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Urine alpha1-microglobulin is a better marker for early tubular dysfunction than beta2-microglobulin among tenofovir-exposed human immunodeficiency virus-infected men who have sex with men[☆]



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ABSTRACT

Objectives: Men who have sex with men are at risk of tenofovir nephrotoxicity due to its wide use in both treatment and prophylaxis for human immunodeficiency virus infection, but little is known about the urinary biomarkers of early renal dysfunction in this population. This study aims to identify useful biomarkers of early renal dysfunction among human immunodeficiency virus-infected men who have sex with men exposed to tenofovir.

Methods: In a cross-sectional study urinary alpha1-microglobulin, beta2-microglobulin, N-acetyl- β -D-glucosaminidase and albumin were measured and expressed as the ratio-to-creatinine in 239 human immunodeficiency virus-infected men who have sex with men who were treatment naïve or receiving antiretroviral therapy with tenofovir-containing or non-tenofovir-containing regimens. Additionally, 56 patients in the non-antiretroviral therapy group started a tenofovir-containing regimen and were assessed after 3 and 6 months on antiretroviral therapy.

Results: Both the frequency of alpha1-microglobulin proteinuria (alpha1-microglobulin-creatinine ratio >25.8 mg/g) and the median urinary alpha1-microglobulin-creatinine ratio were higher in the tenofovir disoproxil fumarate group than the other two groups

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(all $p < 0.05$). A higher frequency of beta2-microglobulin proteinuria (beta2-microglobulin-creatinine ratio > 0.68 mg/g) was also observed in the tenofovir group (28.9%) compared to the non-tenofovir group (13.6%, $p = 0.024$). There were no significant differences between groups for N-acetyl- β -D-glucosaminidase and albumin. In the longitudinal study, the median urinary alpha1-microglobulin-creatinine ratio after 3 and 6 months on tenofovir-containing therapy (16.8 and 17.3 mg/g) was higher than baseline (12.3 mg/g, $p = 0.023$ and 0.011, respectively), while no statistically important changes were observed in urinary beta2-microglobulin-creatinine ratio or in the other biomarkers after 3 and 6 months on antiretroviral therapy (all $p > 0.05$).

Conclusion: Urinary alpha1-microglobulin seems to be a more sensitive and stable indicator of tubular dysfunction than urinary beta2-microglobulin for assessing tenofovir-related nephrotoxicity and can be significantly altered after tenofovir exposure.

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Introduction

The prevalence of HIV-associated kidney disease has decreased in the era of antiretroviral therapy (ART), but drug-induced nephrotoxicity is emerging.¹ Long-term exposure to nephrotoxic antiretroviral drugs, such as tenofovir disoproxil fumarate (TDF), may lead to progression of renal dysfunction and incomplete reversibility of toxicity, even after drug withdrawal.^{2,3} Moreover, some urinary biomarkers of early kidney injury were found to be associated with important clinical outcomes, such as mortality and severe heart disease.^{4,5} Therefore, it is important to detect early renal dysfunction in HIV-infected individuals, particularly in those exposed to TDF.

TDF is widely used as a preferential drug for ART according to worldwide guidelines, and it is also part of the only approved regimen for pre-exposure prophylaxis to HIV infection.⁶ The drug is excreted through glomerular filtration and proximal tubular secretion, and its use has been related to an increased risk of rapid decline in renal function and chronic kidney disease.² Clinically, renal insufficiency is usually assessed by an estimated glomerular filtration rate (eGFR),⁷ but proximal tubular dysfunction is more common and specific for TDF-induced toxicity⁸ and can occur with the absence of glomerular defects.⁹

The evaluation of urinary biomarkers, such as α 1-microglobulin (α 1M), β 2-microglobulin (β 2M) and N-acetyl- β -D-glucosaminidase (NAG), may improve the identification of those at risk of tubular toxicity and may detect early stages of renal dysfunction.^{10,11} However, the evaluation criteria of these proteins in tubular dysfunction have not been established and little is known about the change of these new biomarkers during ART. The role of the aforementioned biomarkers in diagnosing and monitoring early tubular damage still needs to be explored.

HIV transmission is increasing markedly faster among MSM than other high-risk groups in China and some other countries.^{12,13} MSM are at risk of TDF toxicity due to its comprehensive role in treatment and pre-exposure prophylaxis. However, most biomarkers of early renal dysfunction have not been validated in HIV-infected patients let alone in specific key-populations, such as MSM. Our study aims to investigate

the presence of early renal dysfunction among HIV-infected MSM and to find the indicators of early renal injury in MSM with TDF exposure.

