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Authors

Gandhi, Monica
Glidden, David V
Liu, Albert
[et al.](#)

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Strong Correlation Between Concentrations of Tenofovir (TFV) Emtricitabine (FTC) in Hair and TFV Diphosphate and FTC Triphosphate in Dried Blood Spots in the iPrEx Open Label Extension: Implications for Pre-exposure Prophylaxis Adherence Monitoring

Monica Gandhi,¹ David V. Glidden,² Albert Liu,^{1,4} Peter L. Anderson,⁶ Howard Horng,³ Patricia Defechereux,⁵ Juan V. Guanira,⁷ Beatriz Grinsztejn,⁸ Suwat Chariyalertsak,⁹ Linda-Gail Bekker,¹⁰ and Robert M. Grant^{1,5}; for the iPrEx Study Team

Departments of ¹Medicine, ²Epidemiology and Biostatistics, and ³Bioengineering and Therapeutic Sciences, University of California, ⁴Bridge HIV, San Francisco Department of Public Health, and ⁵Gladstone Institutes, San Francisco; ⁶Department of Pharmaceutical Sciences, University of Colorado, Denver; ⁷Investigaciones Medicas en Salud, Lima, Peru; ⁸STD/AIDS Clinical Research Laboratory, Evandro Chagas National Institute of Infectious Diseases-Fiocruz, Rio de Janeiro, Brazil; ⁹Research Institute for Health Sciences, Chiang Mai University, Thailand; and ¹⁰The Desmond Tutu HIV Centre, University of Cape Town, South Africa

Self-reported adherence to pre-exposure prophylaxis (PrEP) has limitations, raising interest in pharmacologic monitoring. Drug concentrations in hair and dried blood spots (DBS) are used to assess long-term-exposure; hair shipment/storage occurs at room temperature. The iPrEx Open Label Extension collected DBS routinely, with opt-in hair collection; concentrations were measured with liquid chromatography/tandem mass spectrometry. In 806 hair-DBS pairs, tenofovir (TFV) hair levels and TFV diphosphate (DP) in DBS were strongly correlated (Spearman coefficient $r = 0.734$; $P < .001$), as were hair TFV/DBS emtricitabine (FTC) triphosphate (TP) ($r = 0.781$; $P < .001$); hair FTC/DBS TFV-DP ($r = 0.74$; $P < .001$); hair FTC/DBS FTC-TP ($r = 0.587$; $P < .001$). Drug detectability was generally concordant by matrix. Hair TFV/FTC concentrations correlate strongly with DBS levels, which are predictive of PrEP outcomes.

Keywords. HIV prevention; pre-exposure prophylaxis; PrEP; pharmacologic monitoring; adherence; tenofovir/emtricitabine; hair concentrations; dried blood spot (DBS) concentrations; iPrEx Open Label Extension (OLE).

The utility of pre-exposure prophylaxis (PrEP) for human immunodeficiency virus (HIV) prevention has been demonstrated in multiple trials. The efficacy of PrEP is highly dependent on adherence to the tenofovir disoproxil fumarate (TDF)/emtricitabine (FTC)-based regimen. However, adherence assessed via self-report or pill counts inconsistently predicts prevention outcomes [1, 2]. Pharmacologic exposure measures, wherein drug levels are monitored in a biomatrix such as plasma, peripheral blood mononuclear cells (PBMCs), or dried blood spots (DBS), are of significant interest to the field [3]. Indeed, substantial discordance between self-reported measures of adherence and drug detection in PrEP trials, such as the global iPrEx study [4], FEM-PrEP [1], Partners PrEP [5, 6], and the Vaginal and Oral Interventions to Control the Epidemic (VOICE) trial [2], have highlighted the critical need to incorporate objective pharmacologic exposure measures into PrEP demonstration projects and real-world settings.

