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Authors

Jotwani, Vasantha
Scherzer, Rebecca
Estrella, Michelle M
et al.

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HIV Infection, Tenofovir Disoproxil Fumarate, and Urine α1-Microglobulin: A Cross-sectional Analysis in the Multicenter AIDS Cohort Study

Vasantha Jotwani, MD[1,2], **Rebecca Scherzer, PhD**[1,2], **Michelle M. Estrella, MD, MHS**[3], **Lisa P. Jacobson, ScD**[4], **Mallory D. Witt, MD**[5], **Frank J. Palella Jr., MD**[6], **Bernard Macatangay, MD**[7], **Michael Bennett, PhD**[8], **Chirag R Parikh, MD, PhD**[9,10], **Joachim H Ix, MD, MAS**[11,12], and **Michael G. Shlipak, MD, MPH**[1,13]

[1]Department of Medicine, San Francisco VA Medical Center, San Francisco, USA

[2]Department of Epidemiology and Biostatistics, University of California, San Francisco, USA

[3]Department of Medicine, Johns Hopkins School of Medicine, Baltimore, USA

[4]Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, USA

[5]Division of HIV Medicine, Department of Medicine, Harbor-UCLA Medical Center and the Los Angeles Biomedical Research Institute at Harbor-UCLA, Torrance, USA

[6]Division of Infectious Diseases, Department of Medicine, Northwestern University, Chicago, USA

[7]Division of Infectious Diseases, Department of Medicine, University of Pittsburgh, Pittsburgh, USA

[8]Division of Nephrology and Hypertension, Cincinnati Children's Hospital Medical Center, Cincinnati, USA

[9]Section of Nephrology, Department of Medicine, Yale University, New Haven, USA

[10]Program of Applied Translational Research, Yale University, New Haven, USA

[11]Division of Nephrology-Hypertension, Department of Medicine, University of California, San Diego, USA

[12]Nephrology Section, Veterans Affairs San Diego Healthcare System, USA

[13]Kidney Health Research Collaborative, San Francisco VA Medical Center and University of California, San Francisco, USA

Corresponding author Vasantha.Jotwani@ucsf.edu.

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Contributions: research idea and study design: VJ, MGS; data acquisition: LPJ, MDW, FJP, BM, MB; data analysis/interpretation: VJ, RS, MME, MGS; statistical analysis: RS; supervision or mentorship: CRP, JHI, MGS. Each author contributed important intellectual content during manuscript drafting or revision and accepts accountability for the overall work by ensuring that questions pertaining to the accuracy or integrity of any portion of the work are appropriately investigated and resolved. VJ takes responsibility that this study has been reported honestly, accurately, and transparently; that no important aspects of the study have been omitted, and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

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Abstract

Background—Tenofovir disoproxil fumarate (TDF) can cause proximal tubular damage and chronic kidney disease in HIV-infected individuals. Urine α1-microglobulin (α1m), a low molecular weight protein indicative of proximal tubular dysfunction, may enable earlier detection of TDF-associated tubular toxicity.

Study Design—Cross-sectional

Setting & Participants—883 HIV-infected and 350 uninfected men enrolled in the Multicenter AIDS Cohort Study

Predictors—HIV infection and TDF exposure

Outcome—Urine α1m levels

Results—Urine α1m was detectable in 737 (83%) HIV-infected and 202 (58%) uninfected men, respectively ($p<0.001$). Among the HIV-infected participants, 573 (65%) were current TDF users and 112 (13%) were past TDF users. After multivariable adjustment including demographics, traditional kidney disease risk factors and eGFR, HIV infection was associated with 136% higher urine α1m levels (95%CI: 104,173) and 1.5-fold prevalence of detectable α1m (95%CI: 1.3,1.6). When participants were stratified by TDF exposure, HIV infection was associated with higher adjusted α1m levels by 164% among current users (95%CI: 127,208), 124% among past users (95%CI: 78,183), and 76% among never users (95%CI: 45,115). Among HIV-infected participants, each year of cumulative TDF exposure was associated with 7.6% higher α1m levels (95%CI: 5.4,9.9) in fully adjusted models, a 4-fold effect size relative to advancing age (1.8% per year; 95%CI: 0.9, 2.7). Each year since TDF discontinuation was associated with 4.9% lower α1m levels (95%CI: −9.4,−0.2) among past users.

Limitations—Results may not be generalizable to women.

Conclusions—Compared with uninfected men, HIV-infected men had higher urine α1m levels. Among HIV-infected men, cumulative TDF exposure was associated with incrementally higher α1m levels, whereas time since TDF discontinuation was associated with progressively lower α1m levels. Urine α1m appears to be a promising biomarker for the detection and monitoring of TDF-associated tubular toxicity.

