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ORIGINAL RESEARCH

Urine assay for tenofovir to monitor adherence in real time to tenofovir disoproxil fumarate/emtricitabine as pre-exposure prophylaxis

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Objectives

Tenofovir disoproxil fumarate/emtricitabine (TDF/FTC) is approved for pre-exposure prophylaxis (PrEP) against HIV infection. Adherence is critical for the success of PrEP, but current adherence measurements are inadequate for real-time adherence monitoring. We developed and validated a urine assay to measure tenofovir (TFV) to objectively monitor adherence to PrEP.

Methods

We developed a urine assay using high-performance liquid chromatography coupled to tandem mass spectrometry with high sensitivity/specificity for TFV that allowed us to determine TFV concentrations in $\log_{10}$ categories between 0 and 10 000 ng/mL. We validated the assay in three cohorts: (1) HIV-positive subjects with undetectable viral loads on a TDF/FTC-based regimen, (2) healthy HIV-negative subjects who received a single dose of TDF/FTC, and (3) HIV-negative subjects receiving daily TDF/FTC as PrEP for 24 weeks.

Results

The urine assay detected TFV with greater sensitivity than plasma-based measures and with a window of measurements within 7 days of the last TDF/FTC dose. Based on the urine log-linear clearance after the last dose and its concordance with all detectable plasma levels, a urine TFV concentration > 1000 ng/mL was identified as highly predictive of the presence of TFV in plasma at > 10 ng/mL. The urine assay was able to distinguish high and low adherence patterns within the last 48 h (> 1000 ng/mL versus 10–1000 ng/mL), as well as nonadherence (< 10 ng/mL) extended over at least 1 week prior to measurement.

Conclusions

We provide proof of concept that a semiquantitative urine assay measuring levels of TFV could be further developed into a point-of-care test and be a useful tool to monitor adherence to PrEP.

Keywords: adherence, HIV prevention, pre-exposure prophylaxis, Truvada, urine tenofovir assay

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Introduction

Pre-exposure prophylaxis (PrEP) has the potential for tremendous public health benefit through decreasing the still high incidence of new HIV infections. Tenofovir disoproxil fumarate/emtricitabine (TDF/FTC) used as PrEP is at least 90% effective in preventing HIV infection when taken daily [1,2], and up to 99% effective when subjects are taking seven daily doses per week [3]. PrEP is recommended in the USA by the Centers for Disease Control and Prevention [4], and by the World Health Organization globally [5], as a powerful tool for millions of individuals

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at substantial risk for HIV infection. Adherence to PrEP is critical for its success, but unfortunately we do not have adequate methods to monitor adherence in real time. Self-report and pill counts are unreliable methods for monitoring adherence [6,7], particularly in populations at high risk of acquiring HIV infection, such as young men of colour who have sex with men (yMSMc) [8].

How to accurately monitor and identify suboptimal adherence to PrEP, and develop strategic interventions to maintain adherence levels necessary for effectiveness, is a key gap in implementing this otherwise highly effective prevention therapy [6,7]. Therapeutic drug monitoring has been useful for assessment of adherence in other fields, specifically adherence to psychiatric medications, treatment for substance abuse disorders, and treatment for improved blood pressure control in patients with resistant hypertension [9–12]. Furthermore, behavioural changes are maximized when feedback is made available close to the behaviour that needs modification [13,14].

With regard to TDF/FTC, previously published means of measuring medication levels in patients receiving PrEP [dried blood spot (DBS) or hair analysis] require sample collection procedures that may not be acceptable to patients outside of clinical trials [15,16], are associated with delays in reporting that prevent implementation of timely effective interventions, and provide information that does not reflect recent PrEP use. Plasma tenofovir (TFV) concentrations have been measured in clinical trials but can only provide information about adherence in the last 24–36 h [17]. DBS has been used for adherence monitoring and typically provides an average adherence over the preceding 3 months [18–20], although preliminary data suggest that FTC measurement in DBS can be a marker of recent dosing [21]. A 3-month window would be useful in the HIV treatment setting, but may be less relevant during PrEP administration where temporal adherence with regard to exposure may be more critical.

Alternative adherence strategies have been proposed, including intermittent PrEP (IPERGAY) timed around exposure periods [22] and prevention-effective adherence where PrEP is used only during periods of risk exposure to maximize prevention effectiveness and minimize unnecessary risk of medication toxicity and cost at times of decreased risk [23,24]. In addition, self-reported adherence to PrEP has correlated more closely with drug levels in open-label studies such as the PROUD study, where all 52 subjects who reported taking PrEP had detectable TFV levels in plasma [3,25,25]. Thus, for the clinician evaluating PrEP use in patients, the key question is to identify real-time lapses in adherence that would leave the user at a higher risk for infection during periods of risk.

