Antiretroviral Therapy Adherence During and Postbreastfeeding Cessation Measured by Tenofovir Levels in Hair

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Background: We examined change in antiretroviral treatment (ART) adherence after breastfeeding (BF) cessation using hair tenofovir (TFV) concentrations as an objective metric of medication consumption.

Methods: A subset of postpartum women in Zimbabwe randomized in IMPAACT PROMISE to take ART while BF and post-BF cessation had hair TFV measured longitudinally. Using linear mixed-effect models, we estimated differences in hair TFV levels after BF cessation, accounting for trends in levels over time regardless of BF status and change in slope after breastfeeding cessation. We also estimated the relative risk of viremia (>50 copies/mL) per doubling of hair TFV concentration.

Results: Among 55 women (median age 26, interquartile range 24–29 years), hair TFV levels (n = 305) were available for a median of 9 visits per woman between 3 and 29 months postpartum. Hair TFV levels ranged from undetected to 0.25 ng/mg (median 0.04 ng/mg). Controlling for trends since delivery [decline of 2.2% per month, 95% confidence interval (CI): −5.3 to 1.0], TFV levels averaged 24.4% higher (95% CI: −5.1 to 63.1) post-BF cessation than during BF, with no change in slope (0.0% per month, 95% CI: −3.8 to 3.9). Postpartum, 42% of women were ever viremic. Higher TFV levels were strongly protective; relative risk of viremia per doubling of TFV was 0.52 (95% CI: 0.43 to 0.63; P < 0.0001).

Conclusions: Leveraging an objective metric of ART use, we observed modestly declining adherence across the postpartum period, but no additional decline associated with breastfeeding cessation. High viremia frequency and varying postpartum TFV levels observed highlight the importance of enhanced adherence support with viral load monitoring among postpartum women.

Key Words: HIV, ART, adherence, breastfeeding, TFV hair levels, viremia

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INTRODUCTION

In sub-Saharan Africa, where breastfeeding is the norm, approximately 1.1 million women living with HIV became pregnant in 2020.1 Adherence to antiretroviral treatment (ART) can reduce the risk of perinatal transmission to almost zero and benefit maternal health.2–4 Despite accessing universal ART, women can experience significant challenges to adherence and engagement to HIV care.5–8 With 20%–40% of women experiencing elevated viral loads postpartum,9–11 Breastfeeding cessation may be critical juncture when intense contact with the health care system ends.12–14 Moreover, when risk of transmission of HIV to the infant passes, women’s capacity to adhere to ART could change. Declining
adherence after pregnancy has been attributed to declining concern about in utero transmission and failure to optimize women’s own health outcomes.15,16 Few studies measure adherence among healthy women, no longer at risk of transmitting HIV to their infant after breastfeeding cessation.17 In addition, breastfeeding cessation is often followed by a subsequent conception (within 2 years) in settings with high fertility rates, underscoring the importance of sustained viral suppression to maximally reduce the risk of HIV transmission.18,19 UNAIDS estimates for 2020 showed that 37% of infants who acquired HIV were born to women who had initiated ART before the pregnancy, signaling the need to better understand adherence in this period.1

Measuring adherence to antiretroviral drugs (ARVs) is vital for monitoring response to treatment. Suboptimal adherence leads to virologic failure, development of resistance, and onward transmission, including transmission of resistant mutations.20–22 Commonly used adherence measures such as pill counts may inadequately predict virologic outcomes.23–25 Drug concentrations in hair provide an objective measure of cumulative adherence.26,27

We assessed how ART adherence changed with breastfeeding cessation by longitudinally measuring hair TFV levels in a subset of postpartum women enrolled in the IMPAACT PROMISE randomized trial in Zimbabwe. In addition, we estimated the association between hair TFV levels and virologic suppression.

**Methods**

**Study Sample and Procedures**

Data are from the International Maternal Pediatric Adolescents AIDS Clinical Trials Network (IMPAACT) Promoting Maternal and Infant Survival Everywhere Breastfeeding Study (PROMISE 1077BF: NCT01061151), conducted between 2011 and 2015 in 7 countries to examine optimal strategies for prevention of perinatal transmission of HIV and improving maternal health among women not yet eligible for treatment at the time. It included a series of open-label, parallel randomization components. In the postpartum component (within 14 days postdelivery), women not requiring ART for their own health were randomized to either maternal ART (TDF/FTC+LPV/r) or daily infant nevirapine prophylaxis during breastfeeding.3 Women on ART in the postpartum component at BF cessation were randomized to either continue or stop ART (maternal health component) (consistent with the country guidelines at that time).28 This analysis includes participants in Zimbabwe who were randomized to ART in both the postpartum and maternal health components and participated in a hair substudy throughout these 2 components at the 3 study clinics. Participants attended study visits at least quarterly, with more frequent visits in the first 3 months of each component. Hair samples and detailed breastfeeding questionnaires were collected at all visits, and HIV viral load was assessed at least every 6 months. All participants provided written informed consent before participation. This study was reviewed and approved by local and collaborating institutional review boards, relevant regulatory authorities, and reviewed for safety and efficacy by an independent Data and Safety Monitoring Board.

