Phosphaturia in HIV-exposed Uninfected Neonates Associated with Maternal Use of Tenofovir Disoproxil Fumarate in Late Pregnancy

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ABSTRACT

Background: Our recent study showed significantly lower bone mineral content (BMC) in HIV-exposed uninfected (HEU) neonates born to HIV-infected (HIV+) mothers who took tenofovir disoproxil fumarate (TDF) in late pregnancy compared to no TDF use. In this cohort we sought to understand possible mechanisms for lower BMC by comparing markers of bone metabolism and renal function with TDF exposure in HEU neonates.

Methods: Among a subset of HEU children in the multicenter (US and Puerto Rico) observational Surveillance Monitoring for ART Toxicities (SMARTT) Cohort study, we enrolled neonates (≥ 36 wks gestational age) of HIV+ mothers who took TDF for ≥ 8 wks in the 3rd trimester (TDF+) or no TDF in pregnancy (TDF-). In addition to BMC measures, we collected a blood and urine sample on each child ≤ 30 days of birth to measure serum creatinine, phosphate, 25-OH vitamin D, parathyroid hormone and urine creatinine, phosphate and N-terminal telopeptide. Standard equations were used to estimate proximal tubular phosphate reabsorption and glomerular filtration rate (eGFR). Comparisons were made by TDF exposure using Wilcoxon and Fisher's exact tests. We fit linear models to compare TDF+ and TDF- for each assay by age in days at sample collection (slope), stratified by age group at sample collection time (0-3 days, 4-30 days).

Results: Of 160 HEU neonates (Black 71%, Hispanic 31%) 82 were TDF+ and 78 TDF-. Sociodemographic and anthropometric characteristics did not differ by TDF exposure in each age group. Within 0-3 days of life, TDF+ had a greater decline in serum creatinine (p=0.04) and a greater increase in eGFR compared to TDF- (p=0.06), but no difference in slope by TDF exposure within 4-30 days of life, nor in serum phosphate in either age group. Proximal tubular phosphate reabsorption was similar for both groups within the first 3 days of life, with a significantly greater decline in phosphate reabsorption between 4-30 days of life in the TDF+ compared to the TDF- group (p=0.006, Figure 1). Bone markers did not differ by TDF exposure for either age group.

Conclusion: Urinary phosphate loss was increased among HEU neonates of mothers who took TDF in late pregnancy. This suggests proximal tubular dysfunction and may explain, at least in part, the decrease in BMC previously described.

BACKGROUND

- Tenofovir disoproxil fumarate (TDF) has been used extensively for the treatment of HIV in adolescents and adults, including pregnant women, with over 7.5 million person-years of worldwide experience[1]
- TDF with emtricitabine forms the nucleoside reverse transcriptase inhibitor backbone that, in combination with other classes of antiretroviral drugs, is indicated as first-line treatment for HIV by the US Department of HHS, World Health Organization and other bodies
- TDF is a component of several fixed drug combination regimens that are dosed as a once-daily tablet, and is generally well tolerated
- Renal toxicity has been described with TDF in adults[1] and children[2], the main effect being proximal tubular dysfunction, with increased phosphaturia, glycosuria, uricosuria and aminoaciduria. Renal toxicity includes asymptomatic proteinuria, with prolonged use declining glomerular filtration rate, and rarely, Fanconi's syndrome
- The effect of TDF on bone mineral density has been studied in treatment naïve and switch studies, as well as PREP studies, confirming significant reductions in bone mineral density. TDF is also associated with fragility fractures[3]
- Tenofovir alafenamide fumarate (TAF) is a newer FDA-approved drug with markedly reduced renal and bone toxicities relative to TDF, but its use is restricted by cost and access, and hence currently limited to high-income countries
- There are few studies describing the outcome of fetal exposure to TDF in HIV-exposed uninfected (HEU) infants. Siberry et al. showed a 12% reduction in whole-body bone mineral content (BMC) measured by dual energy x-ray absorptiometry in HEU neonates who were TDF-exposed compared to TDF-unexposed[4]. **This current analysis investigated the potential mechanisms behind this reduction in BMC in this cohort of HEU neonates.**

OBJECTIVES

1. To compare estimates of renal function in HEU neonates with *in-utero* exposure to TDF in late pregnancy (TDF+) to those without TDF exposure (TDF-)
2. To compare markers of bone metabolism in TDF+ compared to TDF- HEU neonates

METHODS

Study Population

- The Surveillance Monitoring for Antiretroviral Therapy and Toxicities Study (SMARTT) with the Pediatric HIV/AIDS Cohort Study (PHACS) is a prospective cohort study of HEU infants and children, IRB-approved and conducted at 23 sites within the United States and Puerto Rico that studies the long term effects of *in-utero* exposure (and 6 weeks post partum exposure) to antiretroviral drugs on various domains and organs
- The Dynamic Cohort of SMARTT enrolled participants late in pregnancy or within 72 hours of birth. Informed consent for participation was obtained from legal guardians.

