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Comparing pill counts and patient self-reports versus DBS tenofovir concentrations as ART adherence measurements with virologic outcomes and HIV drug resistance in a cohort of adolescents and young adults failing ART in Harare, Zimbabwe

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Abstract

Background: Monitoring adherence presents a challenge in adolescents and it is prudent to explore several options for determining their level of adherence. This study sought to determine

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Authors' contributions

TJM was a main contributor in the design, implementation and writing of the manuscript. TJM performed the tenofovir drug concentrations analysis. VK performed the drug resistance testing. CEN guided the study design and implementation. TJM and VK analysed the data then GDM and CEN reviewed and provided oversight of interpretation. TD edited the manuscript. All authors read and approved the final manuscript.

Ethical approvals

The PESU study was IRB approved and ethical clearance was obtained (JREC/285/15, MRCZ/A/1717). The present study was approved as JREC/129/18 and MRCZ/A/2361

Consent for publication

Not Applicable

Availability of data and materials

The datasets generated and/or analysed during the current study are not publicly available due to confidentiality reasons. The data includes that of minors which did not have explicit consent for data sharing beyond the study teams and sponsors. However, the data are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Trial identification

Participants in the present study were a subset of those in the PESU intervention ClinicalTrials.gov Identifier: NCT02833441

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

ART adherence levels in adolescents and young adults (on a tenofovir-containing regimen) failing ART as measured by self-reports, pill counts and DBS tenofovir concentrations and to compare levels of agreement among the methods and determine the ability of each method to predict virological suppression.

Methods: This was a cohort study involving 107 adolescents and young adults between 10 and 24 years failing ART with viral load >400copies/ml at enrolment. Pill count (PC) records, self-reports (SR) and DBS tenofovir concentrations (done by liquid Chromatography with tandem mass spectrometry (LC-MS/MS) were used to determine adherence in adolescent participants failing ART in Harare. The latter was used as the reference method with a cut-off of 64ng/ml. Determination of DBS tenofovir concentrations was also performed to rule out inadequate viral response due to low cumulative drug exposure despite high adherence (≥90%). Longitudinal analysis was performed to determine the correlation of viral loads (VL) with adherence. The Kappa (*k*) coefficient was used to evaluate the level of agreement among the 3 methods.

Results: Poor level of agreement was found between PC records and DBS tenofovir concentrations (*k*=−0.115). Moderate agreement was found between DBS and SR methods (*k*=0.0557) . Slight agreement was found between PC and SR methods (*k*=0.0078). Adherence was dependent on age at HIV diagnosis (p=0.0184) and ART initiation (p=0.0265). Participants who were adherent were six times more likely to be suppressed at end point than their non-adherent counterparts (OR=5.7 CI 2.1 – 16.5, p<0.0001).

Conclusions: Self-reported measure of adherence and pill counts exhibited poor agreement with the reference method used i.e. DBS tenofovir concentrations and are thus not effective methods of predicting virological suppression.

Introduction

Over the years, the accessibility and affordability of antiretroviral therapy (ART) for eligible patients has increased. Antiretroviral therapy success depends on optimal adherence to the treatment regimen. Adherence is defined as a patient's ability to follow a treatment plan, take medications at prescribed times and frequencies, and follow restrictions regarding food and other medications. ARV adherence rates of at least 90-95% or missing not more than two doses per week are required in order to achieve maximal viral suppression (1–5). When using drug concentrations in biomatrices to measure adherence, it can be defined using cut-off values above or below which one is considered adherent or not respectively. ART success is defined by sustained virologic suppression and immunologic recovery and is dependent on adherence to the treatment regimen, cumulative drug exposure and HIV drug resistance profiles of the individuals. Adherence to medication remains critical in ensuring effectiveness of treatment, preventing opportunistic infections and suppressing HIV viral replication, minimizing and delaying the development of resistant strains, thus increasing durability of first line regimens(1,2).