Materials and methods

Study population

We performed a cross-sectional study and a longitudinal study with a single-center cohort of HIV-infected MSM at the First Hospital of China Medical University in Shenyang, a major city in northern China that has a rapidly increasing incidence of HIV infection among MSM.¹⁴ A total of 239 MSM receiving care at the AIDS clinic were enrolled in a cross-sectional study between August 2012 and June 2014. All participants in the study were adults (age ≥ 18 years) who were treatment-naïve or had received the current ART regimen for no less than six months. The patients who had used other non-ART nephrotoxic drugs in the previous two months before enrollment were excluded from the study.

The ART regimens were based on either Efavirenz (EFV) or Lopinavir/ritonavir (LPV/r). The other two ART drugs were lamivudine (3TC) plus TDF (TDF group) and 3TC plus zidovudine (AZT) (non-TDF group). Fifty-six patients in the non-ART group who started TDF/3TC/EFV were enrolled in a longitudinal study and were assessed after 3 and 6 months on ART. This study was approved by the Medical Research Ethics Committee of the First Hospital of China Medical University. Written informed consent for participation in this study was obtained from each participant.

Data collection

Demographic characteristics; medical history, such as diabetes, hypertension, and prior AIDS-defining illness; and other clinical and laboratory data of each subject were obtained from the clinical records and laboratory database. Body weight was also measured. Fasting blood and mid-stream urine specimens were collected at the routine morning consultation. Blood samples were assayed for full blood count, CD4T cell count, HIV RNA level, β 2M, and routine biochemistry levels,

including renal, liver, glucose, calcium, phosphorus, and lipid profiles.

Spot urine samples were tested for α 1M, β 2M, NAG, creatinine, and albumin within four hours after sample collection in the clinical laboratory of our hospital. The urinary levels of α 1M, β 2M and albumin were determined by a Siemens BN II nephelometer Analyzer II with the kits from Siemens. NAG was measured with a 6-methyl-2-pyridinethiol (MPT) assay (Leadman Biotechnologies Inc., Beijing), and urinary creatinine was measured by enzymatic methods using an automated multianalyzer (HITACHI 7600, Tokyo, Japan). The upper reference limits given by the manufacturer were 0.3mg/L for urinary β 2M, 12mg/L for urinary α 1M, and 11.5IU/L for NAG. All of the urinary proteins were expressed as the ratio-to-creatinine (α 1MCR, β 2MCR, NAGCR, and ACR) to account for the variations in urine concentration among individuals.

Definition

Hypertension was self-reported or diagnosed as a systolic BP \geq 140, diastolic BP \geq 90 mmHg, or current treatment of hypertension with antihypertensive drugs. Diabetes was defined as a fasting blood glucose \geq 7.0 mmol/L or a self-reported history of diabetes. Hypocalcemia and hypophosphatemia were defined based on the reference ranges in the local clinical laboratory as serum calcemia $<$ 2.17 mmol/L and serum phosphate $<$ 0.81 mmol/L. The eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKDEPI).¹⁵ Albuminuria was defined as an ACR $>$ 30 mg/g.¹⁶ Because there are no accepted criteria for the proteinuria of α 1M, β 2M and NAG, we used the upper quartile (UQ) values of α 1MCR, β 2MCR and NAGCR of all participants to define abnormality.

Statistical analysis

All analyses were performed using SPSS software, version 18.0 (SPSS, Inc., Chicago, IL, USA). Data are presented as means \pm standard deviation (mean \pm SD), medians with inter-quartile ranges (IQR) for continuous variables, and as proportions for categorical variables. Appropriate variables were compared by one-way ANOVA and independent-sample t tests for continuous variables; χ^2 test or Fisher exact test for categorical variables. We calculated Spearman rank correlation coefficients to determine the relationship between urinary proteins and serum β 2M. Risk factors of UQ β 2MCR and UQ α 1MCR were identified with multivariate logistic regression analysis. We considered a *p*-value $<$ 0.05 to be statistically significant.

