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Tenofovir use and urinary biomarkers among HIV-infected women in the Women's Interagency HIV Study (WIHS)

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Abstract

Background—Tenofovir has been associated with renal tubular injury. Biomarkers that signal early tubular dysfunction are needed because creatinine rise lags behind tenofovir-associated kidney dysfunction. We examined several urinary biomarkers to determine if rises accompanying tenofovir initiation preceded creatinine changes.

Methods—Three urinary biomarkers of tubular impairment- neutrophil gelatinase-associated lipocalin (NGAL), N-acetyl- β -D-glucosaminidase (NAG), and β-2-microglobulin (β2MG)-were measured across three time points (one pre-tenofovir visit and two post tenofovir visits) in one hundred and thirty two HIV-positive women from the Women's Interagency HIV Study (WIHS). Women initiating HAART containing tenofovir were propensity score matched to women initiating HAART without tenofovir and women not on HAART.

Results—There were no differences between groups for NGAL or NAG but β2MG was 19 times more likely to be elevated among tenofovir users at the 2nd post tenofovir visit compared to non-TDF users at the pre-tenofovir visit ($p<0.01$). History of proteinuria was associated with elevated NGAL ($p <0.01$). Factors associated with elevated NAG were GFR<60 ml/min, history of proteinuria, hepatitis C ($p<0.01$ for all) and diabetes mellitus ($p=0.05$). Factors associated with

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increased odds of elevated β2MG were HIV RNA>100,000 copies/ml, hepatitis C, boosted protease inhibitor (PI) use, and GFR<60 ml/min ($p \leq 0.01$ for all).

Conclusions—β2MG levels are elevated in women on tenofovir indicating probable early renal dysfunction. Biomarker elevation is additionally associated with baseline chronic kidney disease, uncontrolled viremia, and boosted PI use. Future studies are needed to explore urinary biomarker thresholds in identifying treated HIV-infected individuals at risk for renal dysfunction.

Keywords

Tenofovir; urinary biomarkers; HIV infected women

Introduction

Persons with HIV are at high risk for the onset and progression of chronic kidney disease (CKD). In addition to HIV related kidney disease (HIV-associated nephropathy or HIVAN), the population of people living with HIV/AIDS is aging and developing comorbidities that predispose them to CKD such as diabetes mellitus, hypertension, and cardiovascular disease. Renal toxicity from antiretroviral and other medications also contributes to kidney dysfunction in HIV-infected persons. One such antiretroviral drug, tenofovir undergoes renal clearance by a combination of glomerular filtration and active proximal tubular secretion. It has also been implicated as a source of impaired kidney function in HIV-infected individuals [1-3].

While serum creatinine level is a well-accepted marker of kidney function, its disadvantages include its contemporaneous relationship with cellular damage. In addition, it is affected by a number of other factors including muscle mass, protein intake, proteinuria, race and age [4-5]. Therefore identifying biomarkers that reflect renal injury in real time and prior to the rise in creatinine could impact the delivery of care to patients with HIV. Given the multiple processes (direct HIV effects, antiretroviral effects and increased prevalence of CKD risk factors) that may affect kidney function in persons with HIV, markers that could predict kidney function decline would be of paramount interest to clinicians involved in HIV care.

Over the past decade, novel urine biomarkers specific for tubular injury have been found, which provide an earlier indicator of impairment. Urine neutrophil gelatinase-associated lipocalin (NGAL) is a member of the lipocalin family of proteins that is secreted into the urine by the thick ascending limb of Henle as well as the collecting ducts of the kidney. While normally expressed in low levels, increasing concentrations in urine are seen in the presence of epithelial injury and inflammation [6]. N-acetyl-β-D-glucosaminidase (NAG) is a proximal tubule lysosomal enzyme whose presence in the urine suggests proximal tubular damage [7]. β-2-microglobulin (β2MG) is a low molecular weight protein, found in all nucleated cells, freely filtered by glomeruli and catabolized by the proximal tubules [8].

These three biomarkers have been associated with acute kidney injury (AKI formerly acute renal failure) in people without HIV [9-10] and have been found to be elevated in patients with conditions associated with CKD such as diabetes [11]. However it is unclear whether these biomarkers could be clinically useful to monitor nephrotoxicity from antiretroviral therapy. This study sought to explore the relationships between tenofovirinitation and changesin levels of urinary biomarkers using longitudinal data from a stable outpatient populationof women with HIV.