In Zimbabwe, the adherence assessment methods in the national ART program include the use of refill history, pill counts and patient self-reports, which are often unreliable and offer little information about the actual medication-taking habits of patients on ART. On the other hand, the need for effective adherence monitoring cannot be overemphasized in

ART particularly in vulnerable populations such as adolescents, a population that presents an additional unique set of challenges in ART(8–13). Fear of disclosure, stigma, peer pressure and the need to conform including substance abuse and depression, forgetfulness, inconsistent routines, concerns regarding drug safety and adverse effects all remain real challenges within this population(8,9,13–15). There is also an additional set of challenges associated with transitioning from paediatric to adolescent care(4,12,13,16,17). The increased responsibility put on children to take care of themselves assumes they are developmentally adept and have stable routines that will not affect their ability to remember to take their medications(4,16,18). In many cases, clear transitionary links between paediatric and adult services are lacking, further complicating their ability to navigate often complex health systems(16,19). Monitoring adherence, therefore, presents challenges in this population and it is prudent to explore other options for determining their level of adherence. Objective, valid, reliable yet practical measurements of adherence are needed as more reliable markers for adherence to provide a consolidated approach to identifying patients needing adherence intervention measures(2,4,18,20).

To date, there is no gold standard to accurately measure adherence in routine medical practice, although several competing methods are currently in use as adherence assessments(21). Viral load (VL) testing is common practice but is currently limited to those with clinical and immunologic concern for treatment failure and in Zimbabwe it is performed once every six months (8). VL testing is usually supplemented by qualitative methods such as pill counts, refill history and self-reports. While HIV VL has been traditionally used as a surrogate for ART adherence, it cannot provide information about drug exposure or pharmacologic forgiveness. Average adherence to ARVs may be a better predictor of virologic suppression than duration or frequency of missed doses(19). Pharmacologic tests provide platforms to determine adherence objectively(22). Usually, these involve the measurement of drug concentration, or their direct derivatives, in biomatrices to determine the presence of the drugs in relation to the expected concentrations.

Plasma has been extensively used in the measurement of ARV concentrations(21,22). However, limitations of using plasma for therapeutic drug monitoring (TDM) include the complexity of sample processing and storage for preservation of analytes . Moreover, single plasma ARV concentrations present marked day-to-day variation thus limiting their utility in TDM in estimating an average or long-term measure of medication exposure. Plasma concentrations can also be susceptible to “white coat effects”, where adherence improves transiently prior to study visit(28). Drug concentrations e.g. for phosphorylated tenofovir (TFV-DP) in peripheral blood mononuclear cells (PBMCs) relay information on exposure over longer periods (7-14 days), although processing, isolating and counting PBMCs are costly and technically challenging. Dried blood spots are easier to collect and process than PBMCs, and drug concentrations in red blood cells (RBCs) from dried blood spots represent longer-term exposure(23). Zheng *et al* (2013) compared TFV in DBS versus plasma and reported a high correlation between them, indicating that DBS can be used as a plasma alternative for pharmacokinetic analyses in vivo(23,29). A South African study found a correction factor of plasma TFV to DBS of 1.57 (6). Monitoring of drug concentrations allows clinicians to detect pharmacokinetic concerns earlier, potentially at a

lower cost(30,31). Additionally, drug concentration testing will further aid in differentiating causes for elevated HIV-1 viral load.

Previous literature has shown varying strengths of association between virologic and immunologic response with the different adherence measurements in various settings (19–21,32–34). It is likely that each of the methods has limitations that may uniquely apply to adolescents who have questionable adherence characteristics. Drug concentration measurements in biomatrices offers biologic evidence of adherence and is an objective measure of drug exposure.

Objectives

The main objective of this study was to determine the ART adherence levels in a cohort of adolescents on a tenofovir containing regimen as measured by i) self-report , ii) pill count iii) DBS concentrations of tenofovir. We also compared adherence data from self-reports from participants and pill counts done by clinicians to measurement of tenofovir concentration in DBS. We explored factors (age, sex, duration on ART) that may have an impact on the adherence measurements as well as the association of the DBS concentration with virological suppression and drug resistance.