The TDF study was a sub-study of SMARTT conducted at 14 of the 23 sites above between 4/2011 and 6/2013. It enrolled neonates born to mothers who had received ≥ 8 weeks of TDF in the third trimester (TDF+ group) and those born to mothers who had never received TDF during their pregnancy (TDF-, comparator group).

- **Neonates had to have a gestational age (GA) ≥ 36 weeks**
- **Neonates found to be HIV-infected were excluded**

Neonates had blood and urine collected and stored for testing during one study visit that occurred between 0 and 30 days of life. On these samples the following tests were performed:

- **Serum and urine creatinine and phosphate**
- **Serum vitamin D (25-OHD) and parathyroid hormone (PTH)**
- **Spot urine cross-linked N-telopeptide of type 1 collagen (NTx)**
- **Glomerular filtration rate and tubular reabsorption of phosphate were calculated as described below**

For each of the seven laboratory outcomes above, the data set reflects one result per participant at their visit between 0-30 days of life.

Definitions and Statistical Methods

- Estimated glomerular filtration rate (eGFR) was calculated as follows:
  - neonates <37 weeks GA, eGFR = 0.33*infant length/serum creatinine
  - neonates ≥37 weeks GA, eGFR = 0.45*infant length/serum creatinine
- Percentage tubular reabsorption of phosphate (PTRP) was calculated as [1-(urine phosphate*serum creatinine/serum phosphate*urine creatinine)] *100
- The distributions of maternal and infant characteristics by TDF exposure were assessed by Wilcoxon rank sum and Chi-Square tests as appropriate
- LOESS plots were created to describe the distribution of each laboratory parameter by TDF exposure that was measured once on each neonate from 0-30 days of life
- Linear regression models were fit with the robust variance to estimate the slope of each outcome by age in days at sample collection between TDF+ compared to TDF- for each parameter stratified by age group at sample collection time (0-3 days, 4-30 days)

RESULTS

- Of 160 neonates, 82 were in the TDF+ arm and 78 in the TDF- arm; 71 % were black and 31% Hispanic
- There were no differences between maternal socioeconomic variables and infant anthropometric measures by TDF exposure (Table 1)
- Figure 1 shows LOESS plots describing the values from 0-30 days for seven individual parameters by TDF exposure (four renal: serum creatinine, phosphate, eGFR and PRTP; and three bone: serum 25-OHD, PTH, and urine NTx)
- Figure 2 shows regression lines for TDF+ and TDF- groups for three selected renal variables where slopes differed by TDF exposure (serum creatinine, 0-3 days, eGFR, 0-3 days and PTRP, 4-30 days). All other variables did not differ

RESULTS (CONTINUED)

Table 1: Maternal and neonatal socioeconomic and anthropometric characteristics by *in-utero* tenofovir disoproxil fumarate exposure (*Wilcoxon, **Chi-Square)

Characteristic		TDF exposure among neonates between 0-3 days of birth: TDF exposed (N=36)	TDF non-exposed (N=56)	P-Value	TDF exposure among neonates between 4-30 days of birth: TDF exposed (N=46)	TDF non-exposed (N=22)	P-Value
Mother, Age at delivery (years)	Median (Q1, Q3)	29.2 (23.3, 33.5)	28.0 (23.6, 32.9)	0.55*	29.6 (25.9, 34.0)	30.4 (23.2, 33.5)	0.75*
	N	36	55		45	22	
Mother, Education status	Less than High School	13 (37%)	17 (31%)	0.58**	10 (22%)	5 (23%)	0.93**
	At least High School	22 (63%)	37 (69%)		36 (78%)	17 (77%)	
	.	1	2		0	0	
Income < $10,000/year	No	8 (24%)	18 (38%)	0.18**	20 (44%)	12 (57%)	0.34**
	Yes	26 (76%)	30 (63%)		25 (56%)	9 (43%)	
	.	2	8		1	1	
Infant - Black race	No	7 (23%)	15 (31%)	0.43**	8 (17%)	4 (18%)	0.94**
	Yes	24 (77%)	34 (69%)		38 (83%)	18 (82%)	
	.	5	7		0	0	
Infant - Race/ethnicity	White Non-Hispanic	0 (0%)	5 (9%)	0.23**	3 (7%)	0 (0%)	0.39**
	Black Non-Hispanic	16 (44%)	26 (46%)		36 (78%)	18 (82%)	
	Hispanic (Regardless of Race)	17 (47%)	23 (41%)		5 (11%)	4 (18%)	
	More than one race or other	3 (8%)	2 (4%)		2 (4%)	0 (0%)	
Gestational age (weeks)	Median (Q1, Q3)	38.4 (37.9, 39.3)	38.1 (37.9, 39.0)	0.22*	38.7 (37.7, 39.3)	38.3 (37.9, 39.4)	0.84*
	N	36	55		45	22	
Infant - Length (cm)	Median (Q1, Q3)	49.1 (47.3, 50.5)	50.0 (47.3, 50.8)	0.22*	49.7 (48.1, 50.7)	49.1 (47.0, 51.1)	0.58*
	N	36	56		44	22	
Infant - Length z-score	Median (Q1, Q3)	-0.3 (-0.8, 0.4)	0.2 (-0.8, 0.8)	0.17*	-0.1 (-0.7, 0.2)	-0.2 (-1.1, 0.4)	0.65*
	N	36	56		45	22	
Infant - Weight z-score	Median (Q1, Q3)	-0.8 (-1.3, -0.3)	-0.7 (-1.2, -0.0)	0.29*	-0.4 (-0.8, -0.2)	-0.4 (-0.8, 0.2)	0.50*
	N	36	56		45	22	

