Urine Tenofovir Levels Measured by a Novel Immunoassay Predict HIV Protection

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URINE TENOFOVIR LEVELS MEASURED BY A NOVEL IMMUNOASSAY PREDICT HIV PROTECTION

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Abstract

New tools are needed to support PrEP adherence for individuals at risk for HIV, including those that enable provision of real-time feedback. In a large, completed PrEP trial, adequate urine tenofovir levels measured by a novel immunoassay predicted HIV protection and showed good sensitivity and specificity for detectable plasma tenofovir.

Keywords: PrEP, HIV prevention, urine, immunoassay, ELISA

Introduction

Use of oral pre-exposure prophylaxis (PrEP) is a highly efficacious strategy for preventing HIV when taken daily.[1] However, adherence to PrEP has proven challenging among many populations.[2] Due to the limited accuracy of self-reported adherence, objective biological adherence metrics, such as measurement of PrEP drug concentrations in plasma, dried blood spots (DBS) and hair, have become valuable tools for appraising recent or cumulative PrEP adherence.[3] However, these methods can be costly and often require shipment of samples to an external laboratory, skilled laboratory personnel and specialized equipment, making them impractical options for routine use, particularly in resource-limited settings.

Innovative approaches are needed to support PrEP adherence, including those that enable counseling at the point of care (POC). Recently, we reported on the development of a novel antibody with high selectivity for tenofovir (TFV),[4] with the resultant immunoassay demonstrating high sensitivity and specificity relative to the standard liquid chromatography/tandem mass spectrometry (LC-MS/MS) assay. Here we further evaluate the immunoassay by comparing urine TFV measurements to TFV concentrations measured in plasma, which served as the gold standard metric for short-term adherence in most PrEP trials. Further, we assess the novel urine assay's ability to predict protection from HIV in a large completed PrEP trial.

Methods

We used a randomly-sampled, nested cohort of women and men, as well as cases of those who HIV seroconverted, assigned to the use of tenofovir disoproxil fumarate (TDF) or TDF/emtricitabine (FTC) in the Partners PrEP Study (NCT00557245), a randomized, placebo-controlled PrEP efficacy trial conducted among HIV serodiscordant couples in Kenya and Uganda.[5] During study follow-up, urine samples were archived at 3-, 12-, 24-, and 36-month visits. TFV concentrations were measured in all available archived urine samples using a quantitative enzyme-linked immunosorbent assay (ELISA) (lower limit of quantification [LLOQ]=1000 ng/mL) with this novel antibody.[4] TFV concentrations in date-matched plasma specimens were previously measured among the cohort using a validated LC-MS/MS assay (LLOQ=0.31 ng/mL). TFV concentrations below the LLOQ were assigned a value of half the LLOQ for each respective assay. Urine and plasma samples were stored at -80^{o}C upon collection and shipped on dry ice.

Correlation between paired urine and plasma concentrations was assessed and the sensitivity and specificity of detectable TFV in urine for determining detectable TFV concentrations in plasma were calculated. Additionally, we determined the sensitivity and specificity of urine TFV levels ≥1500 ng/mL, a concentration indicative of PrEP dosing in the past day among Thai men and women,[6] for determining plasma TFV concentrations >40 ng/mL, levels consistent with daily PrEP dosing.[7]

To assess the association between urine TFV concentrations ≥1500 ng/mL and protection from HIV acquisition, we conducted a nested case-control analysis. Case samples collected on the date of the first evidence of HIV infection (i.e., first positive test for HIV-1 RNA) were matched with control samples collected at the same study visit month. If the case's first evidence of HIV was observed between regular urine sample archiving, controls from the

nearest archive visit were selected. Control samples were matched 35:1, the ratio where estimates began to stabilize, and randomly sampled from the risk set of participants who were HIV-negative at the case's date of HIV detection, including future seroconverters. Controls could be matched to multiple cases. Conditional logistic regression, adjusted for matched sets, estimated the odds ratio of HIV acquisition given a urine TFV concentration ≥1500 ng/mL, which approximates a rate ratio (RR) given our time-matched risk set sampling approach. Adjusted models controlled for participant sex, age, and report of any condomless sex with their study partner in the prior month at enrollment. All models were replicated to also assess the association of plasma TFV >40 ng/mL with HIV protection. Case samples were too few to conduct adequately powered sex-based subgroup analyses.

The protocol for the parent study received ethical approval from the Institutional Review Board at the University of Washington and ethics review committees at each study site. All participants provided written informed consent.

Results

Of 4,432 individuals randomized to use of TDF or TDF/FTC in the Partners PrEP Study, 292 were included in the nested cohort. Among these participants, 39% were female and the median age was 33 (interquartile range [IQR]=28-39). Participants in the cohort contributed 722 paired urine and plasma samples. Of 52 individuals who seroconverted to HIV while using PrEP in the study, 22 had urine samples available from the visit where HIV was first detected and were included as cases. An additional 69 seroconverter samples collected prior to HIV infection were included as possible controls. Among cases, 55% were female and the median age was 33 (IQR=27-39).