Methods

This was a retrospective observational cohort study which included primary laboratory analysis and secondary analysis of adherence data obtained from the Peer Support (PESU) study described elsewhere (35) . The PESU study was a prospective adherence intervention cohort study conducted in the then UZCHS Department of Medicine. Between April 2016 and April 2018, HIV-infected patients who were failing their ART regimens, aged 10-24 years, were recruited and randomized to a community-based peer counselling support group or clinic-based standard of care. The participants were meant to be followed up for up to 48 weeks but the study monitoring stopped for most participants at 36 weeks due to funding constraints. Viiral loads and CD4+ counts were performed at baseline and then at 12-week intervals for up to 36 weeks to determine the efficacy of the interventions in improving adherence and virologic suppression. Self-report and pill count data collected prospectively, primarily for the PESU study, were used to compare with tenofovir drug concentration measurements determined in corresponding DBS also collected during the PESU study. The drug concentrations were performed primarily for the present study.

Participants and setting

This study was nested within the PESU intervention conducted at Parirenyatwa Hospital and within Harare City Health Clinics in Zimbabwe(35–37). Youth (10-24 years) presenting with an HIV VL >400 copies/mL were recruited and followed up with viral load and adherence measurements being done at 12 week intervals for 36 weeks (37). Participants who missed scheduled follow-up visits were contacted by text message to remind them to reschedule a visit. When that failed, 3 telephone call attempts were made. Where these were unsuccessful, and were participants had consented, a home visit was arranged and attempted. Although the parent study (PESU) had enrolled 214 participants (of a target 250),

purposive/convenience sampling was used to enrol all the 107 participants who were on a TDF containing triple regimen and hence included in this study's analyses. Participants were excluded in the present study if they were not on a TDF containing ART regimen.

All participants received ART through the national treatment program at no cost to the patient. The participants included in this analysis received a tenofovir-containing regimen primarily Tenofovir disoproxil fumarate, lamivudine and efavirenz or nevirapine. All participants were provided with three months' of drug supply as per standard clinic procedures and seen back at the clinic at weeks 12, 24 and 36. Data points for the study were taken at these time points.

Study Outcomes

i) Adherence Measurement—All adherence measurements were taken at each of the study visit time points.

Self-report: Self-report adherence data were obtained from the study questionnaire which asked how many doses they had missed out of the seven possible doses in the week preceding the visit. A week was used as the unit of recall to avoid recall bias as participants were more likely to accurately remember events of the past week than they would of an entire month. The number of missed doses was then expressed as a percentage and subtracted from the possible 100% (where a participant did not miss a single dose). Percentage adherence was therefore calculated as [100%-100(number of missed doses/7)]. This was used as a marker of their performance since their previous clinic visit. Participants with 90% adherence and higher were considered adherent while those below were recorded as non-adherent(1–4).

Pill count: Participants were asked to bring all their medication to each study visit. Counsellors or study nurses performed pill counts. They determined the number of pills taken since the preceding visit by subtracting the number of pills returned from the number dispensed at the previous visit. The percentage pill count adherence was calculated as the number of pills taken, expressed as a percentage of the number of pills prescribed between visit dates. A cut off of 90% was also used to determine adherence or lack thereof (1–4).

Drug concentrations: For this study determination of TFV in DBS was done by liquid chromatography-mass spectroscopy/mass spectrometry (LCMS/MS) using protein precipitation and a method developed and validated in the Clinical Pharmacology Laboratory at the University of Cape Town, South Africa. Whole blood samples spotted and air dried and stored at room temperature for up to 18 months were used. A full 10mm punch was extracted with a working solution containing the internal standard (IS 1000ng/ml TNF-d6) with methanol and reconstituted in water for analysis by LC-MS/MS utilizing stable isotope labelled internal standards. The assay was validated over the range of 10ng/mL to 1,600ng/mL. The method was accurate (within 15% of control). Analytes were stable for up to 72 hours at room temperature. Drug concentrations were categorized as adherent if values were at or above minimum inhibitory concentration (C_{min}) or non-adherent if below C_{min}, using a C_{min} cut off of 64ng/mL.

