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Tenofovir Treatment Duration Predicts Proteinuria in a Multi-Ethnic United States Cohort of Children and Adolescents with Perinatal HIV-1 Infection

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Abstract

Background—Tenofovir is associated with renal proximal tubule injury. Such toxicity has not been extensively studied in HIV-1-infected children, in whom tenofovir is increasingly used.

Methods—History, urine and blood were collected at regular intervals from 448 children and adolescents with perinatal HIV-1 infection followed in the Pediatric HIV/AIDS Cohort study. Relationships between tenofovir use and proteinuria and chronic kidney disease (CKD) outcomes were examined using multivariable logistic regression models. Proteinuria was defined as at least one urine protein/creatinine ratio (uPCR) ≥0.2, and CKD as ≥2 sequential uPCR ≥0.2 or estimated glomerular filtration rates (eGFR) <60 mL/min/1.73 m^2 with no subsequent resolution, or a clinical diagnosis not contradicted by a normal uPCR. Subjects with ≥2 uPCR <0.2, and no abnormal uPCR and eGFR comprised the comparison group.

Results—Subjects were 47% male, 72% black, 24% Hispanic, with entry mean age (±standard deviation) of 11.5±2.5 years. Proteinuria prevalence at entry, and annually during 3 years, ranged from 10.3%–13.7%. The cumulative prevalence of proteinuria was 22% (94/434, 95% CI: 18%–26%) and CKD 4.5% (20/448, 95% CI: 2.7%–6.8%). Duration of tenofovir use was an independent predictor of proteinuria, with >3 years of exposure having the highest risk compared

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with no exposure (OR: 2.53, 95% CI: 1.23- 5.22, overall p=0.01). Overall, duration of tenofovir use did not significantly predict the presence of CKD.

Conclusions—Rates of proteinuria and CKD were lower than those seen in the pre-HAART era. However, prolonged exposure to tenofovir increases risk of renal injury.

Keywords

Tenofovir; proteinuria; chronic kidney disease; proximal tubules; nephrotoxicity; urine protein/creatinine ratio

Tenofovir disoproxil fumarate (tenofovir), in combination with other antiretroviral (ARV) drugs, is recommended as first-line therapy for HIV-1-infected adults [1]. Tenofovir has been increasingly used in ARV treatment-experienced children despite relatively limited evaluation in children and the initial lack of a pediatric formulation [2–4]. In 2010, the Food and Drug Administration (FDA) approved revised labeling for use in adolescents aged 12–18 years and a body weight ≥35 kilograms, and in 2012, tenofovir received FDA approval for use in HIV-1-infected children age ≥2 years, with availability of a powder formulation and lower dose pediatric tablets [5].

Clinical studies of tenofovir conducted on healthy HIV-infected adults and post-marketing data have demonstrated a generally favorable safety profile, with the major concern being nephrotoxicity [6–8]. The reported risk of significant tenofovir nephrotoxicity when estimated using grade 2 or higher elevations in serum creatinine or declines in estimated glomerular filtration rate (eGFR) among adults enrolled in clinical trials is 1%–2% [8–12]. Tenofovir exhibits toxicity for the proximal tubule, likely via deleterious effects on mitochondria [13, 14]. The proximal tubule is responsible for the reabsorption of approximately two-thirds of filtered sodium, a process that requires substantial mitochondrial ATP generation. The reabsorption of many other solutes, among them glucose, bicarbonate, and phosphate, are directly or indirectly coupled to transcellular sodium transport. The initial manifestations of tenofovir tubular toxicity that can be detected with commonly used clinical laboratory testing include glycosuria, phosphaturia, uricosuria, and low molecular weight proteinuria, which may be accompanied by reduced serum phosphate, urate, and bicarbonate concentrations, and can present as a partial or complete Fanconi syndrome [15]. In some cases tubular injury is also associated with reduced glomerular filtration rate (GFR) representing the clinical syndrome of acute kidney injury. If tenofovir therapy is continued, progressive proximal tubular injury leads eventually to tubular atrophy and tubulointerstitial fibrosis, accompanied by progressive reductions in GFR. Using composite endpoints defined by two or more earlier manifestations of tubular dysfunction, adult cohort studies have reported rates of renal tubular toxicity [16–18] ranging from 16.5% to 22%, which are higher than those reported based on serum creatinine or eGFR.

