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Effects of renal tubular dysfunction on bone in tenofovir-exposed HIV-positive patients

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Abstract

Objectives—Tenofovir disoproxil fumarate (TDF) may cause renal tubular dysfunction (RTD) and reduce bone mineral density (BMD). We examined the relationship between RTD and BMD in TDF-exposed HIV-positive men.

Design and methods—We analysed urinary retinol binding protein/creatinine ratio (RBPCR) and fractional excretion of phosphate (FEPO₄) to quantify RTD in a cross sectional sample of randomly selected HIV positive men at a single tertiary outpatient clinic. BMD at the lumbar spine and hip was measured by dual-energy x-ray absorptiometry. Multivariate logistic regression was used to analyse factors associated with RTD, and linear regression to examine the relationship between RTD and BMD.

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Conflict of interest

Lisa Hamzah has received funding to attend conferences from Gilead Sciences and honoraria from Janssen-Cilag.

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Presentation at meetings

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Permission is granted for personal communications

Results—Of 293 men (mean age 48 years, 94% white ethnicity, median TDF exposure 2.1 years), 22.5% had RBPCR-defined RTD and 12.3% had FEPO₄-defined RTD. We observed a negative correlation between RBPCR and BMD at the spine (β -0.2, $p=0.002$) and hip (total: β -0.1, $p=0.02$; femoral neck: β -0.1, $p=0.02$), but not between FePO₄ and BMD. In multivariable analyses, RTD defined by >5 fold elevations in RBPCR was associated with significantly lower BMD of the spine.

Conclusions—RTD was associated with lower BMD of the spine in HIV-positive men. RBPCR quantification may identify patients at increased risk of TDF-associated BMD reduction.

Keywords

HIV; Tenofovir; Renal Tubular Dysfunction; Bone Mineral Density

Introduction

Tenofovir disoproxil fumarate (TDF) is a preferred treatment option for HIV-1 infection and one of the most widely prescribed antiretrovirals [1]. Although TDF has a favourable safety record, a small proportion of patients develop severe renal tubular disease (Fanconi syndrome) [2]. Fanconi syndrome is characterised by glycosuria, phosphate wasting, and markedly increased urinary concentrations of low molecular weight proteins (LMWP), and may be accompanied by bone pain, osteomalacia and fragility fractures [3–6]. TDF exposure may cause or worsen renal tubular dysfunction (RTD), a lesser derangement of renal tubular function [7–10]. RTD is typically asymptomatic, non-progressive and non-treatment limiting, and characterised by mild to moderate increases in urinary LMWP concentration and variable manifestations of impaired reabsorption of phosphate, urate, glucose and other solutes [11]. Using variable definitions, RTD has been reported in 10–22% of HIV-positive patients receiving TDF [12–14].

TDF has an adverse effect on bone mineral density (BMD) [15]. Patients initiating TDF-containing regimens experience greater reductions in BMD compared with those initiating non-TDF containing regimens [16, 17]. In addition, switching to a TDF-containing regimen is associated with reductions in BMD [18–20], and discontinuing TDF with improvements in BMD [21]. Preliminary evidence suggests that TDF-associated BMD reductions may translate into increased fracture risk [22].

It remains unclear whether sub-clinical RTD is associated with altered bone homeostasis and/or reductions in BMD. In this cross-sectional study, we examined the relationship between RTD, bone turnover and BMD in a cohort of TDF-exposed HIV-positive men.

Methods

Data were drawn from a BMD study in HIV-positive men. Individuals had been randomly selected using a randomisation table from a single HIV outpatient clinic in Brighton, UK [23]. At the baseline study visit, biometrics including height, weight and blood pressure (BP) were measured. A self-completed questionnaire evaluated medical history and lifestyle factors including history of fragility fracture, exposure to drugs associated with bone

metabolism (including inhaled, oral and anabolic steroids), co-morbidities associated with bone loss or renal impairment, current alcohol use, smoking status and levels of exercise. Demographics and HIV parameters including age, ethnicity, risk factors for HIV acquisition, hepatitis B and C co-infection, time since HIV diagnosis, current and nadir CD4 cell count, prior AIDS diagnoses, current HIV viral load, time since initiating antiretroviral therapy, current exposure to TDF and protease inhibitors (PI) and length of exposure to TDF and PI were obtained from the clinic database. As the relationship between RTD and bone is most relevant to patients with current TDF exposure, analyses were restricted to those reporting current TDF use.

