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Research Article

Plasma Drug Level Validates Self-Reported Adherence but Predicts Limited Specificity for Nonadherence to Antiretroviral Therapy

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Introduction. Adherence to antiretroviral therapy (ART) in low-income countries is mainly assessed by self-reported adherence (S-RA) without drug level determination. Nonadherence is an important factor in the emergence of resistance to ART, presenting a need for drug level determination. *Objective*. We set out to establish the relationship between plasma stavudine levels and S-RA and validate S-RA against the actual plasma drug concentrations. *Methods*. A cross-sectional investigation involving 234 patients in Uganda. Stavudine plasma levels were determined using high-performance liquid chromatography. We compared categories of plasma levels of stavudine with S-RA using multivariable logistic regression models. *Results*. Overall, 194/234 patients had S-RA ≥ 95% (good adherence) and 166/234 had stavudine plasma concentrations ≥ 36 nmol/L (therapeuticconcentration). Patients with good S-RA were eight times more likely to have stavudine levels within therapeutic concentration (Adjusted Odds Ratio: 7.7, 95% Confidence Interval: 3.5–7.0). However, of the 194 patients with good S-RA, 21.7% had below therapeutic concentrations. S-RA had high sensitivity for adherence (91.6%), but limited specificity for intrinsic poor adherence (38.2%). *Conclusions*. S-RA is a good tool for assessing adherence, but has low specificity in detecting nonadherence, which has implications for emergence of resistance.

1. Introduction

The role of good adherence in the attainment of positive clinical outcomes among HIV-infected patients on the life-long antiretroviral therapy (ART) has been well demonstrated in previous studies [1, 2]. Good adherence has been associated with suppression of the virus, reduction in resistant strains of HIV, delayed progression to AIDS, and improved quality of lifeand reduction in AIDS-related mortality, among other benefits [3–14]. Globally, suboptimal or poor adherence levels to ART are associated with poor viral suppression and rebound plasma viremia, which leads to emergency of drug resistant HIV strains, increasing risk of transmission of multidrug resistant viruses within the population [15]. Studies conducted around the world have reported adherence rates ranging between 40–70% including a rate of 68% for Uganda [15–17].

Accurate assessment of patient adherence to antiretroviral medication is critical to maintain the ART benefits and reduce the risks associated with poor adherence. Among

treatment centres within sub-Saharan Africa, adherence is assessed using various methods including patients' self-reports, announced or unannounced pill counts, use of pharmacy refill records, compliance to clinical appointments, medication diaries, linear analog scales, electronic monitoring systems, and sometimes measurement of plasma drug levels [18–20]. However, nearly all these methods are associated with known limitations [16, 21–23] and thus fail to capture the important dimensions of adherence, which is not commonly done in resource limited countries [22].

In Uganda, adherence to ART is commonly assessed using self-report adherence (S-RA). Recent literature has shown diverse views about the degree of reliability of S-RA [24]. Furthermore, limited information is available regarding the sensitivity and specificity of this adherence assessment method validated using plasma drug concentration levels. In the current study, we aimed at establishing the relationship between plasma drug levels and adherence levels determined by S-RA among HIV/AIDS patients on stavudine-containing regimen in Uganda.

2. Methods

2.1. Study Site and Design. This was a cross-sectional study of patients attending the ART clinic at the Joint Clinical Research Centre (JCRC) in Kampala, Uganda. The study was conducted between June 2006 and June 2007, recruited patients were aged 18 to 50 years, who had been on treatment for at least three months and were willing to give a written consent. At the JCRC clinic, patients on ART are assessed for S-RA at every visit by an adherence counselor basing on the number of pills reported to have been taken. A patient is classified to be adherent to ART if their S-RA is 95% or more. Their CD4 counts are also measured at initiation of therapy and then once every six months, as part of the routine monitoring activities. These data sets are recorded on their clinical charts and later stored in an electronic database.

