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Bone Age and Mineral Density Assessments Using Plain Roentgenograms in Tenofovir-exposed Infants in Malawi and Brazil Enrolled in HIV Prevention Trials Network 057

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Background: Tenofovir disoproxil fumarate (TDF) use during pregnancy has been increasing, and studies linking bone toxicity with exposure to TDF have raised concern for its use in infants.

Methods: Hand/wrist and spine radiographs were obtained at 3 days and 12 weeks of age in infants born to HIV-infected pregnant women enrolled in the HIV Prevention Trials Network 057 pharmacokinetic study of TDF conducted in Malawi and Brazil assigned to 3 TDF dosing cohorts. In cohort 1, mothers received 600 mg of TDF during labor. In cohort 2, infants received 4 mg/kg dose on days 0, 3 and 5. In cohort 3, a 900 mg maternal dose was given during labor, followed by a 6 mg/kg infant dose on days 0, 3 and 5 of life.

Results: Across all 3 cohorts, 89 infants had radiographs performed at either time point, and 85 had radiographs performed at both time points. Metaphyseal lucency was present in 1 case in Brazil and 2 in Malawi. Fifteen percent of infants from Brazil and 9% of infants from Malawi presented bone age discrepancies. No other abnormalities were identified in Brazil, whereas in Malawi, there were 7 more cases of wrist osteopenia, 2 of spine osteopenia and 3 other abnormalities.

Conclusion: Bone abnormalities were not uncommon in the overall cohort of HIV-exposed infants. Because of very limited study drug exposure at the time of birth, it is unlikely that TDF was associated with these findings. Untreated maternal HIV disease and/or maternal nutritional status could potentially be related to fetal bone development. This association should be explored in future cohort studies.

Key Words: tenofovir, bone, HIV-exposed infants, HIV Prevention Trials Network 057

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Combination antiretroviral therapy (cART) has been successful in the prevention of mother-to-child transmission (PMTCT) of HIV, reducing rates from 20% to 1%–2.7%.[1–5] It is now recommended by the World Health Organization for HIV PMTCT.[6]

Tenofovir disoproxil fumarate (TDF) is one of the preferred agents used in cART because of its efficacy, once-daily dosing and favorable tolerability and toxicity profiles.[7–12] TDF is also approved for the treatment of hepatitis B virus (HBV),[13,14] and it is recommended for use as preexposure prophylaxis in uninfected adults at risk for HIV acquisition.[15–20]

The number of pregnant women exposed to TDF has been steadily increasing,[21,22] and although the guidelines from the European AIDS Clinical Society have no specific recommendation against the use of TDF during pregnancy,[23] guidelines in the US consider it an alternative, rather than preferred, therapy for HIV-infected, HBV-uninfected pregnant women. US guidelines also recommend TDF as a first-line treatment for nonpregnant HIV-infected adults and, during pregnancy, as first-line therapy for women with of HBV/HIV coinfection.[24] The more restrictive US recommendations rest in part on the results showing bone toxicity related to the use of TDF, first from animal studies[25–29] and then from human studies[30–41] and the limited data on the safety of in utero TDF exposure in humans. Data on chronic use of TDF in humans, both from studies comparing HIV-infected adults receiving regimens with and without TDF[30–35] and in preexposure prophylaxis studies of uninfected adults at risk,[37,38] showed decreased bone mineral density (BMD) and increased markers of bone metabolism. Also, there are case reports describing potential bone lesions or fractures in adults,[39,40] in association with TDF therapeutic use.

A systematic review of the effects of in utero TDF exposure on infant development, although generally encouraging,[42–50] was inconclusive, suggesting that more studies on the subject are necessary.[51]The HIV Prevention Trials Network (HPTN) 057 study was a phase I open-label clinical trial of the pharmacokinetics (PKs) of TDF during labor and during the neonatal period. PK results of the study have been previously reported.[52] As part of the study, infant BMD was assessed from radiographs taken of the hand and spine at 3 days and 3 months of age, to monitor for potential bone toxicities following infant exposure to the drug. The present study was conducted to evaluate for possible bone abnormalities in infants exposed to tenofovir as part of a perinatal trial, and as a secondary aim, to evaluate the utility of bone radiographs in the assessment of potential antiretroviral-mediated toxicities.