Several studies have described tenofovir nephrotoxicity in pediatric HIV infection [4, 19–23]. We describe the prevalence of proteinuria and chronic kidney disease in a large prospective cohort of children and adolescents with perinatal HIV infection in the highly active antiretroviral therapy (HAART) era, and the association with use of tenofovir.

METHODS

Study population

The Adolescent Master Protocol (AMP) of the Pediatric HIV/AIDS Cohort Study (PHACS) is an ongoing prospective cohort study designed to evaluate the impact of HIV-infection and

antiretroviral therapy on pre-adolescents and adolescents with perinatal HIV-infection. The study commenced March 2007 and recruited subjects from 15 clinical research sites within the United States. Subjects were eligible for enrollment into AMP if they were born to HIV-infected mothers, were age 7- <16 years at enrollment, and were previously enrolled in other pediatric longitudinal cohort studies or had complete medical history since birth, including details of ARV therapy use, HIV plasma RNA concentrations (viral load), and lymphocyte subsets. The AMP protocol was approved by the institutional review boards (IRB) at each participating site and at the Harvard School of Public Health. Written informed consent was obtained from each child's parent or legal guardian and assent obtained from child participants according to local IRB guidelines.

Study design and data collection

Medical history, ARV and other drug history and immunologic and virologic laboratory measurements were performed at study visits, abstracted from medical charts and obtained from databases of studies in which children were enrolled prior to entry into PHACS. Medication classified as nephrotoxic included certain antibiotics (gentamicin, tobramycin, amikacin, rifampicin, sulfadiazine, sulfamethoxazole), antifungals (amphotericin B, pentamidine), antivirals (acyclovir, cidofovir, adefovir), and nonsteroidal anti-inflammatory drugs (ibuprofen, naproxen).

Blood pressure was measured at each AMP study visit using an automated, non-invasive monitor with subjects in the sitting position; the average of at least two readings was standardized for sex, age, and height using methods outlined in the Fourth Report on the Diagnosis, Evaluation, and Treatment of High Blood Pressure in Children and Adolescents [24]. Body mass index (BMI) was calculated and expressed as z-scores, based on age and sex [25]. Serum creatinine was used to calculate eGFR using the Schwartz equation [26]. Fasting lipids, insulin, and glucose were measured on all children annually from the time of AMP entry. Random urine protein and creatinine measurements, obtained annually beginning with the AMP entry visit, were used to calculate urine protein/creatinine ratios (uPCR). These laboratory measurements were performed at each site's local laboratory.

Statistical analysis

The first outcome of interest was proteinuria defined as having at least one uPCR ≥0.2g/g; this was not required to be persistent. The second outcome was chronic kidney disease (CKD) defined by at least one of the following three criteria, whichever presented first:

1. Two or more annual sequential uPCR ≥0.2g/g, not followed by a uPCR <0.2 g/g (persistent proteinuria) or
2. A clinical diagnosis of CKD (such as chronic renal failure, nephropathy, nephritic syndrome) not contradicted by a normal uPCR (<0.2 g/g) or
3. Two or more annual sequential estimated glomerular filtration rates (eGFR) <60 mL/min/1.73 m^2.

Cases with either proteinuria or CKD were compared to the same control comparison group comprised of HIV-infected study subjects with neither proteinuria nor CKD. The normal kidney function comparison group was defined as children with two or more uPCR <0.2g/g, no uPCR ≥0.2g/g and all calculated eGFR ≥60 mL/min/1.73 m^2.

The primary predictor of interest was tenofovir use. For cases meeting the definition of proteinuria and CKD, current tenofovir use was defined as use at the time of becoming a case. For prevalent CKD cases, current use was defined as use at AMP study entry. For the comparison group, current tenofovir use was defined as use at the last visit with an uPCR

measurement. We also reviewed past history to determine whether participants had ever used tenofovir. “Ever tenofovir use” was defined as any use prior to becoming a case (including prior to AMP entry), or, for the comparison group, any use prior to the last visit with an uPCR measurement. Ever use therefore included cases of current tenofovir use. Duration of tenofovir use was calculated as the cumulative duration of tenofovir use until becoming a case, AMP entry, or the last visit with an uPCR measurement for new cases, prevalent cases, and comparison group members respectively.