2.2. Sample Size Calculations and Inclusion Criteria. Given the diversity in the characteristics of patients attending the JCRC clinic, we anticipated their adherence levels to be a little lower than the global adherence levels of 70%, but within Uganda's adherence levels of 68% as documented in other studies [15–17]. In our power calculations, we used the formula for computing proportions of sample sizes as documented by Schaeffer et al. (1990) [25], which gives similar results to the Kish et al. (1965) formula [26]. With the assumption of a 68% adherence rate among the 750 patients on ART, on average, who attended the clinic weekly in 2006, the study would be sufficiently powered to detect a 5% effect if 232 participants would be recruited. In the end, however, this study recruited 234 patients, who were on the Triomune-30 regimen for at least three months and had consistent S-RA assessments during the last three clinic encounters. Clients who had taken their last medication dose before consent or were not willing to consent for a blood draw or were taking concomitant medication which was likely to interfere with the bioavailability of stavudine such as methadone, were excluded from participating.

2.3. Data Collection. Demographic, clinical, and S-RA data were extracted from the patient records using a check list. Patients with mean S-RA of 95% and above on the past three clinic visits were classified as adherent, whereas those with mean S-RA below 95% within the same duration were classified as nonadherent. Predose venous blood samples (4 mls) from each participant were collected in prelabeled EDTA vacutainer for plasma drug level analysis.

2.4. Laboratory Procedures. Reference stavudine and the internal standard (thymidine oxetane) were obtained from Bristol-Myers Squibb. Distilled water was obtained from Mili-Q water purification system. The analytical column was a Nova pak C_{18} 5 micro meter particle size, 150 × 3.9 mm (Waters) with a guard column Nova pak C_{18} (Waters). Varian solid-phase extraction cartridges (Varian, Netherlands: part no. 12102028 lot number 0715906, code 33106t phase C18, 3 cc 500 mg) were used for extraction of the drugs from the plasma samples. HPLC-grade acetonitrile and methanol were purchased from BDH (U) Limited, Kampala. Blank plasma was obtained from the Uganda Blood Bank, Nakasero, Kampala.

Stavudine stock solution of 1.5 mg was prepared in 10 mL methanol, with serial dilutions with distilled water, to provide solutions of 10 μg to 550 μg/mL. Calibration concentrations of 35, 50, 78, 312, 625, 1250, and 2500 nmol/L in blank plasma were used to construct the standard curve from the stock solution. A second stock solution of stavudine was used to prepare the quality control standards in plasma. All the calibration and the quality control standards were contained in polypropylene microtubes and stored at −70°C until assay. Stock solution of internal standard of 1 mg/mL was prepared in methanol and diluted to 100 μg/mL in 50% methanol. The mobile phase comprised of 0.01 M ammonium acetate : acetonitrile : methanol (80 : 10 : 10, V/V/V (%)) at a flow rate of 1.2 mL/min.

Plasma concentrations of stavudine were determined according to the method described by Sarasa et al. [27] utilizing a validated high-performance liquid chromatographic (HPLC) separation [28]. Aliquots of 200 μL of thawed patient plasma samples were added to the solid-phase cartridges and allowed to pass through the cartridge bed into clean dry glass tubes and then washed with two 1-mL water aliquots. The cartridge bed was then dried by gentle suction. The stavudine was eluted from the solid-phase cartridge with 1 mL of methanol. The eluent was then evaporated to dryness under nitrogen stream. The residue was reconstituted with 65 μL of the mobile phase, vortexed and 50 μL of this sample was injected onto the HPLC system. Chromatograph curve peak heights were plotted against concentration to generate the standard curves.

The accuracy and interday precision of the method were estimated by assaying five replicate plasma samples at different concentrations, in three runs. The overall mean precision was defined by the coefficient of variation set at

2.5% from five standards of three different concentrations analyzed on the same day. Recovery of the study drug after the solid phase extraction was determined by comparing the observed stavudine concentrations in the extracted plasma, to those of nonprocessed standard solutions. Any possible interference from the endogenous compounds was investigated by the analysis of six different blank matrices. All reagents were of HPLC grade.

2.5. Statistical Methods. Plasma stavudine concentrations analyzed ranged from zero to 4254 nmol/L. The steady state pharmacokinetic parameters of stavudine 30 mg in HIV-infected adults ranges between 8 ± 2 ng/mL (35.7 ± 8.9 nmol/L) and 536 ± 454 ng/mL (2392.9 ± 2026.8 nmol/L) [29]. Patients with 95% and above self-reported adherence levels are expected to have a trough plasma drug concentration of not less than 36 nmol/L. The 36 nmol/L is the documented cutoff for the lower boundary normal stavudine therapeutic concentrations [29]. A binary variable was created, indicating whether each patient's plasma drug concentrations were below 36 nmol/L or not, and this was the outcome of interest. In this analysis, the primary independent variable was the binary S-RA variable indicating whether the patient was 95% and above adherent to ART or not.