Tenofovir disoproxil fumarate (TDF) is a nucleotide analog reverse transcriptase inhibitor that is prescribed worldwide for the treatment of HIV infection. Due to its efficacy, tolerability and availability as a once daily medication, TDF is recommended by the United States (US) Department of Health and Human Services (http://aidsinfo.nih/gov) and the HIV Medicine Association of the Infectious Diseases Society of America[1] as a component of several first-line combination antiretroviral regimens. In addition to its global use for the treatment of HIV, TDF was recently approved by the FDA for pre-exposure prophylaxis of individuals at high risk for HIV acquisition,[2–4] and it remains an effective therapy for hepatitis B virus infection.[5,6] Although pre-marketing studies suggested a favorable safety profile,[7] TDF use has been associated with the development of acute kidney injury, proteinuria, chronic kidney disease (CKD), and the Fanconi syndrome of proximal tubular dysfunction.[8–12] Kidney biopsy series of patients with TDF-associated kidney injury have demonstrated flattening of proximal tubular epithelial cells and widespread mitochondrial

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abnormalities.[13,14] Notably, the active drug, tenofovir, is eliminated in urine through active secretion by proximal tubular epithelial cells,[15] with tenofovir influx and efflux mediated by organic anion transporters and multidrug resistance proteins, respectively.[16,17]

The serum creatinine concentration is an insensitive marker of early kidney damage, as more than 50% of glomerular filtration function may be lost before serum creatinine is above the normal laboratory range.[18,19] Because the proximal tubule is the primary site of TDF-associated nephrotoxicity, biomarkers that are sensitive markers of proximal tubular dysfunction may enable the detection of tubular toxicity at earlier stages. α1-microglobulin (α1m) is a 26-kDa lipocalin that is freely filtered at the glomerulus but reabsorbed by proximal tubular epithelial cells under healthy conditions;[20] elevated urine α1m levels therefore indicate proximal tubular dysfunction.[21] In the Women's Interagency HIV Study, we recently found that HIV-infected women had higher urine α1m levels compared with HIV-uninfected women.[22] Furthermore, urine α1m levels were associated with subsequent kidney function decline and mortality independent of traditional and HIV-related risk factors, eGFR, and albuminuria, suggesting that proximal tubular dysfunction may lead to irreversible kidney damage. However, because our prior study utilized urine specimens that were collected prior to the widespread use of TDF, we were unable to evaluate the associations of TDF exposure with urine α1m levels.

In this contemporary study of men enrolled in the Multicenter AIDS Cohort Study, we evaluated the associations of HIV infection and TDF exposure with proximal tubular dysfunction, measured by urine α1m levels. Then, we examined the associations of other antiretroviral medications and clinical factors with urine α1m levels.

Methods

Study Population and Design

The Multicenter AIDS Cohort Study (MACS) is an ongoing, prospective cohort study designed to describe the epidemiology and natural history of HIV infection among men who have sex with men. A total of 6,972 HIV-infected and uninfected men were enrolled between 1984 and 2003 from four sites in the US: Baltimore, Chicago, Los Angeles and Pittsburgh.[23] Participants attend semiannual visits that include standardized questionnaires, a physical examination, and collection of biological specimens.

The MACS Kidney Study was designed as a nested cohort study to investigate the onset and progression of kidney disease among HIV-infected men, using stored urine and serum samples. Urine specimens were refrigerated immediately after collection, and centrifuged at 5000×g to remove cellular debris. The supernatant was aliquoted into 1cc vials and then stored at −80°C until biomarker measurement was undertaken. This cross-sectional study of kidney damage included all 883 HIV-infected men with urine samples collected between October 1, 2009 and September 30, 2011, and a random sample of 350 uninfected men with available urine specimens from this time period.

The institutional review boards of participating institutions approved the study protocol (IRB #10-00827), and informed consent was obtained from all study participants. This study was

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also approved by the University of California, San Francisco, and San Francisco VA Medical Center committees on human research.

Antiretroviral Medication Exposure

Antiretroviral (ARV) medication exposure was ascertained using self-reported data from each MACS participant collected at semi-annual visits. ARVs with less than 5% prevalence of use at the time of biomarker measurement were not included as candidate covariates in our analyses. Cumulative exposure was defined as the sum of current and historical exposure durations for each participant. Current duration was defined as duration on therapy at the time of biomarker measurement.

Urine Biomarker Measurements

Urine α1m was measured at the Cincinnati Children's Hospital Medical Center Biomarker Laboratory using a commercially available assay (Siemens BNII nephelometer, Munich, Germany). The detectable limit of the α1m assay was 0.53 mg/dl. Urine specimens were in continuous storage at −80°C until biomarker measurement without prior freeze-thaw. Laboratory personnel performing the biomarker assays were blinded to participants' clinical information.

Covariates

The following demographic and clinical characteristics were tested as candidate covariates in multivariable models: age, race/ethnicity, diabetes mellitus (fasting glucose ≥126mg/dL; hemoglobin A1c ≥6.5%; or self-reported history of diabetes and diabetes medication use), systolic and diastolic blood pressure, hypertension (systolic blood pressure >140 mmHg or diastolic blood pressure >90 mmHg; or self-reported history of hypertension and antihypertensive medication use), cigarette smoking status (current, past, or never), LDL and HDL cholesterol, triglycerides, body mass index (BMI), waist circumference, and hepatitis C virus (HCV) infection (confirmed by detectable HCV RNA following a positive HCV antibody result). Candidate HIV-related characteristics included: current CD4 lymphocyte count, nadir CD4 lymphocyte count, history of clinical AIDS diagnosis,[24] current and peak plasma HIV RNA level, and time-averaged historical HIV RNA level. Urine albumin, total protein, and creatinine were measured using a Siemens Dimension Xpand plus HM clinical analyzer (Siemens, Munich, Germany). Glomerular filtration rate was estimated using the CKD-EPI equation for creatinine (eGFR).[25] CKD was defined by the presence of an eGFR<60 ml/min/1.73m^2. Multiple imputation with the Markov chain Monte Carlo method was used to impute missing covariates, with 5 imputations to yield ~95% relative efficiency.[26] The percentage of missing observations for each covariate ranged from 0% to 26% (Table S1).