Methods

Study Design and Measurements

Women's Interagency HIV Study (WIHS)—The rationale and methods of the WIHS have been previously described [12-13]. Briefly, the WIHS is a multicenter, prospective cohort study of the natural history of HIV-infection among women, conducted in Chicago, Los Angeles, New York City, the San Francisco Bay Area, and Washington DC. From October 1994 through November 1995, 2,623 (2,054 HIV+ and 569 HIV-) study participants were enrolled. From October 2001 through September 2002, an additional 1,143 (737 HIV+ and 406 HIV-) participants were enrolled for a total of 3,766 (2,791 HIV+ and 975 HIV-) women. Participants were recruited from HIV primary care clinics, hospital-based outpatient infectious diseases clinics, research programs, community outreach sites, women's support groups, drug rehabilitation programs, HIV testing sites, and referrals from previously enrolled participants. HIV-infection was determined with ELISA and confirmed via Western Blot. A standardized interview-based survey was used at enrollment to collect demographic data and prior medical, sexual, and drug use history. Women are evaluated semi-annually to obtain weight, CD4 lymphocyte count, HIV RNA level, albumin, creatinine, and other testing. Urine is collected annually and stored in the central repository. Proteinuria was qualitatively assessed during the first seven study visits. Women were described as having proteinuria if at least two urine analyses demonstrated the qualitative presence of ≥+1 protein. Race, hepatitis C antibody status, and history of proteinuria were determined prior to study baseline. All other factors were time-varying and were determined at each visit biomarkers were measured. The study was approved by the institutional review boards (IRBs) at WIHS Institutions and informed consent was obtained from every participant.

Study Population—Among WIHS participants, we included in the current study HIV-infected women initiating a highly active antiretroviral therapy regimen (HAART) containing tenofovir who had available stored urine specimens and serum creatinine measurements. For each included participant, three visits were selected for urine testing: the visit one year prior to the initial report of tenofovir (study baseline), the initial visit that tenofovir was reported (matching visit, 2nd measure) and one year after the initial report of tenofovir (3rd measure). Participant visits contributed by HIV-infected women using HAART without tenofovir (non-TDF HAART users) were matched to the visit that tenofovir was initially reported for each TDF HAART user via propensity score matching with a tolerance of 0.02 [14]. Propensity scores were estimated from a logistic multivariate regression model containing the following predictors: glomerular filtration rate (GFR) calculated using the CKD-EPI equation [15], CD4 cell count (CD4), calendar year, HAART duration, HAART interruptions, body mass index, HIV RNA level, and use of a ritonavir-boosted Protease Inhibitor (PI). The study design and matching structure are shown in Figure 1. A total of 45 person-visits contributed by 45 individual non-TDF HAART users were matched to the visit of the first report of tenofovir for 45 individual TDF HAART users.

As an additional comparison group, participant visits contributed by HIV-infected women who had not used HAART (but who may have used HAART in the past) within the past 3-9 months (non-HAART users) were matched to the visit that tenofovir was initially reported for each TDF HAART user via propensity score matching as described above. For this matched analysis, the propensity scores were estimated from a logistic multivariate model containing the following predictors: race, history of proteinuria, GFR, CD4, calendar year, body mass index, HIV RNA, hepatitis C (HCV) antibody status, and smoking history. A total of 45 person-visits contributed by 42 individual non-HAART users were matched to

the visit of first reported tenofovir use for the same 45 individual TDF HAART users described above.

Assays—Stored urine specimens were measured for NGAL, NAG, and β2MG for each woman at each of the three time points. Urine NGAL level was assessed with the ARCHITECT® assay (Abbott Diagnostics, Abbott Park, IL) that specifically detected human NGAL. Urine NAG activity was assessed using a NAG kit (Roche Diagnostics, Indiana) and β2MG was assayed using enzyme immunoassay kit from ALPCO Ltd., New Hampshire; the intra-assay and interassay variation coefficients were less than 5%. Serum creatinine was measured locally at each WIHS site.