In comparative analyses, the Chi-square test was used to investigate the association between all categorical covariates and the outcome. For continuous variables which were normally distributed, the student t-test with equal variances was used to compare means within the outcome categories.

The primary aim of this analysis was to determine the association between stavudine plasma concentrations and S-RA. This was achieved in classical Mantel Haenszel (MH) and logistic regression models. Crude associations between all categorical exposures and the outcome were determined in univariate logistic models, where statistical significance was assessed using the global likelihood ratio test. Confounding bias introduced in the crude association between plasma concentrations and S-RA from other patient-level covariates was assessed in multivariable logistic regression models. Here, the joint Wald test was used to assess statistical significance. Effects modification was also investigated in these models.

The secondary aim of this analysis was to validate S-RA against the actual-detected plasma drug concentrations. This was achieved by computing the sensitivity, specificity, and negative and positive predictive values.

All analyses were performed using Stata 11/IC (Stata Corp, College Station, TX, USA), all P values were based on the 5% level of precision and all tests were two-tail based.

2.6. Ethical Considerations. All participants gave a written consent. The study protocol was approved by the Faculty of Medicine Research and Ethics Committee, Makerere University and the JCRC Institutional Review Board, while permission to conduct the study was granted by the Uganda National Council of Science and Technology.

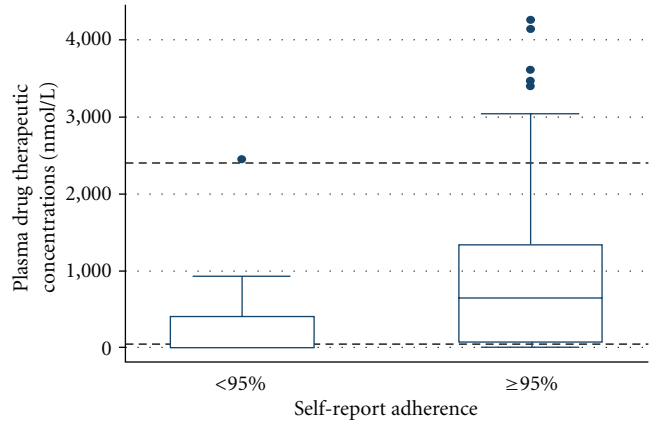

Figure 1: The broken horizontal lines indicate the 36 to 2400 nmol/L normal range levels of stavudine concentrations in steady state. The boxes indicate the lower quartile (Q1), the median (Q2), and upper quartile (Q3) limits of the concentrations, respectively. The upper and lower limits of the Whiskers indicate the minimum and maximum observations, whereas the dots above each upper cap of the whisker represent the concentrations considered to be outliers.

3. Results

All data from the 234 recruited patients were included in the final analysis. Alcohol consumption data was missing for four patients, CD4 measurements were missing for 65 patients, and body weight information was missing for nine patients. While fitting the models, records with missing data were automatically excluded.

Overall, 194/234 (82.9%) patients reported S-RA of 95% and above. The median plasma stavudine concentration for all patients was 517.9 nmol/L (range: 0, 4253.8, Interquartile Range: 0, 1183.0). Overall, 68/234 (29.1%) patients had stavudine plasma concentrations less than 36 nmol/L (classified as below therapeutic range), whereas 166/234 (70.9%) had stavudine plasma concentrations that were 36 nmol/L and above (classified as within or above therapeutic range). Figure 1 shows the distribution of plasma levels of stavudine among patients with S-RA below 95%, compared to those with S-RA of 95% and above. The broken horizontal lines indicate the 36 to 2400 nmol/L normal range levels of stavudine concentrations in steady state. The boxes indicate the lower-quartile (Q1), the median- (Q2), and upper-quartile (Q3) limits of the concentrations respectively. The upper and lower limits of the Whiskers indicate the minimum and maximum observations, whereas the dots above each upper cap of the whisker represent the concentrations considered to be outliers.