Statistical Analysis

Approximately one-fourth of participants had undetectable urine α1m, and the distribution among those with detectable α1m was right-skewed (Figure 1). Due to the left censored nature of the data, we analyzed α1m by two approaches: 1) as a log-transformed continuous

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variable using models that accommodate left censored data, and 2) as a dichotomous variable (detectable vs. undetectable).

We first stratified men into four categories based on HIV status and TDF use (never, past, or current) and compared demographic and clinical characteristics using the chi-square and Kruskal-Wallis tests for categorical and continuous variables, respectively. We then used multivariable generalized gamma regression models to evaluate the associations of HIV infection with urine α1m, and to identify clinical factors associated with α1m. Similar to the Tobit regression method, generalized gamma regression models accommodate left censored data by including undetectable values, and also allow log-transformation of urine α1m to normalize its right-skewed distribution. Results were back-transformed to produce estimated percentage differences in urine α1m attributable to each predictor. As a secondary approach, we used Poisson relative risk regression with a robust variance estimator[27] to assess the association of HIV infection with detectable urine α1m. Models were adjusted sequentially for demographics, traditional kidney risk factors, and eGFR. We then adjusted for albuminuria, a clinical marker of glomerular injury, and urine creatinine, to account for urine tonicity. To assess for effect modification by race, we also performed race-stratified analyses and evaluated interactions of HIV infection and race for the α1m outcomes.

Next, we constructed smoothing splines using generalized additive models in order to examine the relationship of TDF exposure with urine α1m levels. We then used multivariable generalized gamma regression models to examine associations of TDF with urine α1m while controlling for traditional kidney disease risk factors and HIV-related factors, using stepwise backward selection (α=0.05) to remove candidate variables that were not associated with the outcome. We used Bayesian model averaging as an alternative model building approach.[28] Models constructed using the two approaches were very similar. TDF exposure was analyzed continuously (per year of total duration and per year of current duration) and categorically (current, past, or never exposure). We additionally evaluated duration off TDF as a continuous predictor of α1m. We then stratified participants by race to determine whether TDF exposure had similar associations with urine α1m in African Americans and Caucasians.

To evaluate associations of other ARVs with urine α1m levels, we first examined ARVs individually, in models controlling for traditional and HIV-related risk factors. Because individual ARVs are used in combination and therefore inter-correlated, we used the least absolute shrinkage and selection operator (LASSO) method to determine which of multiple ARVs were associated with α1m.[29]

As a sensitivity analysis, we also evaluated the associations of HIV and TDF exposure with urine protein/creatinine ratio, to compare the performance of urine α1m with this clinically available measure. We implemented Bayesian model averaging using the BMA package for R and LASSO using the glmnet package for R (Foundation for Statistical Computing, Vienna). All other analyses were conducted using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA).

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Results

Study population by HIV status and TDF use

Among the 883 HIV-infected and 350 uninfected men included in this study, the median age was 52 years, and approximately one-third of participants were African American (Table 1). Among the HIV-infected participants, 65% (n=573) were receiving TDF at the time of urine collection, while 13% (n=112) were past TDF users. Median TDF exposure duration was 4.4 years among current users (IQR: 2.8, 6.4). Among past TDF users, the median TDF exposure duration was 2.4 years (IQR: 1.0, 4.6) and the median time since TDF discontinuation was 2.3 years (IQR: 1.2, 4.6). Diabetes mellitus was more common among past TDF users (27%) compared with the other study participants (14%), while hypertension and HCV infection were present in approximately 50% and 10% of participants, respectively. Compared with uninfected participants, current TDF users and never TDF users, past TDF users had the lowest eGFR and highest prevalence of albuminuria. HIV-infected men who never received TDF had the highest CD4 lymphocyte counts and lowest historical prevalence of AIDS, while current TDF users had the highest prevalence of HIV viral suppression. Only 5% (n=46) of HIV-infected participants had never received ARV therapy.

Association of HIV infection with urine α1m

Compared with uninfected men, HIV-infected men had higher median α1m levels and prevalence of detectable α1m (Table 2). After multivariate adjustment including demographics, traditional kidney disease risk factors and eGFR, HIV infection was associated with 136% higher urine α1m levels, and a 45% higher prevalence of detectable α1m. When we stratified HIV-infected participants by TDF exposure history, HIV infection remained associated with higher adjusted α1m levels by 76% among never users, 124% among past users, and 164% among current users. Additional adjustment for ACR and urine creatinine only mildly attenuated the effect sizes.

Median urine protein/creatinine ratios were 100 mg/g (IQR: 70, 162) among HIV-infected participants and 68 mg/g (IQR: 51, 92) among uninfected participants (Table S2). In multivariable-adjusted analyses, HIV infection was associated with higher urine protein/creatinine ratio by 54% overall (95% CI: 43, 65), 35% among never users (95% CI: 21, 49), 63% among past users (44, 85), and 59% among current users (95% CI: 47, 72). Urine protein/creatinine ratio was positively correlated with urine α1m in both HIV-infected ($r=0.58$, $p<0.001$) and uninfected ($r=0.25$, $p<0.001$) participants.

