

# Plasma Tenofovir Concentrations and Proximal Tubular Dysfunction in HIV-Infected Adults Receiving Tenofovir in Thailand

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## Abstract

**Background:** HIV-infected Asians may be at risk for tenofovir toxicity due to their relatively low body weight (BW). We assessed the prevalence of proximal tubular dysfunction (PRTD) and risk associated with PRTD in HIV-infected adults receiving tenofovir in Thailand.

**Methods:** A cross-sectional study in HIV-infected adults ( $\geq 18$  years) treated with tenofovir for  $>1$  year. Twenty-four-hour urine samples were collected to assess PRTD. PRTD was defined as the presence of  $>2$  of the following criteria: hyper-phosphaturia (total excretion of phosphate  $>1200$  mg/day or renal tubular reabsorption of phosphate (TmP/GFR)  $< 2.6$  mg/dl), hyper-uricosuria (FE of uric acid  $>15\%$ ), or non-diabetic glucosuria. Mid-dose tenofovir plasma concentrations were determined and concentrations  $>160$  ng/mL were used as a cut-off for assessing risk of PRTD.

**Results:** 351 subjects (52% males) with median age of 40.2 years, BW of 58.9 kg, and duration of tenofovir treatment 4.7 years were included. 93% had a HIV-1 RNA  $<50$  copies/mL and 7% were co-infected with HCV. Fifty-four (15.4%) patients were diagnosed with PRTD. In a multivariate analysis, only a mid-dose tenofovir concentration  $>160$  ng/mL was associated with PRTD [odds ratio: OR 2.02 (95% CI 1.13-3.66)]; 32 of 54 (59.2%) with PRTD had a tenofovir concentration  $>160$  ng/mL; versus 124 of 297 patients (41.7%) without PRTD ( $p = 0.02$ ). Predictors of a tenofovir concentration  $>160$  ng/mL were BW  $<55$  kg [OR 2.32 (95% CI 1.45-3.68)], chronic HCV [OR 2.64 (95% CI 1.13-6.17)], eGFR  $<90$  mL/min/1.73m<sup>2</sup> [OR 2.66 (95% CI 1.64-4.31)], lopinavir/ritonavir [OR 2.47 (95% CI 1.32-4.6)] and PRTD [OR 2.08 (95% CI 1.10-3.92)].

**Conclusion:** Mid-dose tenofovir concentrations  $>160$  ng/mL were independently associated with PRTD. Tubular function should be closely monitored in patients using tenofovir with BW  $<55$  kg, lopinavir/ritonavir use, chronic hepatitis C, or low eGFR.

**Keywords:** Plasma tenofovir concentration; PRTD; Low BW; Hepatitis C; Low eGFR; Lopinavir/ritonavir; Asian

## Introduction

Tenofovir disoproxil fumarate (TDF) is a nucleotide reverse transcriptase inhibitor (N (t) RTI) that has activity against both HIV and hepatitis B virus (HBV). Because of its high efficacy, once-daily dose, and availability within several fixed-dose combination tablets, TDF is one of the most widely used ARV drugs as part of combination antiretroviral treatment (cART). TDF is a prodrug of tenofovir (TFV) that is rapidly hydrolyzed to tenofovir monophosphate in the blood, and then converted to its active tenofovir diphosphate form intracellularly. TFV is primarily eliminated unchanged by a combination of glomerular filtration and proximal tubular secretion [1]. Tenofovir clearance is impaired in patients with abnormal renal function and its accumulation inside the tubular cells may result in further renal damage [2]. Tenofovir is generally well-tolerated but several degrees of renal toxicity, including acute tubular necrosis, nephritis, proximal tubulopathy, proteinuria, diabetes insipidus, hypokalemia and subclinical proximal renal tubular dysfunction (PRTD) has been reported [3-14]. In clinical trials, the risk of clinically significant TDF related renal toxicity is relatively low (0.3-2% Fanconi syndrome) [15-17]. However, trial participants may not represent HIV patients at large as they are generally in good health and have fulfilled strict inclusion criteria.

Several studies conducted in HIV-infected patients found that older age, presence of metabolic disease and concomitant use of TDF and protease inhibitor are associated with an increased risk of tubular dysfunction [18-21]. Recently, several pharmacogenomics studies have reported that PRTD in HIV-infected patients receiving tenofovir were associated with certain genetic polymorphisms within genes of several efflux transporters (ABCC2 or ABCC4 or ABCC10) [22-24]. Higher plasma concentration of tenofovir has also been recently associated with proximal tubular dysfunction [10,25]. Even though the isolated presentation of tubular dysfunction such as hypophosphatemia, tubular proteinuria, normoglycemic glycosuria, and a decrease in phosphate

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reabsorption has been associated with tubular cell damage but the clinical significance of isolated or subclinical tubular dysfunction in the short- and long- term remains largely unclear whether these particular patients are at increased risk for developing Fanconi syndrome, progressive alteration of eGFR, osteomalacia or reduced bone mass density. However, PRTD may indeed contribute to the persistent loss of the phosphate and subsequent prematuration of osteopenia/osteoporosis. Clinical research on tubular function is challenging because of the complexity of kidney exploration. Few studies have assessed prospectively the tubular function of HIV infected patients, and have shown that approximately 6.5-22% of HIV-infected patients may have subclinical PRTD [10,26]. There are limited data on the prevalence of PRTD of HIV infected patients from Asia whom generally have lower body weight than those in resource-rich countries.