Figure 1. LOESS plots for neonatal renal and bone parameters by age at sample collection (0-30 days) for individual TDF-exposed (TDF+) and TDF-unexposed (TDF-) participants

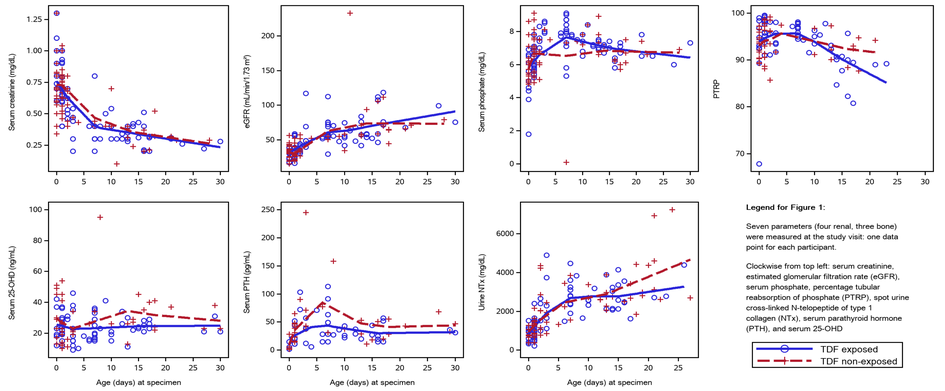
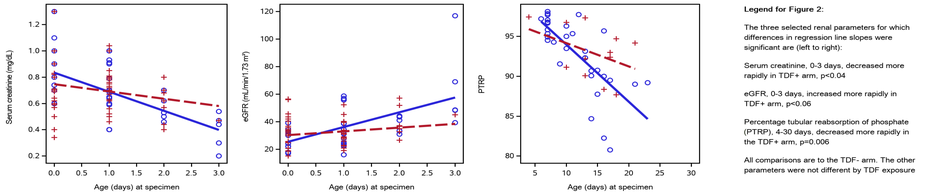


Figure 2: Regression lines for selected renal parameters in TDF-exposed (TDF+) and TDF-unexposed (TDF-) neonates, stratified by age in days (0-3, 4-30)



SUMMARY

- Over the first few days of life, serum creatinine dropped acutely, and then gradually fell to a normal age-appropriate level between 0.2-0.5 mg/dL by 30 days of life. This reduction was from 0-3 days and significantly more acute for TDF+ as compared to TDF- neonates (first panel in Figure 2, p=0.04).
- Changes in eGFR were inverse to serum creatinine, as would be expected. eGFR improved rapidly over the first few days of life, reflecting maturing renal function, and then settled around 75-100 ml/min/1.73 m^2 by 30 days. The increase during the first 3 days was higher in TDF+ compared to TDF- neonates (second panel in Figure 2, p=0.06).
- Although serum phosphate levels did not change much from 0-30 days, PTRP ranged from 90-100% in the first few days and declined at a higher rate between 4-30 days of life in TDF+ compared to TDF- neonates, reflecting greater urinary loss of phosphate in TDF-exposed neonates (third panel in Figure 2, p=0.006)
- Serum 25-OHD concentrations stayed stable through 0-30 days. Serum PTH levels increased over the first few days and then plateaued thereafter. Serum 25-OHD and PTH levels were not different by TDF exposure, suggesting no difference in bone formation or resorption in the two arms
- Urinary NTx levels rose gradually throughout the neonatal period, were not different by TDF exposure, and this is consistent with increasing bone resorption characteristic of the growing infant and child

SUMMARY (CONTINUED)

- Markers of bone metabolism suggested a picture of balanced bone turnover in HEU neonates that was not different by TDF exposure
- The data presented here for various parameters reflect single measures for each individual participant taken at various ages in their first 30 days of life, and are not longitudinal measures performed at serial time points in the same individuals

CONCLUSIONS

- Fetal exposure to TDF in late pregnancy was associated with changes in eGFR and proximal tubular function in the neonate. While GFR appeared to normalize within the first few days of life, phosphaturia, a sign of proximal tubular dysfunction, persisted over the first month of life in HEU neonates
- Post-natal bone metabolism in HEU neonates was not impacted by TDF exposure
- Fetal renal tubular toxicity may explain, at least in part, the reduced BMC previously described in HEU neonates with fetal exposure to TDF in late gestation

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