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ORIGINAL RESEARCH

Lack of effectiveness of adherence counselling in reversing virological failure among patients on long-term antiretroviral therapy in rural Uganda

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Objectives

The current World Health Organization and Uganda Ministry of Health HIV treatment guidelines recommend that asymptomatic patients who have a viral load (VL) ≥ 1000 HIV-1 RNA copies/mL should receive adherence counselling and repeat VL testing before switching to second-line therapy. We evaluated the effectiveness of this strategy in a large HIV treatment programme of The AIDS Support Organisation Jinja in Jinja, Uganda.

Methods

We measured the HIV VL at enrolment, and for participants with VL ≥ 1000 copies/mL we informed them of their result, offered enhanced adherence counselling and repeated the VL measurement after 3 months. All blood samples with VL ≥ 1000 copies/mL were sequenced in the polymerase (pol) region, a 1257-bp fragment spanning the protease and reverse transcriptase genes.

Results

One thousand and ninety-one participants were enrolled in the study; 74.7% were female and the median age was 44 years [interquartile range (IQR) 39–50 years]. The median time on antiretroviral therapy (ART) at enrolment was 6.75 years (IQR 5.3–7.6 years) and the median CD4 cell count was 494 cells/μL (IQR 351–691 cells/μL). A total of 113 participants (10.4%) had VLs ≥ 1000 copies/mL and were informed of the VL result and its implications and given adherence counselling. Of these 113 participants, 102 completed 3 months of follow-up and 93 (91%) still had VLs ≥ 1000 copies/mL. We successfully genotyped HIV for 105 patients (93%) and found that 103 (98%) had at least one mutation: eight (7.6%) had only one mutation, 94 (89.5%) had two mutations and one sample (1%) had three mutations.

Conclusions

In this study, enhanced adherence counselling was not effective in reversing virologically defined treatment failure for patients on long-term ART who had not previously had a VL test.

Keywords: adherence counselling, HIV, resource-limited setting, reversal, virological failure

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Introduction

There has been a dramatic expansion of access to antiretroviral therapy (ART) in sub-Saharan Africa over the past two decades, with 21.7 million people accessing ART by the end of 2017 [1]. Until recently, most of this expanded access occurred through programmes that did not offer routine viral load (VL) monitoring, in keeping with earlier

guidance from the World Health Organization (WHO) which did not explicitly recommend VL testing for patients on ART in resource-limited settings [2,3]. This practice had been supported by the results of randomized clinical trials which found no added clinical benefit of the addition of routine VL monitoring for patients on ART when compared to CD4 cell count monitoring alone [4–7].

However, more recently, WHO guidelines for HIV care and treatment in resource-limited settings were revised to recommend routine VL testing as the preferred tool for diagnosing ART treatment failure [8] as it provides a more accurate indication of ART treatment failure and hence avoids unnecessary switches to second-line therapy [9,10]. It is also expected that treatment failure could be detected earlier and therefore should reduce the number and severity of drug-resistance mutations accumulated in patients who are failing therapy [10]. VL results can also help health care providers to differentiate between non-adherence and ART treatment failure caused by resistance [9]. Additionally, there have been recent findings from a systematic review indicating that clinical and immunological approaches for diagnosing treatment failure have low sensitivity and positive predictive value in identifying persons with virological failure [11].

Consequently, many national ART programmes in sub-Saharan African countries are now adopting the revised WHO recommendations and many programmes that did not provide VL testing may have begun using this as an additional monitoring tool. In sub-Saharan Africa, seven countries, South Africa, Tanzania, Côte d'Ivoire, Kenya, Malawi, Namibia and Uganda, are reported to be at various stages of scaling up routine VL monitoring [12].

In Uganda, the 2013 national ART treatment guidelines recommend that VL testing should be conducted 6 months after initiation of therapy and annually thereafter [13]. Both the WHO and the Ugandan ART guidelines recommend that asymptomatic patients who have been identified with VL $\geq$ 1000 copies/mL should be offered enhanced adherence counselling for 3–6 months and repeat VL testing before switching to second-line therapy [3,14]. Virological treatment failure is confirmed after two consecutive plasma VL test results $\geq$ 1000 copies/mL obtained 3–6 months apart, with enhanced adherence counselling and support following the first VL test [13,14].