Among both HIV-infected and uninfected participants, African Americans had higher median urine α1m levels and higher prevalence of detectable α1m, as compared with Caucasians (Table S3). Median α1m levels were 2-fold higher in HIV-infected men relative to uninfected men among African Americans (1.9 vs. 0.9 mg/dL) and nearly 3-fold higher among Caucasians (1.4 vs. 0.5). In multivariable models adjusting for traditional kidney risk factors, eGFR, ACR and urine creatinine, HIV infection was associated with 48% higher α1m levels among African Americans, and with 120% higher α1m levels among Caucasians (p-value for HIV/race interaction = 0.003).

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Association of TDF exposure with urine α1m

Current and past users of TDF had higher urine α1m levels relative to HIV-infected men who never used TDF and to uninfected men (Figure 1). Duration of TDF exposure was linearly associated with urine α1m ($p<0.001$, Figure 2) and proteinuria ($p<0.001$, Figure S1), although the slope appeared steeper for α1m as compared with proteinuria (8.3% per year of exposure vs. 3.3% in unadjusted analysis). In analyses that adjusted for demographics, traditional kidney risk factors and HIV-related factors (Table 3), each year of cumulative TDF exposure was associated with approximately 8% higher urine α1m levels ($p<0.001$), an approximately 4-fold effect size relative to advancing age (2% per year). There was minimal attenuation of effect size after additional adjustment for eGFR, ACR and urine creatinine. Among current TDF users, each year of TDF exposure was associated with approximately 7% higher urine α1m ($p<0.001$) in fully adjusted models. When TDF exposure was modeled as a categorical variable, current and past TDF users had higher adjusted urine α1m levels by 56% ($p<0.001$) and 29% ($p=0.02$), respectively, compared with HIV-infected men who never received TDF. Among participants previously exposed to TDF, each year since TDF discontinuation was associated with 5% lower urine α1m levels ($p=0.04$).

In multivariable adjusted analyses, each year of cumulative TDF exposure was associated with 3.2% higher urine protein/creatinine ratio (95% CI: 1.7, 4.7). Compared with never users, current and past TDF users had levels of urine protein/creatinine ratio that were 22% (95% CI: 11, 35) and 26% (95% CI: 9, 45) higher, respectively (Table S4).

In race-stratified analyses, each year of cumulative TDF exposure was associated with higher urine α1m by 6.5% (95% CI: 1.6, 11.6; $p=0.009$) among African Americans and 9.3% (95% CI: 5.8, 12.9; $p<0.001$) among Caucasians (p-value for TDF*race interaction = 0.36).

Associations of cumulative ARV exposure and other clinical factors with urine α1m

Next, we evaluated the associations of ARVs other than TDF and clinical factors with urine α1m levels. Compared with the other ARVs, TDF had the highest prevalence of use and largest effect size on α1m levels (Table S5). In multivariable models adjusting for demographics and clinical characteristics, each year of exposure to emtricitabine was associated with 7.7% higher urine α1m ($p<0.001$), while ritonavir and lopinavir were each associated with 2.8% ($p=0.03$) and 6.8% ($p<0.001$) higher urine α1m per year of exposure. Of note, among emtricitabine, ritonavir, and lopinavir users, the proportions of individuals who simultaneously received TDF were 98%, 72%, and 67%, respectively. Zidovudine was the only ARV showing a statistically significant association with lower urine levels of α1m (−2.0% per year exposure, $p=0.02$).

To account for simultaneous use of multiple ARVs, we used the LASSO method to select and adjust for the subset of ARVs most strongly associated with urine α1m levels (Table 4). Using this approach, only TDF and lopinavir use remained significantly associated with higher urine α1m levels, by 7.5% and 4.9% per year of exposure, respectively. Compared to individuals who never received tenofovir or lopinavir, adjusted urine α1m levels were 97%

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higher in participants who received tenofovir and lopinavir simultaneously (95% CI: 58, 145), 42% higher in participants who received tenofovir without lopinavir (95% CI: 17, 72), and 53% higher in participants who received lopinavir without tenofovir (95% CI: −6, 150).

Other clinical factors associated with higher urine α1m included older age, African American race, lower body mass index, diabetes mellitus, and lower CD4 lymphocyte count.

Discussion

With an expanding population of TDF users worldwide, nephrotoxicity has become a common clinical problem in persons with HIV. Early detection of tubular toxicity could enable clinicians to quantify risks of therapy and ensure patient safety. In this large cross-sectional study, we found that HIV-infected men had higher urine levels of α1m compared with uninfected men. Among the HIV-infected participants, current and past TDF users had substantially higher urine α1m levels compared with men who never received TDF, and each year of TDF exposure was incrementally associated with higher urine α1m levels. Notably, among past TDF users, each year since TDF discontinuation was associated with progressively lower α1m levels. In conjunction with our prior work demonstrating the prognostic significance of urine α1m for CKD progression and mortality, these findings highlight α1m as a promising biomarker of TDF-associated tubular toxicity.

