Prevalence and patterns of HIV drug resistance in patients with suspected virological failure in North-Western Tanzania

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Background: More than 15 million people in sub-Saharan Africa receive ART. Treatment failure is common, but the role of HIV drug resistance in treatment failure is largely unknown because drug resistance testing is not routinely done. This study determined the prevalence and patterns of HIV drug resistance in patients with suspected virological failure.

Materials and methods: A single high viral load of >1000 viral RNA copies/mL of plasma at any point during ART was considered as suspected virological failure. HIV-1 RNA was extracted from plasma samples of these patients using the QIAamp Viral RNA kit. The protease and part of the RT regions of the HIV pol gene were characterized.

Results: Viral load was determined in 317 patients; 64 (20.2%) had suspected virological failure. We successfully genotyped 56 samples; 48 (85.7%) had at least one major resistance-associated mutation (RAM). Common mutations in RT were M184V (75%), T215Y (41.1%), K103N (39.3%), M41L (32.1%), D67DN (30.3%), G190A (28.6%) and A98G (26.8%). No RAMs were detected in ART regimens based on a ritonavir-boosted PI.

Conclusions: The Tanzanian national guidelines define ‘virological failure’ as two consecutive viral load measurement results, at 3 month intervals, above the WHO threshold (1000 copies/mL). Here, we show that a single viral load above the WHO threshold is associated with high rates of RAMs. This suggests that a single high viral load measurement could be used to predict virological failure and avoid delays in switching patients from first-line to higher genetic barrier second-line regimens.

Introduction

Among the estimated 37.9 million people living with HIV (PLHIV) worldwide, 70% reside in sub-Saharan Africa (SSA). Since the introduction of antiretroviral drugs (ARVs) for the treatment of HIV, the WHO has gradually implemented several targets for universal access to ARVs in low- and middle-income countries (LMICs), including the 3-by-5 initiative, Treatment 2015 and the very latest global 90–90–90 targets aimed at ending HIV/AIDS by 2030. ART has indeed helped to reduce HIV/AIDS-associated mortality and morbidity, so that HIV infection has become a chronic and manageable condition. In SSA, a systematic review reported optimal virological suppression, defined as <1000 viral RNA copies/mL of plasma, among PLHIV on first-line ART in 76% and 67% after 12 and 24 months of ART, respectively. In the Tanzanian national guideline for the management of HIV/AIDS, virological failure is defined as plasma viral load values of at least 1000 copies/mL in two consecutive measurements 3 months apart, with adherence support in between. Any viral load test result higher than 1000 copies/mL at any point during ART is considered as suspected
viral failure and it is recommended that a patient is enrolled in enhanced adherence sessions to identify barriers to adherence to ART, should there be any, while assessing other possible sources of the rise in viral load. The first-line regimen in Tanzania consists of two NRTIs and one NNRTI; the default combination is tenofovir disoproxil fumarate, lamivudine and efavirenz (TDF/3TC/EFV). Alternative first-line regimens include tenofovir disoproxil fumarate, emtricitabine and efavirenz or zidovudine (TDF + FTC + EFV/3TC), lamivudine and efavirenz/nevirapine (3TC + EFV/NVP) or abacavir, lamivudine and efavirenz (ABC + 3TC + EFV). The default second-line regimen consists of two NRTIs and one boosted PI (bPI); the most commonly used combination is zidovudine, lamivudine and ritonavir-boosted atazanavir (ZDV + 3TC + ATV/r). The proposed third-line regimen consists of one integrase inhibitor (IN), one bPI and one NNRTI; the default combination includes dolutegravir, ritonavir-boosted darunavir and etravirine (DTG + DRV + r/ETV). ARV resistance testing is recommended only when second-line ART failure is observed, and the patient needs to be switched to a third-line regimen.

Studies describe a high virological failure rate as a setback to many ART programs in SSA. Because HIV drug resistance testing is not routinely available, the role of viral resistance in the development of ART failure in SSA is largely unknown and not well documented. If the cause of ART failure is extensive HIV drug resistance, durable re-suppression to a viral load level below 1000 copies/mL is unlikely to be achieved with enhanced adherence counselling. However, patients are switched to pre-defined second-line regimens after first-line ART failure in countries implementing the public health treatment approach, including Tanzania, rather than being based on ARV resistance patterns. Consequently, patients are put on ART regimens that may be suboptimal because of cross-resistance between first- and second-line ARVs. To provide more adequate data about HIV drug resistance, our study aimed to determine the prevalence and pattern of HIV drug resistance in patients with suspected first-line ART failure in North-Western Tanzania. A second aim was to assess the use of a single viral load test result of >1000 copies/mL at any point during treatment to define virological failure and whether genotypic data at this stage can provide evidence to inform treatment decisions.