However, little is known about the effectiveness of counselling and adherence support interventions in reversing virological failure in long-term HIV-positive patients, particularly in those who have been receiving ART for long periods of time without prior VL testing.

We designed a study to evaluate the effectiveness of enhanced adherence counselling and support for patients identified with VL $\geq$ 1000 copies/mL at their first VL test, among individuals who had been receiving ART for $\geq$ 4 years in an HIV treatment programme in which VL testing was not routinely available. Furthermore, we evaluated factors associated with successful reversal of virological failure, including ART drug resistance patterns, in these patients.

Methods

In June 2012, we initiated a prospective cohort study titled the Long-term Outcome on Antiretroviral Therapy Study in Uganda (LTOAU). This study recruited HIV-positive patients who were receiving care at The AIDS Support Organisation (TASO) Jinja service centre, and had been receiving ART for a minimum of 4 years.

The AIDS Support Organisation Jinja is one of the 11 HIV clinics of TASO, the largest nongovernmental organization in Uganda providing HIV care in the country. The TASO Jinja clinic is located in Jinja District, in the east-central region of Uganda, and serves persons in the district and other neighbouring districts within a radius of 75 km. Jinja District has a population of 468 256 and, like its neighbouring areas, is largely rural; the average annual income per household is estimated to be US$100, and the majority of inhabitants are subsistence farmers, with very few working in the formal sector and earning wages [15,16].

The ART programme in TASO Jinja began in 2004, and has over 5000 HIV-positive persons receiving therapy. All patients are commenced on ART in accordance with Ugandan national ART guidelines which recommend first-line regimens with two nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs) plus one nonnucleoside reverse transcriptase inhibitor (NNRTI). Second-line regimens preferentially include two NRTIs not used in the first-line regimen and a protease inhibitor (PI), most commonly lopinavir-ritonavir. During the period of our study, VL monitoring was not provided routinely; however, immunological (CD4 cell count testing) and clinical monitoring was carried out every 6 months in accordance with the Ugandan national ART guidelines [13,14].

We recruited HIV-positive patients aged $\geq$ 18 years who had presented for clinical care at the TASO Jinja clinic or outreach sites between 1 July 2012 and 31 December 2013 and had been receiving first-line ART for $\geq$ 4 years. We excluded patients who had ever received a lopinavir-ritonavir-containing regimen.

From June 2012 to July 2013, we provided an information sheet about the proposed study to all TASO Jinja patients who had been receiving ART for a minimum of 4 years. After providing informed consent, participants completed an interviewer-administered questionnaire and

had blood drawn for VL and CD4 cell count testing. We collected clinical, demographic and behavioural data at the first study visit and all participants continued to receive routine care from the TASO clinical teams every 3 months. After August 2013, we further restricted enrolment to patients whose last CD4 cell count was ≤ 450 cells/μL and continued recruitment until January 2015. This restriction was decided upon following an interim analysis that demonstrated that the prevalence of virological failure among patients with a CD4 cell count of > 450 cells/μL was < 2%. We therefore took this step to increase the diagnostic yield of identifying patients with VL ≥ 1000 copies/mL.

We measured the HIV VL at enrolment and, for participants with a VL ≥ 1000 copies/mL, we informed them of the result and its interpretation and offered enhanced adherence counselling. This consisted of additional monthly face-to-face sessions with the counsellor for three consecutive months. During this session, the counsellor discussed the implications of unsuppressed VL, established the patient's adherence level, and, together with the patient, reviewed the previous adherence strategies to develop a revised adherence plan. Where possible, the 'treatment buddies' were re-contacted during this period. These participants then had a repeat VL measurement 3 months after the first test to determine if they still had virological failure.

In our study, if participants met the criteria for clinical failure, they were offered switches to second-line therapy after the first visit, but otherwise decisions about changing ART regimens (including decisions for patients with immunological failure) were deferred until after the 3 months of enhanced adherence counselling support. Additional social and behavioural data were collected at the 3-month study visit for those with virological failure.

Adherence was measured based on self-reported responses to two questions: (1) 'In general, how often do you miss taking your ARV pills?' (reponses: never; once a month or less; more than once a month but less than once a week; and once a week or more) and (2) 'In this past month how many of your ARV pills have you missed taking?' (responses: 0, 1, 2, 3, 4, 5 and > 5).