For each participant and for each time point, adherence was determined and categorized as described above. The adherence data were categorized into two groups 90% and above (adherent) and less than 90% (non-adherent) for the self-report and pill count methods. Drug concentration data were categorized into two groups: adherent as at or above C_{min} (64ng/ml), and non-adherent as below (64ng/ml).

ii) Treatment response measures—In this study, we evaluated treatment response using viral load data obtained from laboratory analysis performed at the Infectious Disease Research laboratory in the Department of Medicine at the University of Zimbabwe Faculty of Medicine and Health Sciences. Viral loads were performed using GeneExpert (Cepheid, CA, USA) and Roche Amplicor HIV-1 RNA monitor test version 1.5 (Roche Diagnostics Systems, Branchburg, New Jersey, USA). The lower limit of detection for viral load was 20 copies/ml. At study enrolment, virological failure was defined as HIV RNA >400 copies/ml with two consecutive results taken at least a month apart used to define failure. During the study virological suppression was defined as a single HIV RNA of <1000 copies/ ml at weeks 12, 24 and 36(35,37). This cut=off was also in keeping with the WHO definition of treatment failure (14).

iii) HIV drug resistance testing—In this analysis, we used HIV drug resistance profiles obtained from a previous analysis (38). Participants with at least one major resistance mutation causing resistance to ≥1 of the three drugs in their regimen were classified as resistant as per the online Stanford HIV database.

Statistical Analysis

Data from participants of the two arms of the PESU study were analysed separately then pooled into one dataset. The inclusion and exclusion criteria for the two arms were the same.

Data were analysed using Stata 16.1 (Stata Corporation, College Station, USA). Descriptive statistics were used to summarise the baseline characteristics of the participant groups and to tabulate the adherence data. The data followed a normal distribution so parametric statistical tests were used. All means were reported as Mean ± SD. We used *t-tests* to compare means for continuous variables and used chi square test to test for population differences for categorical variables.

Univariate and multivariable logistic regression methods were used to explore the relationship of clinical factors to adherence as well as the impact of adherence on genotypic resistance at end point. Where possible, both pooled data and data disaggregated by arm are presented.

All adherence variables and outcomes were binary and categorised as described above. Linearity was assessed between the variables and the outcomes. We used Chi squared test on baseline data at enrolment (i.e. before any interventions) to compare the two subjective methods (PC and SR) to the reference DBS drug concentration as well as to calculate the levels of agreement between them using the kappa statistic. The Cohen's Kappa coefficient was used to assess level agreement between the adherence measures(39).

We assigned the DBS tenofovir concentration as the reference method as it is an objective method of assessment. Kappa (*k*) statistics was used to define the agreement between adherence by DBS drug concentration and adherence by participant SR and between DBS drug concentration and adherence by PC.

The strength of the agreement was assessed with a commonly used classification scale for kappa 'coefficients. Kappa values range from −1 and 1. −1 to 0.00 = poor, 0.00 to 0.02 = slight, 0.21 – 0.40 = fair, 0.41 – 0.60 = moderate, 0.61 – 0.80 = substantial and 0.81 to 100 = almost perfect (39).

Results

Only data from 107 adolescents and young adults were included in this analysis. The mean age in years at enrolment of this sample was 17.8 ± 3 . About half of the participants (51%) were male. The ages at HIV diagnosis ranged from <1 year to 22 years with a mean age of 10.4 ± 4.5 at HIV diagnosis. The mean duration with known HIV diagnosis was 7.3 ±3.4 years. The mean age at ART initiation was 11.8 ± 3.7 years with a range between 5.4 to 22.6 years. Median duration on ART was 6.25 years (IQR 3.76 – 8.3). Twenty one percent of the participants were on their first ART drug combination, 51% on a second, 18% on a third and 10% on a 4^{th} combination counting from when they had been initiated on ART. The duration on TDF was measured as a median of 86 weeks (IQR 39.6 – 166.4). All data are presented in Table 1.

Participants' adherence outcomes

Complete adherence data by pill count, self-report and by DBS tenofovir concentration were obtained for the 107 participants for all four time points from enrolment through 36 weeks until study end (i.e., enrolment, 12, 24 and 36 weeks).