Results

Two-hundred-thirty-nine HIV-infected MSM were enrolled in the study and stratified by ART into the three groups: TDF group (*n*=97), non-TDF group (*n*=66), and non-ART group (*n*=76). The characteristics of the participants are given in Table 1. In the study sample, the frequency of hypertension, diabetes and hypophosphatemia were 2.9% (*n*=7), 2.5% (*n*=6), and 9.6% (*n*=23), respectively. Although almost half of the

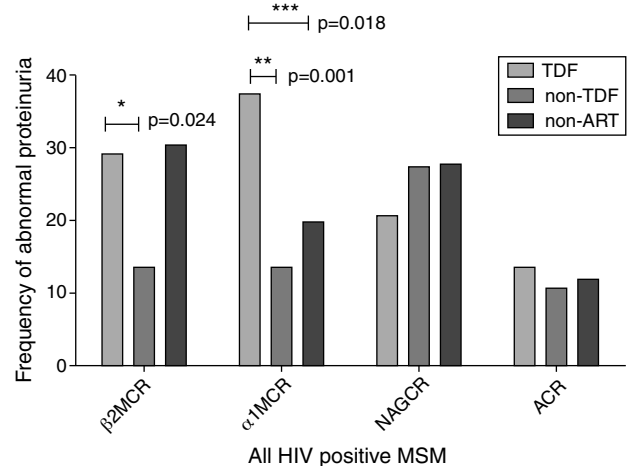


Fig. 1 – Proportion of tubular and glomerular proteinuria among the study groups.

Albuminuria was defined as ACR $>$ 30 mg/g. Other kinds of proteinuria were defined as β 2MCR $>$ 0.68 mg/g, α 1MCR $>$ 25.8 mg/g, and NAGCR $>$ 9.1 IU/g, respectively. ACR, urinary albumin-to-creatinine ratio; β 2MCR, beta2-microglobulin-to-creatinine ratio; α 1MCR, alpha1-microglobulin-to-creatinine ratio; NAGCR, N-acetyl- β -D-glucosaminidase-to-creatinine ratio.

patients in the TDF group were treatment experienced with a non-TDF-containing regimen, the total ART time in both ART groups was similar (*p* $>$ 0.05).

In the cross-sectional study, an eGFR $<$ 90 mL/min/1.73 m² was seen in eight patients (3.3%) and an eGFR $<$ 60 mL/min/1.73 m² was seen in only two patients (0.8%). Albuminuria was present in 12.1% of our participants. As shown in Fig. 1 and Table 2, the TDF group had a higher frequency of α 1M proteinuria (α 1MCR $>$ 25.8 mg/g) and a higher α 1MCR value than the other two groups (all *p* $<$ 0.05). A higher frequency of β 2M proteinuria (β 2MCR $>$ 0.68 mg/g) was also observed in the TDF group compared to the non-TDF group (*p* = 0.024). No significant differences were found in β 2MCR among the three groups. Statistical analysis revealed no significant differences in abnormal frequency or ratio-to-creatinine value of urinary NAG and albumin among the three groups.

β 2MCR and α 1MCR (in urine) were both correlated with serum β 2M (Fig. 2A and B, all *p* $<$ 0.001). In multivariate analyses, TDF exposure (OR 2.62, 95%CI 1.09, 6.30), body weight $<$ 60 kg (OR 3.05, 95%CI 1.45, 6.40), and an eGFR $<$ 90 mL/min/1.73 m² (OR 13.77, 95%CI 2.37, 80.03) were associated with UQ α 1MCR; after adjusting for serum β 2M, TDF exposure (OR 4.16, 95%CI 1.43, 12.10), body weight $<$ 60 kg (OR 3.30, 95%CI 1.43, 7.63), and the cumulative time on ART (OR 1.02, 95%CI 1.00, 1.04) were related to UQ α 1MCR (Table 3). Moreover, protease inhibitor exposure (OR 3.09, 95%CI 1.00, 9.55), CD4 cell count $<$ 200 cells/mm³ (OR 2.96, 95%CI 1.40, 6.25), and eGFR $<$ 90 mL/min/1.73 m² (OR 25.26, 95%CI 2.74, 232.71) were associated with UQ β 2MCR; after adjusting for serum β 2M, only cumulative time on ART (OR 1.02, 95%CI 1.00, 1.04) was related to UQ β 2MCR (Table 4). Factors with

Table 1 – Demographic and clinical characteristics of the HIV-infected MSM.