HIV VL was measured with the COBAS Ampliprep/TaqMan HIV-1 test V2.0 (Roche, Mannheim, Germany) at the Uganda Virus Research Institute (UVRI)/Medical Research Council (UK) laboratory in Entebbe. All blood samples with a VL ≥ 1000 copies/mL were sequenced in the polymerase (pol) region, a 1257-bp fragment spanning the protease and reverse transcriptase genes, as previously described [17]. The study received approval from the Research Ethics Committee of UVRI, the Uganda National Council for Science and Technology in Uganda and the University of British Columbia in Vancouver, Canada. Virological failure was defined as having a VL ≥ 1000 copies/mL at enrolment and after 3 months of enhanced adherence counselling support.

We obtained descriptive statistics of characteristics of the entire study population including those with VL ≤ 1000 copies/mL at enrolment and included all the explanatory and outcome variables of interest. As multiple thymidine analogue mutations (TAMs) have the greatest propensity to reduce the effectiveness of second-line regimens in use in low-income countries, we conducted bivariate analyses comparing participants who had zero or one TAM with those with at least two TAMs using Wilcoxon rank sum tests for continuous variables and χ^2 or Fisher's exact test for categorical variables. We then conducted a subsequent bivariate analysis comparing participants on the basis of correction of virological failure after an additional 3 months of follow-up. All analyses were conducted using SAS Version 9.3 (SAS Corporation, Cary, NC).

Results

A total of 1091 participants were enrolled in the study, of whom 815 (74.7%) were female, and the median age was 44 years [interquartile range (IQR) 39–50 years]. The median time on ART at enrolment was 6.75 years (IQR 5.3–7.6 years) and the median CD4 cell count at enrolment was 494 cells/μL (IQR 351–691 cells/μL). The most common regimen taken by participants at the time of enrolment in this study was nevirapine, lamivudine and zidovudine (72.7% of participants) followed by efavirenz, lamivudine and zidovudine (18.3%) and nevirapine, lamivudine and tenofovir (7.4%). However, 555 participants (50.9%) reported receiving other drug regimens since beginning ART. Of these, the most commonly received ART regimen was nevirapine, lamivudine and stavudine (407 participants or 73.3% of those with previous regimens). A total of 667 (61.3%) participants reported never missing a dose of their ART and 865 (79.6%) reported not missing any doses in the previous month (Table 1).

While we were unable to quantify the number of individuals who were ineligible for this study because of previous switches to lopinavir-containing ART regimens, by the end of 2014 (during our recruitment and enrolment period), there were approximately 265 clients of TASO Jinja who were receiving second-line therapy, out of approximately 5855 patients on ART [18].

Among the 1091 patients screened at enrolment, a total of 113 (10.4%) had VLs ≥ 1000 copies/mL. Of these 1091 patients, 870 were enrolled irrespective of their CD4 cell counts, of whom 61 had virological failure (prevalence of 7.0%). The analysis comparing those participants in our study with VLs ≥ 1000 copies/mL to those with VLs

< 1000 copies/mL is reported elsewhere [19]. When we restricted enrolment to those with CD4 cell counts ≤ 450 cells/μL, we found an additional 52 participants with virological failure out of 221 enrollees (prevalence of 23.5%).

For the 113 (61 plus 52) participants with HIV VL ≥ 1000 copies/mL at enrolment, we were able to successfully genotype 105 (93%) of the samples. Of these, 103 (98%) had at least one mutation, eight (7.6%) had only one mutation, 94 (89.5%) had two mutations and one sample (1%) had three mutations. A total of 100 samples (95.2%) had NRTI mutations and 98 (93.3%) had NNRTI mutations. Only one sample (1%) had a mutation conferring resistance to PIs. Among the 105 samples, M184V was the most frequent mutation found, occurring in 95 samples (90.5%), followed by Y181C which occurred in 42 samples (40.0%). A total of 12 samples (11.4%) had K65R mutations; 69 patients (65.7%) had at least one TAM and 53 (50.5%) had at least two TAMs (Table 2).

Having two or more TAMs was more common among participants with; < 1 year of primary school education (24.5% of those with at least two TAMs versus 7.7% of those with zero or one TAM), previous regimen that included nevirapine, lamivudine and stavudine (96.0% vs. 62.5%; p=0.002) and an enrollment VL ≥100,000 copies/mL (43.4% vs. 26.9%; p=0.001). Participants with at least two TAMs also had lower CD4 cell counts at the first study visit (median of 179 versus 263 cells/μL in those with zero or one TAM; $P = 0.004$) and had been receiving ART for a longer period of time (median of 6.8 versus 6.2 years, respectively; $P = 0.026$) (Table 3).