Covariates included current age; sex; race; ethnicity; current and nadir CD4 count and CD4%; current and peak viral load; fasting total, HDL, and LDL cholesterol; fasting triglycerides; blood pressure; use of nephrotoxic medications; body mass index (BMI); fasting insulin; and fasting glucose. Covariates described as current are defined as the values at the time of becoming a case, or for those that did not become a case, the time of the last uPCR.

Univariable associations between tenofovir use and the other covariates with CKD and proteinuria were assessed using the Kruskal-Wallis tests for continuous parameters, and the Fisher’s exact test for categorical parameters. All univariable predictors at $p<0.10$ were included with tenofovir in final multivariable models for CKD and proteinuria respectively. The linearity assumption for duration of tenofovir was evaluated with subsequent categorization of duration into three groups (0 years, >0–3 years, >3 years of use). Statistical significance was defined as $p<0.05$. Analyses were conducted using SAS version 9.2 (SAS Institute, Cary, NC).

RESULTS

Of the 448 HIV-infected subjects with an entry visit, 209 (47%) were male, 321 (72%) were of black race and 109 (24%) were of Hispanic ethnicity. The mean age at entry into AMP was 11.5 (standard deviation, 2.5) years. Most of the 448 children were ARV treatment-experienced, with 85% on HAART at AMP entry, and had well-controlled HIV disease: 305 subjects (68%) had viral loads ≤400 copies/mL and 351 subjects (78%) had CD4 counts >500 cells/μl. One or more uPCR values were available for 434 subjects over a duration of three years; 94 (cumulative prevalence: 21.7%, 95% confidence interval [CI]: 17.9%, 25.8%) met the criterion (at least one uPCR ≥0.2 g/g) for proteinuria. Of these 94 subjects, eight had only one uPCR measurement; 32 had two uPCR measurements, of whom six had both uPCR values ≥0.2 g/g; 27 had three uPCR measurements, of whom 10 had two uPCR values ≥0.2 g/g and three had all three uPCR values ≥0.2 g/g; and 27 had four uPCR measurements, of whom four had two uPCR values ≥0.2 g/g and one had three uPCR values ≥0.2 g/g (see Fig., Supplemental Digital Content 1, http://links.lww.com/INF/B423). The annual prevalence of proteinuria was stable over follow-up and was 10.3%, 13.7%, 10.6% and 10.6% at entry, and year 1, 2, and 3 visits, respectively. There were 23 (5.3%) subjects who had at least one uPCR ≥0.5 g/g. CKD was identified by clinical diagnosis or laboratory finding (as defined above) in 20 out of the 448 AMP subjects with an entry visit (cumulative prevalence: 4.5%, 95% CI: 2.7%, 6.8%) and comprised the CKD group. There were 270 subjects who met study criteria for the comparison group. The median eGFR (range) at entry for this group was 152.5 (101.9, 490.8) mL/min/1.73 m^2.

Proteinuria group

Subjects in the proteinuria group were significantly younger than the comparison group (median age of 12.6 vs. 14.5 years respectively, $p<0.001$) but did not differ by sex, race or ethnicity (Table 1). The median eGFR (range) at entry was 151 (11.4, 371.7) mL/min/1.73 m^2. There were no significant differences in current or ever tenofovir use between the proteinuria cases and the comparison group in univariable analyses, but duration of

tenofovir, categorized into three groups, was significantly different between the case and comparison groups, with a higher percentage of cases having over three years of tenofovir exposure relative to the comparison group. The proteinuria group had a lower median fasting insulin than did the comparison group (7.0 vs.10.4 mU/mL, $p<0.01$). Otherwise, there was no difference between groups in growth and metabolic parameters or any of the HIV disease measures, including CD4 count, CD4%, or HIV viral load. In the final multivariable logistic models (Table 2) including age and fasting insulin, current or ever use of tenofovir were not significant predictors of proteinuria. However, duration of use of tenofovir was associated with proteinuria, with an over 2-fold increase in the odds of proteinuria (OR: 2.53, 95% CI: 1.23, 5.22, overall p=0.008) for greater than three years of use compared to no use of tenofovir. In a subset analysis involving subjects with at least three urine samples in which proteinuria was frequently transient or intermittent, duration of use of tenofovir remained predictive of proteinuria with an OR of 4.24 (95% CI: 1.75, 10.27, overall p=0.002) for greater than three years of use compared to no use of tenofovir.