Table 1. Primers used in the different steps of sequencing procedure

| PCR Step     | Gene | Primer | Forward/reverse | Oligonucleotide          |
|--------------|------|--------|-----------------|-------------------------|
| cDNA synthesis | PR & RT | RT2    | Forward          | GATAAGCTTGGGGCTTATCTATCCAT |
| Primary PCR   | PR & RT | D1818  | Reverse          | AGAAGAAATGATGACAGCATGTCAGGGAGT    |
| Nested PCR    | RT    | JG103  | Forward          | GATAAGCTTGGGGCTTATCTATCCAT    |
|               |       | JG202  | Reverse          | AACAATGCCCATTGACAGAA       |
|               | PR    | D2213A2| Reverse          | TCAGGATGGATTCA-TAICCCA     |
| Sequencing    | RT    | JG103  | Forward          | AGCCAGATTCGGAAGGACAGGGA    |
|               |       | JG202  | Reverse          | CCATCCTGGGCTTTAATTTC TACTGG |
|               | PR    | D2213A2| Reverse          | AACAATGGCCATTGACAGAA   |
|               |       | R2598L | Reverse          | TCAGGATGGATTCA-TAICCCA    |
|               |       |        | Reverse          | AGCCAGATTCGGAAGGACAGGGA    |

Materials and methods

Study participants
This was a cross-sectional study conducted between 2013 and 2017 whereby patients with suspected virological failure who were attending the routine HIV Care and Treatment Clinic (CTC) at Bugando Medical Centre in Mwanza, Tanzania and met the inclusion criteria were included. The inclusion criteria were: (i) confirmed HIV positive; (ii) suspected virological treatment failure with >1000 copies/mL at any time of treatment; and (iii) accepted to participate in the study by signing an informed consent/assent form depending on the participant's age as defined by the WHO. Those who met the inclusion criteria but declined to participate and/or did not sign the informed consent/assent forms or had known psychiatric disorders were excluded from the study. Participants' demographics were systematically recorded, using EpiData Software version 3.1 (http://www.epidata.dk/structured documentation.php), from various sources including oral interviews and structured questionnaires, as well as from the CTC electronic database.

Viral load testing
Viral load testing was performed at the Bugando Medical Centre clinical laboratory on whole blood collected in 4.5 mL EDTA collection tubes (BD, Franklin Lakes, NJ, USA). Plasma was obtained by centrifuging blood samples at 956 × g for 15 min and was then cryopreserved at −80°C until the time of testing. The viral load estimation was done using the COBAS AmpliPrep/COBAS TaqMan 96 platform (Roche Diagnostics, Branchburg, NJ, USA) following the manufacturer's instructions. Plasma samples with a viral load test result of more than 1000 copies/mL were further analysed for resistance-associated mutations (RAMs).

HIV genotyping and sequencing
HIV-1 RNA extraction was performed using the QIAamp Viral RNA Mini Extraction Kit's Spin protocol, according to the manufacturer’s instructions, and the viral RNA amplification and PCR product purification were performed following a validated internal protocol; primers and PCR conditions are shown in Table 1. PCR product sequencing was performed on a 4-capillary ABI Prism Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) according to the same protocol. All sequences were checked for quality to assess for stop codons, frame shifts, hypermutations and similarity using the HIV-1 Sequence Quality Analysis tool (http://www.hiv.lanl.gov/content/sequence/QC/index.html) before being submitted to the Stanford HIV drug resistance database for analysis and subtyping.

The subtyping process was...
done using online subtyping programmes: Context-based Modelling for Expedient Typing (COMET), Recombinant Identification Program (RIP), Stanford HIV drug resistance database (HIVDRDB) and REGA HIV-1 subtyping tool. All sequences were submitted to GenBank and they were assigned accession numbers from MT418522 to MT418578.

Statistical analysis

The primary outcomes of this study were the prevalence of suspected virological failure, defined as a single viral load test result of more than 1000 copies/mL at any point during ART, and the prevalence and patterns of HIV-1 drug RAMs. Data cleaning and analysis was performed using Stata version 13 statistical software (Stata Corp, College Station, TX, USA). Due to the small sample size, only descriptive statistics were summarized. Median and IQR values were used for continuous variables, while frequency and proportion were used for categorical variables. Fisher’s exact test was used to compare frequencies of resistance mutations detected among patients on different treatment regimens, to check whether mutations were statistically significant.