All the 113 patients were informed of the VL result by the study nurse or physician and given enhanced adherence counselling and support by a counsellor. Of these, 102 completed follow-up at the 3-month study visit and 93 (91%) still had VL ≥ 1000 copies/mL after

Table 1 Characteristics of the 1091 participants at their enrolment visit

Characteristic	Category	*n*	%
Gender	Female	815	74.7
	Male	276	25.3
Income source	Agriculture	335	30.7
	Wage/salaried employment	143	13.1
	Crafts trade	66	6.1
	Petty trade	276	25.3
	None	91	8.3
	Other	180	16.5
Education level	Low	224	20.5
	Medium	493	45.2
	High	374	34.3
Marital status	Legally married	188	17.2
	Cohabiting	269	24.7
	Single/separated/divorced	220	20.2
	Widowed	414	38.0
Years in TASO	< 2 years	10	0.9
	2 to < 5 years	41	3.8
	≥ 4 years	1039	95.3
ARV regimen at enrolment	Efavirenz/3TC/TDF	21	1.9
	Efavirenz/3TC/ZDV	200	18.3
	Nevirapine/3TC/TDF	81	7.4
	Nevirapine/3TC/ZDV	787	72.1
	Other ARV regimen	2	0.2
Been on previous ARV	No	536	49.1
	Yes	555	50.9
Previous ARV regimen	Efavirenz/3TC/TDF	2	0.4
	Efavirenz/3TC/ZDV	27	4.9
	Efavirenz/3TC/d4T	52	9.4
	Nevirapine/3TC/TDF	8	1.4
	Nevirapine/3TC/ZDV	59	10.6
	Nevirapine/3TC/d4T	407	73.3
ARV adherence:	Never	667	61.3
frequency reported of missing	Once a month or less	269	24.7
taking pills	More than once a week but less than once a month	85	7.8
	Once a week or more	67	6.2
Number of ARV pills reported	One or more	222	20.4
missing in past month	None	865	79.6
Viral load ≥ 1000 copies/mL	No	978	89.6
	Yes	113	10.4

ARV, antiretroviral; TASO, The AIDS Support Organisation; 3TC, lamivudine; TDF, tenofovir disoproxil fumarate; ZDV, zidovudine; d4T, stavudine.

Table 2 Frequency of antiretroviral therapy (ART) resistance mutations among participants with viral load ≥ 1000 copies/mL ($n = 105$)

	n	%
ART class mutations		
NNRTI resistance mutations	98	93.3
PI resistance mutations	1	1.0
NRTI resistance mutations	100	95.2
TAMs	69	65.7
Other NRTI resistance mutations	31	29.5
Individual mutations		
M184V	95	90.5
Y181C	42	40.0
K103N	36	34.3
M41 l*	34	32.4
T215Y*	30	28.6
K70R*	25	23.8
G190A	23	21.9
L210W*	21	20.0
T215F*	19	18.1
D67N*	18	17.1
K219Q*	15	14.3
K219E*	13	12.4
K65R	12	11.4
K101E	7	6.7
P225H	6	5.7
Others	33	31.4

NNRTI, nonnucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; TAM, thymidine analogue mutation.

Table 3 Bivariate analysis comparing participants with viral load (VL) ≥ 1000 copies/mL and at least two thymidine analogue mutations (TAMs) with participants with VL ≥ 1000 copies/mL and fewer than two TAMs