Table 1 presents the distribution of S-RA, demographic and clinical characteristics by plasma drug concentration classification. Most 152/194 (78.4%) patients with S-RA of 95% and above were found to have within or above normal therapeutic concentrations of stavudine ($P < 0.0001$). However, 42/194 (21.7%) of patients reporting good adherence actually had below normal drug concentrations. In unadjusted models, patients who reported 95% and above S-RA

Table 1: The association between patients' demographic/clinical characteristics and plasma drug therapeutic concentration among patients on a stavudine-containing regimen in Uganda.

		Plasma drug concentration		Crude association	
		Below normal range[1]	Within or above normal range[2]	Odds ratio (95%CI)	*P*-value
Self-report Adherence	Below 95% ($n = 40$)	26 (65.0%)	14 (35.0%)	[reference]	<0.0001
	95% and above ($n = 194$)	42 (21.7%)	152 (78.4%)	6.72 (3.06, 14.78)	
Gender	Male ($n = 84$)	23 (27.4%)	61 (72.6%)	[reference]	0.6728
	Female ($n = 150$)	45 (30.0%)	105 (70.0%)	0.88 (0.49, 1.59)	
Age in years	Mean (SD)	38.4 (7.0)	38.6 (6.9)	1.00 (0.96, 1.05)	0.833
Employment status	Not Employed ($n = 96$)	23 (24.0%)	73 (76.0%)	[reference]	0.2079
	Employed ($n = 133$)	42 (31.6%)	91 (68.4%)	0.68 (0.38, 1.24)	
Marital Status	Not married ($n = 106$)	24 (22.6%)	82 (77.4%)	[reference]	0.0496
	Married ($n = 128$)	44 (34.4%)	84 (65.6%)	0.56 (0.31, 1.01)	
Education	No Education ($n = 16$)	5 (31.3%)	11 (68.8%)	[reference]	0.8419
	Some Education ($n = 218$)	63 (28.9%)	155 (71.1%)	1.12 (0.37, 3.36)	
Alcohol drinking status	Never ($n = 144$)	42 (29.2%)	102 (70.8%)	[reference]	0.7946
	Stopped drinking ($n = 26$)	8 (30.8%)	18 (69.2%)	0.93 (0.37, 2.29)	
	Still drinking ($n = 60$)	15 (25.0%)	45 (75.0%)	1.24 (0.62, 2.45)	
Current CD4 count in cells/mL	Mean (SD)	289 (180)	292 (141)	1.00 (1.00, 1.00)	0.916
Body weight in kgs	Mean (SD)	61.9 (9.9)	62.9 (10.0)	1.01 (0.98, 1.04)	0.520

[1]Stavudine plasma concentration below 36 nM/mL
[2]Stavudine plasma concentration 36 nM/mL and above.

Table 2: Multivariate models explaining the association between plasma drug therapeutic concentrations and self-report adherence adjusted for demographic/clinical characteristics among patients on a stavudine-containing regimen in Uganda.

Adjusting variable	Adjusted association between plasma drug concentration and self-reported adherence	
	Adjusted odds ratio (95% CI)	*P*-value
None [crude association]	6.72 (3.06, 14.78)	<0.0001
Gender	7.13 (3.18, 16.01)	<0.0001
Age in years	6.72 (3.22, 14.01)	<0.0001
Employment status	6.54 (2.92, 14.65)	<0.0001
Marital status	6.56 (2.98, 14.41)	<0.0001
Education	6.75 (3.06, 14.87)	<0.0001
Alcohol drinking status	6.94 (3.10, 15.52)	<0.0001
Current CD4 count in cells/mL	7.34 (3.39, 15.87)	<0.0001
Body weight in kgs	8.49 (3.21, 22.46)	<0.0001

were almost seven times more likely to have within or above therapeutic plasma stavudine concentrations, compared to those who reported less than 95% S-RA (crude odds ratio: 6.72, 95% confidence interval (CI): 3.06 to 14.78).

Table 2 presents results from multivariable models to determine the association between plasma stavudine concentration and S-RA, after adjusting for each demographic and clinical characteristic. We detected no evidence of effects modification from any of the independent study covariates. The final model was adjusted for gender and body weight, where patients who reported 95% and above S-RA were almost eight times more likely (Adjusted Odds Ratio: 7.7, 95% CI: 3.5 to 7.0, $P < 0.0001$) to have within or above therapeutic plasma stavudine concentrations, compared to those who reported less than 95% S-RA.