		TDF (n=97)	Non-TDF (n=66)	Non-ART (n=76)	All (n=239)	p-Value
Body weight (kg)	Mean ± SD	65.6 ± 9.2	66.7 ± 9.6	67.5 ± 10.7	66.5 ± 9.8	0.459
Age (years)	Mean ± SD	38.7 ± 10.0	39.1 ± 12.5	35.4 ± 12.5	37.7 ± 11.6	0.102
Hepatitis B surface Ag (positive)	N (%)	10 (10.3)	1 (1.5)	6 (7.9)	17 (7.1)	0.100
Hepatitis C Antibody (positive)	N (%)	3 (3.1)	0 (0)	3 (3.9)	6 (2.5)	0.283
Current eGFR (mL/min/1.73 m ²)	Mean ± SD	118.0 ± 11.4	119.4 ± 14.6	116.3 ± 16.5	117.8 ± 14.1	0.409
Baseline eGFR(CKDEPI)(mL/min/1.73 m ²)	Mean ± SD	112.3 ± 18.8	109.3 ± 19.7			0.369
Current CD4 cell count (cells/mm ³)	Mean ± SD	349.4 ± 186.2	301.3 ± 124.9	203.5 ± 152.2	291.6 ± 171.6	<0.001 ^a
Nadir CD4 cell count (cells/mm ³)	Mean ± SD	165.2 ± 124.2	203.1 ± 75.7		178.7 ± 113.4	0.203
Current HIV RNA (log10)	Mean ± SD	0.86 ± 1.36	0.66 ± 1.20	4.79 ± 0.75	2.03 ± 2.18	<0.001 ^a
Serum β2M (mg/L)	Mean ± SD	2.42 ± 0.56	2.13 ± 0.81	3.67 ± 1.39	2.71 ± 1.13	<0.001 ^a
Prior AIDS-defining illness	N (%)	22 (22.7)	10 (15.2)	20 (26.3)	52 (21.8)	0.178
Hypocalcemia	N (%)	14 (14.4)	10 (15.2)	21 (27.6)	45 (18.8)	0.215
Hypophosphatemia	N (%)	13 (13.4)	5 (7.6)	5 (6.6)	23 (9.6)	0.355
Diabetes	N (%)	1 (1.0)	4 (6.0)	1 (1.3)	6 (2.5)	0.097
Hypertension	N (%)	2 (2.1)	3 (4.5)	2 (2.6)	7 (2.9)	0.705
On LPV/r	N (%)	25 (25.8)	1 (1.5)			<0.001 ^a
On initial regimen	N (%)	44 (45.3)	61 (92.4)			<0.001 ^a
Time on current regimen (months)	Mean ± SD	12.9 ± 10.9	21.7 ± 15.9			<0.001 ^a
Time on ART (months)	Mean ± SD	24.1 ± 24.7	21.7 ± 15.9			0.479

p-Value for comparison between the three groups.

TDF, tenofovir disoproxil fumarate; ART, antiretroviral therapy; eGFR, estimated glomerular filtration rate; β2M, beta2-microglobulin; AIDS, acquired immune deficiency syndrome; LPV/r, lopinavir/ritonavir.

^a A significant difference between the groups compared.

Table 2 – The levels of urinary proteins in the subjects.

		TDF (n=97)	Non-TDF (n=66)	Non-ART (n=76)	All (n=239)	p-Value
Urinary β2MCR (mg/g)	Median (IQR)	0.26 (0.14, 0.77)	0.14 (0.09, 0.29)	0.30 (0.15, 0.91)	0.23 (0.13, 0.68)	0.325
Urinary α1MCR (mg/g)	Median (IQR)	17.2 (10.9, 30.0)	12.7 (9.1, 21.6)	14.1 (8.4, 21.0)	15.4 (9.2, 25.8)	0.028 ^a
Urinary NagCR (IU/g)	Median (IQR)	5.6 (4.1, 8.5)	6.2 (4.1, 9.2)	6.1 (3.90, 10.5)	6.0 (4.0, 9.1)	0.411
Urinary ACR (mg/g)	Median (IQR)	8.7 (4.7, 16.1)	6.6 (4.0, 13.6)	7.3 (4.7, 11.4)	7.4 (4.4, 13.9)	0.586

p-Value for comparison between the three groups.

β2MCR, beta2-microglobulin-creatinine ratio; α1MCR, alpha1-microglobulin-creatinine ratio; NAGCR, N-acetyl-β-D-glucosaminidase-creatinine ratio; ACR, albumin-creatinine ratio (urine).

