ORIGINAL ARTICLE

Tenofovir to Prevent Hepatitis B Transmission in Mothers with High Viral Load

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ABSTRACT

BACKGROUND

Few data are available regarding the use of tenofovir disoproxil fumarate (TDF) during pregnancy for the prevention of mother-to-child transmission of hepatitis B virus (HBV).

METHODS

In this trial, we included 200 mothers who were positive for hepatitis B e antigen (HBeAg) and who had an HBV DNA level higher than 200,000 IU per milliliter. Participants were randomly assigned, in a 1:1 ratio, to receive usual care without antiviral therapy or to receive TDF (at an oral dose of 300 mg per day) from 30 to 32 weeks of gestation until postpartum week 4; the participants were followed until postpartum week 28. All the infants received immunoprophylaxis. The primary outcomes were the rates of mother-to-child transmission and birth defects. The secondary outcomes were the safety of TDF, the percentage of mothers with an HBV DNA level of less than 200,000 IU per milliliter at delivery, and loss or seroconversion of HBeAg or hepatitis B surface antigen at postpartum week 28.

RESULTS

At delivery, 68% of the mothers in the TDF group (66 of 97 women), as compared with 2% in the control group (2 of 100), had an HBV DNA level of less than 200,000 IU per milliliter ($P<0.001$). At postpartum week 28, the rate of mother-to-child transmission was significantly lower in the TDF group than in the control group, both in the intention-to-treat analysis (with transmission of virus to 5% of the infants [5 of 97] vs. 18% [18 of 100], $P=0.007$) and the per-protocol analysis (with transmission of virus to 0 vs. 7% [6 of 88], $P=0.01$). The maternal and infant safety profiles were similar in the TDF group and the control group, including birth-defect rates (2% [2 of 95 infants] and 1% [1 of 88], respectively; $P=1.00$), although more mothers in the TDF group had an increase in the creatine kinase level. After the discontinuation of TDF, alanine aminotransferase elevations above the normal range occurred more frequently in mothers in the TDF group than in those in the control group (45% [44 of 97 women] vs. 30% [30 of 100], $P=0.03$). The maternal HBV serologic outcomes did not differ significantly between the groups.

CONCLUSIONS

In a cohort of HBeAg-positive mothers with an HBV DNA level of more than 200,000 IU per milliliter during the third trimester, the rate of mother-to-child transmission was lower among those who received TDF therapy than among those who received usual care without antiviral therapy. (Funded by Gilead Sciences; ClinicalTrials.gov number, NCT01488526.)

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Chronic hepatitis B virus (HBV) infection remains a serious threat to public health and is associated with cirrhosis and liver cancer.[1,2] Although long-term antiviral therapy can reduce the severity of cirrhosis and the incidence of liver cancer, the eradication of HBV is rare.[1,3] Thus, the prevention of HBV transmission is the most effective way to reduce the global burden of hepatitis B infection and liver cancer.[4,5]

Pregnant mothers with chronic HBV infection can vertically transmit HBV to their infants,[6-8] and if untreated, chronic HBV infection will develop in 80 to 90% of infants born to mothers who are positive for hepatitis B e antigen (HBeAg).[9,10] The combination of postnatal passive and active immunization reduces the rate of mother-to-child transmission from 90% to 10%.[4,5,9,11] However, immunoprophylaxis fails in 10 to 30% of infants born to mothers with an HBV DNA level of more than 6 $\log_{10}$ copies per milliliter,[12-18] which contributes to many cases of vertical transmission in areas in which HBV is endemic and further increases the global prevalence of chronic HBV infection.[4,19]

A small but growing body of evidence has suggested that antiviral treatment may reduce the risk of mother-to-child transmission among mothers with an HBV DNA level of more than 6 $\log_{10}$ copies per milliliter, although quality studies are lacking and the existing studies have shown conflicting results.[5,13,14,17,20-23] In a nonrandomized study conducted by Chen et al. that involved 62 mothers treated with tenofovir disoproxil fumarate (TDF) and 56 untreated mothers,[23] 2 infants born to TDF-treated mothers received a diagnosis of chronic HBV infection (at 6 months and 12 months), which resulted in similar rates of mother-to-child transmission in the TDF group and the untreated group at the end of the study (3% [2 of 65 infants] and 11% [6 of 56], respectively; $P=0.14$).[23] Furthermore, these studies have low quality scores, according to recent World Health Organization (WHO) guidelines.[5] Because the panel concluded that the efficacy of adjuvant maternal antiviral treatment during pregnancy remains unclear, the WHO guidelines make no recommendation regarding the use of antiviral therapy to prevent mother-to-child transmission.[5]

In this context, TDF, a nucleotide analogue and a potent inhibitor of HBV polymerase,[1,3,24,25] may be useful for preventing mother-to-child transmission,[21-24,26] which is a critical step toward the global eradication of HBV and a reduction in the incidence of liver cancer.[4,27,28] Therefore, we designed the current randomized, controlled trial to determine the efficacy and safety of TDF therapy in mothers who have an HBV DNA level of more than 200,000 IU per milliliter.