In South East Asia, TDF is now widely used for HIV treatment with increased usage of TDF, many patients with low BW and/or pre-existing renal disease may exhibit clinical symptoms of TDF nephrotoxicity later in life. The studies from Thailand, Vietnam, and Japan in HIV-infected patients with median BW of 55-63 kg showed that the high prevalence of TDF-related renal dysfunction (14.4%-19.6%) was strongly associated with low BW [27-30]. Since TDF-related renal toxicity is linked to an increased plasma concentration, possibly in a dose-dependent manner, Therefore, the Asian population with small body weight is expected to be at higher risk of developing TDF-related renal toxicity [27,28,30,31]. Thus, it is important to understand the relationship between plasma tenofovir concentrations and factors associated with tubular dysfunction among Asian HIV-infected patients. It is also critical to identify subpopulations at risk of developing long-term TDF nephrotoxicity. As a result of this, we assessed the prevalence of PRTD and risk factors associated with proximal tubular dysfunction among HIV-infected patients receiving TDF containing antiretroviral therapy within a large HIV cohort in Thailand.

## Methods

### Study population

HIV infected patients participating in a cohort study (clinicaltrials.gov identifier: NCT 00411983) at the HIV Netherlands Australia Thailand Research collaboration (HIV-NAT), Bangkok, Thailand, were included in this analysis. This sub study was reviewed and registered by the Institutional Review Boards of the Faculty of Medicine, Chulalongkorn University (NCT01138241). All patients provided a written consent. A total of 351 patients treated with TDF-containing cART with a normal estimated glomerular filtration rate (eGFR > 90 ml/min/1.73m<sup>2</sup>) underwent a 24-hour urinary analysis for assessment of proximal tubular dysfunction. Plasma samples were collected "mid-dose (11-13 hours post dose)" to measure the tenofovir plasma concentration. Patients were excluded if they were currently on nephrotoxic agents such as pentamidine, ganciclovir, amphotericin B, adefovir, cidofovir or indinavir. Patient characteristics were extracted from the HIV-NAT 006 cohort database including sex, age, route of HIV transmission, HBV and/or HCV co-infection, hypertension, diabetes, clinically defined lipodystrophy, ARVs, duration of ARVs and concomitant drugs. Chronic hepatitis B (Hep B) and hepatitis C (Hep C) infection were defined as having a two positive tests (i.e., Hep B S antigen (HBsAg or anti-HCV antibody, respectively) more than 6 months apart.

### Assessment of proximal tubular function

Blood and 24-hour urine samples were used to assess the kidney

glomerular and tubular parameters. All patients were required to fast for at least 8 hours before blood samples were collected. Written and verbal instructions for urine collection were provided along with the container. Urine was collected over a 24-hour period, which also included the samples from the morning of the blood draw. Various multi-parameter blood chemistry and hematology profiles were assessed, such as glucose, creatinine, sodium, potassium, chloride, bicarbonate, phosphorus, total calcium, uric acid, albumin, and total protein. Urine pH, glucose, creatinine, phosphorus, uric acid, albumin, proteins,  $\beta$ 2 microglobulin, white and red blood cells in urine was assessed. All parameters for renal proximal tubular function were performed at the laboratory of the Division of Nephrology, Chulalongkorn University.

PRTD was defined on the basis of the presence of at least two of the following criteria [10]:

1. Fractional tubular absorption for phosphorus [ $1 - \{(\text{urine phosphorus} \times \text{plasma creatinine}) / (\text{plasma phosphorus} \times \text{urine creatinine})\}$ ] less than 0.80.
2. Total daily excretion of phosphorus (urine phosphorus x urine volume) more than 1200 mg.
3. Fractional excretion of uric acid [ $1 - \{(\text{urine uric acid} \times \text{plasma creatinine}) / (\text{plasma uric acid} \times \text{urine creatinine})\} \times 100$ ] more than 15%.
4.  $\beta$ 2 microglobulin more than 1 mg/day or  $\beta$ 2 microglobulin/urinary creatinine more than 0.3 mg/l.
5. Non-diabetic glucosuria (urine glucose > 300 mg/day or positive for urine glucose) with normal glycemic levels (plasma glucose < 100 mg/dl).

Immunonephelometry (BN II DADE, Behring, Barcelona, Spain) was used to assess urine  $\beta$ 2 microglobulin.

The eGFR was calculated using the Modification of Diet in Renal Disease (MDRD) equation [32].

### Assessment of tenofovir plasma concentrations

Tenofovir plasma concentrations were measured using a validated reversed-phase high-performance liquid chromatography (HPLC) method with fluorescent detection. This method was validated using the AIDS Clinical Trials Group (ACTG) method validation guidelines over the concentration range of 15 to 1,500 ng/mL [33]. Average accuracy for tenofovir was 99-102% and precision (inter- and intra- assay) was <5% of the coefficient of variation (CV). Samples were assayed at the PHPT-IRD laboratory at Chiang Mai University which participates in the ACTG Pharmacology Quality Control program, which performs standardized inter-laboratory testing twice a year [34].