Statistical analysis

All biomarker values were log transformed and the unadjusted geometric means were examined graphically. For multivariable analyses, NGAL and NAG were treated as log normally distributed while β2MG was treated as a dichotomous outcome (> 0.5 versus ≤ 0.5 μg/mL) as a result of a large percentage of undetectable values (limit of detection =0.06 μg/ml).This dichotomized outcome for β2MG is consistent with previous reports [16], but there is no established cutoff value for either NGAL or NAG in previous studies. The transformed biomarker values were fit using generalized linear models, with generalized estimating equations used to adjust standard errors to account for the repeated measures over time and matched exposure groups [17]. Time trends over the three biomarker visits were modeled piecewise linearly with a spline at the matching visit (2^{nd} measure). Models were simplified to linear if the estimated change from the 2^{nd} to 3^{rd} measure was non-significant, indicating no departure from linearity over time across the three visits. Final models using the non-tenofovir HAART group as the comparator were adjusted for age as continuous, race (white, black and Latina), proteinuria and diabetes history and WIHS baseline HCV antibody status as binary indicators, eGFR<60 ml/min|1.73 m^2, hypertension (systolic>140 mmHg or diastolic>90 mmHg), current smoking status, categorized body mass index (BMI: <18.5, 18.5-25, 25-30, >30 kg/m^2), CD4<200 cells/mm^3, HIV RNA>100,000 copies/ml, years since HAART initiation, proportion of visits no HAART use was reported since initiation, and boosted PI use. For the additional analysis using a non-HAART comparison group, final models used the same covariates with the exception of the HAART use-related variables. All covariates except race, history of proteinuria, and baseline hepatitis C antibody status were time-varying.

Missing data were imputed according to whether the factor was fixed (proteinuria history was missing for 8 women and diabetes history for 4 women) or time-dependent (9 BMIs, 7 systolic and diastolic blood pressures, 6 eGFR measures, 5 CD4 cell counts, and 3 HIV RNA measures). For fixed covariates, the proportion of cases among individuals with complete data was used to randomly assign case status to individuals with missing data. For time-dependent covariates, missing values were interpolated from neighboring values in time using the average between the last non-missing value prior to and the first non-missing value after the missing value.

Results

Urinary biomarkers were measured at three time points for three groups of women based on subsequent exposure to antiretroviral therapy (Figure 1). This comprised 135 person-visits from 132 individual women. At baseline, the median age of the women initiating HAART with tenofovir was 42 years and the racial distribution was 27% white/other, 49% black and 24% Latina (Table 1). Eighteen percent of women in the group had a history of proteinuria at baseline and the average eGFR was 98ml/min. Only 4 women reported use of stavudine

or atazanavir – both of which have been linked to renal tubular dysfunction - in any of the study visits [18-19]. Following propensity score matching all factors were similar between the tenofovir HAART users and the non-tenofovir HAART users except for the proportion of women with unknown history of proteinuria (0% vs. 18%, p<0.01), median HIV RNA (3.5 vs. 1.9, p=0.01), and categorized HIV RNA (p=0.01).

The geometric means and 95% confidence limits (95% CIs) for NGAL did not change over time and were not different among exposure groups in the unadjusted analysis (Appendix Figure, top row). While NAG levels increased over time, there were no notable differences among exposure groups (Appendix Figure, middle row). In contrast, β2MG levels increased over time among women initiating HAART with tenofovir (Appendix Figure, bottom row). The proportion of women with elevated β2MG (>0.5 μg/ml) increased from 7% to 40% among tenofovir HAART users (p < 0.01), from 11% to 16% among non-tenofovir HAART users (p 0.29), and 13% to 18% among non-HAART users, from the 1st to the 3rd measurement. We calculated Spearman correlation coefficients for the log-transformed values of the 3 urinary biomarkers. The correlation between NGAL and NAG was 0.47 (p<0.0001). Restricted to pairs with positive β2MG, correlation between β2MG and NGAL was 0.29 (p<0.0001) and between β2MG and NAG was 0.52 (p<0.01).