Plasma creatinine (Cr), phosphate (PO_4) and alkaline phosphatase (ALP) concentrations were measured in fasting blood samples. Additional aliquots were frozen at -80° and analysed for total 25-hydroxy vitamin D (25[OH]D), parathyroid hormone (PTH), type 1 collagen cross-linked C-telopeptide (CTX, a marker of bone resorption, Uscn Life Science Inc. Wuhan, China), and procollagen type 1 N-terminal propeptide (PINP, a marker of bone formation, Uscn Life Science Inc. Wuhan, China). Fasting urine samples were analysed for protein-creatinine ratio (PCR) and for quantification of urinary phosphate; an aliquot was frozen at -80° for urinary retinol binding protein (RBP, a LMWP) concentrations (DELFI[®] monoclonal antibody assay, Cambridge, UK [24]). Dual energy X-ray absorptiometry (DXA) measurements were obtained using the GE Healthcare Lunar iDXA bone densitometer (GE Healthcare, Madison, Wisconsin, USA). BMD (expressed in g/cm^2) of the lumbar spine (L1-L4 composite), the non-dominant total hip and the non-dominant neck of femur (NOF) were used for analysis.

Definitions

The Chronic Kidney Disease Epidemiology Collaboration (CKD-Epi) formula was used to calculate eGFR [25]. Hypertension was defined as a BP $>140/90$. Renal tubular function was assessed by RBP-creatinine ratio (RBPCR) and by fractional excretion of phosphate (FEPO_4) using the following equation: $(\text{Urine } \text{PO}_4 / \text{Serum Cr}) / (\text{Serum } \text{PO}_4 / \text{Urine Cr})$. Patients were considered to have RTD if their RBPCR was above the laboratory upper limit of normal (ULN, $2.93 \mu\text{g}/\text{mmol}$ [24]); these patients were stratified into those with RBPCR 1-5xULN and $>5\text{xULN}$ ($>14.65 \mu\text{g}/\text{mmol}$). Patients were also considered to have RTD if they had evidence of phosphate wasting as defined by a fasting $\text{FEPO}_4 >20\%$ in the presence of hypophosphataemia (plasma $\text{PO}_4 <0.8 \text{ mmol/L}$).

Statistical methods

Variables were transformed where appropriate to approximate to a normal distribution. Factors associated with raised RBPCR and phosphate wasting were examined categorically using multivariate logistic regression with forward stepwise inclusion of variables with $p < 0.1$. The relationship between RBPCR, FEPO_4 , bone biomarkers and BMD (all as continuous variables; RBPCR also as a categorical variable) was examined using correlation coefficients, and where possible, multivariate linear regression. Assumptions were tested graphically. Exposures, confounders and interactions were chosen *a priori* on the basis of biological plausibility as well as a significant association in univariate analysis with $p < 0.1$.

Data were complete or near complete for the majority of cases. Whole case analysis was used to deal with missing data.

Results

Cohort description

A total of 293 HIV-positive men were eligible for inclusion. The mean (SD) age was 47.7 (8.9) years, 94.2% were of white ethnicity and 92.2% had acquired HIV through sex with men (Table 1). They had been diagnosed HIV positive for a median (IQR) of 9.2 (4.9, 15.0) years and had received TDF for a median of 2.1 (1.2, 4.2) years. Almost all had an undetectable HIV viral load. The mean (SD) eGFR was 92.8 (16.6) mL/min/1.73m². Overall, 3.1% had moderate or severe proteinuria (PCR >50 mg/mmol), 22.5% had RBPCR-defined RTD (16.0% with RBPCR 1-5×ULN, 6.5% with RBPCR >5×ULN) and 12.3% had FEPO₄-defined RTD. The mean (SD) BMD was 1.13 (0.14), 0.99 (0.13) and 0.94 (0.12) g/cm² at the lumbar spine, total hip and NOF respectively.