Plasma or PBMC concentrations of tenofovir (TFV)/FTC or their phosphorylated forms have been most frequently incorporated into PrEP trials as pharmacologic measures [1, 2, 7]. Measurements of plasma antiretroviral concentrations represent more recent dosing (eg, 1–14 preceding days, depending on assay sensitivity) [6] and may be susceptible to “white-coat effects,” wherein adherence improves transiently before visits [8]. Exposure measures for drugs with longer half-lives, such as TFV and its phosphorylated forms, would ideally provide information over prolonged periods. For example, TFV diphosphate (DP) levels in PBMCs [4, 9] relay information on exposure over longer periods (7–14 days), although processing, isolating and counting PBMCs are costly and technically challenging. DBS—made from whole blood—are easier to collect and process than PBMCs [10], and drug levels in DBS (which consist mainly of red blood cells), provide longer-term exposure information (half-life 17 days) [10]; DBS assays, however, require standardization against hematocrit abnormalities for interpretation. Importantly, TFV or TFV-DP concentrations in all 3 matrices (plasma, PBMCs, and DBS) have shown strong relationships with the protective efficacy of PrEP in either global iPrEx [4, 9] or the Open Label Extension follow-up study (iPrEx OLE) [11].

Given the need for low-cost, feasible, longer-term measurements of exposure for PrEP, analyzing concentrations of

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Correspondence: Monica Gandhi, MD, MPH, Division of HIV/AIDS, University of California, San Francisco, 995 Potrero Ave, Fourth Flr, San Francisco, CA 94110 (monica.gandhi@ucsf.edu).

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TFV/FTC in small hair samples has been of emerging interest. Hair collection is noninvasive, nonbiohazardous, and does not require phlebotomy or cold storage, and hair levels reflect uptake from the systemic circulation over weeks to months. Hair concentrations of TFV are strongly and linearly related to dose [12], and hair levels of both TFV and FTC correlate with plasma and PBMC concentrations [3]. Hair and DBS collection both provide feasibility advantages in resource-limited settings, and concentrations in these 2 matrices represent longer-term exposure, although the correlation between hair and DBS measures is unknown. We report for the first time the correlation between drug levels and concordance of drug detection in hair and DBS in a large cohort receiving PrEP.

METHODS

Participants and Sample Collection

The iPrEx Open Label Extension (OLE) enrolled 1603 HIV-negative men who have sex with men and transgender women previously enrolled in 3 PrEP trials [11], of whom 1225 initiated PrEP. Visits were performed at baseline and at weeks 4, 8, 12, 24, 36, 48, 60, and 72. Whole blood was routinely collected for DBS preparation, as described elsewhere [10], at 4 and 8 weeks after PrEP initiation and then every 12 weeks. Hair samples were collected from consenting participants who opted in for this substudy every 12 weeks after PrEP initiation by cutting approximately 100 strands of hair as close as possible to the scalp in the occipital region and labeling the distal end. As described elsewhere [3], each hair sample is stored and shipped in aluminum foil at room temperature. The study protocol was approved by Institutional Review Boards of all participating sites, and all participants provided written informed consent in their preferred language.

Laboratory Methods

The DBS from all relevant visits were stored at -20°C within 24 hours of collection and shipped on dry ice to our University of Colorado laboratory. At the time of analyses, a 3-mm DBS punch sample was extracted and analyzed for TFV-DP and FTC-triphosphate (TP) concentrations by validated liquid chromatography/tandem mass spectrometry (LC/MS-MS) methods, as described elsewhere [10]. The dynamic range of the assay was 2.5–2000 fmol per sample for TFV-DP and 0.1–200 pmol per sample for FTC-TP.

After storage and shipment at ambient temperature to our University of California, San Francisco laboratory, the proximal 1.5 cm of each hair samples (representing approximately 6 weeks of exposure) was cut finely with scissors and 5 mg was processed and analyzed using LC/MS-MS as described elsewhere [3, 12]. These assays have been validated from 0.002 to 0.400 ng/mg hair for TFV and from 0.02 to 4 ng/mg for FTC [12]. Of note, our assays for measuring TFV and FTC in hair samples have

been peer reviewed and approved by the Division of AIDS Clinical-Pharmacology and Quality-Assurance (CPQA) program.

Statistical Analysis

Spearman correlation coefficients and scatterplots were examined to assess relationships between TFV and FTC levels in hair and TFV-DP and FTC-TP concentrations in red blood cells measured via DBS. The concordance of drug detection for each combination was also determined for paired samples.

