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Strengthening HIV therapy and care in rural Tanzania affects rates of viral suppression

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Objectives: To assess viral suppression rates, to assess prevalence of acquired HIV drug resistance and to characterize the spectrum of HIV-1 drug resistance mutations (HIV-DRM) in HIV-1-infected patients in a rural Tanzanian HIV cohort.

Methods: This was a cross-sectional study nested within the Kilombero and Ulanga Antiretroviral Cohort. Virological failure was defined as HIV-1 RNA ≥50 copies/mL. Risk factors associated with virological failure and with the development of HIV-DRM were assessed using logistic regression.

Results: This study included 304 participants with a median time on ART of 3.5 years (IQR = 1.7–5.3 years); 91% were on an NNRTI-based regimen and 9% were on a boosted PI-based regimen. Viral suppression was observed in 277/304 patients (91%). Of the remaining 27 patients, 21 were successfully genotyped and 17/21 (81%) harboured ≥1 clinically relevant HIV-DRM. Of these, 13/17 (76.5%) had HIV-1 plasma viral loads of >1000 copies/mL. CD4 cell count <200 cells/mm^3 at the time of recruitment was independently associated with a close to 8-fold increased odds of virological failure [adjusted OR (aOR) = 7.71, 95% CI = 2.86–20.78, $P < 0.001$] and with a >8-fold increased odds of developing HIV-DRM (aOR = 8.46, 95% CI = 2.48–28.93, $P = 0.001$).

Conclusions: High levels of viral suppression can be achieved in rural sub-Saharan Africa when treatment and care programmes are well managed. In the absence of routine HIV sequencing, the WHO-recommended threshold of 1000 viral RNA copies/mL largely discriminates virological failure secondary to HIV-DRM.

Introduction

About 5% of the Tanzanian adult population is living with HIV.[1] Although this prevalence has recently fallen slightly, the burden of disease differs widely, with some regions reporting a prevalence of <2% and others as high as 16%.[2] The HIV/AIDS epidemic in Tanzania is driven by a complex interweaving of factors that include extreme wealth disparities, cultural and traditional practices (i.e. polygamy, widow inheritance, gender inequality), casual and extramarital relationships, drug and alcohol abuse, and sexual practices. After the roll-out of free ART in 2004 by the National AIDS Control Program, the number of individuals on ART has increased from fewer than 5000 people to 740078 in 2015, corresponding to 53% of all HIV-positive patients.[3]

Provision of ART is the only medical intervention presently able to increase survival of HIV-infected subjects in low-income countries. As in other sub-Saharan African countries, HIV infection in Tanzania is treated via a public health approach with standardized first- and second-line ART regimens. Adherence to ART is a principal determinant of therapeutic success, because insufficient drug levels after suboptimal intake of ART may lead to virological failure. As ART coverage expands, the chances of uncontrolled use or suboptimal doses rise, causing higher rates of acquired HIV drug resistance mutations (HIV-DRM).[4] As a consequence, drug-resistant viruses can also be transmitted to other individuals, potentially fostering an increase of transmitted drug-resistant HIV to ART-naive patients.[5,6]

Early detection of treatment failure and a timely switch to second-line ART could limit the development of drug resistance and reduce clinical progression and cases of advanced disease.[7] In addition, genotypic resistance testing could help to prevent unnecessary drug-class switching, delay the emergence of HIV-DRM and enhance the effectiveness of ART, thus underscoring the growing need for virological monitoring in resource-limited settings.[8] Given the limited ART options in sub-Saharan Africa, evidence from studies assessing the efficacy of ART programmes using virological endpoints and surveillance of HIV-DRM are of particular importance.

In this study, we investigated the rate of viral suppression and prevalence of acquired HIV drug resistance and characterized the spectrum of HIV-DRM in HIV-infected patients enrolled in a rural Tanzanian HIV cohort on ART for at least 6 months.