^a A significant difference between the groups compared.

too low prevalence, such as diabetes (2.5%) and hypertension (2.9%), were not included in the analysis.

To further study the impact of TDF on kidney function, we followed-up 56 MSM from the non-ART group who started TDF/3TC/EFV. As shown in Table 5, α1MCR was the only urinary biomarker that dramatically increased after three and

six months on TDF-containing therapy compared to baseline data ($p=0.023$ and 0.011 , respectively). There was a slight increasing trend of β2MCR (in the urine) at three months, but it disappeared at six months (all $p>0.05$), concurrent with a significant decrease in β2M after six months of ART ($p=0.028$).

Table 3 – Factors associated with upper quartile (UQ) α1MCR in multivariate analysis (unadjusted or adjusted by serum β2M).

	OR (95%CI)	p-Value	Adjusted OR (95%CI)	p-Value
Body weight <60 kg	3.05 (1.45, 6.40)	0.003	3.30 (1.43, 7.63)	0.005
Age >50 years	1.29 (0.49, 3.39)	0.605	0.54 (0.17, 1.71)	0.292
eGFR <90 mL/min/1.73 m ²	13.77 (2.37, 80.03)	0.030	1.50 (0.15, 15.27)	0.732
CD4 cell count <200 cells/mm ³	1.70 (0.80, 3.61)	0.165	0.72 (0.29, 1.77)	0.469
TDF exposure	2.62 (1.09, 6.30)	0.032	4.16 (1.43, 12.10)	0.009
LPV/r exposure	0.88 (0.29, 2.70)	0.820	0.45 (0.13, 1.56)	0.207
Cumulative TDF (increase by year)	1.03 (0.97, 1.08)	0.355	1.05 (0.98, 1.11)	0.189
Cumulative ART (increase by year)	1.01 (0.99, 1.02)	0.452	1.02 (1.00, 1.04)	0.047

eGFR, estimated glomerular filtration rate; TDF, tenofovir disoproxil fumarate; LPV/r, lopinavir/ritonavir; ART, antiretroviral therapy.

Table 4 – Factors associated with upper quartile (UQ) β 2MCR in multivariate analysis (unadjusted or adjusted by serum β 2M).

	OR (95%CI)	p-Value	Adjusted OR (95%CI)	p-Value
Body weight <60 kg	1.65 (0.76, 3.61)	0.208	1.49 (0.65, 3.40)	0.349
Age >50 years	1.29 (0.49, 3.40)	0.607	0.66 (0.22, 1.98)	0.459
eGFR <90 mL/min/1.73 m ²	25.26 (2.74, 232.71)	0.004	5.75 (0.42, 78.07)	0.188
CD4 cell count <200 cells/mm ³	2.96 (1.40, 6.25)	0.004	1.79 (0.77, 4.14)	0.178
TDF exposure	1.31 (0.52, 3.29)	0.569	1.03 (0.96, 1.09)	0.447
LPV/r exposure	3.09 (1.00, 9.55)	0.050	2.00 (0.63, 6.37)	0.240
Cumulative TDF (increase by year)	1.01 (0.96, 1.07)	0.694	1.03 (0.96, 1.09)	0.447
Cumulative ART (increase by year)	1.007 (0.99, 1.03)	0.488	1.02 (1.00, 1.04)	0.052

eGFR, estimated glomerular filtration rate; TDF, tenofovir disoproxil fumarate; LPV/r, lopinavir/ritonavir; ART, antiretroviral therapy.

Table 5 – Longitudinal observation of urinary biomarkers in patients exposed to TDF-containing regimen.

		Baseline	3 months	p-Value	6 months	p'-Value
eGFR (mL/min/1.73 m ²)	Mean \pm SD	116.4 \pm 16.7	121.2 \pm 11.7	0.105	118.4 \pm 14.3	0.523
Urinary ACR (mg/g)	Median (IQR)	6.5 (4.2, 10.6)	8.6 (5.9, 12.8)	0.989	8.7 (5.3, 14.5)	0.171
Serum β 2M (mg/L)	Mean \pm SD	3.43 \pm 1.49	2.80 \pm 1.36	0.052	2.80 \pm 1.19	0.028 ^a
Urinary β 2MCR (mg/g)	Median (IQR)	0.29 (0.15, 0.62)	0.33 (0.19, 1.11)	0.371	0.29 (0.16, 0.65)	0.467
Urinary α 1MCR (mg/g)	Median (IQR)	12.3 (7.2, 19.0)	16.8 (9.4, 26.0)	0.023 ^a	17.3 (8.9, 29.5)	0.011 ^a
Urinary NagCR (IU/g)	Median (IQR)	5.9 (3.8, 10.5)	4.9 (4.0, 8.9)	0.60	5.7 (3.8, 9.0)	0.466

p-Value for comparison between baseline and 3 months of ART, and p'-value for comparison between baseline and 6 months of ART.