Finally, any test intended to be used for repeated monitoring in adolescents and young adults, who have the fastest rising incidence rates for HIV infection, would have to be highly acceptable to this population; youth and young adults have been shown to prefer non-blood draw or needle-requiring assays in HIV testing (50.5% chose rapid oral swab, 30.3% chose traditional venipuncture and 19.2% chose rapid fingerstick blood test) [26], although preferences may differ when monitoring adherence to PrEP. A urine-based test would address many of these concerns.

The goal of this study was to develop and validate a urine assay to measure the concentration of TFV (the active metabolite of the prodrug TDF) in order to objectively monitor adherence to PrEP in a clinical setting.

Methods

Novel urine TFV assay

We developed a high-performance liquid chromatography–tandem mass spectrometry (LC-MS/MS) plasma and urine assay with high sensitivity and specificity for TFV. This assay allowed us to determine urine TFV concentrations in $\log_{10}$ categories between 0 and > 10 000 ng/mL (i.e. 0, 10–100, 100–1000, > 1000 ng/mL). The assay was developed with modifications to a previously reported method [27,28]. Following protein precipitation (0.1 mL human plasma or urine or a diluted urine sample) using 100% acetonitrile with 0.1% formic acid, containing deuterated internal standard ($^2H_6$-tenofovir; 50 ng/mL), the analytes were separated using the gradient mobile phase on a Kinetex PFP (Phenomenex) column (2.6 μm; 4.6 × 100 mm) and analysed by MS/MS (AB Sciex API4000; Foster City, CA). The multiple reaction monitoring of m/z 288.3–176.3 and 288.3–159.2 (sum of multiple ions) for TFV and m/z 294.3–182.2 for $^2H_6$-tenofovir was used for analysis.

Study design

We conducted three sequential cohort studies to validate the assay. The sample size for each cohort was 10 subjects, for a total of 30 subjects across three cohorts. Urine samples were first-catch samples collected at any time of day, and plasma and urine samples collected were approximately 1.5 mL.

The first cohort study was a qualitative and semiquantitative evaluation of the relationship of urine TFV to plasma TFV in 10 HIV-positive subjects who reported excellent adherence to antiretroviral therapy, with undetectable HIV viral loads on a TDF/FTC-based regimen.

Subjects kept a diary of the date/time of antiretroviral therapy (ART) doses for the previous week. For all 10 subjects, plasma and urine samples were collected at a single time-point within a 4-week window after an undetectable HIV viral load. Data were compared to determine the positive and negative predictive values of the presence of TFV in urine using plasma TFV as the gold standard.

The second cohort study was a quantitative evaluation of TFV clearance in plasma and urine over 7 days in 10 HIV-negative subjects who received a single dose of TDF/FTC under direct observation. This would allow us to estimate the relationship between the level of detectable TFV in urine and the interval (of up to 7 days) since the last administration of oral TDF/FTC.

The third cohort study was a 24-week pilot study of 10 HIV-negative individuals on daily TDF/FTC for PrEP wherein urine TFV levels were assessed weekly (as the test provides adherence information over a 1-week period) to obtain a comprehensive view of adherence during the study period, and were used together with monthly plasma TFV levels to determine the concordance between plasma and urine TFV levels. At each study visit, the date and time of the last TDF/FTC dose were collected by self-report. We also administered acceptability surveys to all subjects in this cohort at the beginning and end of the 24-week period.

Study participants

Recruitment, enrollment, and study visits were conducted at Philadelphia FIGHT, a community-based organization that provides comprehensive care to patients living with and at risk for HIV infection (http://fight.org/). Adults (≥ 18 years of age) followed at Philadelphia FIGHT who were HIV-positive with undetectable HIV viral loads in the previous 4 weeks on a TDF/FTC-based regimen were eligible for inclusion in cohort 1. HIV-negative adults with normal baseline creatinine clearance were eligible for inclusion in cohort 2. HIV-negative adults prescribed TDF/FTC as PrEP for HIV prevention at FIGHT's youth clinic (the Youth Health Empowerment Project) were eligible for inclusion in cohort 3. Subjects were recruited through word of mouth and advertising on social media sites. Written informed consent was obtained from all subjects with the use of approved consent forms. This study was approved by the Institutional Review Boards at Philadelphia FIGHT and the University of Pennsylvania.