Chronic kidney disease group

Of the 20 subjects in the CKD group, 12 met the definition based only on persistent proteinuria, four had a clinical diagnosis not contradicted by a normal uPCR, two had both a clinical diagnosis and persistent proteinuria, one had a clinical diagnosis with persistent proteinuria and eGFR values <60 mL/min/1.73 m^2, and one had a clinical diagnosis with eGFR values <60 mL/min/1.73 m^2. The clinical diagnoses were nephropathy in seven subjects and nephrotic syndrome in one subject. The median eGFR (range) at entry was 143.6 (11.4, 328.6) mL/min/1.73 m^2. One subject, with the lowest eGFR, was on hemodialysis. Current, ever, or duration of tenofovir use was not significantly different in the CKD group compared to the comparison group in univariable analyses (Table 1). There were also no differences between the two groups for demographic, immunologic, virologic, growth and metabolic factors, except for age (13.4 years CKD vs. 14.5 years comparison group, p=0.008) and total cholesterol >200 mg/dL (45% CKD vs. 16% comparison group, p=0.005). In multivariable logistic models (Table 2) including age, cholesterol and fasting insulin, tenofovir use defined either as current, ever, or overall duration of use did not significantly predict CKD. However there was an over 3-fold increased odds of CKD with > three years of tenofovir use compared to no use as indicated by the confidence interval (OR: 3.86, 95% CI: 1.06, 14.09). Of the six children with CKD on tenofovir at the time of becoming a case, one child subsequently discontinued it during the study period.

DISCUSSION

Previous studies have described a prevalence of proteinuria assessed by uPCR ranging from 21% to 33% in predominantly untreated children and adolescents with perinatal HIV-1 infection [27, 28], and of AIDS-related glomerulopathy in 8% to 29% of children with perinatal AIDS early in the HIV epidemic, prior to the development of ARV agents [29, 30]. Renal disease in these studies was associated with black race, advanced HIV disease and usually preceded or accompanied a diagnosis of HIV-associated nephropathy (HIVAN). In the present study, in which most of the children and adolescents were receiving HAART and had well-controlled HIV disease, the annual prevalence of proteinuria was markedly lower, but stable, ranging from 10.3% to 13.7% over the 3-year period, with a cumulative prevalence of 21.7%, and for CKD, a prevalence of 4.5%. In the general pediatric population, studies have shown that between 1% and 10% of non-diabetic children may have proteinuria on initial screening using urine dipstick protein testing, but that less than 1% have persistent proteinuria [31, 32]. The reduced prevalence of proteinuria and CKD in our cohort compared with previously-reported HIV-infected pediatric cohorts is probably explained by excellent virologic control of HIV resulting from the widespread use of

HAART, and is a reflection of the impact this has had over the last decade on the decline in the incidence of HIVAN [33, 34]. However, even in this well-treated cohort, proteinuria and CKD rates remain elevated above that observed in the general population.

Several studies have addressed rates of GFR impairment and proteinuria, assessed quantitatively, in children and adolescents with HIV infection. Soler-Palacin et al studied 40 Spanish subjects treated with tenofovir for a mean of 77 months and found reduced GFR in 18 subjects, transient in most, and proteinuria in 89% [22]. In contrast, Vigano et al studied 26 Italian subjects treated with tenofovir for 60 months and found a single instance of reduced GFR and no proteinuria [20]. Given these discrepancies, few conclusions about the role of tenofovir can be drawn from these uncontrolled studies. Two randomized controlled trials, carried out in Italy and Brazil have addressed the risks for reduced GFR or proteinuria of tenofovir therapy in children with HIV infection; these studies showed no increase in serum creatinine or proteinuria over 48 weeks in subjects receiving tenofovir compared to controls [3, 23]. Thus, the contribution of tenofovir to proteinuria and reduced GFR in children and adolescents has remained uncertain, and studies from a multi-ethnic United States cohort employing quantitative measures of proteinuria have been lacking.