^a A significant difference between the groups compared.

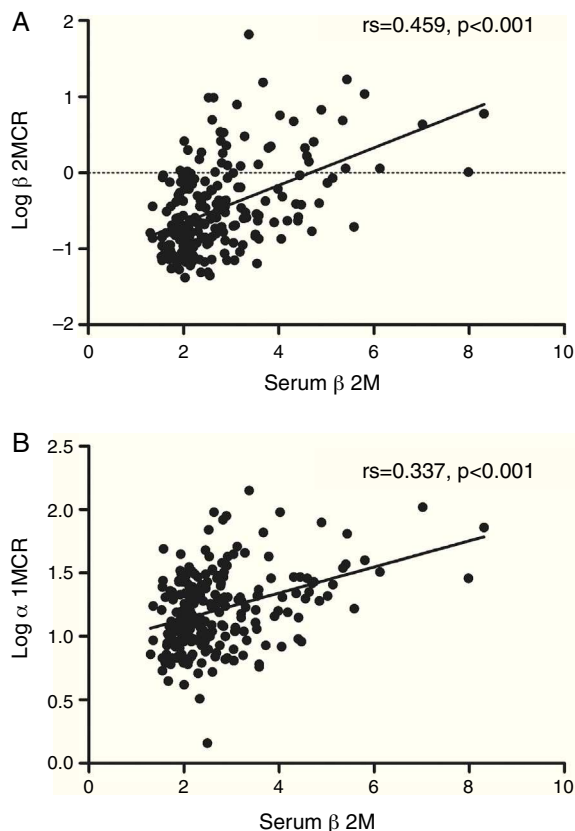


Fig. 2 – Correlations between β 2MCR and serum β 2M (A), α 1MCR and serum β 2M (B). β 2MCR, beta2-microglobulin-to-creatinine ratio; α 1MCR, alpha1-microglobulin-to-creatinine ratio.

Discussion

In an era in which early ART and pre-exposure prophylaxis are generally encouraged,¹⁷ TDF use and its nephrotoxicity are likely to draw increasing attention.¹⁸ In HIV-infected females, some urinary biomarkers were related to TDF use and could forecast declining kidney function.^{19,20} Among HIV-infected MSM, we observed a higher rate of α 1M and β 2M proteinuria in TDF users in a cross-sectional study and confirmed urinary α 1M elevation after TDF exposure in a longitudinal study. HIV-seronegative MSM were found to have a small but significant decrease in creatinine clearance after TDF exposure,²¹ but novel urinary biomarkers among this population have not been evaluated. Our findings underscored the importance of subclinical renal function monitoring in HIV-infected MSM treated with TDF, and the findings might also give clues for early detection of TDF-related toxicity in non-infected MSM.

Here we chose four urinary candidate markers to indicate early renal dysfunction: albuminuria is a classical indicator of early glomerular injury;²² α 1M, β 2M, and NAG are tubular biomarkers, but their role in diagnosing and monitoring early tubular damage is still undetermined. Of all of the selected markers, urinary α 1M seems to be the most reliable for determining TDF-associated renal dysfunction in several ways. First, urinary α 1M was the only biomarker that could distinguish the TDF group from the other two control groups in the cross-sectional study. Second, α 1M was also the only marker with notable elevation after ART initiation assessed in the longitudinal study. In a review paper, TDF-related renal damage usually appears within 5–12 months after initiation of therapy.²³ Our observation confirmed the sensitivity of

urinary α 1M, which increased significantly already after three months of TDF-containing therapy. Third, we observed no significant differences in albumin or NAG in cross-sectional or longitudinal studies. Urinary β 2M might also be a helpful biomarker, which has been recommended along with urinary α 1M as a useful screening tool for TDF-induced kidney tubulopathy in a diagnostic accuracy study,¹⁰ and showed a higher rate of proteinuria in the TDF group than the non-TDF group in our study. However, urinary β 2M is highly dependent on serum β 2M and the pH value of the urine.^{24,25} Unlike β 2M, which is unstable in acidic urine, α 1M has been reported to be very stable in urine at different pHs.²⁶