Adherence rates analysis—Adherence rates were stratified by gender and age. The self-report method consistently showed at least 80% of participants were adherent i.e adherence ≥90% except for week 24 where only 59% reported being adherent. The highest adherence rates (proportion of participants with ≥90% adherence) indicated by pill counts was at enrolment (71%) and showed a downward trend to 31% at W36. Measuring adherence using TDF drug concentrations showed adherence levels (proportion of participants with tenofovir concentrations at or above C_{min} of 64ng/ml) of between 41% and 48% throughout the study except for W12 (63%). There was an improvement in adherence rates from enrolment to W36 using the reference method ($p<0.004$). Table 2 shows the percentage of participants who were adherent using each method throughout the study.

Using bivariate analysis on participant demographic and clinical characteristics, there were no significant differences in adherence rates based on the participants age at enrolment ($p=0.077$), duration on ART($p=0.305$) or gender ($p=0.525$). However, the results show that adherence was dependent on age at HIV diagnosis ($p=0.018$) and age at ART initiation ($p=0.027$). These results are illustrated in Table 3 below.

Levels of agreement between adherence methods

The agreement statistics for the study are presented in Table 4 below:

Relationship analysis of virological suppression and adherence

For each of the participants, viral load determined at corresponding visits, were classified as suppressed or not suppressed based on 400copies/ml as the cut off. Adherence at enrolment was used to determine/predict virological suppression at end point. Virological suppression was not dependent on adherence levels obtained by Self Report (OR=1.083 CI 0.19 - 6.2, p=0.914) and Pill Count methods (OR=1.511 CI 0.2 - 11.1, p=0.605). However, using the DBS tenofovir concentrations method, those who were adherent (≥64ng/ml) at enrolment were almost six times more likely to be virologically suppressed at end point. (OR=5.7 CI 2.1 – 16.5, p<0.0001).

Relationship analysis of adherence and HIV drug resistance

Adherence and HIV drug resistance data from enrolment were analysed to explore the relationship between adherence and drug resistance in this group. There was a statistically significant association between adherence and presence of HIV drug resistance mutations, those who were not adherent were 3 times more likely to have drug resistance mutations (OR=3.4 CI 1.0 −13.7, p=0.03).

Discussion

Adherence assessment

The main aim of this study was to determine the ART adherence levels in adolescents in the PESU study on a tenofovir containing regimen as measured by three different methods (self-reports , pill counts, DBS concentrations of tenofovir) as well as to compare data from them. Overall, the methods showed varied results. The self-reports generally showed higher adherence rates than the other methods 83% of participants being adherent at enrolment and the lowest result being at week 24 where only 59% were adherent. Adherence as measured by pill counts showed a downward trend from 71% at enrolment to 40% at W36. On the other hand, using tenofovir concentrations in DBS showed more consistent adherence levels of between 41% and 48% throughout the study except for W12 (63%). The results also show that in up to 40% of the time, pill count and self-report adherence data were not collected despite the participants presenting at the clinic. It also shows that using the pill count method, adherence was overestimated in up to 5% of the cases.

Despite this being a controlled environment in which the clinicians were expected to perform all study activities, pill count adherence assessments were not done at all in up to 40% of the participant appointments, reflecting the unreliability of the method. It is reasonable to extrapolate that this percentage may be higher in actual clinical settings given the prevailing human resource constraints of clinical staff in the country due to economic constraints with the existing already burdened staff not being able to perform all duties. They are more likely to focus on clinical assessment with more immediate or acute needs.

Although they give unreliable results, it is imperative to acknowledge that the PC and SR methods of adherence assessment are in use because of their practicality and ease of administration. Drug concentration measurements in biomatrices are expensive to implement routinely, require high levels of expertise and therefore have found more use in research than in clinical practice. On the contrary, pill counts and self-reporting questionnaires can be done by cadres such as clinic counsellors hence relieving nurses and doctors of some duties as they are often overwhelmed with advanced patient management in low resource settings where human resources are scarce. For clinical practice, it is clear why the PC and SR methods are common in use but rates of up to 40% of the tools not being used show a gap in the implementation thus reducing the effectiveness of these methods. It must then be emphasised that the workforce administering these tools must be thoroughly and constantly trained on the importance of implementing them and also on how to do so effectively. Where possible, an objective method such as measuring drug concentrations in a biomatrix must be done periodically to complement adherence measures. An example would be to adopt the schedule used for viral load monitoring i.e. twice a year. This would help keep in check the continued efficiency of the adherence assessment methods in common use.