The median duration from collection to assay of plasma and urine samples was 20 months and 103 months, respectively. In the cohort, the median TFV concentration was 37,500 ng/mL (IQR=500-90,000 ng/mL) in urine via ELISA and 65.4 ng/mL (IQR=1.6-103.0 ng/mL) in plasma via LC-MS/MS. Spearman's rank correlation coefficient (ρ) for the two measures was 0.46 ($p<0.001$). Of 558 plasma samples with detectable TFV (≥LLOQ of 0.31 ng/mL), 486 had a paired urine sample with detectable TFV (≥LLOQ of 1000 ng/mL) for a sensitivity of 87% (95% CI=84-90%). There were 164 plasma samples with undetectable TFV, of which 119 had a paired urine sample with undetectable TFV for a specificity of 73% (95% CI=65-79%). Of 468 individuals with plasma TFV >40 ng/mL, 420 had a paired urine sample with TFV ≥1500 ng/mL, for a sensitivity of 90% (95% CI=87-92%). Finally, 254 plasma samples had TFV levels ≤40 ng/mL, of which 146 had a paired urine sample with TFV <1500 ng/mL for a specificity of 57% (95% CI=51-64%).

In total, 770 control samples from 280 individuals were matched to the 22 case samples in our case-control study. Among participants in both active PrEP study arms, urine TFV ≥1500 ng/mL was associated with a 71% (95% CI=30-88%) reduction in HIV risk in the adjusted model (Table 1). By contrast, plasma TFV >40 ng/mL was associated with an 87% (95% CI=54-96%) reduction in HIV risk (Supplemental Table 1).

Discussion

The development of a low-cost POC assay to evaluate PrEP adherence would facilitate the implementation of real-time, drug-level feedback in current PrEP programs in resource-limited settings. Here we demonstrate that urine TFV concentrations, measured by a novel immunoassay, predict protection from HIV acquisition among PrEP users in sub-Saharan Africa. The concentrations measured by the urine assay also had good sensitivity and specificity for plasma levels, the gold-standard metric of adherence in placebo-controlled PrEP trials.

Previous studies have highlighted the advantages of using a urine-based assay to evaluate PrEP use. TARGET, a pharmacokinetic study that randomized Thai adults to directly-observed TDF in arms simulating low, medium and high adherence patterns, demonstrated that paired urine and plasma TFV concentrations measured by LC-MS/MS were highly correlated.[8] The study also showed that urine TFV concentrations can evaluate time since dosing, further demonstrated in other U.S.-based studies.[9,10] Data from the U.S. have also suggested that urine collection is highly acceptable, which may result in high uptake compared to other biological measures.[11] Additional evidence regarding the feasibility and acceptability of urine-based measures of PrEP use in other settings, including sub-Saharan Africa, is needed.

We previously demonstrated that urine levels via this immunoassay were correlated with other biomarkers, including TFV and FTC levels in hair and TFV-diphosphate and FTC-triphosphate concentrations in DBS, among men who have sex with men (MSM) and transwomen in the iPrEx open-label extension (OLE) study. Moreover, low urine concentrations among participants in iPrEx OLE were associated with subsequent HIV seroconversion.[12] The data presented here extend these prior results to heterosexual men and women on PrEP in sub-Saharan Africa.

There are several potential limitations to our results. First, we were unable to account for specific gravity of the urine samples or normalize to creatinine levels.[9] Second, longer storage of urine compared to plasma samples prior to analysis may have resulted in differential rates of sample degradation. Moderate correlation observed between urine and plasma TFV concentrations may be partially explained by these factors. Use of a specified TFV threshold for other analyses, however, may have mitigated the influence of measurement variability. Finally, most seroconverters did not have urine samples available from the visit of first HIV detection, limiting study power, and incomplete availability of control samples at urine archive months may have limited our control matching. However, our overall risk reduction estimates with plasma TFV >40 were nearly identical to those previously identified in this cohort,[7] suggesting bias may have been minimal.

In summary, we show the high predictive utility of an adequate urine tenofovir level for HIV protection among men and women using PrEP in sub-Saharan Africa. The urine immunoassay has been developed into a lateral flow assay (LFA), which is low-cost, easy to perform, can be administered at the POC, and provides results within minutes.[4] The LFA is portable and requires no reagents so it may also be administered in the field by non-medical personnel to help reach stigmatized or hidden populations. The assay should be evaluated in a variety of populations for adherence monitoring and feedback.

NOTES

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Conflicts of Interest

RMS: No conflict

JMB: Serves on advisory boards for Gilead Sciences, Merck and Janssen; reports grants from USAID.

DD: No conflict

MAS: No conflict

DVG: Serves on advisory boards for Gilead Sciences and Merck.

WCR: From Abbott Rapid Diagnostics, the developer of the antibody; has a patent pending

GW: From Abbott Rapid Diagnostics, the developer of the antibody

MV: From Abbott Rapid Diagnostics, the developer of the antibody

NM: No conflict

AM: Received non-financial support from Gilead Sciences

MM: Received grants from NIH, outside the submitted work.

CH: Received grants from Merck, Gilead Sciences, NIH, ViiV/GSK, personal fees from Merck and ViiV/GSK, non-financial support from Gilead Sciences; has a patent issued on a tenofovir PrEP product

MG: No conflict

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Table 1. Percent HIV risk reduction associated with urine TFV concentrations >1500 ng/mL as measured by a novel immunoassay

n (%) with urine TFV ≥1500 ng/mL					
Case samples: First evidence of HIV	**Control samples**	**% HIV risk reduction[a] (95% CI)**	**p-value**	**Adjusted % HIV risk reduction[a,b] (95% CI)**	**Adjusted p-value[b]**
8/22 (36%)	527/770 (68%)	73% (36 to 89%)	0.003	71% (30 to 88%)	0.006

TFV: tenofovir, CI: confidence interval
Analyses include individuals assigned to TDF/FTC or TDF-only PrEP. Estimates were generated using conditional logistic regression.
[a]% risk reduction calculated as follows: (1-RR)*100
[b]Adjusted for sex, age at enrollment, and report of any condomless sex with study partner in the month prior to enrollment