Ethics

Ethical approval to conduct this study was obtained from the joint ethical board committee of Bugando Medical Centre/Catholic University of Health and Allied Sciences (CUHAS) (CREC/021/2013), the National Institute for Medical Research of Tanzania (MR/53/100/397) and the Stellenbosch University Health Research and Ethical Committee, South Africa (S16/08/140). All patients gave written informed consent. The laboratory experiments were conducted according to the ethical guidelines and principles of the international Declaration of Helsinki 2013.

Results

Patient demographic information

A total of 326 patients were enrolled in this study (Table 2). The median age of all study participants was 43.5 years and 66% of the participants were female. Seventy-eight percent of the participants were on first-line ART and about half of the study participants had been on ART for less than 5 years. Of the 288 patients who were assigned WHO clinical stages at the time of the study, the majority were at stages 2, 3 or 4. At the time of the study, 77.6% had CD4+ T cell counts of less than 500 cells/mm³. Poor adherence history was documented in 59 (18.1%) participants’ medical records. ART regimens were established in 313 (96%) of the study participants, of whom 244 (78%) were on first-line and 69 (22%) were on second-line ART regimens. All patients on first-line ART received nevirapine/efavirenz-based treatments, while those on second-line ART received a bPI.

Virological failure

Viral load was determined in 317 (97.5%) participants, of whom 64 (20.2%) had a viral load of more than 1000 copies/mL and were thus suspected of failing ART, with 35 (57.4%) of these patients having been on ART for more than 5 years. There were no major differences in age, sex, WHO clinical stage, CD4+ T cell counts, poor adherence history documentation, ART regimen, between zidovudine- and tenofovir disoproxil fumarate-based first-line regimens or between all patients and failing patients (Table 2).

Table 2. Demographic and medical characteristics

| Characteristics                  | All patients (n = 326) | Patients with suspected virological failure (n = 64) |
|----------------------------------|-----------------------|-----------------------------------------------|
| Age, years                       |                       |                                               |
| 13–24                            | 14 (4.3)              | 5 (7.8)                                       |
| 25–44                            | 166 (51.2)            | 39 (60.9)                                     |
| ≥45                              | 144 (44.4)            | 20 (31.3)                                     |
| Median (IQR)                     | 43.5 (13.0)           | 39.9 (12.9)                                   |
| Sex                              |                       |                                               |
| Male                             | 110 (33.7)            | 22 (34.4)                                     |
| Female                           | 216 (66.3)            | 42 (65.6)                                     |
| WHO stage                        | (n = 288)             | (n = 59)                                      |
| 1                                | 33 (11.4)             | 4 (6.8)                                       |
| 2                                | 81 (28.1)             | 15 (25.4)                                     |
| 3                                | 103 (35.8)            | 24 (40.7)                                     |
| 4                                | 71 (24.7)             | 16 (27.1)                                     |
| CD4 count, cells/mm³             | (n = 232)             | (n = 51)                                      |
| <500                             | 180 (77.6)            | 51 (100)                                      |
| ≥500                             | 52 (22.4)             | —                                              |
| Median (IQR)                     | 287 (323.5)           | 94(238)                                      |
| Poor adherence (from participants’ medical records) | | |
| No                               | 267 (81.9)            | 48 (75.0)                                     |
| Yes                              | 59 (18.1)             | 16 (25.0)                                     |
| Years on ART (n = 324)           |                       |                                               |
| <5 years                         | 167 (51.5)            | 29 (45.3)                                     |
| ≥5 years                         | 157 (48.5)            | 35 (54.7)                                     |
| Median (IQR)                     | 4.68 (6.09)           | 6.0 (4.7)                                     |
| Current regimen line             | (n = 313)             | (n = 62)                                      |
| First line                       | 244 (78.0)            | 4 (79.0)                                      |
| Second line                      | 69 (22.0)             | 13 (21.0)                                     |
| First-line regimen (n = 244)     |                       | (n = 48)                                      |
| TDF + 3TC/FTC + EFV/NVP          | 169 (69.3)            | 25 (52.1)                                     |
| ZDV + 3TC + EFV/NVP              | 69 (28.3)             | 21 (43.7)                                     |
| D4T + 3TC + NVP                  | 6 (2.4)               | 2 (4.2)                                       |
| Second-line regimen (n = 67)     |                       | (n = 13)                                      |
| TDF + FTC + LPV/r                | 32 (47.8)             | 4 (30.8)                                      |
| ZDV + 3TC + ATV/r                | 8 (11.9)              | 3 (23.1)                                      |
| ABC + 3TC + ATV/LVP/r            | 27 (40.3)             | 6 (6.1)                                       |
| Other ART regimen, n             |                       |                                               |
| DRV + 3TC + RAL                  | 1                     | 1                                             |
| ABC + ddI + LPV/r                | 1                     | —                                             |