Measurement agreement

The ability to measure treatment adherence accurately is crucial in early identification of viral load failure and possible emergence of drug resistance. Methods should be reliable and valid for use in various settings being able to provide timely information to facilitate early intervention for the patients when needed. In this study we also assessed agreement between adherence as measured by two different methods in common use (self-report (SR) and pill count (PC)) and DBS tenofovir concentrations in adolescents failing antiretroviral therapy in Harare. The results showed that neither the PC nor the SR method had good strength of agreement with the DBS method with the SR method showing only moderate agreement and the PC method showing poor agreement with the DBS method. The PC and SR methods were only slightly in agreement.

Two of the methods (SR and PC) are common clinical practice in Zimbabwe such that evaluation of these methods against an objective method is an important operational research to provide evidence to enhance policy making in providing ART services particularly for adolescents. To date there is no gold standard for adherence assessment to antiretroviral therapy(21). Different settings use different tools but the aim is to find an optimal tool for practical use(18,33,34). This is the first study in Zimbabwe to systematically evaluate agreement between these three methods in adolescents and young adults failing ART in Zimbabwe and to provide objective evidence of the levels of agreement and differences in these methods.

The cut-off value of 90% for adherence as assessed by prescription refill history was chosen based on literature (21,33,40). In this analysis, the results show that the levels of agreement between the methods are generally low, the two methods in common practice do not compare well with using drug concentrations as an objective measure of adherence. This was not surprising as the former methods are practically subjective methods dependant on both the patient and the health worker. Although the guiding principles and formulae used

to calculate adherence are standard, the results can vary as much as the patient and health worker vary. They depend on the honesty of the participant, whether or not they consistently bring the correct number of leftover pills for their appointments and the abilities of the health worker to accurately count and calculate adherence.

Comparison of the level of adherence between the two subjective methods showed only slight agreement, and neither had good agreement with the reference method. As such, neither the PC or SR methods address adherence in a comparable way. The pill count method also revealed up to 5% overestimation of adherence. This may be white coat adherence in which the participants throw out some of their pills when they know they are due for review and know their pills will be counted; only they discard more pills than expected. This is not unusual in this age group as teenagers can be shrewd in their behaviour. However, it can significantly skew study outcomes and clinical practice by presenting a false picture of compliance and treatment efficacy (19,41). This phenomenon undermines the validity of research and may lead to misguided clinical decisions based on inaccurate adherence data on medication effectiveness and mask true adherence difficulties (41).

To mitigate this behaviour, future studies could implement strategies such as more frequent and unannounced pill counts and use of electronic medication monitoring devices (33,34,41). Additionally, researchers can incorporate biological assays for markers of medication use or directly observed therapy (DOT) to objectively assess adherence more accurately (24,25,32,34,41). Enhancing patient education about the importance of medication adherence and establishing a trusting relationship between healthcare providers and patients may also reduce the incidence of this behaviour (42). By employing these strategies, researchers and clinicians can gain a more accurate understanding of adolescent medication adherence and develop more effective interventions.

Factors affecting adherence

Of the factors analysed for their influence on adherence only age at ART initiation was shown to be a significant factor associated with adherence. While this was identified as a significant factor influencing adherence, further exploration of additional potential determinants is warranted. Investigating the role of psychosocial support systems, family dynamics, and educational interventions could yield a more holistic understanding of adherence behaviours (43,44). Psychosocial support may address emotional and mental health challenges that affect adherence, while positive family dynamics can offer a supportive environment conducive to consistent medication intake (43,45,46). Educational interventions tailored to the needs of individuals and families can enhance knowledge about ART and the importance of adherence, potentially leading to improved outcomes (47,48). Comprehensive studies incorporating these factors could inform more effective adherence strategies and interventions.