Results

This study was conducted from January 2014 to December 2014. The measurement of plasma and urine TFV was found to be linear over the range of 10–1000 ng/mL with the limit of detection (LOD) of 5 ng/mL. The total chromatographic run-time was 5.0 min for each sample. The LOD for TFV in human plasma and urine samples was determined and samples were analysed quantitatively to show whether TFV was present or absent in clinical plasma and urine samples. Calibration curves were calculated for human urine and found to be highly accurate between 10 and 1000 ng/mL, with an accuracy range of 93.6–100% (r^2 = 0.9962) (Fig. 1). When the concentration of TFV in urine samples exceeded 1000 ng/mL, samples were diluted 20- or 50-fold with a control sample of urine (from someone not taking TDF) and reanalysed.

Cohort 1: detection of urine TFV corresponds to plasma levels within a 24-h dosing window

In our first cohort, 10 HIV-positive patients with high adherence to a single-tablet HIV regimen containing TFV (as measured by having an undetectable viral load within 4 weeks of study testing) were asked to provide a single plasma and urine sample within 12–24 h of their last

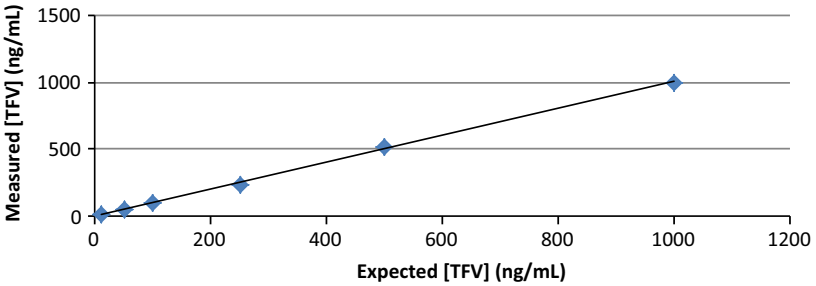

Fig. 1 Tenofovir (TFV) calibration curve in human urine, which was found to be highly accurate over a concentration range of 10–1000 ng/mL, with an accuracy range of 93.6–100% (r^2 = 0.9962). A known concentration of TFV was injected into a human urine sample (*x*-axis) and measured using liquid chromatography–tandem mass spectrometry (LC-MS/MS) (*y*-axis). The measurement of urine TFV was found to be linear over the range of 10–1000 ng/mL with the limit of detection (LOD) of 5 ng/mL. TFV, tenofovir.

dose of medication (see Table 1 for subject characteristics). We observed 100% concordance between the presence of TFV in plasma and urine [positive predictive value (PPV) 100% (95% CI: 0.63–1.0); negative predictive value (NPV) 100% (95% CI: 0.05–1.0)]. The TFV concentration was 3–4 $\log_{10}$ higher in urine than in plasma. Surprisingly, subject 4 had an undetectable viral load yet had no detectable TFV in blood or urine. Follow-up inquiry identified that this subject had stopped taking his ART shortly after his viral load had been collected yet within 4 weeks of sampling for this study (Fig. 2).

Cohort 2: urine TVF concentration predictive of when last dose of TDF/FTC was taken

In our second cohort, we evaluated the kinetics of TVF elimination in plasma or urine over 7 days after a single oral administration of TDF/FTC in 10 healthy subjects (see Table 1 for subject characteristics). Decay rates for signal were tested after a single dose of TDF/FTC to evaluate TFV clearance in plasma and urine (Fig. 3). In this study, TFV was detected for > 7 days in urine and 2–4 days in plasma after a single dose of TDF/FTC. Urine TFV was cleared in a log-linear fashion, with a direct correlation of change in urine levels to time since last dose. The urine assay was 2 $\log_{10}$ more sensitive than serum over 7 days. When TFV was detected in plasma (typically 2-3 days post-dose), urine TFV concentration was > 1000 ng/mL in most subjects, suggesting that a urine tenofovir concentration of > 1000 ng/mL may be predictive of having taken TDF/FTC within the last 2–3 days from sampling.

Table 1 Subject characteristics

Characteristic	Cohort 1 (n = 10)	Cohort 2 (n = 10)	Cohort 3 (n = 10)
Age (years)			
Mean	43.2	37.2	20.4
Range	22–53	25–53	18–23
Sex (n)			
Male	4	8	10
Female	5	2	0
Transgender (M to F)	1	0	0
Race (n)			
African American	7	3	9
Caucasian	3	6	0
Other	0	1	1
HIV status (%)			
HIV-positive	100	0	0
ART regimen			
Atripla	7	NA	NA
Stribild	2		
Complera	1		

ART, antiretroviral therapy; NA, not applicable.