	0 or 1 TAM (52)		≥ 2 TAMs (53)		
Characteristic	***n*** **or median**	**% or IQR**	***n*** **or median**	**% or IQR**	***P*****-value**
Gender					
Female	36	69.2	39	73.6	0.670
Male	16	30.8	14	26.4	
Age	42	37–46	39	34–44	0.046
Income source					
Agriculture	12	23.1	15	28.3	0.057
Wage or salaried employment	11	21.2	10	18.9	
Crafts trade	4	7.7			
Petty trade	12	23.1	17	32.1	
None	7	13.5	1	1.9	
Other	6	11.5	10	18.9	
Education					
No formal education or < 1 year of primary	4	7.7	13	24.5	0.040
Some primary education	29	55.8	20	37.7	
Some secondary school education	19	36.5	20	37.7	
Duration of antiretroviral therapy (years)	6.2	4.7–7.2	6.8	6.0–7.7	0.026
ARV regimen at first study visit					
Efavirenz/3TC/TDF	2	3.9			0.064
Efavirenz/3TC/ZDV	5	9.6	6	11.3	
Nevirapine/3TC/TDF	10	19.2	3	5.7	
Nevirapine/3TC/ZDV	35	67.3	44	83.0	
Previous ARV regimen					
Efavirenz/3TC/TDF	0	0	0	0	0.002
Efavirenz/3TC/ZDV	1	4.2	0	0	
Efavirenz/3TC/d4T	1	4.2	0	0	
Nevirapine/3TC/TDF	0	0	1	4.0	
Nevirapine/3TC/ZDV	7	29.2	0	0	
Nevirapine/3TC/d4T	15	62.5	24	96.0	
CD4 count at enrolment (cells/μL)	263	147–407	179	101–253	0.004
Viral load at enrolment					
1000–5000 copies/mL	7	13.5	0	0	
5000–10 000 copies/mL	4	7.7	4	7.6	
10 000–50 000 copies/mL	24	46.2	14	26.4	
50 000–100 000 copies/mL	3	5.8	12	22.6	
> 100 000 copies/mL	14	26.9	23	43.4	

ARV, antiretroviral; IQR, interquartile range; 3TC, lamivudine; TDF, tenofovir disoproxil fumarate; ZDV, zidovudine; d4T, stavudine.

3 months. Comparing the nine (8.8%) participants who achieved a VL < 1000 copies/mL on the second test with those who did not, we found that a lower proportion of them had two or more drug mutations (50% versus 93%, respectively; $P = 0.010$), NNRTI drug resistance mutations (67% versus 95.5%, respectively; $P = 0.046$) and NRTI resistance mutations (67% versus 98%, respectively; $P = 0.019$). We also found that none of those who achieved virological suppression had two or more TAMs compared to 51% of participants who remained unsuppressed ($P = 0.027$). Furthermore, fewer participants who achieved virological suppression had M184 mutations compared to those who remained unsuppressed (50% versus 95%, respectively; $P = 0.005$). Of the nine participants, four (44%), who had a VL between 1000 and 5000 copies/mL, were able to reverse their virological failure. However, for participants with VL > 5000 copies/mL, enhanced adherence support did not achieve a reduction in VL of > 10% (Table 4).

Of the 11 participants who did not have a repeat VL test conducted at 3 months, three died before their 3-month visit, another four were switched to second-line therapy before their 3-month visit, one was lost to follow-up and another four were more than 1 month late for their repeat VL test. In total, 97 (86%) of those with virological failure were switched to second-line therapy either before or after their 3-month visit.

Discussion

In this study, enhanced adherence counselling was largely ineffective in reversing virologically defined treatment failure for patients on long-term ART (patients on ART for ≥ 4 years) who had not previously had a VL test. Only