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MATERIALS AND METHODS

HPTN 057 was a phase I open-label study of TDF among HIV-infected pregnant women at the time of labor and their infants, with radiographic data collected from the first 3 of 4 cohorts. The fourth cohort did not include radiographic evaluations as it was developed long after PK and safety data from the first 3 cohorts were available and further bone evaluations were not deemed necessary by the protocol team. In cohort 1, no infant dose was given, but mothers received a 600 mg dose during labor. In cohort 2, no maternal dose was given, but infants received 4 mg/kg doses on days 0, 3 and 5. In cohort 3, a 900 mg maternal dose was given during labor and three 6 mg/kg infant doses on days 0, 3 and 5 of life. All women and infants received the local standard antiretroviral therapy (ART) regimen used at the time for PMTCT in addition to the study TDF regimen assigned to each cohort. At the time of study conduct, in Brazil, the specific cART regimen provided to pregnant women for PMTCT purposes consisted of triple ART with a protease inhibitor and 2 nucleoside reverse transcriptase inhibitors or nevirapine (NVP) and 2 nucleoside reverse transcriptase inhibitors starting at 14–28 weeks of gestation. During labor, women were typically given intravenous zidovudine (ZDV) infusion, and infants received 6 weeks of ZDV initiated at birth. In Malawi, the specific ART regimen provided for PMTCT purposes at the time of study conduct depended on when and if women presented for antenatal care. Women who presented for antenatal care were given ZDV starting at 28 weeks. During labor, women were typically given ZDV+lamivudine and single-dose NVP. Postpartum women were given ZDV+lamivudine for 7 days. All infants received single-dose NVP at birth. Infants whose mothers started ZDV at 28 weeks were given ZDV for 7 days initiated at birth. Infants whose mothers did not start ZDV at 28 weeks were given 4 weeks of ZDV initiated at birth. All patients provided written informed consent for study participation. The study was approved by local and national institutional review boards at all participating institutions in the US, Malawi and Brazil.

The study was conducted at 1 clinical site in Malawi and 4 clinical sites in Brazil from December 2006 to November 2010. Inclusion criteria for women consisted of being HIV-infected and pregnant, at least 18 years of age and provision of informed consent. Exclusion criteria consisted of previous treatment with TDF and/or an active medical condition that could affect TDF PK or compromise the study. All infants born to an enrolled mother were included in the study, but they were excluded from the initial dosing if they had a birth weight of less than 2000 g, severe congenital malformations, grade 2 or higher creatinine levels (graded using the division of AIDS adverse reaction grading criteria),[53] other toxicity grade 3 or higher[53] or a medical condition incompatible with life or that would interfere with study participation or interpretation (as judged by the study clinician).

Anteroposterior (AP) views of the left hand and wrist radiographs and AP and lateral views of the spine were obtained at 3 days and 3 months of age. The images were read by a local radiologist in real-time and centrally by a single radiologist at a US institution, and all abnormalities identified by either reader were recorded in the study database. Cortical thinning, osteopenia and other abnormalities were assessed both in the left hand and wrist AP radiographs and the lateral thoracic spine radiograph. Osteopenia was diagnosed when thinning of the cortex was identified, in combination with decreased bone density. In addition, metaphyseal lucency and bone age were also assessed in the left hand and wrist AP radiographs. Bone age was calculated according to the Atlas of Greulich-Pyle, according to gender. The radiographs were compared with the standards for age and gender, considering the normal standard deviation for each age group. Comparisons were made longitudinally, as the child returned for follow-up visits. Results were compared with standard norms applicable to long bones and spine in children of the same age group. Any eventual-associated diagnosis was also recorded in the study database.

Frequencies of abnormal bone findings for infants from Brazil and Malawi (categories at least 1 wrist abnormality, at least 1 spine abnormality, any bone age abnormality and any abnormality) were reported, and statistical significant differences were assessed via χ^2 and Student's t test.