**Study Measures**

**Objectively Measured Adherence**

Small hair samples were collected following established procedures.29 Among women taking tenofovir disoproxil fumarate for at least 60 days, we assayed 1.5 centimeters of hair closest to the scalp for TFV concentrations using validated liquid chromatography/tandem mass spectrometry (LC-MS/MS)–based methods.30 Medications are incorporated from the systemic circulation into hair as it grows, at approximately 1 cm per month; these data reflect cumulative exposure to tenofovir over the prior ~45 days.31

**Breastfeeding Cessation**

The estimated date of breastfeeding cessation is the last date when the baby sucked on the mother’s breast for any reason, recorded only after complete breastfeeding cessation for at least 4 weeks. We created an indicator variable to classify breastfeeding status at each visit as follows: visits >45 days after breastfeeding cessation as postbreastfeeding (value = 1), visits during breastfeeding (value = 0), and visits 0–45 days after breastfeeding cessation, before potential changes in adherence would be fully reflected in hair TFV concentrations, were assigned fractional values, for example, 27 days after cessation/45 days = 0.6.

**Viremia**

We defined viremia as plasma HIV RNA >50 copies/mL following the World Health Organization’s recommendation to consider adherence interventions at this threshold in breastfeeding women.32 Only viral loads collected after 90 days on ART were included.

**Statistical Analysis**

To estimate how much ART adherence changes after breastfeeding cessation, we used mixed linear regression models with a random intercept and assumed an autoregressive covariance structure. The outcome was log_{10}-transformed hair TFV levels, and we included 3 predictors in the model to assess the impact of breastfeeding cessation on hair levels. First, the indicator variable for breastfeeding status described above: This variable quantifies the differences in hair TFV levels at postbreastfeeding visits vs. still-breastfeeding visits; second, a linear term reflecting months since delivery to account for changes in hair TFV levels over time regardless of breastfeeding status; and third, an additional linear term, measuring time since breastfeeding cessation, to quantify how the slope of TFV levels since delivery changed after breastfeeding cessation. Because quadratic terms for these time trends did not have P values <0.10, we only included linear terms in regression models. To facilitate the interpretation of associations between predictor variables and a log-transformed outcome, we back-transformed regression coefficients to reflect percent differences in hair levels associated with a unit change in each predictor.
Potential Confounders
We decided, a priori, to include the following potential confounders in adjusted models estimating the association between breastfeeding cessation and hair TFV levels: maternal age, education, Zimbabwe study clinic, and ART initiation in pregnancy vs at delivery. To ensure a parsimonious model, we screened additional potential confounders, using $P$ value < 0.25 in bivariate analysis as a threshold for inclusion: parity and gravidity at baseline, time-varying (collected at the visit before the hair sample) employment status (any work outside the home vs. none), and food insecurity. None achieved this threshold.

Missing Data
Fifteen (27%) participants were missing educational status, and 4 (7%) were missing parity; we used multiple imputation and assumed that data were conditionally missing at random. We imputed 50 data sets to minimize sampling variability from the imputation process and used multivariate imputation by chained equations. We report adjusted regression analyses from multiple imputed data as our primary results for the association between breastfeeding cessation and hair levels.

Hair TFV Levels and Viremia
To quantify the association between hair TFV levels and viral suppression, we used Poisson regression with generalized estimating equations to estimate relative risks. Because hair levels were log$_2$ transformed in this analysis, the risk ratios represent the relative risk of viremia per doubling of hair TFV levels. We report unadjusted models.

RESULTS
Among 55 eligible women, median age was 26 years [interquartile range (IQR) 24–29 years] and 93% were in WHO Clinical Stage I (Table 1). Baseline characteristics were comparable with that of women enrolled in the main PROMISE study. The median duration of breastfeeding was 14 months (IQR 12–16 months). Hair samples collected at visits ≥60 days since tenofovir disoproxil fumarate initiation (N = 326) were analyzed for TFV concentrations; 21 (6%) had no results because of technical or sample labeling issues. We include 305 hair TFV results from 55 women for a median of 8 visits per woman, collected between 3 and 29 months postpartum (up to 1 year post-BF cessation).