Cohort 3: distinguishing between recent adherence (within 48–72 h), low adherence (within 1 week) and nonadherence to PrEP (last dose > 1 week prior)

We conducted a 24-week study of 10 HIV-negative subjects receiving daily PrEP to evaluate the concordance between plasma and urine TFV concentrations over time (see Table 1 for subject characteristics). TFV was detected in 90% of urine samples collected weekly (concentration range: 10 to > 10 000 ng/mL) and 73% of plasma samples collected monthly (concentration range: > 10–1000 ng/mL). In all of the urine samples in which TFV was not detected (10%), TFV was not detected in plasma; we did not collect data from these patients to determine the reasons for nonadherence. Thirty per cent of subjects had at least one urine sample during the 24-week study period in which drug was not detected. Urine TFV concentration > 1000 ng/mL was highly predictive of the presence of TFV in plasma (> 10 ng/mL) [PPV 0.95 (95% CI: 0.80–0.99); NPV 0.79 (95% CI: 0.49–0.94)]. As TFV is cleared from the plasma in 2–3 days, this suggests that the urine assay could be used

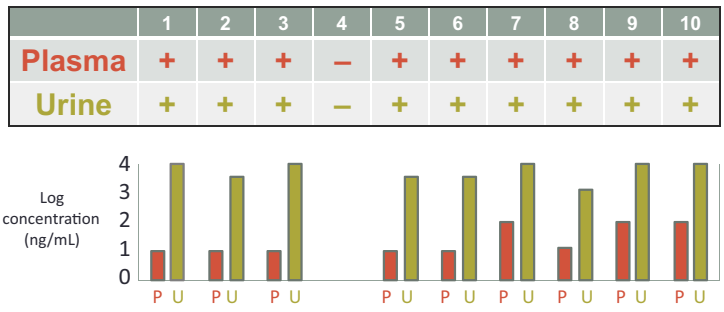

Fig. 2 Qualitative assessment, at a single time-point, of the relationship of urine tenofovir (TFV) to plasma TFV in 10 HIV-positive subjects with undetectable HIV viral loads on a tenofovir disoproxil fumarate/emtricitabine (TDF/FTC)-based regimen. All subjects with TFV in plasma also had detectable TFV in urine. Subject 4 had an undetectable viral load yet had no detectable TFV in blood or urine, and was later found to have stopped taking his antiretroviral therapy shortly after his viral load had been collected yet within 4 weeks of study sampling. Concentrations in plasma (P) and urine (U) are shown for each subject.

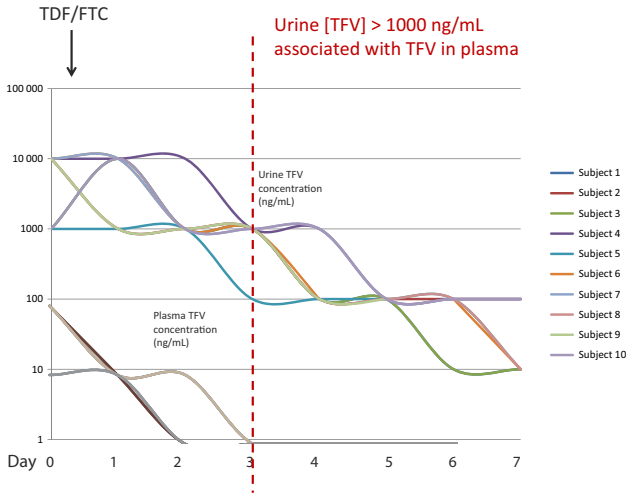

Fig. 3 Measurement of tenofovir (TFV) clearance in plasma and urine over 7 days in 10 HIV-negative subjects who received a single dose of tenofovir disoproxil fumarate/emtricitabine (TDF/FTC). After one dose of TDF/FTC had been taken, tenofovir (TFV) was measurable in plasma for up to 4 days, and in urine for at least 7 days. TFV concentration decays in a log-linear fashion. When TFV was detected in plasma (up to 3 days post-dose), urine TFV concentration was > 1000 ng/mL in almost all subjects. TFV, tenofovir concentration.

to distinguish between recent adherence, as defined by a dose of TDF within 48–72 h (> 1000 ng/mL), low adherence as defined by last dose within the past week but not within the past 72 hours (10–1000 ng/mL), and nonadherence, defined as last dose more than 1 week prior (< 10 ng/mL).