It is worth mentioning that the inflammatory status of HIV infection may disturb the interpretation of urinary biomarkers in tubular dysfunction because α 1M and β 2M are both inflammatory biomarkers.^{26,27} In our observation, urinary α 1MCR and β 2MCR were both correlated to serum β 2M, which is a non-specific marker of chronic inflammation and could be increased even after ART.^{27,28} However, serum β 2M seemed to have a stronger effect on urinary β 2MCR than urinary α 1MCR. In multivariate analyses, TDF exposure was an independent risk factor for UQ α 1MCR before and after serum β 2M adjustment. By contrast, after adjusting for serum β 2M, all traditional risk factors of tubular proteinuria²⁹ lost their significance in association with UQ β 2MCR. In the longitudinal study, we also observed elevated α 1MCR levels after three and six months on TDF exposure, but no obvious change was found in β 2MCR after ART, probably due to the decreased level of serum β 2M. Nevertheless, in another study with a longer observation time after TDF than in our study, urinary β 2M was highly elevated.¹⁹ It reminds us that urinary β 2M may still be a useful marker after long-term TDF exposure and encourages further study.

Our study provided information about the presence of early renal dysfunction in Chinese HIV-infected patients. Compared with another HIV-infected cohort of African Americans and Caucasians in America, our patients had a lower rate of albuminuria (12.1% versus 28%). A recent study in Germany reported a higher rate of albuminuria (20%), but a lower rate of tubular proteinuria (41%) than that seen in our study (54.4%), as measured by their definition (α 1MCR >14 mg/g).³⁰ More studies using similar biomarkers and criteria of early renal dysfunction are needed. Additionally, because TDF only became a first-line drug to treat naive patients in China in 2012, future studies are required to determine its long-term toxicity in Chinese patients.

Conclusion

Urinary α 1M seems to be a promising biomarker for early detection of TDF-associated tubular dysfunction among HIV-infected MSM. Urinary β 2M may help indicate tubular dysfunction, but its clinical interpretation should also take inflammatory conditions into account. More studies will be necessary to address the clinical role of these biomarkers in diagnosing and monitoring TDF-associated tubular injury.

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Conflicts of interest

The authors declare no conflicts of interest.

REFERENCES

1. Calza L. Renal toxicity associated with antiretroviral therapy. *HIV Clin Trials*. 2012;13:189–211.
2. Scherzer R, Estrella M, Li Y, et al. Association of tenofovir exposure with kidney disease risk in HIV infection. *AIDS*. 2012;26:867–75.
3. Wever K, van Agtmael MA, Carr A. Incomplete reversibility of tenofovir-related renal toxicity in HIV-infected men. *J Acquir Immune Defic Syndr* (1999). 2010;55:78–81.
4. Peralta C, Scherzer R, Grunfeld C, et al. Urinary biomarkers of kidney injury are associated with all-cause mortality in the Women's Interagency HIV Study (WIHS). *HIV Med*. 2014;15:291–300.
5. O'Seaghdha CM, Hwang SJ, Larson MG, et al. Analysis of a urinary biomarker panel for incident kidney disease and clinical outcomes. *J Am Soc Nephrol*. 2013;24:1880–8.
6. Buchbinder SP, Glidden DV, Liu AY, et al. HIV pre-exposure prophylaxis in men who have sex with men and transgender women: a secondary analysis of a phase 3 randomised controlled efficacy trial. *Lancet Infect Dis*. 2014;14:468–75.
7. Islam FM, Wu J, Jansson J, Wilson DP. Relative risk of renal disease among people living with HIV: a systematic review and meta-analysis. *BMC Public Health*. 2012;12:234.
8. Danjuma MI, Mohamad-Fadzillah NH, Khoo S. An investigation of the pattern of kidney injury in HIV-positive persons exposed to tenofovir disoproxil fumarate: an examination of a large population database (MHRA database). *Int J STD AIDS*. 2014;25:273–9.
9. Labarga P, Barreiro P, Martin-Carbonero L, et al. Kidney tubular abnormalities in the absence of impaired glomerular function in HIV patients treated with tenofovir. *AIDS*. 2009;23:689–96.
10. Nishijima T, Shimbo T, Komatsu H, et al. Urinary beta-2 microglobulin and alpha-1 microglobulin are useful screening markers for tenofovir-induced kidney tubulopathy in patients with HIV-1 infection: a diagnostic accuracy study. *J Infect Chemother*. 2013;19:850–7.
11. Del Palacio M, Romero S, Casado JL. Proximal tubular renal dysfunction or damage in HIV-infected patients. *AIDS Rev*. 2012;14:179–87.
12. Shang H, Xu J, Han X, Spero Li J, Arledge KC, Zhang L. HIV prevention: bring safe sex to China. *Nature*. 2012;485:576–7.
13. Baral S, Sifakis F, Cleghorn F, Beyrer C. Elevated risk for HIV infection among men who have sex with men in low- and middle-income countries 2000–2006: a systematic review. *PLoS Med*. 2007;4:e339.
14. Zhang M, Chu Z, Wang H, Xu J, Lu C, Shang H. A rapidly increasing incidence of HIV and syphilis among men who have sex with men in a major city of China. *AIDS Res Hum Retroviruses*. 2011;27:1139–40.
15. Post FA, Wyatt CM, Mocroft A. Biomarkers of impaired renal function. *Curr Opin HIV AIDS*. 2010;5:524–30.
16. Campbell LJ, Dew T, Salota R, et al. Total protein, albumin and low-molecular-weight protein excretion in HIV-positive patients. *BMC Nephrol*. 2012;13:85.