Table 4 Virological failure after 3 months of adherence counselling intervention

Characteristic	Category	Virological failure after 3 months: No (*n* = 9)	Virological failure after 3 months: Yes (*n* = 93)	*P*-value
Age (years) [median (IQR)]		45 (36–52)	41 (36–44)	0.193
Gender [*n* (%)]	Female	7 (77.8)	70 (75.3)	1.000
	Male	2 (22.2)	23 (24.7)	
Income source [*n* (%)]	Agriculture	1 (11.1)	23 (24.7)	0.063
	Wage/salaried employment	0 (0)	21 (22.6)	
	Crafts trade	1 (11.1)	3 (3.2)	
	Petty trade	3 (33.3)	30 (32.3)	
	None	2 (22.2)	4 (4.3)	
	Other	2 (22.2)	12 (12.9)	
Education [*n* (%)]	Low	2 (22.2)	13 (14.0)	0.212
	Medium	6 (66.7)	44 (47.3)	
	High	1 (11.1)	36 (38.7)	
≥ 4 years in The AIDS Support Organisation [*n* (%)]	No	1 (11.1)	6 (6.5)	0.487
	Yes	8 (88.9)	87 (93.6)	
CD4 at enrolment [median (IQR)]		364 (308–423)	220 (129–335)	0.053
Time on ARV at enrolment (months) [median (IQR)]		62 (57–90)	79 (61–92)	0.942
ARV regimen at enrolment [*n* (%)]	Efavirenz/3TC/TDF	1 (11.1)	2 (2.2)	0.046
	Efavirenz/3TC/ZDV	3 (33.3)	8 (8.6)	
	Nevirapine/3TC/TDF	1 (11.1)	13 (14.0)	
	Nevirapine/3TC/ZDV	4 (44.4)	70 (75.3)	
Been on previous ARV	No	4 (44.4)	51 (54.8)	0.729
	Yes	5 (55.6)	42 (45.2)	
Previous ARV regimen (*n* = 47)	Efavirenz/3TC/ZDV	1 (20)	2 (4.8)	0.123
	Efavirenz/3TC/d4T	0 (0)	1 (2.4)	
	Nevirapine/3TC/ZDV	2 (40)	6 (14.3)	
	Nevirapine/3TC/d4T	2 (40)	33 (78.6)	
ARV adherence (*n* = 100)	Never	6 (66.7)	49 (53.9)	0.772
Frequency of missing taking pills [*n* (%)]	Once a week		5 (5.5)	
	More than once a week but less than once a month	1 (11.1)	6 (6.6)	
	More than once a month	2 (22.2)	31 (34.1)	
Good ARV adherence (*n* = 100)	No	3 (33.3)	20 (22.0)	0.426
Did not miss any pill in the past month	Yes	6 (66.7)	71 (78)	
Frequency of medicine companions observations in 7 days before enrolment [median (IQR)]		11 (7–14)	7 (4–14)	0.363
Switching to second-line therapy (*n* = 93)	No		3 (3.2)	
	Yes		90 (96.8)	
Baseline viral load [*n* (%)]	1000–5000 copies/mL	4 (44.4)	5 (5.8)	< 0.001
	5000–10 000 copies/mL	1 (11.1)	8 (8.6)	
	10 000–50 000 copies/mL		39 (41.9)	
	50 000–100 000 copies/mL		13 (14.0)	
	> 100 000 copies/mL	4 (44.4)	28 (30.1)	
> 1000 [*n* (%)]	Yes	9 (100)	93 (100)	n/a
> 5000 [*n* (%)]	Yes	5 (55.6)	88 (94.6)	0.003
> 10 000 [*n* (%)]	Yes	4 (44.4)	80 (86.0)	0.008
> 50 000 [*n* (%)]	Yes	4 (44.4)	41 (44.1)	1.0000
Any drug resistance mutations (*n* = 93)	Yes	5 (83.3)	88 (100)	0.064
Two or more drug class mutations [*n* (%)]	Yes	3 (50)	82 (93.2)	0.010
Any NNRTI drug resistance [*n* (%)]	Yes	4 (66.7)	84 (95.5)	0.046
Any PI mutation [*n* (%)]	Yes		1 (1.1)	1.000
Any NRTI mutation [*n* (%)]	Yes	4 (66.7)	86 (97.7)	0.019
Any TAM [*n* (%)]	Yes	2 (33.3)	58 (65.9)	0.185
≥ 2 TAMs [*n* (%)]	Yes	0 (0)	45 (51.1)	0.027
Any other NRTI mutation [*n* (%)]	Yes	2 (33.3)	28 (31.8)	1.000
K65R mutation [*n* (%)]	Yes	1 (16.7)	11 (12.5)	0.570
M184V mutation [*n* (%)]	Yes	3 (50.0)	84 (95.5)	0.0047

ARV, antiretroviral; IQR, interquartile range; 3TC, lamivudine; TDF, tenofovir disoproxil fumarate; ZDV, zidovudine; d4T, stavudine; NRTI, nucleotide reverse transcriptase inhibitor; NNRTI, nucleotide reverse transcriptase inhibitor; TAM, thymidine analogue mutation.

9% of the participants who were found to have a VL of ≥ 1000 copies/mL at enrolment were able to achieve a VL of < 1000 copies/mL after 3 months of enhanced adherence counselling and support. These results suggest that current national and international guidelines recommending that enhanced adherence counselling and repeat VL testing be offered to such patients on long-term ART should be reconsidered. Switching patients who have been receiving ART for long periods of time on the basis of a single VL result would probably result in more rapid reversal of virological failure and a much shorter duration of unsuppressed viraemia and reduced potential transmission of drug-resistant virus. Our study also found a high frequency of NRTI and NNRTI resistance mutations among the participants with virological failure, with M184V being the most common followed by Y181C. This is similar to what has been reported in other studies [20–22].

