, HBV DNA copy was decreased effectively by LdT treatment in CHB mothers at delivery. In addition, BUN in mothers of the LdT group was higher than in those of control and non-LdT group. It has been reported that LdT (and not lamivudine) exerts renal-protective effects, due to increased mean estimated glomerular filtration rate.<sup>[19]</sup> However, there is no data available analyzing the

association between LdT and BUN. Therefore, a study is highly required to confirm the relationship between LdT and BUN.

To date, plenty of studies have examined the effect of LdT on the reduction of MTCT rate in pregnant women. In this study, LdT effectively blocked viral activity to prevent HBV-MTCT based on the large difference in HBV infection markers between the LdT and non-LdT group. However, muscle toxicity is most frequently linked to LdT among nucleoside analogs used to treat CHB infection. Recent reports indicate that LdT facilitates elevation of serum CK, causing myalgia, myopathy, and rhabdomyolysis in adult patients.<sup>[20,21]</sup> The mechanism of LdT-associated muscle toxicity is postulated to be related to



**Figure 3.** Lower serum creatinine and psychomotor development index in offspring of LdT group. Peripheral blood of offspring was collected to detect ALT (A), CK (B), Cre (C), BUN (D) and HBV DNA copy. The Chinese Revision of the Bayley Scales of Child Development (BSCD-CR) was used to evaluate mental (E) and motor (F) development. Horizontal lines and bars represent the mean and standard deviation, respectively, ALT=Alanine aminotransferase, BUN=blood urea nitrogen, CK=creatin kinase, Cre=creatinine, MDI=mental development index, NS.=not significant, PDI=psychomotor development index.

**Table 3****The incidence of low scores (<85) in children in the control, non-LdT and LdT group.**

	MDI		RR* (95% CI)	P value	RR† (95% CI)	P value
	>85	<85				
Control	60	1	Ref.		–	
non-LdT	26	3	6.31 (0.685–58.11)	.097	Ref.	
LdT	30	0	NA	NA	NA	.112

	PDI		RR* (95% CI)	P value	RR† (95% CI)	P value
	>85	<85				
Control	57	4	Ref.		–	
Non-LdT	25	4	2.10 (0.565–7.826)	.265	Ref.	
LdT	18	12	6.10 (2.147–17.33)	<.001	2.90 (1.056–7.964)	.039

LdT = CHB mother with LdT therapy, MDI = mental developmental index, Non-LdT = CHB mother without any antiviral therapy, NA = not applicable, PDI = psychomotor developmental index, RR = risk ratio, Ref = reference.

\* Control group as reference.

† HBsAg positive group as reference.

RR and its 95% confidence interval (95% CI) were calculated to estimate the risk of mental or motor impairment using the Cochran–Mantel–Haenszel  $\chi^2$  test.

P values were calculated using Fisher's exact test.

the effect on mitochondrial DNA polymerase. Recent evidences indicated that DNMT1 expression was significantly higher in NA (lamivudine, adefovir, telbivudine, entecavir, and tenofovir) treated patients than untreated patients, which might suggest an epigenetic alteration that could be involved in mitochondrial dysfunction during NAs therapy.<sup>[22]</sup>

Concerned about these side effects, it is essential to evaluate the impact on offspring that experience intrauterine exposure to LdT, which has been found to be able to cross the placenta in animal study.<sup>[15]</sup> Different from previous research, we measured CK levels in fetal UCAB instead of peripheral blood, because peripheral blood CK level increases dramatically in the neonatal period which may cover the difference.<sup>[23]</sup> We found higher CK levels in the LdT group than in the control group and non-LdT group, which implied that prenatal LdT exposure might impair CK-rich tissue in the fetus. Moreover, the serum Cre in LdT group was significantly lower than control group in follow-up period, but no difference in UCAB. Serum Cre is primarily a metabolite of creatine phosphate and reflection of skeletal muscle mass. Lower serum Cre not only reflects lower skeletal muscle, also associates with low bone mineral and metabolic disorder.<sup>[24,25]</sup> Thus, we hypothesized that LdT prenatal exposure impacted on muscle mass and may delay the motor development.

The BSCD is known to be of high reliability and validity, and has been used worldwide to assess the motor and intellectual development of children. To our knowledge, the present study is the first to use the BSCD to systematically evaluate offspring's development with prenatal LdT exposure. Recently, based on Gesell Developmental Schedules scores, Zeng et al<sup>[26]</sup> conducted a study to evaluate the development of children with prenatal exposure to LdT, which suggested the combined developmental delay and suspicious developmental delay rates were significantly higher than the control group without LdT treatment. In our study, lower PDI scores and a higher incidence of low PDI (<85) were observed in the prenatal LdT-exposure group. Moreover, risk ratio show the delay of PDI score was strong correlation with exposure of prenatal LdT. Combination with higher CK of UCAB after delivery and lower serum Cre at follow-up period in offspring of LdT group hint us muscle toxicity also happens after intrauterine LdT exposure, which adversely affect offspring's muscle development in their later life and consequencely delay their PDI scores. Prenatal events may contribute to permanent structure and physiology changes and increase the risk of chronic

diseases in later life referring to the Development Origins of Health and Disease model (DOHaD), also known as “Barker Hypothesis.”<sup>[27]</sup> In the past decade, increasing studies have provided evidence to this theory.<sup>[28–30]</sup> Given to this concept, the question of whether motor disability caused by LdT prenatal exposure will develop into diseases in adulthood awaits future long-term study.

Previous studies found no significant difference between different trimesters of LdT treatment in pregnancy safety and adverse outcome.<sup>[10,31]</sup> Therefore, we divided LdT group into LdT treatment during entire pregnancy and later pregnancy (start at second or third trimester). Further comparison displayed no statistic difference (Supplemental Table 1, <http://links.lww.com/MD/C149>). However, the limitation of sample size needs to be considered and researches with abundant subjects ought to be conducted to in the future to explore whether impair of motor development is associated with a particular trimester when mothers begin LdT treatment.

There are limitations in the present study. Some parental information was not obtained, such as social class, income and smoking history, which could be confounding factors in this study. In addition, children in 12 to 24 months old were in the rapid development stage and the follow-up period was too short to document long-term motor development disorders. As mentioned above, long-term follow-up with more samples is necessary to validate our findings.

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