METHODS

TRIAL DESIGN

We used a multicenter, open-label, randomized, parallel-group design for this trial. From March 2012 through June 2013, patients were recruited from academic tertiary care centers (their standard of care is described in Section 1 in the Supplementary Appendix, available with the full text of this article at NEJM.org) in five geographic regions of China (east, south, west, north, and southwest). Enrollment at each center was performed with the use of blocks and randomized for sample balance. Using a randomization table, we randomly assigned 200 mothers, in a 1:1 ratio, to receive usual care without antiviral therapy or to receive an oral dose of 300 mg of TDF (Viread, Gilead Sciences) daily, starting from 30 to 32 weeks of gestation until postpartum week 4. The participants were followed until postpartum week 28. Adherence to the TDF regimen was monitored by means of pill counts at each visit.

Before delivery, all the mothers were followed every 4 weeks for assessment of adverse events and laboratory results (chemical and hematologic tests, liver-function tests, and HBV DNA levels). After delivery, all mother–infant dyads were evaluated at postpartum weeks 4, 12, 24, and 28. In addition, mothers in the TDF group attended visits at postpartum weeks 8 and 16 to monitor any adverse events after the cessation of TDF at postpartum week 4. Mothers were instructed not to breast-feed their infants during the period in which they were receiving TDF treatment.[29] All the infants received 200 IU of hepatitis B immune globulin intramuscularly and 10 μg of the HBV vaccine (GlaxoSmithKline) within 12 hours after birth.[4,5,11] The same dose of active or passive immunization was administered at week 4, which was followed by an additional HBV vaccination at week 24.[4,5,11]

Resistance surveillance was performed with the use of direct HBV genome sequencing when patients had a virologic breakthrough or prema-

turely discontinued TDF between baseline and postpartum week 4.[1] Patients who discontinued TDF and had an alanine aminotransferase level that was above the upper limit of the normal range (40 U per liter) were monitored every 4 weeks for 12 weeks or until the alanine aminotransferase level normalized, whichever was longer. Antiviral treatment was resumed in patients who had a persistently elevated alanine aminotransferase level.[1,30]

TRIAL OVERSIGHT

This trial was designed by the first author and was approved by the independent ethics review board at each site (Section 2 in the Supplementary Appendix). The trial was performed in accordance with the International Conference on Harmonisation Good Clinical Practice guidelines and the Declaration of Helsinki. During the trial period, an external independent data and safety monitoring committee reviewed the safety results. All the authors vouch for the veracity and completeness of the data and analyses presented. There was no agreement regarding data confidentiality between the sponsor (Gilead Sciences) and the authors or the participating institutions. TDF was provided by Gilead Sciences, which had no part in the design or performance of the trial, in the data analysis, in the writing or editing of the manuscript, or in the decision to submit the manuscript for publication.

PARTICIPANT POPULATION

Eligible participants were pregnant women 20 to 35 years of age who had chronic HBV infection, were HBeAg-positive, had an HBV DNA level of more than 200,000 IU per milliliter, and were willing and able to provide written informed consent and adhere to the trial protocol (available at NEJM.org). The exclusion criteria were the following: coinfection with human immunodeficiency virus (HIV) type 1, hepatitis C virus, or hepatitis delta virus; a history of abortion, pregnancy loss, or congenital malformation in a previous pregnancy; previous treatment for HBV infection (except when antiviral agents were used for the prevention of mother-to-child transmission during a previous pregnancy and discontinued >6 months before the current pregnancy); a history of renal dysfunction; evidence of hepatocellular carcinoma or liver decompensation; a creatinine clearance of less than 100 ml per minute; a hemoglobin level of less than 8 g per deciliter; a neutrophil count of less than 1000 per cubic millimeter; an alanine aminotransferase level of 5 or more times the upper limit of the normal range; a total bilirubin level of 2 mg or more per deciliter (34 μmol per liter); an albumin level of less than 2.5 g per deciliter; clinical signs of threatened miscarriage; ultrasonographic evidence of fetal deformity; concurrent treatment with nephrotoxic drugs, glucocorticoids, cytotoxic drugs, nonsteroidal antiinflammatory drugs, or immune modulators; and presence of chronic HBV infection in the biologic father. For full details regarding the trial design, see the protocol and the statistical analysis plan.

OUTCOMES

The primary outcomes were the rates of mother-to-child transmission and birth defects with or without TDF exposure. The rate of mother-to-child transmission was defined as the proportion of infants who had a serum HBV DNA level of more than 20 IU per milliliter (i.e., above the lower limit of detection) or who were positive for hepatitis B surface antigen (HBsAg) at 28 weeks. During the prenatal period or the postnatal period up to 28 weeks of age, cases of a structural defect in newborns or infants were reported as birth defects. The surveillance of birth defects was conducted by a clinical examination during each visit, and further imaging or other tests were performed if indicated. The birth-defect rate represented the proportion of infants with a defect among all live births.

The secondary efficacy outcomes were the percentage of mothers who had an HBV DNA level of less than 200,000 IU per milliliter at delivery and the percentage of mothers with HBeAg or HBsAg loss or seroconversion at postpartum week 28. The secondary safety outcomes were the adverse-event profile of TDF in the mothers and safety events in the mother–infant dyads, which included all adverse events and drug discontinuations in patients who received at least one dose of TDF.