We also noted a very high prevalence of TAMs, with 65% of those with virological failure having at least one TAM and 50% having two or more TAMs; similar prevalences have been reported in other studies for patients receiving regimens containing stavudine, zidovudine, lamivudine, efavirenz and nevirapine [22–24]. However, a lower prevalence of TAMs of between 5 and 25% has been reported in a retrospective multicentre cohort study in sub-Saharan Africa among patients using tenofovir [25]. As many second-line regimens in sub-Saharan Africa will by necessity include didanosine and abacavir, which can have reduced effectiveness in the presence of increasing numbers of TAMs [26], these are probably the most concerning mutation patterns we found. Previous exposure to stavudine was associated with having two or more TAMs in our study. However, given that stavudine is no longer being prescribed in Uganda [13,27] and in most resource-limited settings in accordance with the WHO ART guidelines [8], this issue will probably be less of a problem in the future, which is somewhat reassuring. Fortunately, we also found a fairly low prevalence of the K65R mutation (in 11% of individuals with virological failure), which is another NRTI resistance mutation that can confer pan-NRTI resistance, although one study reported that K65R mutations are likely to increase with increased use of tenofovir as a first-line option [17]. VL measurement with enhanced adherence counselling has been demonstrated to be effective for individuals who are in the first few years of ART in South Africa [20,28,29]. However, our findings regarding adherence counselling for patients who had been receiving ART for longer periods of ≥ 4 years are similar to the findings from a study in Swaziland which reported that, among patients receiving ART for a median of 2.9 years (IQR 1.6–4.6 years), those who were found to have raised VLs (VL ≥ 1000 copies/mL) and received enhanced adherence counselling by counsellors were no more likely to achieve VL suppression at subsequent VL test than those who did not receive counselling [30]. Another study in Khayelitsha in South Africa reported higher rates of virological re-suppression among patients failing on second-line ART following enhanced adherence support [31]. However, patients in this study had been on second-line ART for a short period of time, a median of 1.7 years (IQR 0.9–2.5 years), and were using lopinavir-based second-line therapy rather than NNRTI-based first-line therapy. A systematic review of 29 studies on rates of emergence of HIV drug resistance in resource-limited settings revealed that the rates increased steadily with time on ART. Enhanced adherence counselling may not be effective in situations where virological failure is likely to have occurred months or years before diagnosis, and patients will probably already have developed resistance mutations that even good adherence may not overcome. It is worth noting that, in our study, in cases where virological failure was reversed, the initial median VL was < 10 000 copies/mL and CD4 cell counts tended to be higher.

The occurrence of three deaths in the 3-month period between the first and second VL results implies that mortality may be quite high among this population. While it is not clear whether switching medications earlier would have prevented these deaths, it is also not known if more deaths would have occurred had the study used the recommended upper limit of 6 months for the period between the two results, according to Uganda MoH ART guidelines [13,14].

This study had a number of limitations. Firstly, adherence measures were self-reported and could reflect underestimates of true nonadherence, as has been reported in studies elsewhere [32]. However, adherence to therapy among participants in this study must have been sufficient to allow participants to survive ≥ 4 years on ART. Secondly, while we had a relatively large sample size of patients on long-term ART (> 1000), the proportion of those with virological failure was quite low at 7.0%, leaving a fairly small sample in which to study the effect of enhanced adherence counselling. This 'limitation', however, again demonstrates that TASO has done an excellent job in promoting ART adherence among programme participants. Programmes with greater proportions of nonadherent participants and those with treatment failure may find more value in promoting adherence. However, given the persistent impact of the NNRTI and NRTI resistance mutations that develop among individuals failing first-line therapy, this may not, in fact, be true.

In conclusion, our study found that enhanced adherence counselling was not effective in reversing virologically defined treatment failure for patients on long-term ART who had not previously had a VL test. Thus, recommendations that all patients with virological failure should undergo a period of enhanced counselling and re-measuring of VL before changing their therapy should be revisited. Our findings also highlight the need for timely HIV resistance testing for patients receiving ART in sub-Saharan Africa with unsuppressed VL at first measurement. It remains to be seen whether the resistance patterns we observed in this cohort will impact the longer term clinical and virological outcomes of these patients.

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