RESULTS

Hair Collection in iPrEx OLE

Concentration results from 806 paired hair-DBS samples from 217 participants were available for comparison. Among 1603 HIV-negative individuals enrolled in iPrEx OLE, DBS were analyzed among all seroconverters and a random sample of seronegative participants, as described elsewhere [11]. All available hair and DBS samples from seroconverters and the random sample of non-seroconverters were used in the present analysis. Although 75% of participants in the overall study agreed to hair sampling, not enough hair samples were collected from the seroconversion visit in the 28 participants who acquired HIV during PrEP to perform a meaningful analysis of the protective effect of hair concentrations on HIV acquisition (12 declined or didn't have enough hair, 6 had hair collected but only at HIV RNA-negative time points; only 10 had hair collected at HIV RNA-positive time points).

Correlation Between DBS and Hair Drug Concentrations

Scatterplots of the correlation between hair TFV or FTC levels and DBS TFV-DP or FTC-TP levels in the 806 paired samples are depicted in Figure 1. TFV concentrations in hair and TFV-DP levels in DBS were strongly correlated ($r = 0.734$; $P < .001$), as were hair TFV levels and DBS FTC-TP concentrations ($r = 0.781$; $P < .001$). FTC concentrations in hair were more strongly correlated with DBS TFV-DP levels ($r = 0.742$; $P < .001$) than with DBS FTC-TP levels ($r = 0.587$; $P < .001$).

Levels of TFV/ FTC Hair Concentrations Estimated to Confer Protective Efficacy

In iPrEx OLE [11], the protective levels of TFV-DP in DBS were quantified as 700 fmol per punch sample. From the correlation analysis, these DBS levels correspond to a median hair TFV concentration of 0.023 ng/mg (95% confidence interval [CI], .019–.025) and a hair FTC concentration of 0.37 ng/mg (95% CI, 28–.58).

Concordance of Detection of Hair and DBS Drug Levels

A comparison of detectability of the relevant antiretroviral or metabolite in the 2 matrices is shown in Table 1. Concentrations of drugs were mostly concordant in terms of detectability, with