Consistent with prior literature, we observed positive associations between tenofovir exposure and proteinuria, but the relative effect sizes were substantially stronger for urine α1m as compared with proteinuria. For example, relative to never users of tenofovir, current users had 50% higher urine α1m levels, compared with 22% higher protein/creatinine ratios. Notably, the majority of tenofovir users in this cohort had urine protein/creatinine ratios in the normal range (below 200 mg/g), despite their having substantially higher urine α1m levels compared with nonusers. Our observations suggest that subclinical tubular dysfunction is common among tenofovir users, and that proteinuria is not an optimally sensitive marker of TDF-associated tubular damage. Future studies should rigorously compare urine α1m with other markers of tubular dysfunction (such as glucosuria, phosphaturia, uricosuria, and metabolic acidosis) to determine whether urine α1m is the earliest indicator of tubular dysfunction.

α1-microglobulin was first isolated in urine samples of patients with tubular damage from chronic cadmium poisoning.[30] Subsequent studies characterized α1m as a size- and charge-heterogeneous lipocalin that circulates in plasma in free and protein-bound forms.[20] The 26-kDa unbound α1m is freely filtered at the glomerulus and reabsorbed by proximal tubular epithelial cells, via the endocytic receptor megalin.[31,32] In the presence of proximal tubular damage, the filtered loads of α1m and other low molecular weight proteins, including β2-microglobulin and retinol binding protein, are incompletely reabsorbed, resulting in higher levels in urine.[21,33] Wu *et al.* observed higher urine α1m levels among individuals with drug-induced interstitial nephritis, as compared with age- and sex-matched controls.[34] Among participants with drug-induced interstitial nephritis, urine α1m levels correlated positively with the severity of inflammatory infiltration, interstitial edema, and tubular atrophy on kidney biopsy samples. Furthermore, in a study of children with acute tubular

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necrosis following cardiopulmonary bypass surgery, α1m was one of the earliest biomarkers detectable by proteomic analysis of urine samples, with levels rising by 3-fold within 2 hours of surgery, compared with controls who did not develop acute kidney injury ($p<0.01$).[35]

TDF-associated kidney injury localizes to the proximal tubule, due to active secretion of tenofovir by proximal tubular epithelial cells.[13,15] Hence, markers of proximal tubular dysfunction, may be particularly useful in detecting toxicity from TDF. Nishijima *et al.* reported a 10% prevalence of kidney tubular dysfunction (defined as three or more abnormalities in: urine α1m, β2-microglobulin, N-acetyl-beta-D-glucosaminidase, fractional excretion of phosphorus, or fractional excretion of uric acid) among 190 HIV-infected individuals receiving tenofovir.[36] Labarga *et al.* previously found that tenofovir users had a higher prevalence of tubular dysfunction (defined by at least two of the following: glucosuria, hyperaminoaciduria, hyperphosphaturia, or β2-microglobulinuria), compared with antiretroviral-naïve individuals (22% vs 12%, $p<0.001$).[37] Hall *et al.* also reported higher urine levels of retinol-binding protein and N-acetyl-beta-D-glucosaminidase among HIV-infected tenofovir users, compared with non-users or antiretroviral-naïve patients.[38] In contrast to the markers of tubular dysfunction examined by these studies, urine α1m levels have been associated with elevated risks for subsequent kidney function decline and mortality. In a large cohort of HIV-infected women who were not exposed to tenofovir at the time of biomarker measurement, we previously reported a 2.1-fold risk of incident CKD and 1.6-fold mortality risk over 8 years, for HIV-infected women in the highest vs lowest tertiles of urine α1m.[22] The current study builds upon prior literature by demonstrating a dose response between tenofovir exposure and urine α1m levels, and it supports a potential mechanistic link between TDF use, tubular dysfunction, and the subsequent development of CKD.

Our finding that lopinavir exposure was associated with higher urine α1m levels is consistent with prior literature reporting synergistic nephrotoxicity when TDF is co-administered with protease inhibitors. In a trial of 741 HIV-infected women randomized to receive TDF/emtricitabine with either lopinavir/ritonavir or nevirapine, lopinavir/ritonavir users had an adjusted odds ratio of 3.1 (95%CI: 1.2, 8.1) for renal events (defined as: creatinine rise to ≥2 mg/dL or creatinine clearance <50 ml/min, causing interruption or discontinuation of TDF) compared with nevirapine users, over 2 years of follow-up.[39] Protease inhibitors may interfere with tenofovir efflux from proximal tubular cells by inhibiting the multidrug resistance proteins responsible for tenofovir transport into the tubular lumen.[17,40,41] Other studies have suggested that enhanced intestinal absorption of tenofovir accounts for this drug interaction.[42–44] We also observed elevated urine α1m levels among individuals who received lopinavir without concomitant TDF, although the association did not reach statistical significance. Further studies are needed to verify this finding and to elucidate potential mechanisms by which lopinavir might exert direct kidney toxicity.