Age at enrolment, duration on ART, gender were not associated with adherence. The former two findings may be explained by that adolescents, mostly teenagers are still not yet completely in control of their lives and consequently of their well-being. Much of it is influenced by their caregivers and living environment and it is possible that someone else has been in charge of their pill taking however long it had been since they have been taking

ART. The fact that adherence did not depend on gender may be because in this cohort, the assumption was that most of the participants had perinatal transmission, and coupled with the fact that this was a randomized study there are no differences between males and females because the outcomes are not behavioural.

Viral suppression predictive value

Contrary to a recent study done in Kenya by Nyawira et al (year) who found that self-reported adherence was a reliable measure of adherence, the present study showed that these adherence assessment methods in common use are poor proxies for predicting virological suppression over time(49). The poor agreement between pill count and self-reported adherence in one hand compared with drug concentrations in blood and viral load in the other is a cause for concern regarding the interpretation of these measurements in clinical practice, yet they are the methods in common clinical practice. This means that at enrolment where all participants had detectable viral loads (>400 copies/ml), adherence as indicated by drug concentrations found that only 59% had optimal drug concentrations. In contrast, up to one quarter of the adolescents (12-24%) were otherwise considered as adherent by the health worker (83% by SR and 71% by PC). The accuracy with which these methods identify non-adherence is, therefore, critical.

Furthermore, while adherence assessment results by SR and PC showed that virological suppression was not dependent on adherence, the DBS drug concentration method showed that participants who were adherent were actually 6 times more likely to be virologically suppressed over time. As such, data obtained from these methods for programmatic purposes would result in gross underestimation of treatment responses in this group.

In a previous study done on Zimbabwean adolescents failing second line therapy, drug concentrations in a biomatrix as an adherence assessment method, self-reports and virological suppression were investigated (27). Our results are consistent with this previous study, showing that in adolescents failing ART in Zimbabwe, the adherence as measured by drug concentrations does not show the same virological outcomes as self-reports (27).

Adherence and HIVDRMs

The present study confirms that, decreased adherence is associated with the emergence of drug resistance mutations as previously postulated; reduced adherence to antiretrovirals reduces effectiveness of the treatment and prompt the emergence of resistant strains thereby decreasing durability of first line regimens(6,7). The acquisition and transmission of drug resistance in HIV-1 subtype C impacts ART treatment of adults and children(7,50). Adherence monitoring is particularly critical in preserving the historic first line NNRTI-based regimens as they have a lower genetic barrier to resistance and are prone to cross-resistance between agents, particularly with the K103N and Y181C mutations(51–54).

Sustained drug exposure, remains a reliable predictor of efficacy in HIV treatment(2–4). Poor adherence will result in treatment failure and drug resistance resulting in the requirement for more complex treatment regimens which give way to more toxicity and a more complex prognosis(55). It becomes imperative to monitor adherence in order to preserve first line NNRTI-based regimens which have a lower genetic barrier to resistance

and are prone to cross-resistance between them (7,50,51,53). In addition, the transmission of a resistant virus within the population becomes cause for concern from a public health perspective and the cumulative increased morbidity and mortality is concerning from a clinical outcome as well as an economic viewpoint.

Generalizability, applicability and limitations

This study had some notable limitations. For instance, we acknowledge that our methods for minimizing loss to follow-up have limitations. Text message reminders may not reach all participants, and telephone calls may not always be successful. These limitations could introduce non-response bias, where those lost to follow-up may differ systematically from those who remained in the study. Additionally, even after successful contact, some participants may have been hesitant to disclose complete information during follow-up.