Acceptable to young men of colour who have sex with men

The acceptability of the assay was assessed in cohort 3 before and after the 6-month study period using a Likert scale from 1 to 6, where 1 is lowest and 6 is highest acceptability. Subjects were satisfied with the urine test as a means of monitoring adherence (mean before 5.7; after 5.6), with the side effects of urine testing (mean before 4.3; after 4.8), and with the demands of weekly testing (mean before 5.6; after 5.8). Subjects found it to be convenient (mean before 5.6; after 5.5) and were satisfied with their understanding of the importance of the test (mean before 5.8; after 5.9). They would recommend urine testing to others on PrEP (mean before 4.7; after 6.0) and would be interested in continuing urine testing after the study (mean before 5.9; after 5.8). The acceptability of the urine test in comparison to plasma testing was not assessed.

Discussion

In this proof-of-concept study, we demonstrate that a semiquantitative urine assay that measures TFV concentration could be used as an innovative noninvasive strategy to monitor real-time recent adherence to PrEP. Results of urine testing are available within 1 week, approximately the same turnaround time as viral load testing for HIV-positive patients, and there are no special processing requirements for urine samples after collection. Recent temperature stability studies demonstrate that samples are stable at room temperature for up to 14 days (data not shown). The urine assay is highly acceptable among yMSMc, provides high sensitivity and specificity relative to plasma TFV, predicts the approximate time when the last dose of TDF/FTC was taken, and can distinguish between low, medium and high adherence to PrEP. The urine TFV assay may also be easier to implement clinically as a consequence of its expected ease of use (no specific laboratory processing or shipping requirements) and cost to collect and process compared with alternative tests. It can also be performed at the same time (and using the same sample of urine) as other urine tests, including urine testing to monitor for nephrotoxicity and sexually transmitted infections.

Patient self-report may be more accurate in some populations than others, and an objective marker of adherence (akin to viral load in HIV-positive patients) could be extremely useful in certain settings. Importantly, urine TFV assessment can quickly provide information about whether someone is taking PrEP at all, and whether drug levels are expected to protect them from HIV infection at the time of testing. While TFV is known to be excreted in the urine, the assay described here represents the first to utilize urine to monitor adherence in the PrEP setting.

Our study also shows that monitoring urine TFV is feasible in a community health environment, which is the place where PrEP will have to be implemented in order to be effective. At our practice, at the present time, more than 50 patients who are receiving PrEP in a study setting are being monitored using urine TFV measurements [29]. This approach allows us to flag clinical records that are identified as indicating that the patient is either not protected (urine TFV concentration < 10 ng/mL) or incompletely protected (urine TFV concentration > 10 or > 100 ng/mL) based on their most recent urine TFV concentrations. This information allows clinicians to focus their efforts on individuals currently on PrEP yet remaining at higher and immediate risk of acquisition of HIV. Results of urine monitoring could be used, much like viral load testing in HIV-positive patients, to engage patients in larger questions of risk awareness and stigma around use of PrEP.

Importantly, we document that clinical implementation of this assay is acceptable to a young high-risk MSM urban population. These results may also act as an impetus to develop additional urine-based assays for future candidate medications considered for PrEP programmes, or to develop a point-of-care derivative of this assay (results available during the clinic visit) that could be used in a variety of clinical settings, particularly at resource-limited sites.

Our proof-of-concept study is limited by sample size and by not addressing additional variables that might affect urine drug concentration, including urine flow, frequency of bladder emptying, and urinary pH. While these elements are not included in this analysis, this is a first-pass analysis of the reliability of a urine assay in patients taking daily TDF/FTC. However, the high concentrations of TFV in the urine as compared with plasma limit the potential impact of urine volume, if any. Larger studies will be needed to define more accurate thresholds for evaluation of adherence than those provided here. Another limitation of our study is that our sampling addresses the extent of excretion of TFV in a patient who has only taken one dose of TDF/FTC. Futures studies will need to confirm whether the decay rates in urine levels described here are also observed in subjects stopping TDF/FTC after a prolonged adherence period.

Urine TFV assessment can provide information within 1 week about whether someone is taking PrEP at all, and whether someone is taking PrEP well enough to protect them from HIV infection at the time of testing. Future studies should be carried out to develop point-of-care testing and to determine whether utilizing this type of assay to target adherence interventions ultimately improves adherence to PrEP and decreases the risk of HIV infection at the population level.

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Conflicts of interest: HCK has served as a consultant to and has received a research grant from Gilead, the manufacturer of Truvada™.

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