In the trial protocol, an alanine aminotransferase level that was more than 5 times as high as the baseline level or more than 10 times above the upper limit of the normal range, with or without associated symptoms, was considered to be a clinically significant adverse event that required more frequent monitoring. Any con-

firmed elevation in the alanine aminotransferase level was considered to be a serious adverse event if it occurred in conjunction with an increase in the total bilirubin level of 2 mg or more per deciliter from baseline, an increase in the prothrombin time of 2 seconds or more from baseline, an increase in the international normalized ratio of 0.5 or more from baseline, or a decrease in the albumin level of 1 g or more per deciliter from baseline. The direct genome-sequencing method was used to monitor viral genotypic mutations in the patients. Both perinatal and postpartum complications were included in the safety analysis.

STATISTICAL ANALYSIS

In the intention-to-treat analysis of the rates of mother-to-child transmission, we included all enrolled patients, except those who withdrew consent before initiation of the assigned treatment. Patients who were lost to follow-up or who discontinued treatment were counted as having treatment failure. In addition, we performed a per-protocol analysis that excluded patients who withdrew consent, were lost to follow-up, or discontinued treatment for any reason. To analyze the rate of mother-to-child transmission with a reasonable sample size, we calculated that a cohort of 200 patients would need to be enrolled for the trial to have at least 85% power to detect an absolute difference of 18 percentage points in the rate of infection among infants, assuming a rate of mother-to-child transmission of 20% in the control group, at a two-tailed alpha level of 0.05.[14,17,20]

The characteristics of the patients at baseline, the number of infants with birth defects, and adverse events were reported with the use of descriptive statistics, which included percentages, means with standard deviations, medians with interquartile ranges, and 95% confidence intervals. Student's t-test, the chi-square test, and Fisher's exact test were used to compare qualitative and categorical variables, and P values of less than 0.05 were considered to indicate statistical significance. All P values and confidence intervals were based on two-tailed tests. In addition, the effects of TDF on maternal viremia and serologic results were evaluated by comparing the TDF group with the control group, and we used the pooled data from each group. All the data were analyzed with the use of SPSS software, version 17.0 (SPSS). No interim analyses were performed, other than summaries of the safety data for the monitoring committee.

RESULTS

PARTICIPANTS

Among 216 mothers who were screened, 200 were enrolled in the trial and underwent randomization. The most common reason for nonenrollment was a serum HBV DNA level of less than 200,000 IU per milliliter. After randomization, 3 women in the TDF group withdrew consent before the baseline visit; therefore, 97 patients received at least one dose of TDF. A total of 180 mother–infant dyads completed the study (Fig. 1).

The maternal characteristics at baseline were similar in the TDF group and the control group (Table 1). The mean (±SD) duration of TDF therapy before delivery was 8.57±0.53 weeks. The characteristics of the infants at birth were similar in the two groups (Table 1), except for HBeAg-positive status and a detectable level of HBV DNA in serum, which were observed in significantly fewer infants in the TDF group than in the control group.

EFFICACY ASSESSMENT IN MOTHERS

At delivery, the median HBV DNA level was significantly lower in the TDF group than in the control group (4.7 $\log_{10}$ IU per milliliter [interquartile range, 4.1 to 5.3] vs. 8.0 $\log_{10}$ IU per milliliter [interquartile range, 7.5 to 8.3], P<0.001). A total of 66 of 97 mothers in the TDF group (68%; 95% confidence interval [CI], 59 to 77), as compared with 2 of 100 mothers in the control group (2%; 95% CI, 0 to 6), had an HBV DNA level that was less than 200,000 IU per milliliter at delivery (P<0.001); Section 3 in the Supplementary Appendix shows the changes in the HBV DNA and alanine aminotransferase levels. The percentage of women in the TDF group who had an HBV DNA level of 200,000 IU or more per milliliter at delivery was higher among women who had had a baseline HBV DNA level of more than 8 $\log_{10}$ IU per milliliter than among women who had had a baseline HBV DNA level of 8 $\log_{10}$ IU or less per milliliter (39% [19 of 49 women] and 25% [12 of 48], respectively; P=0.19). Two patients who withdrew consent were in the subgroup of patients with a low viral load and were considered to have treatment failure with respect