NGAL and NAG

In adjusted models, no differences were seen for NGAL between groups in the main comparison (tenofovir HAART versus non-tenofovir HAART) at baseline or over time. However, NAG rose among tenofovir HAART users by 20% per year on average (p<0.05) but did not differ between groups over time (figure 2). NGAL and NAG were both elevated among women with renal parameters suggestive of CKD at baseline (Table 2). Values of both were between 69% and 62% higher, respectively, among women with a history of proteinuria as compared to women without a history of proteinuria (p<0.01 for both). Similarly, women with eGFR less than 60 ml/min at study baseline had approximately a 60% greater value of NGAL (p0.10) and 80% greater value of NAG (p0.01). With respect to medical history, two other factors were marginally associated with higher NGAL and NAG levels. These include years since initiation of HAART for NGAL (p0.04), and a history of diabetes mellitus (p0.05) and hepatitis C (p<0.01) for NAG. Race was also highly associated with NGAL and NAG level. Latina women had NGAL and NAG levels two times as high as white women (p0.01 for both).

In the secondary comparison, levels and change over time for NGAL and NAG among HAART users initiating tenofovir were compared to a group not taking HAART. NGAL and NAG levels were found to be similar and there was no significant change over time. However, NAG increased among tenofovir users by 21% per year on average compared to baseline (p <0.05) (consistent with results from the main analysis) and also rose among non-tenofovir HAART users by 33% per year on average compared to baseline (p<0.05) (compared to non-HAART group). African-American women as well as Latina women had higher NAG levels compared to whites (p0.01). Levels of NGAL and NAG were not significantly different among women with a history of proteinuria in this analysis, but were more than twice as high among women with estimated GFR<60ml/min (p0.01).

β2MG

In the adjusted model of β2MG, tenofovir HAART users had 19 times the odds of having an elevated β2MG at the 3rd time point compared to 2.8 for non-tenofovir HAART users at the 3rd time point (p < 0.01). Furthermore, CKD risk factors were associated with an elevated β2MG. Women with GFR<60 ml/min (OR 5.67 p 0.01) and HCV (OR 9.3, p <0.01) were more likely to have elevated β2MG while smokers (OR0.27, p0.04) and Black women

(OR0.2, p0.02) were less likely to have elevated β2MG. However, controlling for eGFR, proteinuria was not associated with likelihood of elevated β2MG (p0.22). With respect to HIV specific parameters, women with HIV RNA >100,000 copies/ml (OR 23.89, p<0.01) and boosted PI use (OR 8.97, p <0.01) were more likely to have elevated β2MG.

In the secondary comparison, a different pattern of predictors of elevatedβ2MG was noted. The estimated odds of having an elevated β2MG level at the 3rd time point was 3.9 times higher among tenofovir initiators compared to 1.1 times higher for non-HAART users at the 3rd time point (p0.05). The CKD risk factors of an estimated GFR<60 and HCV were still significant factors. CD4 ≤ 200 cells/mm^{3}was also a significant predictor of elevated β2MG (OR 9.0, p<0.05) and obesity was associated with a decreased odds of elevated β2MG (OR 0.22, p<0.05)

Discussion

In this cross-sectional analysis of a cohort of HIV infected women (WIHS), we evaluated the changes in three significant urinary biomarkers over time in a group of women who initiate a tenofovir-containing antiretroviral regimen compared to women not initiating tenofovir or not on HAART. At study baseline, there were no differences between groups for NGAL, NAG and β2MG. The increase over time for NGAL and NAG was not significant among TDF users compared to the non-TDF users. β2MG was more likely to be elevated among TDF users at the 3rd time point compared to non-TDF users, indicating that this marker may be an important indicator of TDF related kidney dysfunction. Even though increases over time for NGAL and NAG was not significant among TDF users compared to the non-TDF users , our results suggest that elevations in NGAL and NAG among TDF users are more generally related to comorbid diseases commonly associated with chronic kidney disease (CKD). In contrast, the factors specific to HIV infection such as antiretroviral regimen (HAART with tenofovir and regimen including a boosted PI), lower CD4 and higher HIV RNA level were associated with greater urinary β2MG levels.