D4T, stavudine; RAL, raltegravir; ddI, didanosine.

HIV-1 drug RAM analysis

Genotyping was attempted on samples from patients with suspected virological failure. Figure 1 shows how those with analysed sequences (56) were derived from those initially enrolled. Of the 56 patients whose sequences were analysed, 48 (85.7%) had at least one major RAM associated with the ARVs commonly used in
mutations were not statistically significant. This indicates that the different frequencies between observed and expected frequencies were not significant. Through our test, mutations showed a significant difference between the observed frequencies and the expected frequencies at a 2% significance level.

Figure 2. All RAMs that occurred at a frequency of more than 2% were included. Among them, 20 (35.7%) clustered with subtype A1, 13 (26.9%) were identified as subtype C, 13 (26.9%) were identified as subtype D, and potential recombinants for subtypes A (A1) and recombinant forms of CA, DA and AD were each identified in one (1.79%) set of nucleotide sequences.

Discussion

Virological failure

This study investigated the prevalence of HIV drug resistance in patients with suspected virological failure as defined by a single high viral load result (>1000 copies/mL) at any point during ART. First-line ART regimens consisted of two NRTIs (primarily tenofovir disoproxil fumarate or zidovudine, and lamivudine) and one NNRTI (efavirenz or nevirapine). Second-line ART regimens for patients failing first-line ART consisted of two NRTIs (tenofovir disoproxil fumarate or zidovudine, and lamivudine and/or emtricitabine) and one PI (either atazanavir or lopinavir). Virological failure using the single-test criterion was observed in 20.2% of the patients, higher than the 11.0% (95% CI: 9.6%–12.4%) reported by Hawkins et al. from Tanzania in 2016 and a study on patients on a tenofovir disoproxil fumarate-based regimen in Kenya with 10%, yet lower than observed in a cohort of children in Tanzania with virological failure in 25.4% of patients.

Our study showed that a single high viral load result (greater than 1000 copies/mL) at any point during ART is associated with a high probability of RAMs. This might suggest that a single high viral load result at any point could be used as a predictor of virological failure. This conclusion is supported by studies from developed and developing countries. A study conducted in Austria showed that early virological failure detection (using only a single viral load) leads to an earlier switch to second-line ART in patients with NNRTI mutations, with 65% of the patients switching, versus 48% using the WHO-recommended criteria for virological failure. A study conducted in South Africa indicated that a single high viral load result could be used to define virological failure, especially in patients on an efavirenz-based first-line regimen, and could greatly help in simplifying the switch to a second-line regimen and avoid delays caused by the longer time taken to diagnose virological failure. This study, using a simulation of an individual-based model of HIV transmission, progression and effect of ART, showed that early virological failure detection (using only a single viral load) leads to an earlier switch to second-line ART in patients with NNRTI mutations, with 65% of the patients switching, versus 48% using the WHO-recommended criteria for virological failure, which are also adopted in Tanzania. This study also showed that an early switch would lead to a reduction in AIDS-related mortality.

We screened 56 HIV-1 RT and protease (PR) sequences for the presence of RAMs associated with RT inhibitors (RTIs) and PIs. We detected HIV-1 RTI drug RAMs in 85.7% (n = 48) of the analysed samples. Major RAMs associated with NRTIs and NNRTIs were present at a rate of 76.8% (n = 43) and 85.7% (n = 48), respectively. Despite some patients being on a bPI, none of the study participants harboured RAMs associated with bPIs. These findings are similar to other studies from Tanzania, which show high rates of RAMs, not only in patients virologically failing ART, but also in treatment-naïve patients. Our findings are in line with another study, also conducted in Tanzania, which reported high RAMs and virological failure rates in cohorts of children due to different challenges that group is facing.

