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Does Adherence Change When No One is Looking? Comparing Announced and Unannounced Tenofovir Levels in a PrEP Trial

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Abstract Differences between unannounced and announced tenofovir levels as measures of PrEP adherence are not well understood. In an ancillary adherence study involving one urban site (Kampala) and two rural sites (Kabwohe and Tororo) from the Partners PrEP study, 268 specimen pairs from chronologically proximal clinic and home visits were tested for plasma tenofovir levels. Comparing clinic and home specimens, 89 versus 89 % were classified as detectable ($\geq$0.31 ng/ml; p = 0.77), 87 versus 86 % as recent dosing ($\geq$10 ng/ml; p = 0.80), and 82 versus 80 % as steady-state ($\geq$40 ng/ml; p = 0.44). Mean difference between announced and unannounced drug levels, adjusted for specimen collection time was 3.2 ng/ml (p = 0.50) for Kabwohe, 23.2 ng/ml (p = 0.003) for Kampala and −3.3 ng/ml p = 0.69) for Tororo. In the setting of high adherence, plasma tenofovir levels tested at the clinic were categorically similar as levels tested at home; however, differences were seen between urban and rural settings.

Resumen Las diferencias entre los niveles de tenofovir no anunciadas y anunciadas como medidas de adherencia PrEP no se conocen bien. En un estudio de la adherencia de auxiliar con un sitio urbano (Kampala) y dos sitios rurales (Kabwohe y Tororo) del estudio Partners PrEP, 268 muestras pares de visitas clínicas y domiciliarias cronológicamente proximales fueron analizadas para niveles de tenofovir plasma. Comparando muestras de la clínica y el hogar, 89 frente a 89 % se clasificaron como detectable ($\geq$0.31 ng/ml; p = 0.77), 87 frente a 86 % como dosificación reciente ($\geq$10 ng/ml; p = 0.80), y 82 frente a 80 % como en estado estacionario ($\geq$40 ng/ml; p = 0.44). Diferencia media entre los niveles de fármaco anunciadas y no anunciadas, ajustado por el tiempo de recogida de muestras fue 3.2 ng/ml (p = 0.50) para Kabwohe, 23.2 ng/ml (p = 0.003) para Kampala y −3.3 ng/ml p = 0.69) para Tororo. En el escenario de alta adherencia, los niveles de tenofovir plasma analizado en la clínica eran categóricamente similar a los niveles analizados en hogar; sin embargo, se observaron diferencias entre entornos urbanos y rurales.

Keywords Announced adherence · Unannounced adherence · Prep · Tenofovir · Emtricitabine

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Background

When taken consistently, pre-exposure prophylaxis (PrEP) can be >90 % effective in preventing HIV acquisition [1, 2]. PrEP adherence, however, can be difficult to measure

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[3] and has varied widely across studies [4]. While tenofovir levels have been the most commonly used objective measure of drug exposure in clinical trials [4–9], it reflects a short window of exposure (e.g., less than 7 days for plasma specimens [10] and may be subject to "white coat bias" [11]. That is, individuals may alter their routine adherence behavior for the few days prior to a clinic visit, because they know a drug level will be performed. The risk of such behavioral alteration is mitigated if the drug levels are performed during unannounced visits at the research subject's home as has been shown in prior studies of pill counts for antiretroviral therapy [12, 13]

In this brief report, we compare announced tenofovir levels drawn in clinic and unannounced tenofovir drug levels drawn in the home for participants in the Partners PrEP Ancillary Adherence Study, a clinical trial of PrEP. We aim to estimate the extent to which clinic-based drug levels differ, if at all, from unannounced home-based drug levels which reflect routine adherence behavior in this population.

Methods

The Partners PrEP Study and Ancillary Adherence Study

The Partners PrEP Study was a phase III, randomized controlled trial of tenofovir (TDF) and emtricitabine/tenofovir (FTC/TDF) PrEP provided to the HIV-uninfected partner in 4747 serodiscordant couples. It was conducted between July 2008 and December 2012 in 9 sites in Kenya and Uganda [4]. In July 2011, the Independent Data and Safety Monitoring Board recommended public report of the results and discontinuation of the trial placebo arm due to demonstration of 67 % efficacy for HIV protection with TDF and 75 % efficacy with FTC/TDF.