We found that African-Americans have higher urine α1m levels than Caucasians, an observation that is supported by our previous findings in the Women's Interagency HIV Study.[22] Although the relative associations of HIV infection with urine α1m levels appeared

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stronger in Caucasians than in African Americans, these results may have been driven by elevated urine α1m levels among the uninfected African American participants. Large cohort studies have demonstrated that HIV-infected African Americans have a higher incidence of ESRD relative to Caucasians, and experience faster progression from CKD to ESRD.[45–47] Although specific polymorphisms on the *APOL1* gene appear to account for a portion of this racial disparity,[48–50] we recently reported that the high-risk *APOL1* genotype was not associated with urine α1m levels in a cross-sectional evaluation of HIV-infected African-American women with well-preserved kidney function.[51] In the present study, we performed race-stratified analyses of TDF with urine α1m levels to examine potential differences in susceptibility to TDF-associated proximal tubular dysfunction, and we observed no statistically significant interactions by race. Further studies are needed to validate these findings and to evaluate alternate mechanisms leading to tubular dysfunction among HIV-infected African Americans.

Our study has several implications for clinical care. The measurement of urine α1m, in combination with other biomarkers of tubular damage, could constitute a novel method for the detection and monitoring of tubular toxicity while on therapy with TDF. Future studies should evaluate longitudinal changes in α1m levels among TDF users, and whether these changes are associated with the development of CKD. Second, little is known regarding the potential reversibility of tubular dysfunction following cessation of TDF. Although we found that time since TDF exposure was associated with lower α1m levels, the persistence of high α1m levels among past TDF users suggests incomplete recovery. Finally, recognition of nephrotoxicity at its earliest stages may be particularly important for the growing population of HIV-uninfected individuals receiving TDF as pre-exposure prophylaxis.

There are important limitations to this study. First, because this was a study of men, the results may not be generalizable to women. However, our earlier work in the WIHS cohort revealed that urine α1m levels in HIV-infected women were strongly predictive of incident CKD and all-cause mortality.[22] Additionally, there is no known pathophysiologic basis for a gender-based interaction between TDF exposure and kidney injury. Second, we did not have access to the clinical reasons for TDF discontinuation. However, the presence of lower eGFR and higher prevalence of CKD in past TDF users, as compared with current or never users, suggests that nephrotoxicity may have led to the discontinuation of TDF. Finally, although we adjusted for multiple potential confounders, we cannot exclude the possibility of residual confounding.

In conclusion, in this large cohort of men with predominantly normal kidney function, HIV infection and TDF exposure were associated with higher urine levels of α1m, a marker of proximal tubular dysfunction. If these findings are validated in future studies, urine α1m may be a useful indicator of TDF-associated tubular dysfunction.

Supplementary Material

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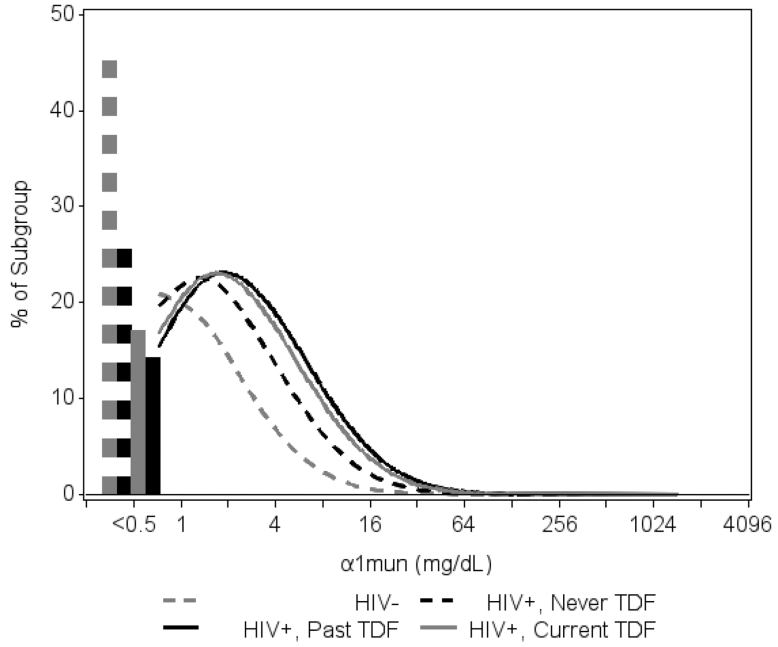

Figure 1. Distribution of urine α1m levels in MACS participants (N=883) by HIV status and TDF use

Empirical distributions of urine α1m levels with model-based density from Tobit regression. Test for difference in location: $p<0.001$. Test for homogeneity of variance: $p=0.013$. Proportions with undetectable values are represented as vertical bars.

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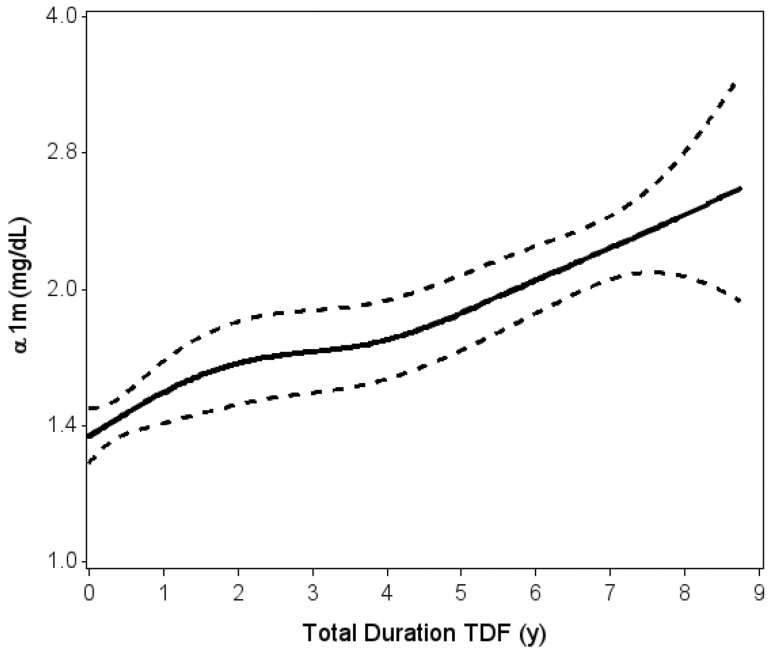