For this analysis we chose two outcomes of interest: an inclusive outcome of at least one episode of proteinuria, and a more specific outcome of CKD. The proteinuria group was heterogeneous, with some subjects having only a single uPCR measurement performed that was abnormal, while others (apart from those who met the criteria for CKD, who were also part of this group) had two or three abnormal uPCR values, either preceded, interspersed, or followed by a normal uPCR. This outcome was based on the premise that pathologic proteinuria can be intermittent especially in mild disease, or when a nephrotoxic agent is discontinued. Further, tubular proteinuria, an indicator of proximal tubular injury, is found in most patients with demonstrated toxicity secondary to tenofovir as part of a partial or complete Fanconi syndrome, and that this can be adequately assessed using uPCRs [35].

Comparing these outcome groups to a population with no indication of proteinuria, we found that there was a duration effect of tenofovir exposure on proteinuria, with > three years of exposure significantly increasing the risk of proteinuria. In contrast, Kelly et al reported a 29%, and Scherzer et al a 34% increased risk for proteinuria with each year of tenofovir use in adults [36, 37]. Prolonged treatment with tenofovir can result in progressive tubular damage leading to an eventual decline in GFR [38], supporting the association of proteinuria with duration of treatment with tenofovir. Recent adult literature further suggests that tenofovir-related renal toxicity may not always be reversible [36, 39, 40], and that markers of renal disease can persist well beyond the immediate period following drug discontinuation.

There are overlapping features that make it difficult to distinguish CKD attributable to drug toxicity from chronic glomerular disease complicating HIV, such as HIVAN and immune complex glomerulonephritis. Moreover although the incidence of HIVAN has declined in the HAART era [33, 34], it has been described even in well controlled HIV-infected children and adolescents [41]. Therefore the lack of a statistically significant association of tenofovir with CKD may be explained by the fact that some of the cases of CKD we identified may be of glomerular origin, or alternatively, by the much smaller number of CKD cases compared to proteinuria cases, resulting in loss of power to detect a difference.

The present study has a number of limitations. Examining the association of tenofovir with other features of proximal tubular dysfunction and Fanconi syndrome that may be perturbed in tenofovir -related toxicity, such as serum and urine phosphate, uric acid and urine glucose was not possible, as these laboratory evaluations were not part of the study-required tests in

the AMP cohort. Collecting consistent first morning void samples instead of urine specimens obtained at the clinic visit would have been ideal, as it would have eliminated other causes of proteinuria, such as postural and exercise-induced proteinuria. However, it has been shown that random uPCR values correlate well with timed measures of protein excretion, are a reliable predictor of progression of renal disease, have been validated as a means of quantifying proteinuria in HIV-infected children, and constitute a practical and reliable predictor of progression of renal disease [35]. Another limitation of our study is the potential for exposure misclassification for the prevalent proteinuria and CKD outcomes for which the relative timing of exposure and outcome are unclear. However the inclusion of prevalent cases increases power to evaluate potential associations for safety concerns.

This study also has important strengths. The cohort size makes it the largest pediatric study of tenofovir renal outcomes that assessed urine protein quantitatively, and included a control comparison group. The multi-ethnic population, including a majority of black children, extends the findings of prior pediatric studies. Study centers were widely distributed across the United States, and thus the practice patterns and the renal outcomes probably reflect the status of pediatric HIV medicine during the time that study was conducted.

In conclusion, we have shown that the longer duration of tenofovir use is associated with proteinuria in children and adolescents with perinatal HIV infection. Our findings support the current pediatric clinical practice guideline recommending that a spot uPCR be performed every 6–12 months to screen for renal dysfunction in patients receiving tenofovir [1]. With increasing use of tenofovir in this population, careful attention to monitoring for early signs of renal toxicity with uPCR is important. Longer follow-up will provide a more complete assessment of the long-term effects of tenofovir on kidney function in ARV-exposed children and adolescents.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Characteristics of subjects in the proteinuria, chronic kidney disease and comparison groups.