### Statistical analysis

Continuous values are presented as median (interquartile range: IQR) and categorical data as percentages. A multivariable analysis using logistic regression models evaluated factors associated with PRTD. A mid-dose plasma tenofovir concentration cut-off of 160 ng/mL was used as a threshold to test for an association with PRTD [25]. Variables obtained from the univariate analysis with a p-value < 0.10 were included in the full model. A stepwise selection procedure was used to assess the relative role of the risk factors. P-values of <0.05 were considered significant. All statistical analyses were carried out using STATA version 12.0 (StataCorp, College Station, TX, USA)

## Results

### Characteristics of the study patients

Baseline characteristic of study participants are presented in Table 1. Between April 2011 and August 2012, 351 TDF-treated HIV-1-infected patients (52% male) were enrolled (Table1). Median age was 40.2 (IQR 36.3-45.6) years. Body weight (BW) was 58.9 (IQR 52-67.5) kg, 19% and 4% of patients had a body mass index (BMI) > 25 kg/m<sup>2</sup> or <18 kg/m<sup>2</sup>, respectively, and 21% were ARV-naïve at the time TDF was initiated. Median duration of TDF use was 4.7 (IQR 2.9-5.4) years for ARV-naïve patients and 2.9 (IQR 1.8-4.9) years for ARV-experienced patients. CD4 cell count was 554 (IQR428-749) cells/mm<sup>3</sup> and 93% had a HIV RNA <50 copies/mL. Eight percent of patients had previously used indinavir boosted with ritonavir (IDV/r), with a median duration of 2.2 (IQR0.9-5.8) years exposure; 53% of the patients were co-treated with a NNRTI (42% on efavirenz (EFV) and 11% on nevirapine (NVP)), and 47% with a ritonavir boosted protease inhibitor (boosted PI; 17% atazanavir/ritonavir (ATV/r), 17% lopinavir/ritonavir (LPV/r), 10% saquinavir/ritonavir (SQV/r), and 3% darunavir/ritonavir (DRV/r)). Chronic HBV and HCV were identified in 22% and 7% of the patients, respectively. Nine percent and 3% of the patients had hypertension and diabetes mellitus, respectively.

### Proximal renal tubular function

Fifty-four (15.4%) patients were diagnosed with PRTD. A higher percentage of patients with PRTD had previous IDV exposure (14.8 % versus 7.1%), current ATV exposure (27.7% versus 15.5%), boosted PI exposure (59.3% versus 45.1%), hypertension (14.8% versus 7.4%), higher serum creatinine levels and lower median eGFR (86.8 versus 98.5 ml/min/1.73m<sup>2</sup>). Overall, the median eGFR was 97.8 (IQR 84.9-108.1) ml/min/1.73m<sup>2</sup> and 29 (8.3%) patients had serum phosphorus

< 3 mg/dl. Median eGFR for TDF ARV-naïve and TDF-switch subjects were not significantly different (99.1 (86.9-107.8) and 97.4 (84.2-108.1) ml/min/1.73m<sup>2</sup>, respectively (p = 0.39) and the prevalence of PRTD was comparable between the two groups (13.9% versus 15.8%, p = 0.69).

### Tenofovir plasma concentrations

Median tenofovir plasma concentrations were 168 (IQR 120-198) ng/mL and 149 (IQR 120-193) ng/mL for patients with and without PRTD, respectively (Figure1, Table1). Overall, 156 (44.4%) patients had tenofovir plasma concentrations > 160 ng/ml (a reported threshold to predict PRTD) and among these patients, 32 of 54 (59.2%) had PRTD; versus 124 of 297 patients (41.7%) without PRTD (p = 0.02).

### Risk factors associated with kidney tubular dysfunction

In a univariate analysis, previous IDV exposure, current ATV exposure, hypertension and a TDF plasma concentration >160 ng/mL were associated with PRTD (Table 2). However, in the multivariate analysis, only a tenofovir plasma concentration >160 ng/mL remained significantly associated with PRTD (OR 2.02, 95% CI 1.13-3.66, p = 0.02). Low body weight, chronic HCV, lopinavir/r use and duration of TDF were not associated with PRTD.

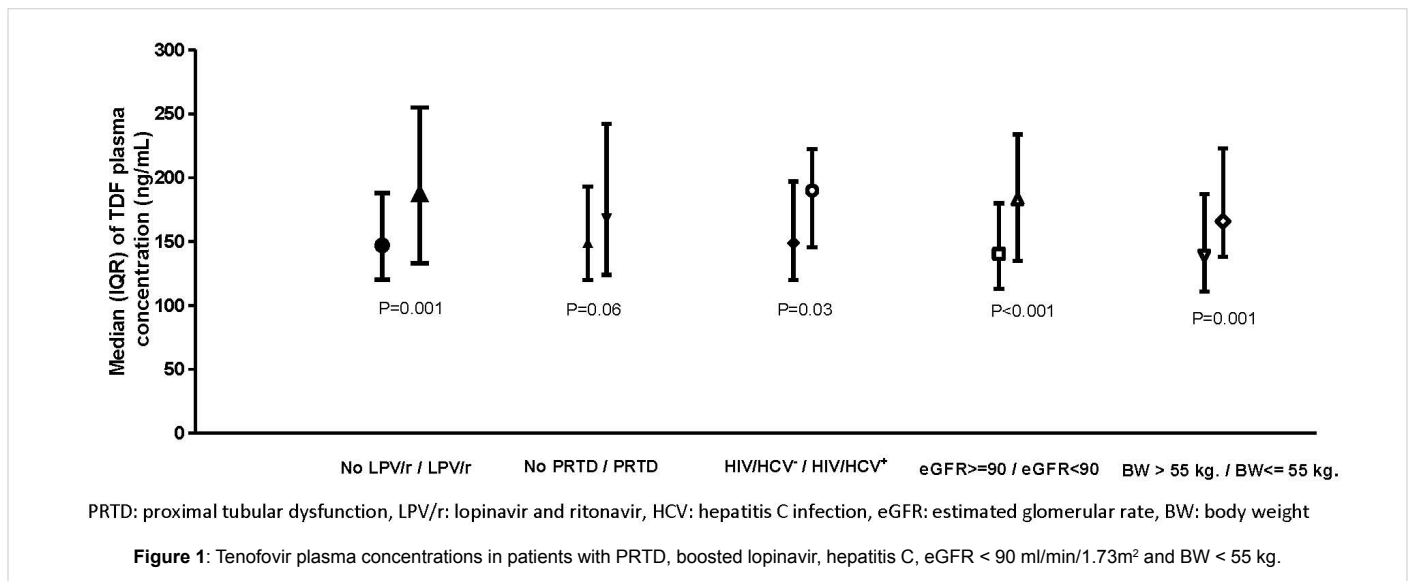
### Risk factors for tenofovir plasma concentrations >160 ng/mL