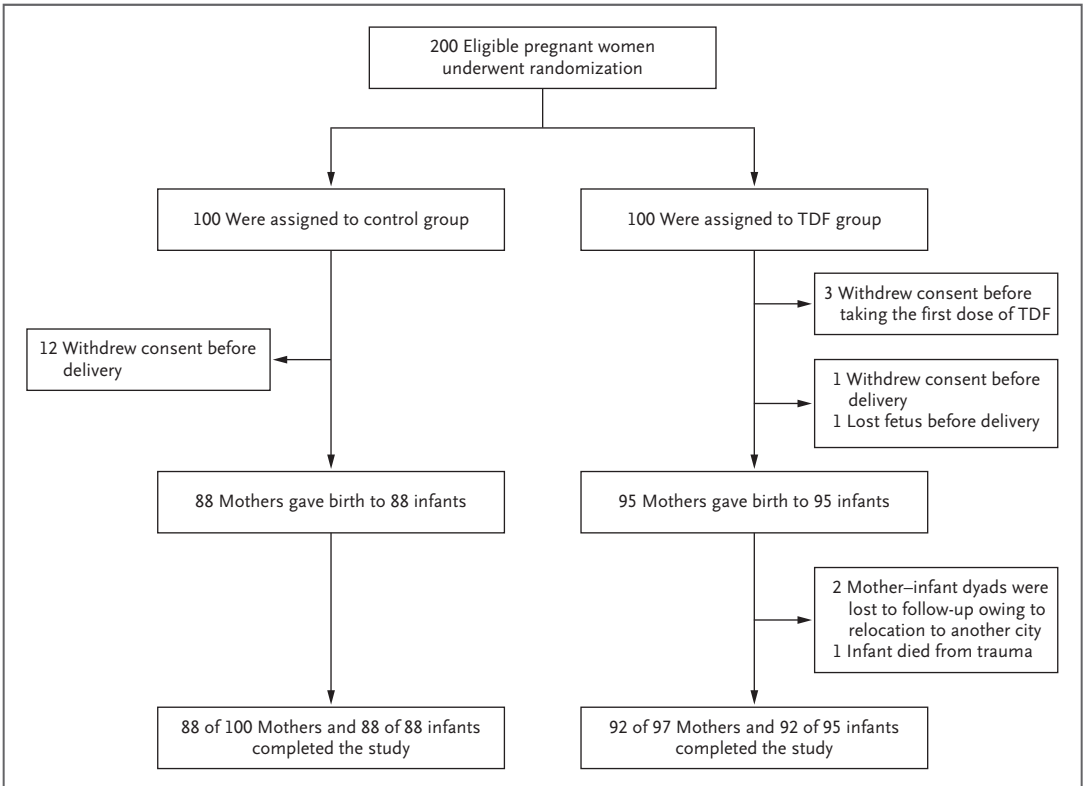

Figure 1. Enrollment and Randomization of the Trial Participants.
All births were singleton births. TDF denotes tenofovir disoproxil fumarate.

to reduction in viral load. Among the 31 patients who did not have an HBV DNA level of less than 200,000 IU per milliliter at delivery, 19 (61%) had had a baseline HBV DNA level of more than 8 $\log_{10}$ IU per milliliter and 6 (19%) had missed two to seven doses of TDF before delivery. The mean decrease in the HBV DNA level in these 31 patients was 1.7 $\log_{10}$ IU per milliliter, and 39% (12 women) had a level of 200,000 to 1,000,000 IU per milliliter.

During the trial, one mother in the TDF group had HBeAg seroconversion and HBsAg loss. Four patients in the control group had HBeAg loss, including three in whom antibodies to HBeAg developed and one who had HBsAg seroconversion. The serologic outcomes did not differ significantly between the groups.

EFFICACY ASSESSMENT IN INFANTS

Among the 183 delivered infants, 180 completed the trial, including 92 infants in the TDF group and 88 in the control group. All the infants who completed the trial received 10 μg of the HBV vaccine within 6 hours after birth, two additional vaccinations at weeks 4 and 24, and 200 IU of hepatitis B immune globulin intramuscularly at birth and at week 4. At 28 weeks, the rate of mother-to-child transmission was significantly lower among infants born to mothers in the TDF group than among infants born to mothers in the control group (Fig. 2). In the intention-to-treat analysis, the rate was 5% (95% CI, 1 to 10; 5 of 97 infants) in the TDF group versus 18% (95% CI, 10 to 26; 18 of 100 infants) in the control group (P=0.007); in the per-protocol analysis, the rate was 0% (95% CI, 0 to 3; 0 of 92 infants) in the TDF group versus 7% (95% CI, 2 to 12; 6 of 88 infants) in the control group (P=0.01).

In the intention-to-treat analysis, there were five cases of treatment failure in the TDF group (in 5 of 97 women), including one case of stillbirth at 36 weeks of gestation, one case of withdrawal from the study, one case of newborn death, and two cases in which the infants were lost to follow-up (both were HBsAg-negative at the last visit). Among 18 infants in the control

Table 1. Characteristics of the Mothers and Infants.*

Characteristic	TDF	Control
Maternal characteristics at baseline		
No. of women	97	100
Age — yr	27.4±3.0	26.8±3.0
Gravidity	1.0±0.2	1.0±0.2
HBV DNA — $\log_{10}$ IU/ml	8.2±0.5	8.0±0.7
Alanine aminotransferase — U/liter†	23.0±22.4	20.5±15.4
Infant characteristics at birth		
No. of infants	95	88
Gestational age — wk	39.2±1.0	38.9±1.3
Delivery by means of cesarean section — no. (%)	47 (49)	50 (57)
Length — cm	50.1±1.1	50.2±1.8
Head circumference — cm	33.2±3.6	33.5±3.4
Weight — kg	3.6±2.7	3.4±0.5
Apgar score at 1 min	9.6±1.0	9.7±0.7
Laboratory variables‡		
Alanine aminotransferase — U/liter	14.1±9.5	15.0±20.8
Positive for hepatitis B e antigen — no. (%)	73 (77)	85 (97)
Positive hepatitis B surface antigen — no. (%)	6 (6)	4 (5)
Detectable HBV DNA — no. (%)	3 (3)	15 (17)

* Plus–minus values are means ±SD. The only significant differences between the two groups were positivity for hepatitis B e antigen at birth ($P<0.001$) and detectable hepatitis B virus (HBV) DNA at birth ($P=0.002$). TDF denotes tenofovir disoproxil fumarate.
† The upper limit of the normal range for maternal alanine aminotransferase is 40 U per liter.
‡ Blood samples were obtained from the newborns' peripheral vein or artery. The lower limit of detectable HBV DNA was 20 IU per milliliter. The upper limit of the normal range for alanine aminotransferase in infants is 40 U per liter.

group who were considered to have had immunoprophylaxis failure, 6 were infected with HBV at the age of 28 weeks and 12 were lost to follow-up because the mother withdrew consent before delivery. All the infants who were infected at week 28 were born to mothers in the control group whose HBV DNA level was more than 200,000 IU per milliliter at delivery. At birth, all 6 of these infants had viremia, including 4 who were HBsAg-positive. At week 28, all 6 infants had viremia and were HBsAg-positive.