Starting in November 2009, an ancillary adherence study of 1147 couples was conducted to objectively measure adherence at three of the Ugandan sites (one urban [Kampala] and two rural [Kabwohe and Tororo]). A convenience sample was selected from all trial arms (which were blinded at the time) involving participants with at least 6 months of remaining follow-up in the main study. In the ancillary adherence study, adherence was monitored using unannounced pill counts at home visits and electronic monitoring (MEMS) initiated and downloaded at clinic visits. Adherence was calculated as the number of pills taken divided by the number of days during which the participant was not on a drug hold. "Pills taken" using unannounced pill counts was defined as pills given minus pills remaining, whereas by electronic monitoring it was the number of cap openings recorded electronically.

Among other procedures in the Partners PrEP Study, blood was drawn during routine monthly visits and stored for later determination of plasma tenofovir levels. In the ancillary adherence study, blood was drawn during unannounced home visits at approximately 6 and 12 months of follow-up (i.e., participants were informed home visits would take place, but the date and time were not scheduled). For the present analysis, a random sample of 268 visits was selected from all unannounced home visits with plasma available from participants randomized to active PrEP. Each home visit selected was paired with the closest monthly clinic visit and the resulting specimen pairs tested for plasma tenofovir levels. Since the random selection of visits was independent of which participant contributed the visit, it was possible for the random sample to contain more than one visit per participant. The sample size was selected to detect a 20 % difference between clinic and home-based drug levels, with 80 % power. Specimens were collected before July 2011 (when efficacy of PrEP was announced in the main study) and excluded samples when drug was not available to the participant (e.g., due to a protocol-driven study hold, like pregnancy). Tenofovir levels were determined by previously described ultra-performance liquid chromatography-mass spectrometry assay methods [14]. Tenofovir assays met the FDA bioanalysis guidance values of within 15 % for precision and accuracy. Calibration standards for assay ranged from 0.31 to 1280 ng/mL [14].The lower limit of detection was 0.31 ng/mL.

Statistical Analyses

We compared specimens collected in clinic and at home by both categorized and continuous drug levels. Drug levels were categorized as (a)$<$0.31 versus $\geq$0.31 ng/ml (i.e., any tenofovir detection, consistent with any dosing in the past week); (b)$<$10 versus $\geq$10 ng/ml (i.e., the concentration consistent with dosing in the past 2-3 days) and, (c)$<$40 versus $\geq$40 ng/ml (i.e., the concentration consistent with steady-state daily dosing) [14–18]. For each of the categories, we assessed agreement between clinic and home specimens using conditional logistic regression accounting for potential clustering by participant. Continuous drug concentrations were compared between clinic and home specimens using mixed models of the paired difference (clinic-home), with a random effect for participant to account for potential correlation in multiple pairs within participant. The mean paired difference is represented by the model intercept and the null hypothesis tested was intercept term = 0. Adjusted models accounted for differences in hour of specimen collection between the clinic and home visit pair. Variations in paired difference by site were estimated in the same manner, adding site as predictor

and adjusting for demographics differing by site. Analyses were conducted using SAS 9.3 and Stata 12.

Ethical Approval

Ethical approval for both the main and sub-studies was obtained from the University of Washington, Partners Healthcare, the Ugandan National Council for Science and Technology, the Ugandan Virus Research Institute, and the US Centers for Disease Control and Prevention.

Results

Participant Characteristics

The 268 randomly selected visit pairs for this analysis came from 228 participants, of whom 127 (56 %) were male and the median age was 34 (IQR 30-39). Sixty-one (27 %) were from Kabwohe, 80 (35 %) from Kampala, and 87 (38 %) from Tororo. Median [interquartile range (IQR)] adherence was 100 % (96.9–100 %) by unannounced pill count for the period since the prior home visit, and 100 % (90.9–100 %) by electronic monitoring for the 30-day period prior to the home visit. These findings are similar to adherence from all participants in the ancillary adherence study, in which the median adherence was 99.1 % (IQR 96.9–100 %) by unannounced pill counts at home visits and 97.2 % (90.6–100 %) by electronic monitoring. Likewise, gender, age, education and adherence were similar to that in the total ancillary adherence cohort [1]. Site populations in this analysis varied: those from Kampala were more likely to be male (73 vs 44 % in Kabwohe and 48 % in Tororo), younger (median (IQR) 33 (29, 37) years vs 34 (30, 40) in Tororo and 36 (31, 39) in Kabwohe), and more highly educated (median (IQR) 7 (7,11) years vs 5 (2, 7) in Kabwohe and 4 (0, 7) in Tororo).