In univariate analysis, body weight < 55 kg, LPV/r, chronic HCV, eGFR < 90 ml/min/1.73m<sup>2</sup>, duration of ART per 5 years, and PRTD were associated with higher tenofovir plasma concentrations >160ng/mL (Table 3). In multivariate analysis; BW < 55 kg [OR 2.19 (95% CI 1.37-3.50), p = 0.001], chronic HCV [OR 3.63(95% CI 1.18-11.18, p = 0.024)], eGFR < 90 ml/min/1.73m<sup>2</sup> [OR 2.56 (95% CI 1.58-4.16), p < 0.001], LPV/r [OR 2.47 (95% CI 1.32-4.6), p = 0.004], and PRTD [OR 1.98 (95% CI 1.05-3.73), p = 0.03] were associated with plasma

	Total N = 351	PRTD N = 54	Non PRTD N = 297	p-value
Sex: Male N (%)	184 (52.4)	31 (57.4)	153 (51.5)	0.43
Age (years), median (IQR)	40.2 (36.3-45.6)	40.6 (37.9-47.4)	40.2 (35.9-45.3)	0.16
BW (kg), median (IQR)	58.9 (52-67.5)	60.2 (53-68.1)	58.5 (52-67.5)	0.22
Duration of ART exposure (month)	7.1 (4.6-10.3)	9.1 (4.7-11.9)	6.8 (4.6-10.2)	0.31
TDF exposure, years				
ARV-naïve	4.7 (2.9-5.4)	4.2 (2.8-6.0)	4.7 (2.9-5.4)	0.74
ARV-experienced	2.9 (1.8-4.9)	2.8 (1.5-4.7)	2.9 (1.8-4.9)	0.33
Indinavir exposure N (%)	29 (8.3)	8 (14.8)	21 (7.1)	0.06
Atazanavir exposure, N (%)	61 (17.3)	15 (27.7)	46 (15.5)	0.03
Stavudine exposure, N (%)	198 (56.4)	29 (53.7)	169 (56.9)	0.66
Current ARV regimen N (%)				0.06
NNRTI	185 (52.7)	22 (40.7)	163 (54.9)	
Boosted PI	166 (47.3)	32 (59.3)	134 (45.1)	
Current CD4 cell count (cells/mm <sup>3</sup> ), median (IQR)	554 (428-749)	538 (429-730)	554 (428-749)	0.53
Proportion with HIV RNA < 50 copies/mL, N (%)	327 (93.1)	52 (96.3)	275 (92.5)	0.32
Hypertension, N (%)	30 (8.6)	8 (14.8)	22 (7.4)	0.07
Diabetes Mellitus, N (%)	9 (2.6)	1 (1.9)	8 (2.7)	0.72
Lipodystrophy, N (%)	133 (37.9)	14 (25.9)	119 (40.1)	0.05
Chronic HBV, N (%)	76 (21.6)	10 (18.5)	66 (22.2)	0.54
Chronic HCV, N (%)	26 (7.4)	2 (3.7)	24 (8.1)	0.26
Median plasma creatinine, mg/dl, median (IQR)	0.8 (0.7-0.9)	0.9 (0.8-1)	0.8 (0.7-0.9)	0.001
Median eGFR (ml/min/1.73m <sup>2</sup> )	97.8 (84.9-108.1)	86.8 (75.4-100.5)	98.5 (85.6-109.3)	0.002
Plasma TDF concentrations: ng/ml	152 (120-198)	168 (124-242)	149 (120-193)	0.06
Proportion of patients with plasma TDF >160ng/ml, N (%)	156 (44.4)	32 (59.2)	124 (41.7)	0.02

Data is expressed as median and interquartile range (IQR). TDF: Tenofovir Disoproxil Fumarate; BW: Body Weight; BMI: Body Mass Index; NNRTI: Non-Nucleoside Reverse Transcriptase; PI: Protease Inhibitor; ART: Antiretroviral Therapy; eGFR: Estimated Glomerular Filtration Rate; HBV: Hepatitis B; HCV: Hepatitis C; PRTD: Proximal Renal Tubular Dysfunction

**Table 1:** Baseline characteristics of the study population with PRTD and without PRTD.



			Univariate analysis		Multivariate analysis	
	PRTD	No PRTD	OR (95%CI)	p	OR (95%CI)	p
Indinavir exposure (%)						
No	46 (14.2)	276 (85.8)	1	0.07		
Yes	8 (27.6)	21 (72.4)	2.29 (0.96-5.46)			
Atazanavir exposure (%)						
No	39 (13.4)	251 (86.6)	1	0.03		
Yes	15 (24.5)	46 (75.5)	2.1 (1.07-4.11)			
Other boosted PI (maily lopinavir/r (%)						
No			1	0.27		
Yes	20 (13)	134 (87)	1.39 (0.71- 2.54)			
Hypertension, n (%)						
No	46 (14.3)	275 (85.7)	1	0.09		
Yes	8 (26.6)	22 (73.4)	2.17 (0.91-5.17)			
Chronic HCV (%)						
No	52 (16)	273 (84)	1	0.22		
Yes	2 (7.7)	24 (92.3)	0.44 (0.1-1.90)			
TDF plasma concentrations						
<160 ng/ml	23 (11.6)	175 (88.4)	1	0.02	1	0.02
>160 ng/ml	31 (20.3)	122 (79.7)	2.02 (1.13-3.66)		2.02 (1.13-3.66)	

Age, sex, body weight, duration of ART, duration of tenofovir, diabetes mellitus, and chronic hepatitis B were not associated with PRTD.