MATERNAL AND FETAL SAFETY

One mother in the TDF group (1%) withdrew from the trial owing to grade 2 nausea. No patients discontinued TDF owing to a lack of efficacy. Before postpartum week 4, a total of 5 women in the TDF group (5%) had viral rebound, including 4 who did not adhere to the treatment regimen and 1 who discontinued TDF voluntarily. Direct genome sequencing of samples obtained from these patients revealed that their isolates were all genotype C wild-type with no genotypic mutations. At postpartum week 4, all the mothers in the TDF group discontinued therapy per protocol, and viral rebound was observed in 89% (85 of 95 women), although no clinically significant events other than alanine aminotransferase flares as defined by the protocol were reported.

The maternal adverse events are summarized in Table 2. Two significant differences between the groups were detected. The first significant difference was a higher frequency of an elevation in the creatine kinase level among mothers in the TDF group than among those in the control group (7% vs. 0, $P=0.006$); all the elevations were of grade 1 or 2, with levels increasing to 3 or fewer times the upper limit of the normal range.[31] These events were reported as nonclini-

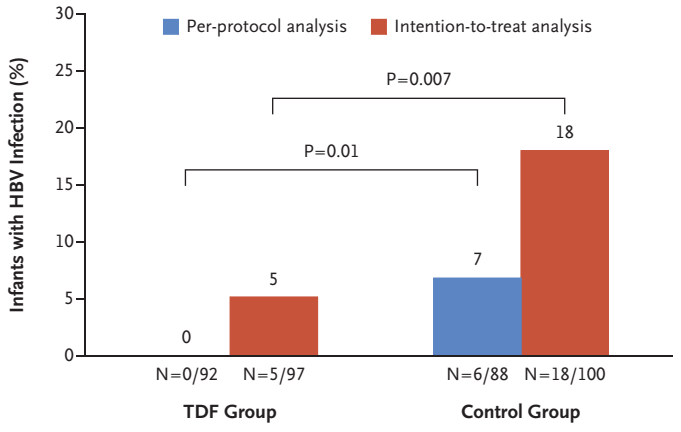

Figure 2. Rate of Hepatitis B Virus (HBV) Infection among Infants.

The intention-to-treat analysis included the infants born to all enrolled participants except for women who withdrew consent before the initiation of treatment. The per-protocol analysis excluded infants born to women who withdrew consent, were lost to follow-up, or discontinued treatment for any reason.

cally significant events because all patients were asymptomatic and had normal electrocardiograms. The second significant difference was a higher frequency of elevations in the alanine aminotransferase levels in the TDF group than in the control group after women in the TDF group ceased taking TDF (45% in the TDF group vs. 30% in the control group, P=0.03). Section 3 in the Supplementary Appendix shows the dynamic changes in the alanine aminotransferase levels.

In the TDF group, a 21-year-old woman had a stillbirth (with no observed congenital defects); this patient had had a pregnancy that ended in fetal death 2 years earlier, although she did not disclose this information during the screening. In the current pregnancy, pruritus and jaundice developed in this patient at 32 weeks of gestation; these symptoms abated after ursodiol treatment. However, fetal death occurred at 36 weeks of gestation, after the mother had episodes of lower abdominal pain. Another patient in the

Table 2. Adverse Events and Laboratory Abnormalities in Mothers.*

Variable	TDF (N=97)	Control (N=100)
	number (percent)	
Adverse event		
Any event	61 (63)	47 (47)
Grade 1 or 2 event	14 (14)	24 (24)
Fatigue	1 (1)	2 (2)
Headache	1 (1)	1 (1)
Cough	2 (2)	2 (2)
Diarrhea	2 (2)	1 (1)
Fever	0	1 (1)
Nausea	2 (2)	1 (1)
Pruritus	2 (2)	5 (5)
Palpitation	0	1 (1)
Dyspepsia	1 (1)	0
Rash	1 (1)	1 (1)
Insomnia	1 (1)	0
Dizziness	1 (1)	2 (2)
Abdominal pain	1 (1)	0
Jaundice	2 (2)	4 (4)
Upper respiratory infection	1 (1)	3 (3)
Grade 3 or 4 event		
Fetal loss or stillbirth	1 (1)	0
Preeclampsia	0	1 (1)

Table 2. (Continued.)