Some of the most common mutations in this study were M184V, K103N, Y181C and G190A, which occurred at similar frequencies in our study.
Table 3. PI/RT mutations based on the 56 sequences analysed

| Mutations                | Frequency n (%) | ART regimens                          | Frequency n (%) |
|--------------------------|-----------------|----------------------------------------|-----------------|
| Major NRTI resistance mutations |                 |                                        |                 |
| M184V                    | 42 (75)         | TDF + 3TC/FTC + EFV/NVP                | 18 (4.2)        |
|                          |                 | ZDV + 3TC + EFV/NVP                    | 13 (30.9)       |
|                          |                 | TDF + FTC + LPV/r                      | 4 (9.5)         |
|                          |                 | ABC + 3TC + LPV/rr/ATV/rr              | 3 (7.1)         |
|                          |                 | ZDV + 3TC + ATV/r                      | 2 (4.8)         |
|                          |                 | d4T + 3TC + NVP                        | 1 (4.2)         |
|                          |                 | DRV + 3TC + RAL                        | 1 (2.4)         |
| T215Y                    | 23 (41.1)       | TDF + 3TC/FTC + EFV/NVP                | 8 (34.8)        |
|                          |                 | ZDV + 3TC + EFV/NVP                    | 8 (34.8)        |
|                          |                 | TDF + FTC + LPV/r                      | 3 (13)          |
|                          |                 | ABC + 3TC + ATV/rr/ATV/rr              | 2 (8.7)         |
|                          |                 | d4T + 3TC + NVP                        | 1 (4.3)         |
|                          |                 | ZDV + 3TC + ATV/r                      | 1 (4.3)         |
|                          |                 | ABC + 3TC + ATV/r                      | 1 (5.5)         |
|                          |                 | TDF + 3TC/FTC + EFV/NVP                | 7 (38.9)        |
|                          |                 | ZDV + 3TC + EFV/NVP                    | 6 (33.3)        |
|                          |                 | TDF + FTC + LPV/r                      | 2 (11.1)        |
|                          |                 | d4T + 3TC + NVP                        | 1 (5.5)         |
|                          |                 | ZDV + 3TC + ATV/r                      | 1 (5.5)         |
|                          |                 | ABC + 3TC + ATV/r                      | 1 (5.5)         |
|                          |                 | D67DN                                  |                 |
| K219KQ                   | 14 (25)         | TDF + 3TC/FTC + EFV/NVP                | 7 (50)          |
|                          |                 | ZDV + 3TC + EFV/NVP                    | 5 (35.7)        |
|                          |                 | ZDV + 3TC + ATV/r                      | 1 (7.1)         |
|                          |                 | ABC + 3TC + ATV/rr/ATV/rr              | 1 (7.1)         |
|                          |                 | ABC + 3TC + ATV/r                      |                 |
|                          |                 | K70R                                   |                 |
|                          | 12 (21.4)       | TDF + 3TC/FTC + EFV/NVP                | 5 (41.7)        |
|                          |                 | ZDV + 3TC + EFV/NVP                    | 4 (33.3)        |
|                          |                 | ZDV + 3TC + ATV/r                      | 2 (16.7)        |
|                          |                 | ABC + 3TC + LPV/rr                     | 1 (8.3)         |
|                          |                 | ABC + 3TC + ATV/rr/ATV/rr              |                 |
|                          |                 | V75M                                   |                 |
|                          | 9 (16.1)        | TDF + 3TC/FTC + EFV/NVP                | 4 (44.4)        |
|                          |                 | ZDV + 3TC + EFV                        | 2 (22.2)        |
|                          |                 | ZDV + 3TC + ATV/r                      | 2 (22.2)        |
|                          |                 | ABC + 3TC + ATV/rr/ATV/rr              | 1 (11.1)        |
| Major NNRTI resistance mutations |                 |                                        |                 |
| K103N                    | 22 (39.3)       | TDF + 3TC/FTC + EFV/NVP                | 8 (36.4)        |
|                          |                 | ZDV + 3TC + EFV/NVP                    | 8 (36.4)        |
|                          |                 | TDF + FTC + LPV/r                      | 3 (13.6)        |
|                          |                 | ABC + 3TC + ATV/rr/ATV/rr              | 3 (13.6)        |
|                          |                 | G190A                                  |                 |
|                          | 16 (28.6)       | TDF + 3TC/FTC + EFV/NVP                | 9 (56.2)        |
|                          |                 | ZDV + 3TC + EFV/NVP                    | 4 (25)          |
|                          |                 | TDF + FTC + LPV/r                      | 1 (6.2)         |
|                          |                 | DRV + 3TC + RAL                        | 1 (6.2)         |
|                          |                 | ZDV + 3TC + ATV/r                      | 1 (6.2)         |
|                          |                 | A98G                                   |                 |
|                          | 15 (26.8)       | TDF + 3TC/FTC + EFV/NVP                | 6 (40)          |
|                          |                 | ZDV + 3TC + EFV/NVP                    | 5 (33.3)        |
|                          |                 | TDF + FTC + LPV/r                      | 2 (13.3)        |
|                          |                 | ZDV + FTC + ATV/r                      | 1 (6.7)         |
|                          |                 | d4T + 3TC + NVP                        | 1 (6.7)         |
|                          |                 | K101E                                  |                 |
|                          | 12 (21.4)       | TDF + 3TC/FTC + EFV/NVP                | 6 (50)          |