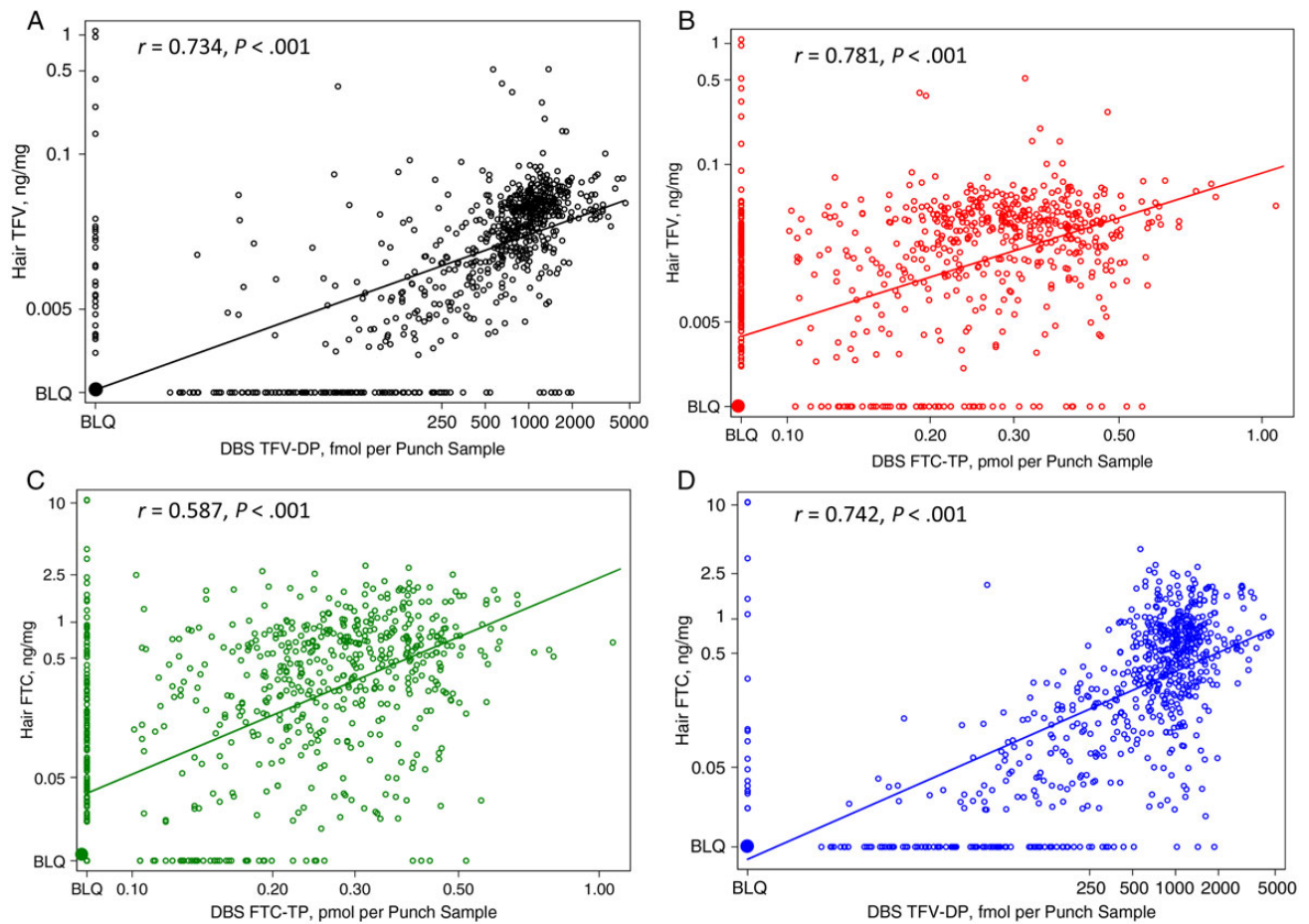


Figure 1. Scatterplots showing correlations between hair tenofovir (TFV) and dried blood spot (DBS) TFV-diphosphate (DP) levels (A); hair TFV and DBS emtricitabine (FTC)-triphosphate (TP) levels (B); hair FTC and DBS FTC-TP levels (C); and hair FTC and DBS TFV-DP levels (D). Abbreviation: BLQ, below limit of quantification.

concordance of 82% for hair TFV and DBS TFV-DP levels, 90% for hair TFV and DBS FTC-TP levels, 81% for hair FTC and DBS FTC-TP levels, and 85% for hair FTC and DBS TFV-DP levels.

DISCUSSION

Given that pharmacologic measures of drug exposure have been crucial for trial interpretation in several PrEP studies [1,2,4], the

incorporation of objective markers of drug adherence/exposure into PrEP settings is of growing interest. Easily stored samples that do not require a cold chain, such as hair, could be useful for drug monitoring in resource-constrained settings. Our study showed strong and significant correlations between concentrations of PrEP drugs in hair and DBS, as well as high levels of concordance (>80% for all combinations) for drug detectability in the 2 matrices. The current study is the first to analyze correlations between antiretroviral concentrations in hair and DBS.

Table 1. Concordance of Drug Detection of PrEP Drugs and Their Metabolites in Hair and DBS (806 Paired Samples)

Drug Level	Detectable in Hair and DBS, %	Detectable in Hair but Not DBS, %	Detectable in DBS but Not Hair, %	Undetectable in Hair or DBS, %
TFV in hair, TFV-DP in DBS	72	3	15	10
TFV in hair, FTC-TP in DBS	71	5	5	19
FTC in hair, FTC-TP in DBS	62	14	5	19
FTC in hair, TFV-DP in DBS	74	2	13	11

Abbreviations: DBS, dried blood spots; DP, diphosphate; FTC, emtricitabine; PrEP, pre-exposure prophylaxis; TFV, tenofovir; TP, triphosphate.