RESULTS

The total number of infants enrolled across the 3 cohorts totaled 89, with 65 enrolled in Malawi (63%) and 33 enrolled in Brazil (37%). Cohort 1 enrolled 30 infants (34%), cohort 2 enrolled 23 (26%) and cohort 3 enrolled 36 (40%). The median birth weight was 3 kg. Clinical, demographic and laboratory characteristics as well as pregnancy outcomes are outlined in Table 1. Out of the total, 87 infants had radiographs performed on day 3 of life, 85 had radiographs performed at 12 weeks of life, 89 had radiographs performed at either time point, and 85 had radiographs performed at both time points.

Ten infants had abnormal wrist radiographs, and 3 had abnormal spine radiographs (Table 2). Ten infants had bone age abnormalities. In total, 19 infants had at least 1 abnormality in either wrist, bone age or spine. Bone age differences were seen both in babies from Malawi (9%) and Brazil (15%). However, clinically significant changes such as osteopenia or metaphyseal lucencies were seen almost exclusively in infants from Malawi, and in only 1 infant from Brazil. Despite a larger number of infants from Malawi having abnormalities noted, the findings were not statistically significant (Table 2).

Out of the 10 infants presenting bone age abnormalities, 6 had delayed and 4 had advanced bone age (Table 3). Metaphyseal lucency of the wrist was seen in 3 infants, whereas osteopenia was seen in the wrist of 7 and in the spine of 2 infants, with 1 of those infants having osteopenia of both the wrist and spine. Unrelated abnormal findings were present in the wrist radiographs of 2 infants at 12 weeks: 1 with mild distal osteosclerosis of the radius and ulna and the other with sclerosis and widening of the distal radius and ulna. The spine radiograph at 3 days of age of 1 infant demonstrated enlargement of the proximal femur consistent with congenital syphilis (further supported by serologic findings). Some infants presented more than 1 abnormality. No cases of fractures or cortical thinning were reported.

From day 3 of life to 12 weeks, the prevalence of osteopenia increased from 3.4% (3/87) to 5.8% (5/87) (Table 4). The prevalence of delayed bone age increased from 1.2% (1/87) to 5.8% (5/87), and the prevalence of advanced bone age increased from 1.2% (1/87) to 4.6% (4/87). Two cases of metaphyseal lucency were present at day 3, but only 1 at week 12. In total, the prevalence of abnormal radiographs increased from 9.2% (8/87) to 17.2% (15/87). Out of the 8 abnormalities that were initially present at birth (from the 85 patients who had radiographs performed at both time points), 4 resolved and 4 persisted in the follow-up visit. Ten infants with previously normal roentgenograms at day 3 presented to week 12 visit with new abnormalities. This included 2 cases of osteopenia, 4 cases of delayed bone age, 3 cases of advanced bone age, 1 case of mild distal osteosclerosis of the radius and ulna and 1 case of sclerosis and widening of the distal radius and ulna. Sixty-seven infants (79%) had no abnormalities detected at either visit. Bone abnormalities were equally distributed between infants enrolled in cohorts 1, 2 and 3 within HPTN 057.

TABLE 1. Characteristics of Mothers and Infants Enrolled in the Study (n = 89)