While this study offers valuable localized data on ART adherence patterns of a cohort in Harare, Zimbabwe, generalizability is limited by socio-economic and healthcare context variations. Disparities in income, education, and healthcare access can significantly influence adherence and virological outcomes (42,56–58). Harare's socio-economic landscape may not reflect wealthier regions, potentially underestimating adherence challenges in underprivileged populations. In areas with better healthcare infrastructure and higher socio-economic status, adherence rates may be higher due to improved access to medications and support (57,59,60). Conversely, robust healthcare systems and higher socio-economic status in other settings can higher adherence(58,61–63). Therefore, while these localized findings are crucial, broader studies across diverse settings are necessary to fully understand the spectrum of adherence determinants.

Conclusions

Self-reported measure of adherence and pill counts exhibited poor agreement with DBS drug concentrations and are thus, not effective method of predicting virological suppression. A good measure of adherence should consider factors such as the purpose of measuring adherence, the characteristics of the population being studied, and the feasibility of each measure for the purpose at hand.

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List of abbreviations

ART	antiretroviral therapy/treatment
DBS	dried blood spot
DRM	drug resistance mutations
HIV	Human immunodeficiency syndrome
NNRTI	non-nucleoside reverse transcriptase inhibitor
PBMC	peripheral blood mononuclear cells
PC	pill count
SR	self-reports
TDF	tenofovir disoproxil fumarate
TDM	therapeutic drug monitoring
TFV	tenovofir
VL	viral load

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Table 1:

Demographics and descriptive clinical characteristics for participants

Parameter	Participants
Age,	N=107
meant± SD,	17.8 ±3
Gender,	N=107
Female	52,(49)
Male	55, (51)
*Number of prior ART combinations taken including current, (n,%)	N=107
1	22 (21)
2	55 (51)
3	19 (18)
4	11 (10)
Participants virologically suppressed (<400copies/ml) per visit, (*N#*,%)	
Day 1 visit (D1)	*N#*=107 (0)
Week 12 visit (W12)	*N#*=97 (33)
Week 24 visit (W24)	*N#*=91 (46)
Week 36 visit (W36)	*N#*=91 (48)

* - this denotes the number of ART regimens taken by the participant prior to enrolment, counting from when they had been initiated on ART and including the one they were on at enrolment.

N#, - this denotes the number of participants analysed at each visit

Table 2:

Outcomes by adherence assessment method per visit (n,%)

N=107	n,% participants per outcome using SR				% participants per outcome by PC					% participants per outcome by TDF concentrations (DBS)		
	♦A	NA	ND	M	♦A	NA	ND	O	M	*A	NA	M
Enrolment	89,83	9,8.5	9,8.5		76,71	9,8	17,16	5,5		44,41	63,59	
W12	86,80	0,0	13,12	8,8	59,55	15,14	23,21	2,2	8,8	67,63	31,29	9,8
W24	63,59	6,7	27,25	10,9	45,42	16,15	32,30	4,4	10,9	51,48	42,39	14,13
W36	86,80	3,3	12,11	6,6	33,31	12,11	43,40	3,3	16,15	51,48	42,39	14,13

SR=Self report PC=pill count

♦A=adherent (defined as follows: i) for PC ≥90%, ii)for SR ≥ 90% iii) for DBS tenofovir ≥64ng.ml) NA=Nonadherent ND=not done O=overadherent/overestimated M=missed visit (participant did not turn up for scheduled visit)

Table 3:

Adherence (measured by DBS tenofovir concentrations) at enrolment and association with demographic and clinical characteristics

Characteristics in bivariate analysis with Adherence	p value
Gender	0.525
Age at enrolment vs adherence	0.0767
Age at HIV diagnosis	0.018
Age at ART initiation	0.027
Duration on ART	0.305
Number of prior ART combinations/regimens	0.256

Table 4:

Agreement statistics

	Agreement Statistics		
Adherence measure*	**p value**	**Kappa value**	**Strength of agreement**
Adherence from DBS tenofovir concentrations vs Adherence from Self report	0.632	0.0559	Moderate
Adherence from DBS tenofovir concentration vs Adherence from Pill count	0.100	−0.1115	Poor
Adherence from Self report vs Adherence from pill count	0.936	0.0078	Slight

* Adherence defined as follows: i) for PC ≥90%, ii) for SR ≥ 90% iii) for DBS tenofovir ≥64ng.ml