Continued
Table 3. 

| Mutations | Frequency n (%) | ART regimens | Frequency n (%) |
|-----------|----------------|--------------|----------------|
| Y181C     | 11 (19.6)      | ZDV + 3TC + EFV/NVP 3 (25) |
|           |                | ZDV + 3TC + ATV/r 1 (8.3) |
|           |                | DRV + 3TC + RAL 1 (8.3) |
|           |                | TDF + FTC + LPV/r 1 (8.3) |
| E138A     | 10 (17.8)      | ZDV + 3TC + EFV/NVP 4 (36.4) |
|           |                | ZDV + 3TC + ATV/r 1 (9.1) |
|           |                | ABC + 3TC + LPV/r 1 (9.1) |
| K238T     | 7 (12.5)       | TDF + 3TC/FTC + EFV/NVP 2 (28.5) |
|           |                | ZDV + 3TC + NVP 2 (28.5) |
|           |                | TDF + FTC + LPV/r 1 (14.3) |
|           |                | ZDV + 3TC + ATV/r 1 (14.3) |
|           |                | ABC + 3TC + LPV/r 1 (14.3) |
| V108I     | 7 (12.5)       | TDF + 3TC/FTC + EFV 5 (71.4) |
|           |                | TDF + FTC + LPV/r 1 (1.43) |
|           |                | ABC + 3TC + LPV/r 1 (1.43) |
| Major PI mutations | 0 | | |

D4T, stavudine; RAL, raltegravir. RAMs observed and ARV regimens used by 56 patients whose viral sequences were analysed. M184V and T215 were the most prevalent mutations.

Figure 2. Levels of resistance against the commonly used ARVs in Tanzania observed in patients receiving first-line therapy, second-line therapy and PIs as part of their ART. High-level resistance to NNRTIs and NRTIs was observed in 47.4% and 41.8% of cases, respectively, while no RAMs associated with PIs were found. Resistance was also found to drugs that are not yet in use in Tanzania, 21% against doravirine (DOR), 9% against etravirine (ETR) and 38% against rilpivirine (RPV). This figure appears in colour in the online version of JAC and in black and white in the print version of JAC.
frequencies to those reported in studies from other SSA and Tanzanian settings.\textsuperscript{14,17,24–26,28} The presence of M184V was in line with a study done in Tanzania that showed a high prevalence of this mutation among virological failure patients.\textsuperscript{10} The presence of these mutations in both suspected and confirmed cases of virological failure suggests the need for performing resistance testing for suspected virological failure patients, especially treatment-experienced ones.

None of the study participants who were receiving bPIs exhibited any RAMs associated with PIs, consistent with earlier findings on pre-treatment HIV drug resistance at the same study site.\textsuperscript{27} This shows that bPIs still work well in our settings and switching to bPI-containing second-line regimens must not be delayed to overcome challenges that might be brought about by having longer periods of viraemia, such as further accumulation of RAMs, increased risks in mortality, decreased CD4+ cell counts and rises in viral load.\textsuperscript{1,23,30}

The presence of thymidine analogue mutations (TAMs) among patients with tenofovir disoproxil fumarate-based regimen failure has also been observed in a meta-analysis in SSA.\textsuperscript{31} The most plausible explanations for the occurrence of T215Y, D67N, M41L, K219K/Q and K70R TAMs among patients with tenofovir disoproxil fumarate-based ART regimen failure are that these TAMs were transmitted drug resistance RAMs (TDR-RAMs) or were selected by exposure to undisclosed thymidine analogue-based regimens.\textsuperscript{27} Likewise, the selection of NNRTI-associated mutations such as G190A and Y181C among patients on failing bPI ART regimens can be explained by either TDR-RAMs or by previous undisclosed exposure to NNRTI-based regimens.