Advanced immunosuppression (CD4 <50 cells/mm^3) has been associated with a decline in creatinine clearance in a prior observational study [20]. However, another study did not find an association between CD4<200 cells/mm^3 and higher β2MG excretion in HIV-infected patients on TDF compared to other nucleoside reverse transcriptase inhibitors [21]. Therefore, our study uniquely demonstrates that immunosuppression affects β2MG levels which has not previously been described. Similarly, uncontrolled HIV viremia has been linked to renal dysfunction in a large randomized trial [22]. Our findings of the association between uncontrolled HIV viremia with urinary biomarker elevation supports the findings from a recent cohort study which noted an association between detectable HIV RNA (> 400 copies/ml) and elevated cystatin C, another biomarker of renal function [23]. This suggests that the development of these biomarkers as clinical guides will need to take into account the medical history of the patient as well as the effectiveness of their HIV directed care.

Our findings regarding β2MG confirm prior work demonstrating higher levels of urinary β2MG among persons on tenofovir containing antiretroviral regimen and the impact of concurrent PI use. In the ASSERT study, subjects receiving TDF/FTC fixed dose combination (FDC) had a 72% greater increase at week 24 and a 133% greater increase at week 48 in urinary β2MG than subjects taking ABC/3TC FDC [24]. In addition, a greater decline in kidney function has been noted in persons receiving tenofovir with a boosted PI compared to tenofovir without a PI [25]. Furthermore, an observational cohort of patients receiving tenofovir demonstrated that subgroups receiving tenofovir with ritonavir-boosted lopinavir had greater urinary β2MG levels than those receiving tenofovir without a PI [16].

In addition, our findings confirm prior reports that metabolic factors may play a role in modulating biomarker levels. Low body weight (defined by <60 kg) has been associated with renal function decline in persons on TDF compared to abacavir containing regimen [26] and greater urinary β2MG levels [16]. Likewise, our study found that obesity was protective against elevated β2MG in the TDF group compared to the non-HAART group. Furthermore, we found an association between current smoking and reduced β2MG levels in the TDF group compared to non-TDF HAART users contradicting prior reports of smoking and elevated β2MG in an environmental cohort [27].

Prior studies have been limited in their ability to examine the relationships between CKD risk factors and these biomarkers due to the exclusion of patients with proteinuria or preexisting history of renal disease, our results reveal the importance of preexisting kidney disease in the interpretation of urinary biomarker elevations in patients on tenofovir. These results thus add to the literature on the clinical utility of these markers for screening for sub-clinical renal toxicity in HIV-infected patients.

The interpretation of the results of this study may be limited to women because of gender differences in these biomarker levels. In an HIV negative population with type 1 diabetes, urinary NGAL levels were significantly higher in females compared to males [28], which may reflect an estrogen-mediated difference in protein expression in renal or urinary tract tissues [29]. Furthermore, gender and race differences have been noted in NAG levels. In a cross-sectional study of the early natural history of cardiovascular disease, Black women had the higher levels of urinary NAG compared to Black men but White men and women had similar levels [30]. Studies assessing β2MG levels in HIV-infected persons have mostly been in men and have not assessed for gender differences [16, 24, and 31].

Other limitations to our analysis should be noted. Our conclusion regarding the impact of GFR <60 on biomarkers is limited by the small sample of women with GFR<60. Further the level of urinary biomarkers is influenced by urine concentration. Urine creatinine measurements, typically used to standardize for urine concentration, were unavailable in this study. However, the impact of not accounting for differences in urine concentration would likely be to attenuate effects by introducing additional variability into the analysis and thus our results may be conservative.