Figure 2. Association of cumulative TDF exposure with urine α1m levels in HIV-infected MACS participants (N=883)

Spline plot displaying unadjusted association of TDF exposure duration with urine α1m levels, calculated from generalized additive models. Solid line denotes predicted urine α1m level; dotted lines represent 95% confidence bounds. Highest 2.5% of values were truncated. $p<0.001$ for association of TDF duration with urine α1m; $p=0.41$ for tests of non-linearity.

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Table 1

Characteristics of MACS participants, stratified by HIV status and tenofovir use

	HIV-negative	HIV-positive		
		Never TDF	Past TDF	Current TDF
N	350	187	112	573
Age (y)	54 (49–62)	53 (49–58)	53 (48–59)	51 (45–57)
Race				
Black	102 (29%)	65 (35%)	36 (32%)	169 (29%)
White	229 (65%)	116 (62%)	72 (64%)	344 (60%)
Other	19 (5%)	6 (3%)	4 (4%)	60 (10%)
Diabetes mellitus	44 (15%)	20 (14%)	25 (27%)	65 (14%)
Systolic BP (mm Hg)	128 (116–136)	130 (120–139)	126 (114–137)	125 (115–134)
Diastolic BP (mm Hg)	78 (71–84)	81 (75–86)	75 (68–83)	77 (71–84)
Hypertension	155 (47%)	95 (53%)	57 (56%)	222 (43%)
Antihypertensive use	117 (34%)	80 (43%)	49 (44%)	175 (31%)
Hepatitis C	33 (9%)	17 (9%)	13 (12%)	57 (10%)
Cigarette smoking				
Current	82 (24%)	52 (28%)	33 (31%)	174 (31%)
Past	174 (51%)	86 (47%)	48 (45%)	244 (43%)
Never	85 (25%)	45 (25%)	26 (24%)	145 (26%)
LDL (mg/dL)	115 (92–137)	105 (88–132)	104 (79–130)	108 (88–132)
HDL (mg/dL)	50 (41–60)	46 (38–56)	48 (39–54)	45 (38–54)
TG (mg/dL)	108 (76–157)	133 (94–199)	166 (113–257)	134 (94–202)
Body Mass Index (kg/m^2)	27 (24–32)	26 (23–29)	26 (23–33)	26 (24–30)
Waist Circumference (cm)	97 (89–107)	92 (84–101)	93 (87–103)	94 (87–102)
eGFR ($ml/min/1.73m^2$)	89 (78–100)	92 (81–104)	77 (58–97)	92 (77–104)
eGFR<60ml/min/1.73m^2	13 (4%)	14 (8%)	31 (28%)	31 (5%)
Albuminuria *	29 (8%)	34 (18%)	25 (23%)	88 (16%)
Current CD4 ($cells/mm^3$)		607 (450–808)	515 (347–641)	572 (405–741)
Nadir CD4 ($cells/mm^3$)		317 (207–432)	260 (149–369)	287 (177–415)
History of AIDS		20 (11%)	29 (26%)	74 (13%)
HIV Viral Load (copies/mL)				
<80		114 (62%)	85 (77%)	496 (87%)
80–2,000		24 (13%)	9 (8%)	44 (8%)
2,000–9,999		17 (9%)	10 (9%)	3 (1%)
>10,000		30 (16%)	6 (5%)	27 (5%)

Data are presented as median (interquartile range) or numbers (percent). Interquartile ranges and percentages were calculated among participants with non-missing data.

Abbreviations: eGFR, estimated glomerular filtration rate; TDF, tenofovir disoproxil fumarate.

* defined as a positive urine dipstick result (≥1+) or urine albumin-creatinine ratio >30 mg/g

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Table 2

Association of HIV infection with urine α1m levels, overall and stratified by tenofovir use