Of the 268 randomly selected specimen pairs, 174 (65 %) were collected at the 6-month unannounced home visit and the remainder at the 12-month visit. Forty participants (18 %) had a specimen at both the 6 and 12-month visits. The median (IQR) time between clinic and home visits was 16 (10–25) days. The mean (SD) specimen collection time of day was 12:13 (2:12) at clinic and 12:54 (2:33) at home ($p = 0.02$).

As shown in Table 1, the specimens were 96, 95, and 86 % concordant at the <0.31 ng/ml, <10 ng/ml and <40 ng/ml cutoffs, respectively. Comparing clinic and home specimens, 89 versus 89 % were classified ≥0.31 ng/ml ($p = 0.77$), 87 versus 86 % were ≥10 ng/ml ($p = 0.80$), and 82 versus 80 % were ≥40 ng/ml ($p = 0.44$).

The overall mean tenofovir levels at clinic and at home visits were 87.5 and 78.3 ng/mL, respectively (mean difference, 9.2, 95 % CI, (0.7, 17.8; $p = 0.04$). After adjusting for hour of specimen collection, the overall mean (95 % CI) difference was 8.1 ng/ml (−0.5, 16.7; $p = 0.07$).

Table 1 Cross tabulations of home versus clinic visits

Home visits	Clinic visits		
	<0.31 ng/ml	≥0.31 ng/ml	Total
Cut-off for detection of 0.31 ng/ml			
<0.31 ng/ml	24 (9 %)	5 (2 %)	29 (11 %)
≥0.31 ng/ml	6 (2 %)	233 (87 %)	239 (89 %)
Total	30 (11 %)	238 (89 %)	268 (100 %)
Home visits	Clinic visits		
	<10 ng/ml	≥10 ng/ml	Total
Cut-off for detection of 10 ng/ml			
<10 ng/ml	29 (11 %)	8 (3 %)	37 (14 %)
≥10 ng/ml	7 (3 %)	224 (84 %)	231 (86 %)
Total	36 (13 %)	232 (87 %)	268 (100 %)
Home visits	Clinic visits		
	<40 ng/ml	≥40 ng/ml	Total
Cut-off for detection of 40 ng/ml			
<40 ng/ml	33 (12 %)	21 (8 %)	54 (20 %)
≥40 ng/ml	16 (6 %)	198 (74 %)	214 (80 %)
Total	49 (18 %)	219 (82 %)	268 (100 %)

The mean (95 % CI) difference between clinic-based and home-based tenofovir levels was 6.6 ng/ml (−11.0, 24.2; $p = 0.46$) for Kabwohe, 23.3 ng/ml (9.3, 37.4; $p = 0.002$) for Kampala and −3.3 ng/ml (−17.5, 10.8; $p = 0.64$) for Tororo (Fig. 1). The mean (95 % CI) difference adjusted for hour of specimen collection, age, gender, and years of education was 5.4 ng/ml (−12.7, 23.5; $p = 0.55$) for Kabwohe, 18.6 ng/ml (2.9, 34.3; $p = 0.02$) for Kampala and −0.6 ng/ml (−15.3, 14.4; $p = 0.95$) for Tororo. While the effect of education was not significant overall (1.3 ng/ml (−1.2, 3.9; $p = 0.30$) per each additional year of education), we found that education was associated with a larger discrepancy in Kampala of 5.8 ng/ml per year (0.4, 11.2; $p = 0.03$), but not at the other sites [2.2 (−4.8,9.1; $p = 0.53$) for Kabwohe and −1.9 (−6.1, 2.4; $p = 0.38$) for Tororo]. No such relationship was seen with age and gender.

Discussion

In an analysis of HIV-uninfected members of serodiscordant couples who have high adherence to PrEP by other objective measures, plasma tenofovir levels tested at the clinic were largely consistent with levels tested at home.