In summary, this analysis examined the value of the urinary biomarkers NGAL, NAG and β2MG as indicators of kidney injury resulting from tenofovir use. Our results demonstrate the potential utility of urinary β2MG levels in predicting patients at risk for loss of kidney function due to tenofovir use. Further studies are needed to determine when it is appropriate to use this urinary biomarker as well as how frequently to monitor. If validated, this biomarker may have clinical utility in identifying higher risk individuals allowing appropriate diagnostic and therapeutic interventions to be delivered earlier with a potential positive impact on kidney function.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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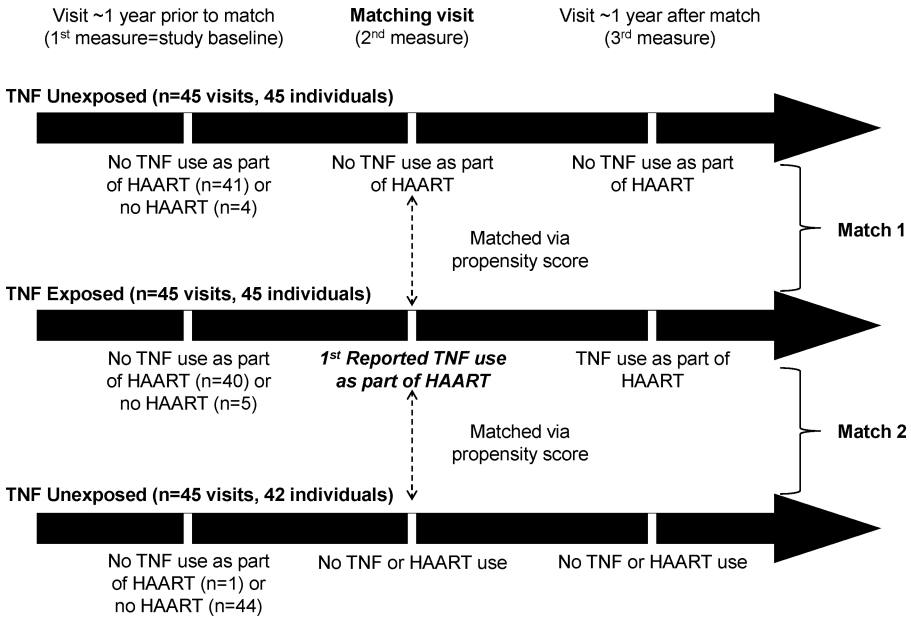

Figure 1.

The matched design of the study is represented by the three arrows, which highlight the three groups of HIV-infected women (women on HAART initiating tenofovir and two comparison groups) and the three time points at which the urinary biomarkers were measured. Participant visits were matched at the second time point which corresponded to the initiation of a tenofovir-containing HAART regimen. Different visits from a participant not exposed to tenofovir could be matched to multiple tenofovir initiators.

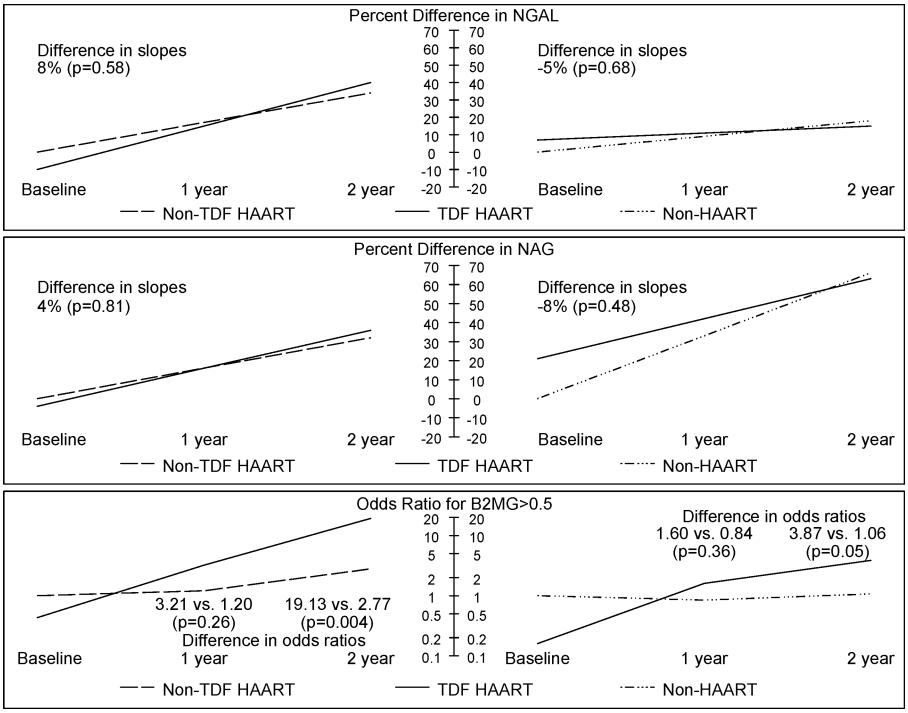

Figure 2.

Estimated changes over time in biomarker level or odds of an increased level from the multivariate generalized linear models adjusted for confounding factors. All odds ratios are relative to baseline for the control group, non-TDF HAART on the left and non-HAART on the right.