Factors associated with renal tubular dysfunction

A statistically significant correlation between RBPCR and FEPO₄ was observed (r^2 0.2, $p < 0.0001$). No correlation was seen between RBPCR and total (25[OH]D) (r^2 -0.04, $p = 0.4$) or PTH (r^2 -0.08, $p = 0.1$). Factors associated with RBPCR and FEPO₄-defined RTD are shown in Table 2. In multivariate analyses, a lower eGFR was significantly associated with RBPCR 1-5×ULN (aOR [95% CI] 1.2 [1.0, 1.5] $p = 0.03$), RBPCR >5×ULN (aOR 1.8 [1.3, 2.5] $p = 0.001$) and FEPO₄-defined RTD (aOR 1.4 [1.2, 1.8] $p = 0.001$). In addition, more prolonged exposure to a boosted PI was independently associated with RBPCR >5×ULN (aOR 1.2 [1.0, 1.3] $p = 0.02$). Other factors, including time since HIV diagnosis, duration of TDF exposure, current or nadir CD4 cell count and hepatitis B or C co-infection, were not associated with RTD. When eGFR was considered categorically (<90, 90-75, 75-60, <60 mL/min/1.73m²), an eGFR <60 mL/min/1.73m² was associated with RBPCR >5×ULN (aOR 9.1 [2.2, 36.6], $p = 0.002$), and FEPO₄-defined RTD (aOR 3.8 [1.5, 9.4], $p = 0.004$).

Relationship between renal tubular dysfunction and bone

A significant negative correlation was observed between RBPCR and BMD at the spine (β -0.2, $p = 0.002$), total hip (β -0.1, $p = 0.02$) and NOF (β -0.1, $p = 0.02$). By contrast, we found no such correlation between FEPO₄ and BMD at any of the three sites (β -0.006, -0.03 and -0.04, $p > 0.5$ for the lumbar spine, total hip and NOF, respectively) (Figure 1 a-f).

The relationship between RTD and BMD was further explored using multivariate linear regression (Table 3). When RBPCR was considered as a continuous independent variable, after adjustment for eGFR, age, ethnicity, time on TDF, PI use and BMI, RBPCR remained significantly associated with BMD of the spine (β [95%CI] -0.02 [-0.03, -0.006] $p = 0.004$). When RBPCR was considered as a categorical variable, we again observed a significant association between RBPCR >5×ULN and BMD at the spine (β [95%CI] -0.1 (-0.2, -0.08) $p < 0.001$). Using this model, the presence of RBPCR >14.65 μ g/mmol predicted an approximate 13% lower BMD at the spine as compared to a subject of similar age without

RTD (predicted mean spine BMD [95% CI] 0.99 [0.92, 1.06] vs. 1.14 [1.12, 1.16] g/cm² respectively).

In univariate linear regression models, we observed no significant association between FEPO₄ (as continuous or categorical variable) and BMD at the spine, total hip or NOF (data not shown). When maximal tubular phosphate reabsorption (TmPO₄) [26], an alternative marker to assess renal tubular phosphate handling [26–28], was used as a continuous variable, we observed a significant association with spine BMD in univariate analysis; however, this association was no longer present after adjustment for other covariates and confounders including eGFR, cumulative exposure to TDF, cumulative exposure to a PI, current PI use, current smoking status, steroid use and body mass index (β -0.08 [-0.2, 0.03], $p=0.1$). No correlation was observed between RTD and bone turnover, neither when RBPCR was used to define RTD (ALP: β 0.03, $p=0.7$; CTX: β 0.003, $p=1.0$; P1NP: β 0.0, $p=1.0$), nor when FEPO₄ was used to define RTD (ALP: β 0.04, $p=0.6$; CTX: β -0.06, $p=0.3$; P1NP: β -0.06, $p=0.3$).

Discussion

In HIV-positive men receiving TDF-containing antiretroviral therapy, we observed a significant negative correlation between RBP-defined RTD and BMD of the lumbar spine and the hip. This association remained significant at the spine after adjusting for both HIV-associated and traditional risk factors, and for eGFR as a crude measure of renal function. Our results suggest that mild derangement of renal tubular function (RBPCR $\leq 5 \times \text{ULN}$) has little effect on BMD. By contrast, more severe RTD (RBPCR $> 5 \times \text{ULN}$) was associated with significant reduction at the spine only.