HIV-1 subtypes identified in this study were mainly subtypes A, C and D, similar to other studies conducted in Tanzania.\textsuperscript{10,14,24,26}

**Study limitations**

Our study did not attempt to find the association between adherence and other factors and the presence of drug resistance mutations. This study could also not establish the circumstances
under which a single higher-than-threshold (>1000 copies/mL) viral load at any point during treatment could be used to define virological failure since follow-up testing was not conducted to identify which of the study participants were confirmed to have virological failure.

Conclusions

The findings from this study show a high rate of RAMs in patients with suspected virological treatment failure and mutations associated with multiple ARVs. This indicates that sustainable virological suppression is not guaranteed even if it is achieved during enhanced adherence counselling. This study also shows that one HIV viral load test result of >1000 copies/mL is associated with a high probability of HIV drug resistance. This suggests that waiting for another 3 months for a second high viral load over the 1000 copies/mL threshold might risk the further accumulation of HIV RAMs, further limiting options for standard second-line ART regimens. Furthermore, the high prevalence of TAMs among PLHIV on tenofovir disoproxil fumarate-based regimens also calls for HIV drug resistance testing before switching to predetermined second-line ART regimens.

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Transparency declarations

All authors declared no conflict of interest.

Author contributions

S.H., C.K. and G.B.J. conceptualized and designed the study. S.H., K.K. and E.L. did the laboratory analyses, captured demographic information and performed the statistical analysis. S.H., J.M.B., C.K. and S.A. performed the laboratory experiments. S.H., J.M.B. and G.V.Z. drafted and revised the manuscript. All authors read and approved the final manuscript.

References

1. UNAIDS. 2018 Global HIV Statistics. 2019. https://www.unaids.org/sites/default/files/media_asset/UNAIDS_FactSheet_en.pdf.

2. WHO. The 3 by 5 Initiative. 2003. https://www.who.int/3by5/publications/documents/zambia/en/.

3. UNAIDS. Access to Antiretroviral Therapy in Africa. Status Report on Progress towards the 2015 Targets. 2013. https://www.unaids.org/sites/default/files/media_asset/20131219_AccessARTAfricaStatusReportProgressTowards2015Targets_en_0.pdf.

4. Sidibe M, Loures L, Samb B. The UNAIDS 90–90–90 target: a clear choice for ending AIDS and for sustainable health and development. J Int AIDS Soc 2016; 19: 21133.

5. UNAIDS. Global AIDS Update. 2016. http://www.unaids.org/sites/default/files/media_asset/UNAIDS-update-2016_en.pdf.

6. Barth RE, van der Loeff MFS, Schuurman R et al. Virological follow-up of adult patients in antiretroviral treatment programmes in sub-Saharan Africa: a systematic review. Lancet Infect Dis 2010; 10: 155–66.

7. National AIDS Control Programme (NACP), National Guidelines for the Management of HIV and AIDS. Sixth edition. 2017. https://www.departementdeservicedelivraison/Portals/0/adami/Content/NqOqGyocrU2RTjS8IR37uA/File/Tanzania_NATIONAL%20GUIDELINES%20FOR%20MANAGEMENT%20OF%20HIV%20AND%20AIDS%206TH%20EDITION%202017.pdf.

8. Jaka H, Mshana S, Liwa A et al. Prevalence of immunological failure and durability of first line antiretroviral therapy at Bugando Hospital Mwanza, Tanzania. Tanzania Med J 2010; 2: 24.

9. Gunda DW, Kidenya BR, Mshana SE et al. Accuracy of WHO immunological criteria in identifying virological failure among HIV-infected adults on first line antiretroviral therapy in Mwanza, North-western Tanzania. BMC Res Notes 2017; 10: 45.

10. Hawkins C, Ulenga N, Liu E et al. HIV virological failure and drug resistance in a cohort of Tanzanian HIV-infected adults. J Antimicrob Chemother 2016; 71: 1966–74.

11. UNAIDS. Ending AIDS. Progress towards the 90–90–90 Targets. 2017. http://www.unaids.org/sites/default/files/media_asset/UNAIDS_2017_ENDINGAIDS_Slides_en.pdf.

12. Phillips AN, Stover J, Cambiano V et al. Impact of HIV drug resistance on HIV/AIDS-associated mortality, new infections, and antiretroviral therapy program costs in sub-Saharan Africa. J Infect Dis 2017; 215: 1362–5.