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Table 1

Comparison of characteristics at study baseline

	TDF HAART (n=45 women)	Non-TDF HAART[1] (45 visits for 45 women)		Non-HAART[2] (45 visits for 42 women)	
Characteristic	**% or Med (IQR)**	**% or Med (IQR)**	***p*-value[3]**	**% or Med (IQR)**	***p*-value[3]**
Calendar year	2002.0 (2001.8-2001.1)	2002.0 (2001.8-2001.1)	0.12	2002.0 (2001.2-2002.1)	0.50
Age	42 (39-47)	40 (36-45)	0.18	41 (37-47)	0.54
Race					
African-American	49	60	0.26	53	0.92
Latina	24	11		22	
White/other	27	29		24	
CKD-EPI-estimated GFR	98 (86-114)	91 (78-114)	0.29	103 (82-112)	0.91
<60	4	4	1.00	2	1.00
Hx Proteinuria					
Unknown	0	18	<0.01	0	
Dipstick reading of ≥1+ at ≥2 of 1st 7 WIHS visits	18	14	0.76	16	1.00
HCV Ab+ @ BL	38	40	1.00	22	0.17
DM	11	16	0.82	4	0.44
SBP≥140 or DBP≥90	13	7	0.48	22	0.41
Current smoker	42	44	0.83	31	0.38
BMI	24.4 (22.5-28.5)	25.3 (21.4-30.2)	0.85	26.4 (22.9-29.4)	0.27
Underweight (<18.5)	7	9	0.42	0	0.36
Normal Weight (18.5-24.9)	47	40		40	
Overweight (25-29.9)	31	22		38	
Obese (≥30)	16	29		20	
CD4	411 (292-587)	324 (237-579)	0.29	465 (315-581)	0.51
<200	18	18	1.00	11	0.47
200-500	49	47		44	
>500	33	33		40	
Log_{10} RNA	3.5 (1.9-4.5)	1.9 (1.9-3.5)	0.01	3.3 (1.9-3.8)	0.25
HIV RNA (copies/ml)					

	TDF HAART (n=45 women)	Non-TDF HAART[1] (45 visits for 45 women)		Non-HAART[2] (45 visits for 42 women)	
Characteristic	**% or Med (IQR)**	**% or Med (IQR)**	***p*-value[3]**	**% or Med (IQR)**	***p*-value[3]**
<500	31	62	0.01	36	0.40
500-5,000	27	13		36	
5,000-50,000	24	18		22	
>50,000	18	4		7	
Years since HAART initiation	4.0 (2.9-5.0)	4.5 (3.6-5.0)	0.38	N/A	
Proportion visits not on HAART	0.17 (0-0.27)	0.11 (0-0.27)	0.45	N/A	
Boosted PI use	22	29	0.63	N/A	

[1] Characteristics used in final propensity score model were CKD-EPI-estimated glomerular filtration rate, CD4 cell count, Calendar year, years since HAART initiation, HAART interruptions, BMI, $\log_{10}$-transformed HIV viral load, and boosted PI use.

[2] Characteristics used in final propensity score model were race, history of proteinuria, CKD-EPI-estimated glomerular filtration rate, , CD4 cell count, calendar year, BMI, $\log_{10}$-transformed HIV viral load, baseline HCV antibody status, current smoking status.

[3] Reported *p*-values were obtained from Fisher's exact tests for percentages and from Wilcoxon rank sum tests for medians. *P*-values represent statistical differences between tenofovir users and nonusers (HAART and non-HAART users).