Variable	TDF (N=97)	Control (N=100)
	number (percent)	
Obstetrical complication		
Pregnancy-induced hypertension	3 (3)	0
Placenta previa	2 (2)	0
Fetal growth retardation	1 (1)	1 (1)
Intrahepatic cholestasis of pregnancy	3 (3)	2 (2)
Membrane prerupture	4 (4)	5 (5)
Preterm delivery	2 (2)	1 (1)
Breech delivery	1 (1)	2 (2)
Protracted active-phase dilatation	1 (1)	0
Long umbilical cord or cord around the infant's neck	3 (3)	2 (2)
Macrosomia	3 (3)	0
Postpartum hemorrhage	4 (4)	4 (4)
Laboratory abnormality		
Grade 1 or 2		
Alanine aminotransferase level 1.1 to 5× ULN	54 (56)	32 (32)
Creatine kinase level 1.3 to 3× ULN	7 (7)	0
Anemia	0	3 (3)
Grade 3 or 4		
Confirmed increase in creatinine level of ≥0.5 mg/dl from baseline	0	1 (1)
Confirmed creatinine clearance <50 ml/min	0	0
Severe alanine aminotransferase flare†	5 (5)	6 (6)
Serious alanine aminotransferase flare†	1 (1)	3 (3)
Increased alanine aminotransferase level		
At any time point during the trial	60 (62)	41 (41)
Between baseline and postpartum wk 4	16 (16)	22 (22)
Between postpartum wk 5 and postpartum wk 28	45 (46)	30 (30)

* The only significant differences between the two groups were the rates of any adverse events (P=0.03), elevation of the alanine aminotransferase level to 1.1 to 5 times the upper limit of the normal range (ULN) (P=0.001), elevation of the alanine aminotransferase level at any time point during the trial period (P=0.004), elevation of the alanine aminotransferase level between postpartum week 5 and postpartum week 28 (P=0.03), and elevation of the creatine kinase level (P=0.006). The upper limit of the normal range for alanine aminotransferase was 40 U per liter. The upper limit of the normal range for creatine kinase is 195 U per liter. To convert values for creatinine to micromoles per liter, multiply by 88.4.

† A severe alanine aminotransferase flare was defined as a level that was 5.1 to 10 times the ULN, and a serious alanine aminotransferase flare as a level that was more than 10 times the ULN.

TDF group had an alanine aminotransferase level that was more than 10 times the upper limit of the normal range at postpartum week 28, although the level normalized after she resumed antiviral therapy.

Three patients in the control group had an alanine aminotransferase level that was more than 10 times the upper limit of the normal range, and all cases occurred within 4 weeks post partum. These alanine aminotransferase flares resolved after the initiation of antiviral therapy, although none of these patients had HBeAg seronegativity or seroconversion during or after the alanine aminotransferase flares.

Table 3. Birth Defects, Malformations, and Adverse Events in Infants.*

Variable	TDF (N=95)	Control (N=88)
	number (percent)	
Birth defect or malformation†	2 (2)	1 (1)
Torticollis	1 (1)	0
Umbilical hernia	1 (1)	0
Hypospadias	0	1 (1)
Adverse event		
Grade 1 or 2 event		
Fever	7 (7)	3 (3)
Skin abnormality		
Rash, including diaper rash	4 (4)	4 (5)
Café-au-lait spots	1 (1)	0
Cough	10 (11)	6 (7)
Diarrhea	6 (6)	1 (1)
Vomiting	2 (2)	1 (1)
Jaundice	2 (2)	1 (1)
Grade 3 or 4 event	3 (3)	1 (1)
Forceps-induced intracranial hemorrhage	1 (1)	0
Pneumonia	2 (2)	0
Bronchitis	0	1 (1)

* There were no significant differences between the two groups in these variables (all P≥0.05).

† The frequency of congenital deformities or defects among infants who had exposure to TDF (one infant each with umbilical hernia and torticollis) did not differ significantly from that among infants in the control group (2% [95% CI, 0 to 7] vs. 1% [95% CI, 0 to 6], P=1.00).

INFANT SAFETY

Among the 183 liveborn infants, there were no significant differences between the TDF group and the control group with regard to fetal development and infant growth. The gestational age, delivery mode, weight, height, and Apgar score at birth were similar in the two groups (Table 1). At postpartum week 28, the mean body weight of the infants with exposure to TDF was similar to that of the infants in the control group (9.12±1.33 kg and 9.25±1.46 kg, respectively; P=0.51), as were the height (69.41±3.57 cm and 69.21±3.84 cm, respectively; P=0.72) and the head circumference (44.22±2.37 cm and 44.0±2.16 cm, respectively; P=0.52).

The frequency of congenital deformity or defect was 2% (95% CI, 0 to 7) among infants with TDF exposure and 1% (95% CI, 0 to 6) among those in the control group (P=1.00). One full-term newborn was delivered with the use of forceps from a mother in the TDF group who had declined a cesarean section; this infant died on day 2 from intracerebral hemorrhage and aspiration pneumonia. All the mothers reported following the instruction not to breast-feed. The incidences and natures of the adverse events among the infants were similar in the two groups (Table 3). Three infants with pneumonia or bronchitis required hospitalization but subsequently recovered after a short course of antibiotic treatment.