Characteristic	Control Comparison Group (N=270)	Chronic Kidney Disease Cases[1] (N=20)	P-Value[2]	Proteinuria Cases[3] (N=94)	P-Value[2]
Antiretroviral therapy parameters					
Current[4] tenofovir use – N (%)	95 (35)	6 (30)	0.81	24 (26)	0.10
Ever tenofovir use – N (%)	110 (41)	7 (35)	0.81	30 (32)	0.14
Duration of tenofovir use (years) – Median (Q1, Q3) N (%)	0.0 (0.0, 2.0)	0.0 (0.0, 2.7)	0.83	0.0 (0.0, 2.1)	0.43
0	160 (59)	13 (65)		64 (68)	
>0–3	69 (26)	2 (10)	0.21	9 (10)	0.002
>3	41 (15)	5 (25)		21 (22)	
Demographics					
Current[4] Age (years) – Median (Q1, Q3)	14.5 (12.0, 16.5)	13.4 (10.4, 14.5)	0.008	12.6 (10.2, 14.5)	<0.001
Sex – N (%)					
Male	126 (47)	8 (40)	0.65	43 (46)	0.90
Female	144 (53)	12 (60)		51 (54)	
Race – N (%)					
White/Other	59 (22)	2 (10)	0.26	24 (26)	0.57
Black	193 (71)	18 (90)		67 (71)	
Missing	18 (7)	0 (0)		3 (3)	
Ethnicity – N (%)					
Non-Hispanic	202 (75)	18 (90)	0.18	70 (74)	0.89
Hispanic	67 (25)	2 (10)		24 (26)	
Missing	1 (0)	0 (0)		0 (0)	
Metabolic parameters (Current[4])					
Fasting Insulin (mμ/mL) – Median (Q1, Q3)	10.4 (6.0, 17.8)	8.3 (5.6, 10.6)	0.05	7.0 (3.7, 10.8)	<0.001

Characteristic	Control Comparison Group (N=270)	Chronic Kidney Disease Cases[1] (N=20)	P-Value[2]	Proteinuria Cases[3] (N=94)	P-Value[2]
Fasting Glucose (mg/dL) – Median (Q1, Q3)	81.0 (75.0, 87.0)	79.0 (73.0, 83.5)	0.34	81.0 (75.0, 85.0)	0.56
Total Cholesterol >200 mg/dL – N (%)	43 (16)	9 (45)	0.005	21 (22)	0.21
LDL cholesterol >130 mg/dL – N (%)	23 (9)	4 (20)	0.10	13 (14)	0.16
Triglycerides >110 mg/dL (age <10 years) or > 150 mg/dL (age ≥10 years) – N (%)	47 (17)	6 (30)	0.24	20 (21)	0.44
HDL cholesterol <35 mg/dL – N (%)	18 (7)	2 (10)	0.65	9 (10)	0.36
Nephrotoxic medications[5] – N (%)	51 (19)	1 (5)	0.14	15 (16)	0.64
Blood pressure ≥90th percentile – N (%)	36 (13)	1 (5)	0.49	14 (15)	0.86
Growth parameters (Current[4])					
Body Mass Index – N (%)					
Underweight (<5th percentile)	9 (3)	1 (5)	0.40	6 (6)	0.31
Healthy weight (5– <85th percentile)	174 (65)	16 (80)		66 (70)	
Overweight (85– <95th percentile)	44 (16)	2 (10)		11 (12)	
Obese (≥95th percentile)	42 (16)	1 (5)		11 (12)	
Missing	1 (0)	0 (0)			
Laboratory parameters					
Current[4] CD4 (cells/cubic mm)					
<200	16 (6)	0 (0)	0.78	2 (2)	0.24
200–350	18 (6)	1 (5)		4 (4)	
351–499	37 (14)	4 (20)		9 (10)	
≥500	199 (74)	15 (75)		79 (84)	
Nadir CD4 (cells/cubic mm)					
<200	70 (26)	5 (25)	0.84	24 (26)	0.46
200–350	72 (27)	6 (30)		20 (21)	