HCV: Hepatitis C; HBV: Hepatitis B; OR: Odd Ratio; 95% CI: 95% Confidential Interval; TDF: Tenofovir Disoproxil Fumarate; PRTD: Proximal Renal Tubular Dysfunction; boosted PI: Protease Inhibitor

**Table 2:** Predictors of PRTD in HIV-1 infected Thai patients.

concentrations of tenofovir >160ng/mL. Age, sex, duration of ART, duration of tenofovir, diabetes mellitus, hypertension and chronic hepatitis B were not associated with tenofovir concentrations >160ng/mL.

## Discussion

In this study, 15.4% of 351 TDF-treated patients had subclinical PRTD. We found that: 1) patients with PRTD had higher tenofovir plasma concentrations compared to those without (168 ng/mL versus 149 ng/mL); 2) tenofovir plasma concentrations of >160 ng/mL was associated with PRTD; 3) BW < 55 kg, eGFR < 90 ml/min/1.73m<sup>2</sup>, chronic HCV, PRTD, and use of LPV/r were associated with high tenofovir plasma concentrations. The overall prevalence of PRTD in TDF-treated patients (15.4%) in this study is relatively lower when compared to the study from Spain (22%) and higher than study from Italy (Aquitaine cohort) (6.5%) and the United States (7.6%) [10,26,35]. Our findings are in agreement with prior studies performed in Europe which reported

that high tenofovir plasma concentrations were associated with PRTD [25,31]. In the current study, tenofovir plasma concentrations were 2.5-fold higher in patients with eGFR < 90 ml/min/1.73m<sup>2</sup> and 2-fold higher in patients with subclinical PRTD. Likewise, patients with plasma tenofovir concentrations >160ng/mL were associated with a 2-fold higher risk of developing PRTD, consistent with previous findings showing tenofovir plasma concentrations was independently associated with tubular dysfunction [25,31,36]. It has been theorized that high intracellular concentrations of tenofovir can disrupt the mitochondrial function and damage the proximal tubular cells [37]. The higher the tenofovir plasma concentrations were, the greater the accumulation of tenofovir in the proximal renal tubular cells. This can subsequently result in having a higher risk for tubular cell dysfunction. In this study, we observed that patient with low eGFR had significantly higher plasma tenofovir concentrations and patients with PRTD had lower eGFR than patients without PRTD. Because tenofovir is secreted via glomerular filtration and proximal tubular secretion, thus when the

	TDF plasma concentration >160ng/ml		Univariate analysis		Multivariate analysis	
	Yes	NO	OR (95%CI)	p	OR (95%CI)	p
Gender						
Male	73 (39.7)	111 (60.3)	1	0.06		
Female	84 (50.3)	83 (49.7)	1.5 (0.98-2.29)			
BW						
Weight > 55kg	81 (38.2)	131 (61.8)	1	0.004	1	0.001
Weight ≤ 55kg	75 (54)	64 (46)	1.9 (1.23- 2.92)		2.19 (1.37-3.50)	
BW/creatinine			0.96 (0.95-.97)	<0.001		
boosted PI						
No	60 (39)	94 (61)	1	0.07		
Yes	96 (48.7)	101 (51.3)	1.49 (0.97-2.28)			
LPV/r						
No	118 (40.3)	175 (59.7)	1	0.001	1	0.004
Yes	38 (65.5)	20 (34.5)	2.82 (1.56-5.08)		2.47 (1.32-4.60)	
Chronic HCV (%)						
No	145 (43.2)	190 (56.8)	1	0.05	1	0.02
Yes	11 (68.7)	5 (31.3)	2.88 (0.98-8.48)		3.63 (1.18-11.18)	
eGFR , ml/min/1.73m <sup>2</sup>						
>90	86 (37.1)	146 (62.9)	1	<0.001	1	<0.001
≤90	70 (58.8)	49 (41.2)	2.49 (1.54-3.81)		2.56 (1.58-4.16)	
PRTD						
No	124 (41.7)	173 (58.3)	1	0.02	1	0.03
Yes	32 (59.3)	22 (40.7)	2.02 (1.13-3.66)		1.98 (1.05-3.73)	

Age, duration of ART, duration of tenofovir, diabetes mellitus, chronic hepatitis B, and hypertension were not associated with tenofovir concentrations >160ng/mL. HCV: Hepatitis C; HBV: Hepatitis B; OR: Odd Ratio; 95% CI: 95% Confidential Interval; TDF: Tenofovir Disoproxil Fumarate; LPV/r: Lopinavir/Ritonavir; PRTD: Proximal Tubular Dysfunction; BW: Body Weight; BMI: Body Mass Index; PI: Protease Inhibitor; eGFR: Estimated Glomerular Filtration Rate

**Table 3:** Predictors for plasma tenofovir concentration >160ng/ml in HIV-1 infected Thai adults..

patients have tubular dysfunction/low eGFR, this will possibly cause higher plasma tenofovir concentrations [1,2]. Therefore, it is possible that PRTD could be caused by cumulative exposure to tenofovir. On the other words, PRTD with low renal function can possibly cause high plasma tenofovir concentrations. Our results support data that the effect of tenofovir on PRTD is dose-dependent. However, in this study we could not confirm whether high plasma concentration is mainly caused by PRTD or it is directly caused by low renal clearance.