Table 2

Factors associated with elevated NGAL and NAG in multivariable analysis

	NGAL				NAG			
	Controls: Non-TDF HAART		Controls: Non-HAART		Controls: Non-TDF HAART		Controls: Non-HAART	
Factor	**% diff**	**95% CI**	**% diff**	**95% CI**	**% diff**	**95% CI**	**% diff**	**95% CI**
Race/ethnicity (vs. white/other)								
Black	31	(-10,92)	74	(18,157)†	45	(-2,115)	89	(26,183)†
Latina	100	(21,230)†	106	(36,211)†	98	(26,212)†	79	(18,171)†
Age, per 10 years	-17	(-34,5)	-16	(-31,3)	2	(-17,26)	-13	(-29,7)
History of proteinuria	69	(20,138)†	28	(-21,108)	62	(17,124)†	22	(-20,86)
Hepatitis C antibody positive at WIHS baseline	14	(-19,63)	19	(-15,67)	55	(16,107)†	53	(10,113)†
Diabetes mellitus	33	(-9,92)	11	(-31,79)	44	(1,105)*	7	(-31,68)
Estimated GFR<60	60	(-9,181)	133	(19,359)†	80	(20,169)†	79	(18,172)†
High blood pressure (systolic>140 or diastolic >90)	-4	(-39,51)	-17	(-44,23)	3	(-18,31)	8	(-22,48)
Current smoker	3	(-20,33)	25	(-7,68)	-6	(-28,23)	15	(-16,59)
BMI (vs. normal weight)								
Underweight (<18.5)	7	(-40,91)	38	(-41,225)	26	(-29,123)	20	(-43,150)
Overweight (25-30)	-2	(-33,42)	1	(-29,42)	30	(-4,77)	17	(-15,62)
Obese (>30)	-3	(-31,35)	-30	(-51,1)	15	(-12,50)	-17	(-40,15)
CD4 cell count ≤200 cell/mm^3	28	(-13,89)	-1	(-42,68)	10	(-26,64)	18	(-27,92)
HIV-1 viral load >100,000 copies/ml	1	(-58,144)	-2	(-51,99)	40	(-28,173)	-6	(-46,63)
Years since HAART initiation	-15	(-27,-1)†	N/A		-3	(-12,8)	N/A	
Proportion of visits not on HAART since initiation	0	(-1,1)			0	(-1,1)		

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	NGAL				NAG			
	Controls: Non-TDF HAART		Controls: Non-HAART		Controls: Non-TDF HAART		Controls: Non-HAART	
Factor	**% diff**	**95% CI**	**% diff**	**95% CI**	**% diff**	**95% CI**	**% diff**	**95% CI**
Boosted PI use	-15	(-36,12)			19	(-5,49)		

TDF: Tenofovir

Percent difference refers to TDF HAART users compared to controls

† p-values <0.05

* p-values=0.05, other p-values >0.05

Table 3

Factors associated with elevated β2MG level in multivariable analysis

	Controls: Non-TDF HAART users		Controls: Non-HAART users	
Factor	**OR**	**95% CI**	**OR**	**95% CI**
Race/ethnicity				
White/other (ref)	1.00	Ref	1.00	Ref
Black	0.21	(0.05,0.82)†	1.06	(0.29,3.82)
Latina	0.46	(0.08,2.48)	0.46	(0.09,2.35)
Age, per 10 years	0.97	(0.34,2.75)	1.29	(0.61,2.75)
History of proteinuria	2.36	(0.6,9.34)	1.76	(0.44,7.12)
Hepatitis C antibody positive at WIHS baseline	9.33	(2.81,31.01)†	5.83	(1.84,18.47)†
Diabetes mellitus	1.21	(0.34,4.31)	0.73	(0.17,3.23)
Estimated GFR<60	5.67	(1.48,21.69)†	9.96	(1.32,75.06)†
High blood pressure (systolic>140 or diastolic >90)	1.51	(0.52,4.4)	1.65	(0.64,4.24)
Current smoker	0.27	(0.08,0.92)†	0.41	(0.13,1.35)
BMI (vs. normal weight)				
Underweight (<18.5)	0.67	(0.08,5.3)	2.75	(0.18,42.02)
Overweight (25-30)	0.34	(0.1,1.13)	0.50	(0.15,1.67)
Obese (>30)	0.54	(0.16,1.76)	0.22	(0.06,0.87)†
CD4 cell count ≤200 cell/mm^3	2.58	(0.68,9.74)	8.99	(2.91,27.82)†
HIV-1 viral load >100,000 copies/ml	23.89	(2.89,197.87)†	1.39	(0.28,6.82)
Years since HAART initiation	0.62	(0.39,0.99)*		
Proportion of visits not on HAART since initiation	1.02	(0.99,1.05)	N/A	
Boosted PI use	8.97	(3.56,22.59)†		

TDF: Tenofovir

Odds Ratio refers to TDF HAART users compared to controls

† p-values <0.05

* p-values=0.05, other p-values >0.05