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Characteristic	Control Comparison Group (N=270)	Chronic Kidney Disease Cases[1] (N=20)	P-Value[2]	Proteinuria Cases[3] (N=94)	P-Value[2]
351–499	66 (24)	6 (30)		21 (22)	
≥500	62 (23)	3 (15)		29 (31)	
Current[4] CD4%					
<15	21 (8)	0 (0)		2 (2)	
15–24	40 (15)	3 (15)	0.63	17 (18)	0.12
≥25	209 (77)	17 (85)		75 (80)	
Nadir CD4%					
<15	95 (35)	10 (50)		34 (36)	
15–24	113 (42)	8 (40)	0.30	38 (41)	0.96
≥25	62 (23)	2 (10)		22 (23)	
Current[4] HIV Viral Load (copies/mL)					
≤400	186 (69)	12 (60)	0.46	66 (70)	0.90
>400	84 (31)	8 (40)		28 (30)	
Peak HIV Viral Load (copies/mL)					
≤10,000	8 (3)	1 (5)		4 (4)	
10,001–100,000	58 (21)	5 (25)	0.54	20 (21)	0.78
>100,000	204 (76)	14 (70)		70 (75)	

[1] Chronic kidney disease (CKD) defined as either: A) Two or more sequential urine protein/creatinine ratios (uPCR) ≥0.2g/g not followed by a uPCR <0.2 g/g **OR** B) A clinical diagnosis of CKD not contradicted by normal uPCR (such as: chronic renal failure, nephropathy, nephrotic syndrome, segmental glomerulosclerosis), **OR** C) Two or more sequential eGFR<60 mL/min/1.73 m^2, whichever came first.

[2] Kruskal-Wallis p-value for continuous parameters, Fisher's exact p-value for categorical parameters. P-values compare cases to control comparison group. P-values compare non-missing values.

[3] Proteinuria defined as having at least one uPCR≥0.2g/g.

[4] Current defined as the measurement at the last follow-up visit. For CD4 and HIV Viral Load measures, current was defined as the closest measurement within 1.5 years prior to or at the last follow-up visit. For incident cases, the last follow-up visit was the visit at which they met the case definition. For prevalent cases, the last follow-up visit was defined as the AMP entry visit. For controls, the last follow-up visit was the last visit with an uPCR measurement.

[5] Nephrotoxic medications included (1) antibiotics (gentamicin, tobramycin, amikacin, rifampicin, sulfadiazine, sulfamethoxazole); (2) antifungals (amphotericin B, pentamidine); (3) non-ARV antivirals (acyclovir, cidofovir, adefovir); (4) nonsteroidal anti-inflammatory drugs (NSAIDs) (ibuprofen, naproxen).

Table 2

Multivariable results of tenofovir use with proteinuria and chronic kidney disease

	Chronic Kidney Disease			Proteinuria		
Characteristic	**N[1]**	**Multivariable Odds Ratio (95% CI)[2]**	**P-Value**	**N[3]**	**Multivariable Odds Ratio (95% CI)[4]**	**P-Value**
Current tenofovir (use vs. no use)	274	1.46 (0.49, 4.36)	0.50	344	0.98 (0.54, 1.79)	0.94
Ever tenofovir (use vs. no use)	274	1.68 (0.57, 4.97)	0.35	344	1.27 (0.70, 2.28)	0.43
Duration of tenofovir use	274		0.08	344		0.01
>0–3 vs. 0 years		0.74 (0.15, 3.63)			0.62 (0.28, 1.40)	
>3 vs. 0 years		3.86 (1.06, 14.09)			2.53 (1.23, 5.22)	

[1] Comparison and chronic kidney disease groups combined, with 16 of the comparison group missing values (12 missing both total cholesterol and insulin, 1 missing total cholesterol, and 3 missing insulin values);

[2] Included age, insulin, and total cholesterol;

[3] Comparison and proteinuria groups combined, with 20 missing insulin values (15 from the comparison group, 5 from the proteinuria group);

[4] Included age and insulin.