Methods

Study design and setting

All data were prospectively collected from participants enrolled in the Kilombero and Ulanga Antiretroviral Cohort (KIULARCO). This ongoing, open, prospective cohort is comprised of all patients enrolled at the Chronic Diseases Clinic of Ifakara (CDCI), which serves as a Care and Treatment Centre for HIV/AIDS patients within the Saint Francis Referral Hospital, implementing care and treatment for HIV/AIDS patients according to the National AIDS Control Program (NACP). This is the largest healthcare facility in the Kilombero and Ulanga District of the Morogoro region in southern Tanzania and provides treatment and care for a population of approximately 600000 inhabitants and an estimated 30000 patients living with HIV/AIDS. Established in 2004, it was the first rural clinic accredited to be a Care and Treatment Centre of the NACP in Tanzania and over 9000 patients have been enrolled into care.

Blood samples are drawn at routine clinic visits prior to ART initiation, 2 weeks and 3 months after ART initiation and every 3–6 months thereafter. Plasma is cryopreserved at −80°C. Further details of the KIULARCO cohort are given elsewhere.[9–12]

Ethics

Written informed consent was sought from all participants at registration at the CDCI and all data were anonymized. Samples were collected during routine clinical visits and ethical approval for their cryopreservation and retrospective analysis was obtained from the Institutional Review Board of the Ifakara Health Institute (IHI), the Tanzanian National Institute for Medical Research (NIMR/HQ/R.8c/Vol.I/378) and the Ethics Committee of the University and Canton of Basel (EKBB 160/09).

Participants and ART regimens

This cross-sectional study was nested within the KIULARCO cohort and included HIV-infected adults on ART for at least 6 months and attending the CDCI between October and December 2013. Data were extracted from the KIULARCO electronic medical records.

Currently, the most widely used first-line regimens consist of the combination of tenofovir disoproxil fumarate, lamivudine or emtricitabine, and efavirenz, co-formulated in a fixed-dose combination single tablet. Available alternative NRTIs include zidovudine, lamivudine and abacavir. The only other available NNRTI is nevirapine. The recommended second-line ART regimens are based on boosted PIs, preferentially co-formulated atazanavir/ritonavir or lopinavir/ritonavir. ART initiation is recommended at CD4 counts of <500 cells/mm^3 and in those with WHO stage 3 or 4 regardless of CD4 counts. In addition, ART initiation is recommended to all pregnant women, regardless of CD4 counts.[13]

Laboratory testing

Blood samples were collected in BD Vacutainer EDTA collection tubes and CD4 cell count, plasma HIV-1 viral load and HIV-DRM genotyping were performed at the IHI Laboratory. CD4+ cell counts were determined by flow cytometry (FACS Calibur, BD Company, Franklin Lakes, NJ, USA), and analysed within 3 h after blood drawing. Cell-free plasma was collected by centrifugation at 1950 **g** for 5 min and frozen at −80°C until testing for viral load or drug resistance. HIV RNA from plasma was extracted using a QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany), using the manufacturer's protocol. Viral RNA quantification was done using TaqMan® Real-Time PCR Master Mixes (Thermo Fisher Scientific), on a StepOne Real Time PCR system (Applied Biosystems, ABI, Thermo Fisher Scientific, Waltham, MA, USA). For all samples with detectable viral load, HIV-DRM genotyping was performed on an ABI Genetic Analyser (four-capillary model 3130) using a validated in-house PCR protocol.[14]

HIV-1 drug resistance was determined according to the Stanford University HIV Drug Resistance Database Program version 6.2.0 (http://hivdb.stanford.edu). Drug resistance mutations conferring low-, intermediate- or high-level resistance were considered.

The reported protease and reverse transcriptase sequences are available in GenBank (accession numbers KY014602–KY014621).

Outcome

The primary outcome of this study was virological failure defined as HIV-1 RNA levels $\geq$50 copies/mL in a single determination. The secondary outcome was the prevalence of drug resistance defined as the presence of mutations determined by HIV-1 genotyping and defined in the WHO 2009 HIV resistance mutation list.

Predictor variables

The following variables were considered *a priori* as predictors of viral failure: CD4 <200 cells/mm^3 at recruitment into this study, time on ART, ART regimen, non-adherence (defined as reporting missing any dose of ART in the previous 4 weeks), WHO stage III/IV, gender, age, distance to the health centre, education level and BMI <18.5 kg/m^2.