Three studies from Asia evaluated the risk of developing TDF nephrotoxicity in HIV infected patients with median BW of 55-63 kg. They found that lower body weight was significantly associated with TDF related nephrotoxicity [28-30]. This is consistent with our findings that plasma tenofovir concentrations were 2.2-fold higher among patients with BW < 55 kg. Higher tenofovir plasma concentrations in patients with low BW < 55 kg would lead to further renal damage. In addition, this study found that BW/serum creatinine (Scr) ratio was negatively correlated with tenofovir plasma concentrations (Correlation Coefficient = -0.28; P < 0.001) which was consistent with the findings reported by Rodriguez-Novoa et al. and Calcagno et al. in which tenofovir plasma concentrations decreased as the BW/Scr ratio increased [25,38].

However it is unclear why HCV co-infection was strongly correlated with higher tenofovir plasma concentrations in our study. Patients co-infected with HIV and HCV had higher tenofovir plasma concentrations [median 190 (IQR 146-223) ng/mL] compared to those with HIV mono-infection [median 146 (IQR 120-197) ng/mL] whereas only 2 out of 54 (4%) patients co-infected with HIV/HCV had PRTD. In addition, HCV was strongly associated with higher tenofovir plasma concentration (3.6-fold). Currently, pharmacokinetic data of TDF in HIV and HCV co-infection is largely unknown. Since TDF is not a substrate of CYP450 enzyme, HCV co-infection is not expected to influence the plasma concentrations of tenofovir. There is a higher risk of renal impairment in HIV and hepatitis B co-infection with advanced liver fibrosis (fibrosis score >F3). However, in our study, 20% of the

patients co-infected with HIV and HCV had advanced liver fibrosis (fibrosis score > 9.5kPa) based on transient elastography scores. Median plasma tenofovir concentrations did not differ between those with or without advanced liver fibrosis (191 ng/mL versus 136 ng/mL; p = 0.15). In addition, advanced liver fibrosis was not significantly associated with lower eGFR and higher tenofovir plasma concentrations. Liver damage can lead to impaired blood flow and production of toxins that can have a detrimental effect on kidney function. However, this study did not show any correlation between liver fibrosis and eGFR which may be due to its relatively small sample size. Thus, we cannot ascertain what factors are causing the patients co-infected with HCV to have high TDF plasma concentrations.

Although almost half of the study population (48%) was female, we did not find any association between female sex and high tenofovir plasma concentrations. This finding disagrees with a previous Spanish pharmacokinetic study conducted in 2009 which reported that the female sex was associated with high tenofovir plasma concentrations [25]. Other studies also failed to find an association of sex on tenofovir plasma concentrations. Recently, Gervasoni et al evaluated tenofovir plasma concentrations among 22 post-menopausal and 28 pre-menopausal women from Italy and found that tenofovir plasma concentrations were not difference between pre- and post-menopausal women [39].

Several studies conducted in Caucasians found an association between high plasma tenofovir concentrations with unboosted or boosted PI and a higher risk of developing renal toxicity when compared to EFV, NVP or RAL [21,39-43]. Our results did not observe a correlation of boosted PI and tubular dysfunction, but LPV/r use was associated with higher tenofovir plasma concentrations (2.5 fold). No correlation between ATV/r use and plasma tenofovir concentrations was observed, unlike those patients on unboosted ATV from Calcagno's study which found an association with the high tenofovir plasma concentration [38]. As our ATV/r patients were using ATV/ 200/100 mg once daily, we speculate that receiving a lower dose may lower the

impact on inhibition of multiple resistance protein (MRP) transporters at the apical side of renal tubular cells, and consequently less tenofovir intra-tubular accumulation.

Our study has several limitations. Firstly, due to the nature of the cross-sectional design, we could not foresee whether high plasma tenofovir concentrations and PRTD will predict future renal impairment. Secondly, only patients with eGFR > 60 ml/min/1.73m<sup>2</sup> were enrolled and approximately 50% of patients using tenofovir at our research center were included in this analysis so it is possible that patients with more serious renal toxicity were excluded. Thirdly, most of the patients on boosted ATV were on ATV/r 200/100 mg once daily instead of the standard dose of ATV/r 300/100 mg once daily. Hence we cannot draw strong conclusion whether boosted ATV/r use is associated with high tenofovir plasma concentration. Lastly, this study had only a single tenofovir plasma measurement at 12-hours post-drug administration. Oral apparent clearance of TDF was not determined. High intra-individual variation of tenofovir clearance have been reported in Caucasians, but little is known of tenofovir clearance in Asians [44].

In conclusion, mid-dose tenofovir concentrations >160 ng/mL was independently associated with PRTD. Low BW, chronic HCV, low eGFR, LPV/r use and PRTD were associated with higher tenofovir plasma concentration and tubular function should be closely monitored in these patients. In addition, the association of higher plasma tenofovir concentrations and PRTD need to be further explored in a large prospective cohort to determine whether this association is linked to rapid renal deterioration in the future. Furthermore, lower dosing strategies of TDF in patients at highest risk of TDF associated renal toxicity should be further explored.

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#### Author Contributions

AA, DB, TRC, JS, KP, YA and KR designed the study. AA, RAR and SP conducted the study. JS performed all statistical analysis. NT performed all basic laboratory tests such as the multi-parameter blood chemistry and hematology profiles. KP and YA performed all renal proximal tubular function. TRC performed all PK tests. All authors finalized the manuscript.

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#### Conflict of Interest

RAR is supported by the ART AIDS Foundation. All other authors declare no conflict of interest.