In this population, the sensitivity and specificity of S-RA in determining actual adherence measured by the plasma concentration of stavudine within the therapeutic range was 91.6% and 38.2%, respectively, while the respective positive and negative predictive values were computed as 78.4% and 65.0%.

4. Discussion

The present study found that slightly over 80% of the patients reported to be adherent (S-RA ≤ 95%) to ART, a finding consistent with what has been reported elsewhere [15–17]. We also found out that patients with good S-RA were almost seven to eight times more likely to have therapeutic drug levels within or above therapeutic range. This positive association is critical for treatment success [1, 2, 30, 31]. However, 21.7% of patients reporting good adherence actually had insufficient plasma drug concentrations (false positives) (Table 1). These results reveal that, whereas high S-RA rates to ART correlated well with normal therapeutic plasma drug levels among these patients, S-RA as a tool may have some limitations while identifying nonadherent patients. The specificity calculations implied that, for true

nonadherent patients assessed for ART adherence, the S-RA method is cable of identifying only 38 out of every one hundred nonadhering patients. The false positives subgroup of "good S-RA, but poor drug levels" would thus constitute thecategory of "intrinsic poor adherence". These may have higher risks of contributing to emergence of resistance to antiretroviral therapy. Furthermore, the pharmacokinetic characteristics of this group of patients need to be investigated, especially their variability of stavudine metabolism. It may be possible that these are fast metabolizers of stavudine, hence presenting with low plasma concentrations.

Out of the 40 S-RA nonadherent participants in this study, 14 (35.0%) had plasma stavudine concentrations within or above normalrange (Table 1). This may be attributed to the fact that, prior to sample collection, patients may have taken their dose for that morning, even though they might have been classified as nonadherent according to the three-month S-RA calculations. Alternatively, this finding may be attributed to pharmacokinetic factors, including the fact that these patients may be slow ART metabolizers, which is linked to genetic variability [32, 33]. These false negatives do present an opportunity for further scientific investigations.

Generally, nonadherence to ART has been viewed as a significant public health concern based on the perception that nonadherence would speed the development and transmission of drug-resistant strains to the masses [2, 3, 11]. However, while focus of interventions to address nonadherence is aimed at populations thought to have lowest adherence rates, these are sometimes not the populations in which actual resistance occurs [34]. The existence of virologic failures among adhering patients may need further scientific investigations [35]. Our recommendation would be to encourage patient care providers, main stake holder ministries, and legislators in Sub-Saharan Africa into considering occasional drug bioavailability monitoring to ensure success of ART programs in the region.

Recently, there has been increasing recognition of proper and adequate adherence assessment as a crucial factor in improving treatment outcomes [11]. In this investigation, we recognize that improving adherence and subsequently treatment outcomes will require a combination of methods appropriate to the patient and clinical settings [22, 36]. Timely interventions should include dedicated educational and collaborative efforts offered to every patient, and adherence monitoring in the home setting by community outreach programs. In this way, the true nonadherent patients will quickly be identified for timely intervention to improve their adherence rates even where drug level analysis is not possible such as in resource-limited countries like Uganda.

5. Conclusions

Patients who report high S-RA are most likely to adhere to therapy, but some of them were probably nonadherent basing on the suboptimal plasma drug levels, indicating that S-RA lacks sufficient specificity for detecting nonadherence. We thus recommend that, whenever possible drug level determination should be performed since use of S-RA may result in failure to identify the non adherent patients which could have potential implications for drug resistance. Since some patients in this study showed very high levels of the drug in plasma, drug level determination would be necessary in patients who show signs of adverse effect to ART before switching medication.

Limitations of the Study

The cross-sectional study design provided no opportunity for a complete measurement of all CD4 cell counts and to monitor all patients' body weight gain, as measures of clinical response to therapy.

Authors' Contribution

R. Balikuddembe, C. Obua and P. Waako conceptualized and designed the study, and participated in the interpretation of the data. R. Balikuddembe, D. Musoke, M. Ntale and S. Baveewo conducted the study and performed the drug level analysis. J. Kayiwa and C. Obua performed the statistical analysis and drafted the manuscript.All authors read and approved the final manuscript.

Conflict of Interests

All authors declare no conflict of interests regarding the findings presented in this paper.

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