We noted an independent association with PI use and increased RBPCR. A recent review of PI use and renal function in HIV [29] supports the effect of PIs on renal tubular creatinine secretion and highlight the possible association between Atazanavir (ATV), Lopinavir and CKD. ATV has been shown to be a risk factor for developing proximal renal tubular dysfunction in the Aquitaine cohort [30]. When stratified by treatment exposure, individuals with ongoing ATV exposure of ≥ 4 years were 3 times more likely to have PKTD than those with prior or no exposure. We were not able to distinguish between ATV and other PIs in this cohort due a limited sample size (35/117 (30%) on ATV vs. other PIs).

A previous cross-sectional study, in which only 11 of 153 participants had RTD, found no association between RTD and BMD [31]. Our study, which included 66 cases of RTD, suggests that RBP-defined RTD is associated with reduced BMD. The putative mechanism that links RTD to BMD is phosphate wasting [32]. Although RBPCR was correlated with FEPO₄, we observed no association between this measure of RTD and BMD. This may relate to the cut-offs used in the present analyses to define phosphate wasting, or suggest that reduced fasting plasma phosphate concentrations may not reliably reflect total body phosphate depletion, a signal for tubular phosphate reabsorption. Our data are consistent with a previous study which found superior specificity of LMWP (beta-2 and alpha-1 microglobulin) compared with FEPO₄ to diagnose RTD [14], and suggest that concurrent

plasma and urine phosphate measurements may be of limited use in identifying patients at greatest risk of TDF-associated bone loss.

Reductions in BMD observed in clinical trials with TDF have been associated with increased bone turnover [16, 19, 20]. In keeping with the study of Calmy *et al.* [31], we observed no correlation between RBPCR and markers of either bone resorption or bone formation. This lack of association is somewhat puzzling although the cross-sectional nature of our study may have limited our ability to detect such a relationship. The use of bone turnover markers in clinical practise remains controversial other than to monitor responses to bisphosphonate treatment [33]. It is possible that the mechanisms of TDF-associated initial bone loss are mediated through the parathyroid axis [34] or via a direct effect of TDF on bone [20]. Indeed, the initial reductions in BMD observed with TDF in the ASSERT study did not correlate with LMWP concentrations [16]. While these initial reductions in BMD tend to stabilise after 48 weeks of TDF exposure [16–19], the effects of persistent RTD may be masked by the magnitude of the initial BMD decline and only become apparent with chronic TDF exposure.

We hypothesize that the strength of the relationship between RBP-defined RTD at the spine versus the hip may be partly explained by the difference in bone composition at the spine (predominantly trabecular) [35] and hip (predominantly cortical) [36]. The metabolic rate per unit volume bone is higher in trabecular bone, and this may render this type of bone more sensitive to endogenous insults [37]. Consequently, BMD changes may differ between bone sites, and high bone turnover states such as HIV-associated osteoporosis may involve trabecular bone at an earlier stage than cortical bone [38].

The strengths of this study include the large, homogeneous and contemporaneous study population and the use of a robust assay to measure RBP. However, as we studied almost exclusively white men, our observations cannot necessarily be extrapolated to women or those of other ethnicities. Variables determined on questionnaire were not reconciled with medical records and may therefore have been subject to recall and/or over-reporting bias. Patients who had permanently stopped TDF after developing renal tubular disease (9 in the parent cohort) were not included in the analyses, which may have underestimated the impact of TDF on the renal tubular function. It is also unclear whether other LMWPs would have rendered similar results. Our cross-sectional study design precludes inference of causality, and the clinical significance of the observed BMD reductions remains to be defined. DXA cannot distinguish BMD reductions due to osteoporosis or osteomalacia [39]. The observed correlation between RTD and phosphate wasting suggest that the BMD reductions in patients with RBP-defined RTD may be due to osteomalacia even though (25[OH]D) concentrations did not correlate with RBPCR. Our observations should be confirmed in more ethnically diverse cohorts and women, and perhaps with a broader panel of tubular biomarkers. Finally, longitudinal studies are required to confirm that the relationship between RBP-defined RTD and reduced BMD, and that TDF discontinuation, improves both RTD and BMD.