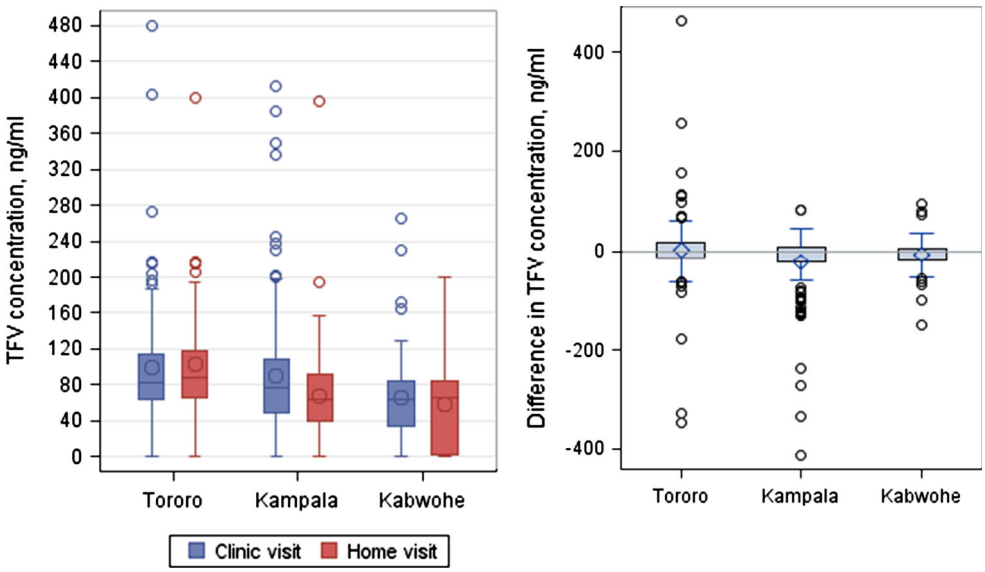

Fig. 1 Tenofovir levels and paired differences by site

When assessing categories of drug concentration, the degree of concordance was statistically similar. When analyzing continuous drug levels, the mean drug concentration was slightly lower at home visits compared to clinic visits; however, statistical significance was lost when accounting for the hour of specimen collection (clinic specimens were collected somewhat earlier in the day than home specimens).

While clinic and home-based drug levels were statistically similar at Kabwohe and Tororo, we did find that clinic-based drug levels were significantly higher than home-based drug levels among participants living in Kampala. Importantly, all sites performed specimen collection and processing according to the same protocols. While adjustment for differences among the sites by hour of specimen collection, age, gender, and education did not influence the discrepancies in home and clinic-based drug levels in multivariable modeling, levels of education at Kampala were higher than the other sites. Because these education and site were highly correlated, statistical adjustment may not have been able to separate effects of these two variables. One potential explanation for the higher clinic drug levels in Kampala may be an increased social desirability pressure (i.e., taking more medication than usual in advance of a clinic visit to please the study staff) in an urban setting, which could have been influenced by the education level. Factors influencing social desirability are poorly understood [19] and further research is needed in this area.

The major limitation of this analysis is the high adherence observed in the study, which creates a ceiling effect. Our ability to detect a difference that might be seen in populations with lower or more variable adherence is thereby limited. Additional comparisons of announced and unannounced drug levels are needed in other settings and populations to determine the generalizability of our findings. Indeed, to our knowledge, this study is unique in the direct comparison of announced and unannounced drug levels.

Our results suggest clinic-based drug levels are a generally unbiased adherence measure in highly adherent populations, particularly in rural settings. Given the resource intensity of performing unannounced home-based drug levels, clinic-based drug levels are likely to be a more efficient measure. Drug levels, however, only reflect a 7-day window of adherence behavior and must be interpreted accordingly. Other objective measures of adherence, such as electronic monitoring, provide more information on daily adherence behavior over time and should be considered a preferable strategy to understand long-term adherence behavior when feasible.

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Compliance with Ethical Standards

Conflicts of Interest All authors report no conflicts of interest.

Ethical Approval All procedures performed on participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent Informed consent was obtained from all individual participants included in the study.

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