DISCUSSION

Studies have shown a linear association between vertical transmission and maternal $\log_{10}$ viral load,[7,12,15] although mother-to-child transmission is much less likely when the maternal HBV DNA level at delivery is less than 200,000 IU per milliliter than when the level is 200,000 IU or more per milliliter.[7,12,13,15-17,20-22,32-34] Six nonrandomized prospective studies and one randomized trial have evaluated antiviral therapy during pregnancy.[13,14,17,20,22,23,35] However, these studies were of low quality and showed conflicting results regarding whether antiviral therapy prevented mother-to-child transmission.[5] For example, in a randomized trial of lamivudine, more than 30% of the patients were lost to follow-up and the per-protocol analysis showed that lamivudine was not effective.[14] The finding of Chen et al. that TDF was effective remains questionable[23] because mother-to-child transmission was evaluated within the month after vaccination.[11,36] In addition, TDF was not significantly effective if the intention-to-treat analysis would have been performed at 6 months (3% [2 of 66 participants] in the TDF group and 12% [7 of 57] in the control group, P=0.08, as calculated by the first author of the present trial), and TDF did not affect the rate of mother-to-child transmission in the per-protocol analysis by the authors at the 12-month follow-up.[23]

In contrast, the current study showed that 68% of the TDF-treated mothers had the target HBV DNA level at delivery, which indicates that TDF reduced maternal viremia. Furthermore, the intention-to-treat analysis and the per-protocol analysis both showed that TDF was effective in lowering the rate of mother-to-child transmis-

sion. Although the serologic status of the mother did not differ between the trial groups, this result is not unexpected, because patients received only approximately 12 weeks of antiviral therapy. In previous phase 3 trials of TDF, after 1 year of treatment, the HBeAg seroconversion rate was 21% and the HBsAg seronegativity rate was 3%.[25]

The Antiretroviral Pregnancy Registry study recently analyzed data from 17,332 mothers who received antiviral therapy, including data from 4013 women who received TDF during pregnancy.[37] The rate of birth defects among the infants with exposure to TDF was 2.4% (95% CI, 1.8 to 3.0), which was similar to the rate in the general population (2.7%).[37] However, the mothers in that registry were typically infected with HIV type 1, and only 1.5% had HBV monoinfection. Although we observed a similar rate of birth defects of 2% (95% CI, 0 to 7) among infants with exposure to TDF, the current study was underpowered to evaluate potentially small differences in birth-defect rates. Furthermore, the postpartum cessation of TDF requires close monitoring.

In conclusion, during a 28-week postpartum follow-up, we found that in a cohort of mothers with an HBV viral load of more than 200,000 IU per milliliter at 28 weeks of gestation, the rate of mother-to-child transmission of HBV was lower among mothers who received TDF therapy than among those who received usual care without antiviral therapy.

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REFERENCES

1. European Association for the Study of the Liver. EASL clinical practice guidelines: management of chronic hepatitis B virus infection. J Hepatol 2012;57:167-85.
2. Pan CQ, Zhang JX. Natural history and clinical consequences of hepatitis B virus infection. Int J Med Sci 2005;2:36-40.
3. Pan CQ, Hu KQ, Tsai N. Long-term therapy with nucleoside/nucleotide analogues for chronic hepatitis B in Asian patients. Antivir Ther 2013;18:841-52.
4. Hepatitis B fact sheet no. 204. Geneva: World Health Organization, July 2015 (http://www.who.int/mediacentre/factsheets/fs204/en/).
5. Guidelines for the prevention, care and treatment of persons with chronic hepatitis B infection. Geneva: World Health Organization, March 2015 (http://apps.who.int/iris/bitstream/10665/154590/1/9789241549059_eng.pdf?ua=1).
6. Stevens CE, Beasley RP, Tsui J, Lee W-C. Vertical transmission of hepatitis B antigen in Taiwan. N Engl J Med 1975;292:771-4.
7. Xu DZ, Yan YP, Choi BC, et al. Risk factors and mechanism of transplacental transmission of hepatitis B virus: a case-control study. J Med Virol 2002;67:20-6.
8. Wong VC, Lee AK, Ip HM. Transmission of hepatitis B antigens from symptom free carrier mothers to the fetus and the infant. Br J Obstet Gynaecol 1980;87:958-65.
9. Beasley RP, Trepo C, Stevens CE, Szmuness W. The e antigen and vertical transmission of hepatitis B surface antigen. Am J Epidemiol 1977;105:94-8.
10. Lee C, Gong Y, Brok J, Boxall EH, Gluud C. Hepatitis B immunisation for newborn infants of hepatitis B surface antigen-positive mothers. Cochrane Database Syst Rev 2006;2:CD004790.
11. Centers for Disease Control and Prevention. Hepatitis B vaccination recommendations for infants, children, and adolescents. December 2005 (http://www.cdc.gov/hepatitis/hbv/vaccchildren.htm).
12. Zou H, Chen Y, Duan Z, Zhang H, Pan C. Virologic factors associated with failure to passive-active immunoprophylaxis in infants born to HBsAg-positive mothers. J Viral Hepat 2012;19(2):e18-25.
13. Zhang H, Pan CQ, Pang Q, Tian R, Yan M, Liu X. Telbivudine or lamivudine use in late pregnancy safely reduces perinatal transmission of hepatitis B virus in real-life practice. Hepatology 2014;60:468-76.
14. Xu WM, Cui YT, Wang L, et al. Lamivudine in late pregnancy to prevent perinatal transmission of hepatitis B virus infection: a multicentre, randomized, double-blind, placebo-controlled study. J Viral Hepat 2009;16:94-103.
15. Wiseman E, Fraser MA, Holden S, et al. Perinatal transmission of hepatitis B virus: an Australian experience. Med J Aust 2009;190:489-92.
16. Pan CQ, Zou H-B, Chen Y, et al. Cesarean section reduces perinatal transmission of hepatitis B virus infection from hepatitis B surface antigen-positive women to their infants. Clin Gastroenterol Hepatol 2013;11:1349-55.
17. Pan CQ, Han GR, Jiang HX, et al. Telbivudine prevents vertical transmission from HBeAg-positive women with chronic hepatitis B. Clin Gastroenterol Hepatol 2012;10:520-6.
18. del Canho R, Grosheide PM, Schalm SW, de Vries RR, Heijtink RA. Failure of neonatal hepatitis B vaccination: the role of HBV-DNA levels in hepatitis B carrier mothers and HLA antigens in neonates. J Hepatol 1994;20:483-6.
19. Perumalswami PV, Factor SH, Kapelusznik L, et al. Hepatitis Outreach Network: a practical strategy for hepatitis screening with linkage to care in foreign-born communities. J Hepatol 2013;58:890-7.
20. Han GR, Cao MK, Zhao W, et al. A prospective and open-label study for the efficacy and safety of telbivudine in pregnancy for the prevention of perinatal transmission of hepatitis B virus infection. J Hepatol 2011;55:1215-21.
21. Pan CQ, Mi LJ, Bunchorntavakul C, et al. Tenofovir disoproxil fumarate for prevention of vertical transmission of hepatitis B virus infection by highly viremic pregnant women: a case series. Dig Dis Sci 2012;57:2423-9.
22. Greenup AJ, Tan PK, Nguyen V, et al. Efficacy and safety of tenofovir disoproxil fumarate in pregnancy to prevent perinatal transmission of hepatitis B virus. J Hepatol 2014;61:502-7.
23. Chen HL, Lee CN, Chang CH, et al. Efficacy of maternal tenofovir disoproxil fumarate in interrupting mother-to-infant transmission of hepatitis B virus. Hepatology 2015;62:375-86.