17. Franco RA, Saag MS. When to start antiretroviral therapy: as soon as possible. *BMC Med.* 2013;11:147.
18. Ananworanich J, Avihingsanon A. HIV and noncommunicable diseases: the Asian perspective. *J Acquir Immune Defic Syndr (1999).* 2014;67 Suppl. 1:S99-103.
19. Oboho I, Abraham AG, Benning L, et al. Tenofovir use and urinary biomarkers among HIV-infected women in the Women's Interagency HIV Study (WIHS). *J Acquir Immune Defic Syndr (1999).* 2013;62:388-95.
20. Shlipak MG, Scherzer R, Abraham A, et al. Urinary markers of kidney injury and kidney function decline in HIV-infected women. *J Acquir Immune Defic Syndr (1999).* 2012;61:565-73.
21. Solomon MM, Lama JR, Glidden DV, et al. Changes in renal function associated with oral emtricitabine/tenofovir disoproxil fumarate use for HIV pre-exposure prophylaxis. *AIDS.* 2014;28:851-9.
22. Choi A, Scherzer R, Bacchetti P, et al. Cystatin C, albuminuria, and 5-year all-cause mortality in HIV-infected persons. *Am J Kidney Dis.* 2010;56:872-82.
23. Pannu N, Nadim MK. An overview of drug-induced acute kidney injury. *Crit Care Med.* 2008;36 4 Suppl.:S216-23.
24. Kabanda A, Vandercam B, Bernard A, Lauwerys R, van Ypersele de Strihou C. Low molecular weight proteinuria in human immunodeficiency virus-infected patients. *Am J Kidney Dis.* 1996;27:803-8.
25. Davey PG, Gosling P. beta 2-Microglobulin instability in pathological urine. *Clin Chem.* 1982;28:1330-3.
26. Penders J, Delanghe JR. Alpha 1-microglobulin: clinical laboratory aspects and applications. *Clin Chim Acta.* 2004;346:107-18.
27. Bethea M, Forman DT. Beta 2-microglobulin: its significance and clinical usefulness. *Ann Clin Lab Sci.* 1990;20:163-8.
28. Ronsholt FF, Ullum H, Katzenstein TL, Gerstoft J, Ostrowski SR. Persistent inflammation and endothelial activation in HIV-1 infected patients after 12 years of antiretroviral therapy. *PLoS ONE.* 2013;8:e65182.
29. Reynes J, Cournil A, Peyriere H, et al. Tubular and glomerular proteinuria in HIV-infected adults with estimated glomerular filtration rate ≥ 60 ml/min per 1.73 m². *AIDS.* 2013;27:1295-302.
30. Gravemann S, Brinkkoetter PT, Vehreschild JJ, et al. Low-grade proteinuria is highly prevalent in HIV-positive patients on antiretroviral treatment. *AIDS.* 2014;28:1783-9.