In conclusion, we observed a negative correlation between RBP-defined RTD and BMD of the spine. Quantification of RBP may help identify HIV-positive patients who are at risk of

TDF-associated reductions in BMD and in whom TDF discontinuation may result in improved BMD and reduced fracture risk.

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Figure 1. Correlations between a) natural log RBPCR and lumbar spine BMD, b) FEPO₄ and lumbar spine BMD, c) natural log RBPCR and non-dominant total hip BMD d) FEPO₄ and non-dominant total hip BMD e) natural log RBPCR and femoral neck BMD f) FEPO₄ and femoral neck BMD

Table 1
(i) Demographic and (ii) clinical characteristics of HIV-infected study participants, and the proportion of each group currently experiencing pain (n=859 unless otherwise stated)

(i) Demographic characteristics		Number (% of total sample)	Number with current pain (%)	p-value ^I
n		859 (100.0)	341 (39.7)	
Sex	Male	775 (90.2)	303 (39.1)	0.33
	Female	84 (9.8)	38 (45.2)	
Age (years)	Median (range)	42 (19 - 77)		0.002
	19-34	171 (19.9)	49 (28.7)	
	35-39	164 (19.1)	56 (34.2)	
	40-44	186 (21.7)	80 (43.0)	
	45-49	143 (16.7)	65 (45.5)	
	50	195 (22.7)	91 (46.7)	
Ethnicity (n=833)	White	631 (75.8)	261 (41.4)	0.35
	Black African	84 (10.1)	27 (32.1)	
	Other	118 (14.2)	43 (36.4)	
BMI (kg/m ²) (n=810)	Underweight (<18.5)	40 (4.9)	17 (42.5)	0.99
	Normal (18.5, <25)	497 (61.4)	197 (39.6)	
	Overweight (25, <30)	204 (25.2)	81 (39.7)	
	Obese/morbidly obese (30)	69 (8.5)	26 (37.7)	
Smoking status (n=850)	Current	348 (40.9)	138 (39.7)	0.71
	Ex-smoker	266 (31.3)	112 (42.1)	
	Never smoked	236 (27.8)	88 (37.3)	
Consume alcohol	No	198 (23.0)	88 (44.4)	0.14
	Yes	661 (77.0)	253 (38.3)	
Hepatitis B +ve	No	843 (98.1)	334 (39.6)	0.94
	Yes	16 (1.9)	7 (43.8)	
Hepatitis C +ve	No	848 (98.7)	336 (39.6)	0.93
	Yes	11 (1.3)	5 (45.5)	
Working status (n=852)	Working	486 (57.0)	145 (29.8)	0.0001
	Not working	366 (43.0)	195 (53.5)	
SF-36				
Mentality	Median (IQR)	14 (5, 30)		0.0001
	5-11	264 (34.1)	68 (25.8)	
	12-16	254 (32.8)	97 (38.2)	
	17-30	256 (33.1)	144 (56.3)	
Physical functioning	Median (IQR)	14 (4, 24)		0.0001
	4-11	266 (34.6)	59 (22.2)	
	12-16	281 (36.5)	121 (43.1)	
	17-24	222 (28.9)	126 (56.8)	