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#### References

- Goicoechea M, Liu S, Best B, Sun S, Jain S, et al. (2008) Greater tenofovir-associated renal function decline with protease inhibitor-based versus non-nucleoside reverse-transcriptase inhibitor-based therapy. *J Infect Dis* 197: 102-108.
- Pavie J, Scemla A, Bouldouyre MA, Pillebout E, Verine J, et al. (2011) Severe acute renal failure in an HIV-infected patient after only 2 weeks of tenofovir-based antiretroviral therapy. *AIDS Patient Care STDS* 25: 457-460.
- Earle KE, Seneviratne T, Shaker J, Shoback D (2004) Fanconi's syndrome in HIV+ adults: report of three cases and literature review. *J Bone Miner Res* 19: 714-721.
- de la Prada FJ, Prados AM, Tugores A, Uriol M, Saus C, et al. (2006) Acute renal failure and proximal renal tubular dysfunction in a patient with acquired immunodeficiency syndrome treated with tenofovir. *Nefrologia* 26: 626-630.
- Cicconi P, Bongiovanni M, Melzi S, Tordato F, d'Arminio Monforte A, et al. (2004) Nephrolithiasis and hydronephrosis in an HIV-infected man receiving tenofovir. *Int J Antimicrob Agents* 24: 284-285.
- Campbell LJ, Ibrahim F, Fisher M, Holt SG, Hendry BM, et al. (2009) Spectrum of chronic kidney disease in HIV-infected patients. *HIV Med* 10: 329-336.
- Breton G, Alexandre M, Duval X, Prie D, Peytavin G, et al. (2004) Tubulopathy consecutive to tenofovir-containing antiretroviral therapy in two patients infected with human immunodeficiency virus-1. *Scand J Infect Dis* 36: 527-528.
- Blaas S, Schneidewind A, Glück T, Salzberger B (2006) Acute renal failure in HIV patients with liver cirrhosis receiving tenofovir: a report of two cases. *AIDS* 20: 1786-1787.
- Kinai E, Hanabusa H (2009) Progressive renal tubular dysfunction associated with long-term use of tenofovir DF. *AIDS Res Hum Retroviruses* 25: 387-394.
- Labarga P, Barreiro P, Martin-Carbonero L, Rodríguez-Novoa S, Solera C, et al. (2009) Kidney tubular abnormalities in the absence of impaired glomerular function in HIV patients treated with tenofovir. *AIDS* 23: 689-696.
- Peyrière H, Reynes J, Rouanet I, Daniel N, de Boever CM, et al. (2004) Renal tubular dysfunction associated with tenofovir therapy: report of 7 cases. *J Acquir Immune Defic Syndr* 35: 269-273.
- Barrios A, García-Benayas T, González-Lahoz J, Soriano V (2004) Tenofovir-related nephrotoxicity in HIV-infected patients. *AIDS* 18: 960-963.
- Gaspar G, Monereo A, García-Reyne A, de Guzmán M (2004) Fanconi syndrome and acute renal failure in a patient treated with tenofovir: a call for caution. *AIDS* 18: 351-352.
- Karras A, Lafaurie M, Furco A, Bourgarit A, Droz D, et al. (2003) Tenofovir-related nephrotoxicity in human immunodeficiency virus-infected patients: three cases of renal failure, Fanconi syndrome, and nephrogenic diabetes insipidus. *Clin Infect Dis* 36:1070-1073.
- Izzedine H, Hulot JS, Vittecoq D, Gallant JE, Staszewski S, et al. (2005) Long-term renal safety of tenofovir disoproxil fumarate in antiretroviral-naïve HIV-1-infected patients. Data from a double-blind randomized active-controlled multicenter study. *Nephrol Dial Transplant* 20: 743-746.
- Nelson MR, Katlama C, Montaner JS, Cooper DA, Gazzard B, et al. (2007) The safety of tenofovir disoproxil fumarate for the treatment of HIV infection in adults: the first 4 years. *AIDS* 21: 1273-1281.
- Jones R, Stebbing J, Nelson M, Moyle G, Bower M, et al. (2004) Renal dysfunction with tenofovir disoproxil fumarate-containing highly active antiretroviral therapy regimens is not observed more frequently: a cohort and case-control study. *J Acquir Immune Defic Syndr* 37: 1489-1495.
- Fux CO M, Cavassimi M (2009) TDF and protease inhibitor use are associated with an increased prevalence of proximal renal tubular dysfunction in the Swiss HIV cohort study. Program and abstracts of the 16th Conference on Retroviruses and Opportunistic Infections. Montreal, CA, Feb 8-11.
- Kearney BP, Flaherty JF, Shah J (2004) Tenofovir disoproxil fumarate: clinical pharmacology and pharmacokinetics. *Clin Pharmacokinet* 43: 595-612.
- Kiser JJ, Aquilante CL, Anderson PL, King TM, Carten ML, et al. (2008) Clinical and genetic determinants of intracellular tenofovir diphosphate concentrations in HIV-infected patients. *J Acquir Immune Defic Syndr* 47: 298-303.
- Kiser JJ, Carten ML, Aquilante CL, Anderson PL, Wolfe P, et al. (2008) The effect of lopinavir/ritonavir on the renal clearance of tenofovir in HIV-infected patients. *Clin Pharmacol Ther* 83: 265-272.
- Rodríguez-Nóvoa S, Labarga P, Soriano V, Egan D, Albalater M, et al. (2009) Predictors of kidney tubular dysfunction in HIV-infected patients treated with tenofovir: a pharmacogenetic study. *Clin Infect Dis* 48: e108-116.
- Nishijima T, Komatsu H, Higasa K, Takano M, Tsuchiya K, et al. (2012) Single nucleotide polymorphisms in ABCB2 associate with tenofovir-induced kidney tubular dysfunction in Japanese patients with HIV-1 infection: a pharmacogenetic study. *Clin Infect Dis* 55: 1558-1567.
- Pushpakom SP, Liptrott NJ, Rodríguez-Nóvoa S, Labarga P, Soriano V, et al. (2011) Genetic variants of ABCB10, a novel tenofovir transporter, is associated with kidney tubular dysfunction. *J Infect Dis* 204: 145-153.
- Rodríguez-Nóvoa S, Labarga P, D'Avolio A, Barreiro P, Albalater M, et al. (2010) Impairment in kidney tubular function in patients receiving tenofovir is associated with higher tenofovir plasma concentrations. *AIDS* 24: 1064-1066.