13. Basson AE, Charalambous S, Hoffmann CJ et al. HIV-1 re-suppression on a first-line regimen despite the presence of phenotypic drug resistance. PLoS One 2020; 15: e0234937.

14. Muri L, Garnell A, Ntamaturungiro AJ et al. Development of HIV drug resistance and therapeutic failure in children and adolescents in rural Tanzania: an emerging public health concern. AIDS 2017; 31: 61–70.

15. Roche. COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test. 2007. https://www.fda.gov/media/73824/download.

16. QIAGEN. Blood Mini Handbook, Sample and Assay Technologies. 2012. https://moodle.ufsc.br/pluginfile.php/1379318/mod_resource/content/0/QIAamp_DNA_Mini_Blood.pdf.

17. Masimba P, Kituma E, Klimkait T et al. Prevalence of drug resistance mutations and HIV type 1 subtypes in an HIV type 1-infected cohort in rural Tanzania. AIDS Res Hum Retroviruses 2013; 29: 1229–36.

18. Stanford University. HIV Drug Resistance Database. https://hivdb.stanford.edu/hivdb/by-mutations/.

19. Pineda-Peña AC, Faria NR, Imbretts S et al. Automated subtyping of HIV-1 genetic sequences for clinical and surveillance purposes: performance evaluation of the new REGA version 3 and seven other tools. Infect Genet Evol 2013; 19: 337–48.

20. Kumar S, Stecher G, Li M et al. MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol 2018; 35: 1547–9.

21. Brooks K, Diero L, Delong A et al. Treatment failure and drug resistance in HIV-positive patients on tenofovir-based first-line antiretroviral therapy in western Kenya. J Int AIDS Soc 2016; 19: 20798.
Leierer G, Grabmeier-Pfistershammer K, Steuer A et al. A single quantifiable viral load is predictive of virological failure in human immunodeficiency virus (HIV)-infected patients on combination antiretroviral therapy: the Austrian HIV Cohort Study. *Open Forum Infect Dis* 2016; 3: ofw089.

Shroufi A, Van Cutsem G, Cambiano V et al. Simplifying switch to second-line antiretroviral therapy in sub-Saharan Africa. *AIDS* 2019; 33: 1635–44.

Kasang C, Kalluvya S, Majinge C et al. HIV drug resistance (HIVDR) in antiretroviral therapy-naïve patients in Tanzania not eligible for WHO threshold HIVDR survey is dramatically high. *PLoS One* 2011; 6: 2–12.

Masha F, Urassa W, Aboud S et al. Prevalence of genotypic resistance to antiretroviral drugs in treatment-naive youths infected with diverse HIV type 1 subtypes and recombinant forms in Dar es Salaam, Tanzania. *AIDS Res Hum Retroviruses* 2011; 27: 377-82.

Barabona G, Mahiti M, Masoud S et al. Pre-treatment and acquired HIV drug resistance in Dar es Salaam, Tanzania in the era of tenofovir and routine viral load monitoring. *J Antimicrob Chemother* 2019; 74: 3016–20.

Rudovick L, Brauner JM, Englert J et al. Prevalence of pretreatment HIV drug resistance in Mwanza, Tanzania. *J Antimicrob Chemother* 2018; 73: 3476–81.

Hamers RL, Sigaloff KCEE, Wensing AM et al. Patterns of HIV-1 drug resistance after first-line antiretroviral therapy (ART) failure in 6 sub-Saharan African countries: implications for second-line ART strategies. *Clin Infect Dis* 2012; 54: 1660–9.

Petersen ML, Tran L, Geng EH et al. Delayed switch of antiretroviral therapy after virologic failure associated with elevated mortality among HIV-infected adults in Africa. *AIDS* 2014; 28: 2097–107.

Ssempijja V, Nakigozi G, Chang L et al. Rates of switching to second-line antiretroviral therapy and impact of delayed switching on immunologic, virologic, and mortality outcomes among HIV-infected adults with virologic failure in Rakai, Uganda. *BMC Infect Dis* 2017; 17: 582.

Gregson J, Kaleebu P, Marconi VC et al. Occult HIV-1 drug resistance to thymidine analogues following failure of first-line tenofovir combined with a cytosine analogue and nevirapine or efavirenz in sub-Saharan Africa: a retrospective multi-centre cohort study. *Lancet Infect Dis* 2017; 17: 296–304.