Parameters	Cohort 1, n = 30	Cohort 2, n = 23	Cohort 3, n = 36
Age at delivery (yr)	26 (18–38)	25 (20–36)	27 (19–37)
Weight at delivery (kg)	62 (47–84)	64 (46–83)	67 (49–116)
CD4+ at delivery (cells/mL)	459 (145–1145)	440 (96–737)	393 (128–1315)
HIV-1 RNA at delivery (copies/mL), n (%)			
Missing	0 (0)	2 (9)	1 (3)
<400	8 (27)	10 (43)	25 (69)
400–1000	2 (7)	0 (0)	0 (0)
1001–10,000	6 (20)	1 (4)	2 (6)
10,001–100,000	7 (23)	6 (26)	8 (22)
>100,000	7 (23)	4 (17)	0 (0)
Study site, n (%)			
Malawi	24 (80)	17 (74)	15 (42)
Brazil	6 (20)	6 (26)	21 (58)
Ethnicity, n (%)			
Non-Hispanic	24 (80)	17 (74)	15 (42)
Hispanic	6 (20)	6 (26)	21 (58)
Race, n (%)			
Black	27 (90)	19 (82)	26 (72)
White	1 (3)	2 (9)	5 (14)
Multiracial	2 (7)	2 (9)	5 (14)
Time from maternal dose to delivery (h)	2.9 (0.3–14.6)	—	3.3 (0.4–39.3)
Number of mothers who delivered vaginally, n (%)	23 (77)	18 (78)	23 (64)
Number of mothers who received background ART during pregnancy,* n (%)	15 (50)	13 (52)	25 (69)
Gestational age at delivery (wk)	40 (32–42)	40 (35–42)	39 (36–42)
Birth weight (kg)	2.97 (1.50–4.00)	3.00 (2.47–3.94)	3.01 (2.30–3.80)
Time from birth to initial infant dose (h)	—	9.4 (2.9–11.3)	4.5 (1.5–18.3)

*The type of ART regimen received is available for 84 mothers. At some time during the pregnancy, 76 received a protease inhibitor, 34 received a nonnucleoside reverse transcriptase inhibitor and 41 received only nucleoside reverse transcriptase inhibitors.
CD4 indicates CD4+ T cells (cells/mm^3).

TABLE 2. Overview of Study Findings

Abnormalities at Any Visit [Number of Cases (%)]	Brazil (n = 33 Infants), n (%)	Malawi (n = 56 Infants), n (%)	P*	Total (n = 89 Infants), n (%)
At least 1 wrist abnormality	1 (3)	9 (16)	0.19	10 (11.2)
At least 1 spine abnormality	0	3 (5.4)	0.054	3 (3.4)
Any bone age abnormality	5 (15.2)	5 (8.9)	0.24	10 (11.2)
At least 1 abnormality	5 (15.2)	14 (25)	0.40	19 (21)

χ^2: 0.138.
*Student's *t* test.

TABLE 3. Findings Across Clinical Sites

Abnormalities at Any Visit [Number of Cases (%)]	Brazil (n = 33 Infants), n (%)	Malawi (n= 56 Infants), n (%)	Total (n = 89 Infants), n (%)
Metaphyseal lucencies	1 (3)	2 (3.6)	3 (3.4)
Wrist osteopenia	0	7 (12.5)	7 (7.9)
Other wrist findings	0	2 (3.6)	2 (2.2)
Spine osteopenia	0	2 (3.6)	2 (2.2)
Other spine findings	0	1 (1.6)	1 (3.4)
Delayed bone age	2 (6)	4 (7)	6 (6.7)
Advanced bone age	3 (9)	1 (1.8)	4 (4.5)

DISCUSSION

There have been conflicting data regarding the potential effects of in utero TDF exposure on infants. In primate studies, administration of high concentration of TDF during pregnancy was toxic to the mother and resulted in fetal toxicity with proximal tubular disorders; however, postnatal exposure of infant rhesus macaques to low concentrations of TDF had no effect on bone growth and density.[25–29] Studies in human infants have also been conflicting. Data from the Pediatric HIV/AIDS Cohort study have suggested no effect of TDF exposure on anthropomorphic parameters at birth but a slight

TABLE 4. Findings Across Visits

Abnormality at Any Study Site [Number of Cases (%)]	Day 3 (n = 87 Infants), n (%)	Week 12 (n = 87 Infants), n (%)
Osteopenia	3 (3.4)	5 (5.7)
Delayed bone age	1 (1.2)	5 (5.8)
Advanced bone age	1 (1.2)	4 (4.6)
Metaphyseal lucency	2 (2.3)	1 (1.2)
Other findings	1 (1.2)*	2 (2.3)†‡
At least one abnormality	8 (9.2)	15 (17.2)