24. Pan CQ, Trinh H, Yao A, Bae H, Lou L, Chan S. Efficacy and safety of tenofovir disoproxil fumarate in Asian-Americans with chronic hepatitis B in community settings. PLoS One 2014;9(3):e89789.
25. Marcellin P, Heathcote EJ, Buti M, et al. Tenofovir disoproxil fumarate versus adefovir dipivoxil for chronic hepatitis B. N Engl J Med 2008;359:2442-55.
26. Pan CQ, Chan S, Trinh H, Yao A, Bae H, Lou L. Similar efficacy and safety of tenofovir in Asians and non-Asians with chronic hepatitis B. World J Gastroenterol 2015;21:5524-31.
27. Mast EE, Margolis HS, Fiore AE, et al. A comprehensive immunization strategy to eliminate transmission of hepatitis B virus infection in the United States: recommendations of the Advisory Committee on Immunization Practices (ACIP) part 1: immunization of infants, children, and adolescents. MMWR Recomm Rep 2005;54(RR-16):1-31.
28. Rapicetta M, D'Ugo E, Argentini C, et al. New perspectives for hepatitis B vaccines and immunization. Vaccine 2009;27:3271-5.
29. Benaboud S, Pruvost A, Coffie PA, et al. Concentrations of tenofovir and emtricitabine in breast milk of HIV-1-infected women in Abidjan, Cote d'Ivoire, in the ANRS 12109 TEmAA Study, Step 2. Antimicrob Agents Chemother 2011;55:1315-7.
30. Keeffe EB, Dieterich DT, Han SH, et al. A treatment algorithm for the management of chronic hepatitis B virus infection in the United States: 2008 update. Clin Gastroenterol Hepatol 2008;6:1315-41.
31. Guidance for industry: toxicity grading scale for healthy adult and adolescent volunteers enrolled in preventive vaccine clinical trials. Rockville, MD: Food and Drug Administration, September 2007 (http://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Vaccines/ucm091977.pdf).
32. Park JS, Pan C. Current recommendations of managing HBV infection in preconception or pregnancy. Front Med 2014;8:158-65.
33. Pan CQ, Lee HM. Antiviral therapy for chronic hepatitis B in pregnancy. Semin Liver Dis 2013;33:138-46.
34. Pan CQ, Duan ZP, Bhamidimarri KR, et al. An algorithm for risk assessment and intervention of mother to child transmission of hepatitis B virus. Clin Gastroenterol Hepatol 2012;10:452-9.
35. Wu Q, Huang H, Sun X, et al. Telbivudine prevents vertical transmission of hepatitis B virus from women with high viral loads: a prospective long-term study. Clin Gastroenterol Hepatol 2015;13:1170-6.
36. Rysgaard CD, Morris CS, Drees D, et al. Positive hepatitis B surface antigen tests due to recent vaccination: a persistent problem. BMC Clin Pathol 2012;12:15.
37. The Antiretroviral Pregnancy Registry — interim report. 1 January 1989 through 31 July 2015 (issued December 2015) (http://www.apregistry.com/forms/interim_report.pdf).