Although plasma measures of TFV and/or FTC have been most commonly analyzed in PrEP trials [1, 2, 4–7], drug concentrations in plasma represent recent drug ingestion (eg, yes/no for taking drug or not). However, TFV and its moieties exhibit long half-lives in various matrices, enabling assessment of longer-term measures of cumulative drug exposure, such as those in DBS [10] and hair, that may be more relevant for PrEP monitoring. PBMC measurements of TFV-DP/ FTC-TP have proved useful [9], but PBMCs are generally difficult to collect, process, and transport. DBS are easy to prepare from whole-blood samples [10], although they must be shipped and stored at cold temperatures to allow measurement. Hair samples are easy to collect in real-world settings, and samples are stored and shipped at room temperature under nonbiohazardous conditions. However, it should be noted that current assays to quantify drug levels for PrEP, including those in both DBS and hair, use LC/MS-MS, which requires expensive equipment. Widespread applicability of biologic adherence measures in the context of PrEP rollout will rely on the availability of lower-cost methods to analyze drug levels, currently in development for hair assays [13].

Although whole blood was routinely collected at all time points in iPrEx OLE, hair collection was performed only on an opt-in basis; sampling required participants to sign an extra consent form and was only performed quarterly. The lack of hair sampling at relevant visits for participants who seroconverted during PrEP impeded our ability to directly analyze this measure in relationship to HIV seroconversion. However, from the correlation data [11], we can estimate that hair TFV concentrations of 0.023 ng/mg (95% CI, .019–.025 ng/mg) and hair FTC concentrations of 0.37 ng/mg (95% CI, .28–.58 ng/mg) are likely to confer protective benefit in the context of PrEP. The TFV hair concentration estimated to confer protection in this study is similar to that achieved by volunteers taking 4 doses of TFV per week in a separate study (median value, 0.023 ng/mg) [12].

Hair collection has been shown to be feasible and acceptable (>95%) among HIV-infected or at-risk individuals in Africa [3, 14]. A recent study from rural Kenya showed that community information campaigns and field staff training early in protocol development was likely to enhance hair collection rates [14]. A qualitative study from South Africa on the acceptability of hair collection underscored this point [15]. Of note, TFV concentrations in pubic hair samples were not reliably associated with dose in another study [12], which corroborates other findings that pubic hair, with its erratic growth rates and urine contamination, is not an informative biomatrix.

Two phase II PrEP studies in Africa collected multiple biologic samples for exposure monitoring (plasma, PBMCs, and hair) and correlated drug levels in hair with drug or metabolite concentrations in plasma and PBMCs [3]. These studies did not collect DBS. In these phase II PrEP studies, the correlation

between TFV levels in hair and plasma after 8 weeks was moderate ($r = 0.41$), as was the correlation between FTC levels in hair and plasma ($r = 0.51$). The coefficients for correlation between TFV or FTC levels in hair and their relevant phosphorylated concentrations in PBMCs at 8 weeks were similar ($r = 0.43$ and $r = 0.50$, respectively). In the current study, the correlation between TFV concentrations in hair and TFV-DP levels in DBS (both longer-term measures of exposure) was high ($r = 0.734$), and DBS measures of TFV-DP in the overall cohort were strongly associated with protective efficacy [11], implying that TFV hair levels may also have predicted efficacy.

The more moderate correlation observed between hair FTC hair and DBS FTC-TP levels may reflect the relatively short half-life of FTC-TP in DBS (approximately 30 hours). The discordance between drug detection in hair and DBS in 19% of pairs could be secondary to differential half-lives of TDF/FTC-based PrEP in different matrices; another study will examine the elimination kinetics of TFV in hair and DBS in the context of directly observed therapy (ClinicalTrials.gov, NCT02022657). Undetectable drug concentrations in hair (25% of samples for TFV) suggest no appreciable dosing over a significant duration before a visit.

The current study adds to the growing literature around the utility of hair monitoring in TDF/FTC-based PrEP. A large ongoing PrEP demonstration trial in 3 US cities has incorporated multiple pharmacologic measures of drug exposure, including hair assays, and will provide further data on the utility of hair measures in monitoring adherence. Future studies of pharmacologic monitoring in PrEP should probably examine the predictive utility of multiple measures of exposure simultaneously, because each measure reflects a different duration of exposure. Given the well-acknowledged limitations of self-report in monitoring adherence to PrEP, novel biologic measures of assessing exposure, including hair assays, should be further examined in real-world settings.

Notes

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Potential conflicts of interest. Gilead Sciences donated emtricitabine/tenofovir disoproxil fumarate for participants in the study but provided no other financial support and did not contribute to data interpretation or manuscript development. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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