Osteopenias were only identified in Malawi; only 6 infants from Brazil had bone abnormalities including 1 case of metaphyseal lucency in the first visit, 2 cases of delayed bone age in the second visit and 3 cases of advanced bone age, 2 in the first and 1 in the second visit.
*Enlargement of the proximal femur (congenital syphilis).
†Mild distal osteosclerosis of radius and ulna.
‡Sclerosis and widening of distal radius and ulna.

decrease in length and head circumference growth parameters at 1 year of age. A separate study evaluating dual-energy radiograph absorptiometry (DEXA) in children up to 5 weeks of age found bone mineral content was 12% lower in TDF-exposed than unexposed infant controls.[22,54] In contrast, other studies have not observed differences in growth parameters in children with in utero TDF exposure compared with unexposed infants either at birth or 2 years of follow-up or differences in bone quantitative ultrasound.[43,44]

Infants in the present study did not have in utero TDF exposure and were exposed for only a limited time period—intrapartum with or without 3 infant doses in the first week of life—at doses resulting in low TDF concentration compared with the doses used in primate studies. Therefore, while and idiosyncratic TDF bone toxicity phenomenon cannot be excluded, a dose-dependent TDF bone toxicity is unlikely to be associated with the abnormal radiographic findings observed. It is more likely that problems with nutrition or factors in the environment led to the noted bone changes.[55] Abnormal bone radiographs were more common than expected in infants born to HIV-infected women in Malawi but they were infrequent in infants from Brazil, which could be due to a different prevalence of baseline abnormalities in the general population of both countries, although such local data are unavailable for comparison. Interestingly, all infants in Malawi were breast-fed, whereas all infants in Brazil were formula-fed. In addition, mothers in Brazil universally received comprehensive cART during prenatal care as a standard of care, whereas in Malawi, the standard of care at the time of study conduct was single-dose NVP to the mother and to the infant for HIV PMTCT. Because of the differences in maternal ART regimens between both countries, mothers delivering in Malawi were more likely to have had longstanding viremia during pregnancy as opposed to mothers in Brazil, as a result of untreated HIV infection. It is possible that poor maternal nutritional status potentially furthered by maternal HIV disease may have played a role contributing to the higher number and severity of bone abnormalities observed in Malawi.[55–57]

While bone radiographs were included in our study as a safety monitoring measure because TDF was being administered to pregnant women and infants in a PK study, the findings as stated were unlikely due to maternal or infant TDF drug exposure. Nevertheless, radiographic screening did note a larger than expected number of bone changes at birth which did not normalize over time. In fact, the presence of abnormalities nearly doubled from 9% to 17% by 12 weeks. The clinical significance of these findings remains to be determined.

It is important to note that plain radiographs are a sensitive way to detect osteopenia. In a study performed in 1988 comparing quantitative computerized tomography to radiographs of the spine in children with cystic fibrosis,[58] we were able to show that patients with low bone density (below 2 standard deviation) had abnormal plain films. Metabolic bone changes are most notable in the most vascularized regions of the bones which in children consists of metaphysis of long bones. Therefore, hand radiographs provide not only information about bone maturation but also about changes related to diet/nutritional factors or congenital/neonatal infections. In addition, the lateral view of the spine is also a helpful view in the diagnosis of osteopenia. As there are no standards for normal bone density values using DEXA in newborns while there are standards for plain films, and also because no DEXA machines were available in Malawi for our study, the protocol team opted to evaluate bone density in this study using plain roentgenograms.

Study limitations include the diverse infant population, the sample size, the impossibility of a non-HIV non-TDF exposed group and the brevity of both in utero and infant TDF exposure. Nevertheless, our findings are relevant in that there are very limited data on bone abnormalities in young HIV-exposed infants. The fact that abnormalities were frequently detected in the first 3 months of age in this population using plain roentgenograms also highlights the potential magnitude of the problem and the complexity of evaluating bone disease in heterogenous infant populations. Further prospective trials of infant TDF exposure with more sensitive evaluations of BMD and longer duration of follow-up are underway and may provide additional information about the long-term effects of TDF on infant BMD. Long-term studies will assist in determining the clinical significance of our findings and possible associations with untreated maternal HIV disease and/or maternal/infant nutritional status.

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