(i) Demographic characteristics				
		Number (% of total sample)	Number with current pain (%)	p-value ¹
(ii) Clinical characteristics				
		Number (% of total sample)	Number with current pain (%)	p-value ¹
n		859	341 (39.7)	
Mode of infection	MSM ²	217 (25.3)	84 (38.7)	
	Other	60 (7.0)	21 (35.0)	
	Not known	582 (67.8)	236 (40.6)	0.66
Latest CD4 (cells/mm ³) (n=837)	Median (range)	475 (12, 1407)		
	<200	59 (7.1)	24 (40.7)	
	200-349	164 (19.6)	66 (40.2)	
	350	614 (73.4)	242 (39.4)	0.99
Latest viral load (copies/ml) (n=842)	Median (range)	40 (40, 834687)		
	40	569 (67.6)	242 (42.5)	
	>40	273 (32.4)	93 (34.1)	0.02
Time since HIV diagnosis (years) (n=839)	1	88 (10.5)	24 (27.3)	
	>1, 3	132 (15.7)	38 (28.8)	
	>3, 5	133 (15.9)	51 (38.4)	
	>5, 10	216 (25.7)	98 (45.4)	
	>10, 20	220 (26.2)	99 (45.0)	
	>20	50 (6.0)	26 (52.0)	0.0009
CDC status (n=839)	A – asymptomatic	489 (58.3)	171 (35.0)	
	B – symptomatic	190 (22.7)	89 (46.8)	
	C – AIDS	160 (19.1)	71 (44.4)	0.01
cART naive	Never had cART	154 (17.9)	46 (29.9)	
	Have had cART	705 (82.1)	295 (41.8)	0.008
Current ART status	Not on cART	202 (23.5)	66 (32.7)	
	On cART	657 (76.5)	275 (41.9)	0.02
	PI based – No	580 (67.5)	209 (36.0)	
	PI-based - Yes	279 (32.5)	132 (47.3)	0.002
	NNRTI based - No	503 (58.6)	201 (40.0)	
	NNRTI based – Yes	356 (41.4)	140 (39.3)	0.91
	NRTI-based – No	259 (30.1)	90 (34.8)	
	NRTI-based - Yes	600 (69.9)	251 (41.8)	0.06

¹ p-value obtained from a univariate comparison of the proportions currently experiencing pain in each group (Chi-squared test).

² MSM: men having sex with men; CDC: Centers for Disease Control; cART: combination antiretroviral therapy; ART: antiretroviral therapy; PI: protease inhibitor; NNRTI: non-nucleoside reverse transcriptase inhibitor; NRTI: nucleoside reverse transcriptase inhibitor

Table 2
Musculoskeletal pain and its impact among 859 HIV-infected adults

	Total (n=859)	% of total
Ever had pain lasting at least 1 day in the last month	539	62.8
Number of painful sites (range)	6 (1-25)	-
Seen a Rheumatologist for pain	110	12.8
Seen an orthopaedic surgeon for pain	102	11.9
Attended Emergency Department for pain	76	8.9
Use analgesics most days for pain	194	22.6
Had an operation because of pain	40	4.7
Of those in pain:		
Currently in pain	341	39.7 (63.3 of those with pain)
Pain lasting >3 months (chronic pain)	431	50.2 (80.0 of those with pain)
Time off work due to pain	44	5.1 (16.2 of those with pain)
Seen a doctor because of the pain	236	27.5 (43.8 of those with pain)
Median duration of pain (years)(range)	3 (1-51)	-
Median pain score (range)	5 (1-10)	-
Mean pain score (SD)	5.2 (2.2)	-
Median disability score (range)	4 (0-10)	-
Mean disability score (SD)	4.4 (2.9)	-

Table 3
Associations between demographic and clinical factors and current pain from univariate and multivariable logistic regression analyses

Characteristic		Unadjusted OR (95% CI)*	p-value	Adjusted OR (95% CI)	p-value
Age	/5 years older	1.12 (1.05, 1.20)	0.0008	1.10 (1.02, 1.19)	0.02
Time since HIV diagnosis	/5 years	1.26 (1.13, 1.41)	0.0001	1.17 (1.03, 1.32)	0.02
Viral load (copies/ml)	>40	1			
	40	1.43 (1.06-1.93)	0.02	-	
HIV stage	Asymptomatic (CDC stage A)	1			
	Symptomatic (CDC stages B/C)	1.57 (1.18, 2.07)	0.002	-	
cART naive	No	1			
	Yes	0.59 (0.41, 0.86)	0.006	-	
Currently on cART	No	1			
	Yes	1.48 (1.06, 2.07)	0.02	-	
On NRTI	No	1			
	Yes	1.35 (1.00, 1.83)	0.05	-	
On PI	No	1		1	
	Yes	1.59 (1.19, 2.13)	0.002	1.39 (1.01, 1.91)	0.04

* OR: odds ratio; CI: confidence interval; CDC: Centers for Disease Control; cART: combination antiretroviral therapy; NRTI: nucleoside reverse transcriptase inhibitor; PI: protease inhibitor.