Hair TFV Levels
Average TFV levels were highly variable over time (Fig. 1). Across all samples, the median TFV concentration was 0.04 ng/mg (IQR 0.03–0.06, range: undetected-0.25). After accounting for the estimated change in hair levels over time since delivery (2.2% decline per month, 95% CI: −5.3 to 1.0; $P$ value 0.17), hair TFV levels in the post-BF period averaged 24.4% higher compared with levels during breastfeeding but with a wide confidence interval (95% CI: −5.1 to 63.1; $P$ value 0.11). The rate of decline since delivery, however, did not improve post-BF, with an estimated change in slope of 0.0% per month (95% CI: −3.8 to 3.9, $P$ value 0.99). To address the possibility that women who stopped BF before or after the majority (outside the IQR of breastfeeding duration: 12–16 months) might be meaningfully different, we conducted a sensitivity analysis excluding them and found a very similar estimate for the effect of BF cessation. The results from a complete case analysis were comparable.

Hair TFV Levels and Viremia
Throughout follow-up and after 90 days on ART, 42% of women experienced viremia at least once and 25% in 2 consecutive samples. In the analysis of hair TFV levels and viremia, 54 women had 237 viral load measurements performed >90 days on ART. Hair TFV levels predicted viremia; the relative risk of viremia was 0.52 (95% CI: 0.43–0.63; $P < 0.0001$) per doubling of hair TFV.

| TABLE 1. Characteristics of Postpartum Women Enrolled in the Hair Substudy of the PROMISE Trial in Zimbabwe |
|---------------------------------------------------------------|
| **N = 55**                                                   |
| **Median (IQR) or n (%)**                                    |
| **Baseline characteristics**                                 |
| Age                                                          | 26 (24–29) |
| Education*                                                   |
| None or some primary                                         | 1 (2%)     |
| Completed primary but not secondary                          | 10 (20%)   |
| Completed secondary                                          | 29 (72%)   |
| Site                                                        |
| St Mary’s CRS                                               | 15 (27%)   |
| Seke north CRS                                              | 24 (44%)   |
| Harare family care CRS                                       | 16 (29%)   |
| On ART in pregnancy                                         | 29 (53%)   |
| Parity                                                      |
| 1 (–2)                                                      |
| Gravida                                                     |
| 3 (2–3)                                                     |
| Gestational age at enrollment, wk                           | 31 (26–35) |
| Nadir CD4 count                                             | 502 (438–591) |
| WHO clinical classification                                  |
| Clinical stage I                                            | 51 (93%)   |
| Clinical stage II                                           | 4 (7%)     |
| **Follow-up characteristics**                               |
| Breastfeeding duration, mo                                  | 13.7 (12.0–16.0) |
| Experienced viremia (>50 copies/mL)                         |
| At least once                                               | 23 (42%)   |
| In 2 consecutive samples                                    | 14 (25%)   |
| Virologic failure (>1000 copies/mL)                         |
| At least once                                               | 12 (22%)   |
| In 2 consecutive samples                                    | 7 (13%)    |
| Number of hair samples collected                            |
| During breastfeeding                                         | 3 (2–4)    |
| After breastfeeding                                          | 5 (3–6)    |
| Total                                                       | 8 (5–9) (max = 11) |
| *15 missing education.                                      |           |

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Within our study, we were not able to assess the role of pharmacokinetic variability. To counter this, we analyzed multiple hair samples collected at various time points and demonstrated a strong association between higher hair levels and reduced risk of viremia. Women who stayed on ART for their own health were not included in this analysis because they were deemed to be naturally different from those who were eligible for randomization. In addition, the ART regimen in this study was different. It included a protease inhibitor and multitablet dosing. The currently recommended single daily tablet of tenofovir disoproxil fumarate, emtricitabine, and dolutegravir may promote better adherence and viral suppression, warranting further study of hair TFV trends postpartum and their associations with viremia. Tenofovir disoproxil fumarate remains a common component of ART regimens globally; thus, the findings remain relevant. Future research on cost-effectiveness is warranted.

In conclusion, we observed modestly declining adherence across the postpartum period, but no additional decline associated with breastfeeding cessation, which is reassuring. Nevertheless, the frequency of viremia we observed highlights the vulnerability of the postpartum period, indicating the need for added focus on long-term ART adherence to achieve the ambitious pediatric HIV elimination target by 2030.

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