26. Dauchy FA, Lawson-Ayayi S, de La Faille R, Bonnet F, Rigotherier C, et al. (2011) Increased risk of abnormal proximal renal tubular function with HIV infection and antiretroviral therapy. *Kidney Int* 80: 302-309.
27. Nishijima T, Kawasaki Y, Tanaka N, Mizushima D, Aoki T, et al. (2014) Long-term exposure to tenofovir continuously decreases renal function in HIV-1-infected patients with low body weight: results from 10 years of follow-up. *AIDS* 28: 1903-1910.
28. Nishijima T, Komatsu H, Gatanaga H, Aoki T, Watanabe K, et al. (2011) Impact of small body weight on tenofovir-associated renal dysfunction in HIV-infected patients: a retrospective cohort study of Japanese patients. *PLoS One* 6: e22661.
29. Chaisiri K, Bowonwatanuwong C, Kasettratrat N, Kiertiburanakul S (2010) Incidence and risk factors for tenofovir-associated renal function decline among Thai HIV-infected patients with low-body weight. *Curr HIV Res* 8: 504-509.
30. Mizushima D, Tanuma J, Dung NT, Dung NH, Trung NV, et al. (2014) Low body weight and tenofovir use are risk factors for renal dysfunction in Vietnamese HIV-infected patients. A prospective 18-month observation study. *J Infect Chemother* 20: 784-788.
31. Ezinga M, Wetzels JF, Bosch ME, van der Ven AJ, Burger DM (2014) Long-term treatment with tenofovir: prevalence of kidney tubular dysfunction and its association with tenofovir plasma concentration. *Antivir Ther* 19: 765-771.
32. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, et al. (1999) A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. *Ann Intern Med* 130: 461-470.
33. Delahunty T, Bushman L, Robbins B, Fletcher CV (2009) The simultaneous assay of tenofovir and emtricitabine in plasma using LC/MS/MS and isotopically labeled internal standards. *J Chromatogr B Analyt Technol Biomed Life Sci* 877: 1907-1914.
34. Diane T Holland, Robin DiFrancesco, Judith Stone, Fayez Hamzeh, James D Connor, et al. (2004) Quality assurance program for clinical measurement of antiretrovirals: AIDS clinical trials group proficiency testing program for pediatric and adult pharmacology laboratories. *Antimicrob Agents Chemother* 48: 824-831.
35. Horberg M, Tang B, Towner W, Silverberg M, Bersoff-Matcha S, et al. (2010) Impact of tenofovir on renal function in HIV-infected, antiretroviral-naive patients. *J Acquir Immune Defic Syndr* 53: 62-69.
36. Poizot-Martin I, Solas C, Allemand J, Obry-Roguet V, Pradel V, et al. (2013) Renal impairment in patients receiving a tenofovir-cART regimen: impact of tenofovir trough concentration. *J Acquir Immune Defic Syndr* 62: 375-380.
37. Herlitz LC, Mohan S, Stokes MB, Radhakrishnan J, D'Agati VD, et al. (2010) Tenofovir nephrotoxicity: acute tubular necrosis with distinctive clinical, pathological, and mitochondrial abnormalities. *Kidney Int* 78: 1171-1177.
38. Calcagno A, Gonzalez de Requena D, Simiele M, D'Avolio A, Tettoni MC, et al. (2013) Tenofovir plasma concentrations according to companion drugs: a cross-sectional study of HIV-positive patients with normal renal function. *Antimicrob Agents Chemother* 57: 1840-1843.
39. Gervasoni C, Meraviglia P, Landonio S, Riva A, Galli M, et al. (2013) Tenofovir plasma concentrations in post-menopausal versus pre-menopausal HIV-infected women. *J Antimicrob Chemother* 68: 1206-1207.
40. Mocroft A, Kirk O, Reiss P, De Wit S, Sedlacek D, et al. (2010) Estimated glomerular filtration rate, chronic kidney disease and antiretroviral drug use in HIV-positive patients. *AIDS* 24: 1667-1678.
41. Scherzer R, Estrella M, Li Y, Choi AI, Deeks SG, et al. (2012) Association of tenofovir exposure with kidney disease risk in HIV infection. *AIDS* 26: 867-875.
42. Ryom L, Mocroft A, Kirk O, Worm SW, Kamara DA, et al. (2013) Association between antiretroviral exposure and renal impairment among HIV-positive persons with normal baseline renal function: the D:A:D study. *J Infect Dis* 207: 1359-1369.
43. Young J, Schäfer J, Fux CA, Furrer H, Bernasconi E, et al. (2012) Renal function in patients with HIV starting therapy with tenofovir and either efavirenz, lopinavir or atazanavir. *AIDS* 26: 567-575.
44. Gagnieu MC, Barkil ME, Livrozet JM, Cotte L, Mialhes P, et al. (2008) Population pharmacokinetics of tenofovir in AIDS patients. *J Clin Pharmacol* 48: 1282-1288.