	HIV-negative	HIV-positive			
		Overall vs HIV-negative	Never TDF vs HIV-negative	Past TDF vs HIV-negative	Current TDF vs HIV-negative
N	346	878	184	111	572
Urine α1m (mg/dL), Median (IQR) [1]	0.61 (<0.53–1.25)	1.51 (0.72–3.19)	1.02 (0.55–2.35)	2.13 (0.71–4.27)	1.62 (0.79–3.32)
% Difference [2]**, HIV+ vs HIV− (95% CI)**					
Models					
Demographic-adjusted[3]	Ref	146 (111, 186)	76 (44, 116)	174 (115, 247)	174 (115, 247)
Multivariable-adjusted[4]	Ref	136 (104, 173)	76 (45, 115)	124 (78, 183)	164 (127, 208)
Multivariable-adjusted+ACR+U_{Cr}[5]	Ref	94 (71, 119)	39 (18, 64)	80.9 (48, 121)	119 (93, 149)
Detectable urine α1m, *n*(%)	202 (58%)	737 (83%)	144 (77%)	92 (82%)	490 (86%)
Prevalence Ratio, [6] **HIV+ vs HIV− (95% CI)**					
Models					
Demographic-adjusted[3]	Ref	1.46 (1.33, 1.60)	1.33 (1.18, 1.50)	1.42 (1.26, 1.60)	1.51 (1.37, 1.66)
Multivariable-adjusted[4]	Ref	1.45 (1.32, 1.59)	1.34 (1.19, 1.51)	1.35 (1.19, 1.54)	1.50 (1.37, 1.65)
Multivariable-adjusted+ACR+U_{Cr}[5]	Ref	1.33 (1.23, 1.45)	1.22 (1.09, 1.35)	1.24 (1.10, 1.39)	1.38 (1.27, 1.50)

Abbreviations: α1m, α1-microglobulin; ACR, albumin-creatinine ratio; CI, confidence interval; eGFR, estimated glomerular filtration rate; IQR, interquartile range; TDF, tenofovir disoproxil fumarate; U_{Cr}, urine creatinine.

[1] Median (IQR) estimates include those with undetectable α1m values. The detectable limit for the α1m assay was 0.53 mg/dL.

[2] Estimated percentage difference in α1m attributable to HIV infection, with HIV-negative participants as reference group.

[3] Adjusted for age and race

[4] Adjusted for age, race, diabetes mellitus, hypertension, antihypertensive use, history of cardiovascular disease, illicit drug use, hepatitis C infection, and eGFR

[5] Adjusted for albumin-creatinine ratio and urine creatinine in addition to factors listed above

[6] Prevalence ratio for detectable α1m in HIV-positive vs HIV-negative participants, with HIV-negative participants as reference group.

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Table 3

Association of TDF exposure with levels of urine α1m among HIV-infected MACS participants (N = 883)

	Model 1: Adjusted for traditional kidney and HIV-related risk factors[1]		Model 2: Adjusted for Model 1 + eGFR, Urine Creatinine, and ACR	
TDF Exposure[2]	**% Estimate (95% CI)**	**P Value**	**% Estimate (95% CI)**	**P Value**
Cumulative TDF exposure (y)	8.4 (5.6, 11.2)	<0.001	7.6 (5.4, 9.9)	<0.001
Current TDF duration (y)	6.8 (4.1, 9.5)	<0.001	6.9 (4.7, 9.1)	<0.001
Current vs never TDF use	50.2 (25.3, 79.9)	<0.001	55.6 (33.9, 80.8)	<0.001
Past vs never TDF use	42.9 (10.8, 84.2)	0.006	28.5 (3.9, 58.9)	0.02
Duration off TDF[3] (y)	−2.5 (−8.2, 3.6)	0.41	−4.9 (−9.4, −0.2)	0.04

Abbreviations: α1m, α1-microglobulin; ACR, albumin-creatinine ratio; CI, confidence interval; eGFR, estimated glomerular filtration rate; TDF, tenofovir disoproxil fumarate; y, years.

[1] Estimated percentage difference in biomarker attributable to TDF exposure. Models adjust for age, race, diabetes mellitus, CD4 lymphocyte count, body mass index (BMI) and TDF exposure

[2] TDF exposure variables enter the model individually, not simultaneously

[3] Analyses restricted to current and past TDF users (n=685)

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Table 4

Demographic and clinical factors associated with urine α1m in HIV-infected MACS participants (N=883)

	Univariate		Multivariate[1]	
Parameter	**% Estimate[2] (95% CI)**	**P Value**	**% Estimate (95% CI)**	**P Value**
Age (per year)	1.9 (0.9, 2.8)	<0.001	1.8 (0.9, 2.7)	<0.001
African-American vs Caucasian	38.0 (17.1, 62.5)	<0.001	39.3 (18.4, 63.9)	<0.001
Other/Latino vs Caucasian	3.2 (−22.4, 37.1)	0.83	3.3 (−2.2, 37.2)	0.82
Body mass index (kg/m^2)	−3.1 (−4.9, −1.2)	0.001	−2.9 (−4.7, −1.1)	0.002
Diabetes mellitus	26.0 (1.7, 56.0)	0.03	24.7 (0.9, 54.1)	0.04
CD4 lymphocyte count (per doubling)	−17.6 (−24.6, −9.9)	<0.001	−18.3 (−25.2, −10.8)	<0.001
Tenofovir (per year exposure)	5.2 (1.5, 8.9)	0.005	7.5 (4.6, 10.3)	<0.001
Zidovudine (per year exposure)	−1.5 (−3.1, 0.2)	0.09		
Abacavir (per year exposure)	1.6 (−0.9, 4.3)	0.21		
Emtricitabine (per year exposure)	3.2 (−1.3, 7.8)	0.17		
Ritonavir (per year exposure)	1.2 (−1.4, 3.8)	0.38		
Lopinavir (per year exposure)	5.5 (2.1, 8.9)	0.001	4.9 (1.7, 8.2)	0.003

[1] Multivariate models include demographic and clinical factors listed, and antiretroviral medications selected by LASSO method.

[2] Estimated percentage difference in α1m attributable to each factor