Statistical analysis

Demographic characteristics were summarized using medians and IQRs for continuous data and frequencies and percentages for categorical data. Multivariate logistic regression models were used to estimate the association between virological failure/development of HIV-DRM and the predictors of interest. ORs and 95% CIs are presented. All analyses were performed using Stata v. 13 (StataCorp LP, College Station, TX, USA).

Results

Characteristics of study participants

Table 1 summarizes the characteristics of the 304 patients enrolled in the study. Almost all patients were living in the Kilombero district and 58% were residents of Ifakara town. The majority were women (70.4%) and the median age at enrolment was 41 years (IQR = 35–48 years). Ninety-one percent had received primary or higher education. The median CD4 cell count at baseline was 414 cells/mm^3 (IQR = 267–610 cells/mm^3) and 158 patients (54%) were diagnosed as having clinical AIDS (WHO stages III and IV). Advanced immunosuppression, defined as a single CD4+

Table 1. Baseline characteristics of the study participants

Characteristic	Study population ($N = 304$)
Age (years), median (IQR)	41 (35–48)
Male, *n* (%)	90 (29.6)
Education, *n* (%)	
none	27 (8.9)
primary	254 (84.1)
secondary	18 (6.0)
college/other	3 (1.0)
BMI (kg/m^2), median (IQR)	22.6 (20.3–58.5)
Years living with HIV, median (IQR)	4.2 (2.2–6.0)
WHO clinical stage, *n* (%)	
I	75 (25.7)
II	59 (20.2)
III	104 (35.6)
IV	54 (18.5)
CD4+ cell count (cells/mm^3)	
median (IQR)	414 (267–610)
<200, *n* (%)	45 (15.0)
200–349, *n* (%)	68 (22.6)
350–499, *n* (%)	76 (25.3)
≥500, *n* (%)	112 (37.2)
ART regimen, *n* (%)	
ZDV/3TC/NVP	71 (23.7)
ZDV/3TC/EFV	89 (29.7)
TDF/FTC/EFV	107 (35.7)
TDF/FTC/NVP	1 (0.3)
TDF/3TC/EFV	5 (1.7)
TDF/FTC/LPV/r	23 (7.7)
other second line	4 (1.3)
Non-adherence, *n* (%)	10 (3.3)

ZDV, zidovudine; 3TC, lamivudine; NVP, nevirapine; EFV, efavirenz; TDF, tenofovir disoproxil fumarate; FTC, emtricitabine; LPV/r, lopinavir/ritonavir.

count measurement <200 cells/mm^3, was observed in 45 (15%) patients. The ART regimen received was tenofovir disoproxil fumarate/emtricitabine/efavirenz in 107/300 (35.7%), zidovudine/lamivudine/efavirenz in 89/300 (29.7%), zidovudine/lamivudine/nevirapine in 71/300 (23.7%) and tenofovir disoproxil fumarate/lamivudine/efavirenz in 5/300 (1.7%). Second-line PI-based ART regimens were used for 27/300 patients (9%), 23 of those (7.7%) on tenofovir disoproxil fumarate/emtricitabine/lopinavir/ritonavir (Table 1).

Virological results

After a median time of 3.5 years on ART (IQR = 1.7–5.3 years), 277/304 (91.1%) of patients had suppressed viraemia, defined as achieving viral load <50 copies/mL. Among the 27 patients with detectable plasma HIV-1 viral load, 6 (22%) had low-level viraemia (50–499 copies/mL), 3 (11%) had a viral load of 500–999 copies/mL, 9 (33%) had a viral load of 1000–9999 copies/mL and 9 (33%) had a viral load of >10000 copies/mL.

Among participants with virological failure, 21/27 (78%) were successfully genotyped and 17 of these (81%) harboured at least one clinically relevant resistance mutation in the reverse transcriptase gene (Table 2). Thirteen of the 17 participants (76.5%) infected with virus harbouring HIV-DRM had a plasma HIV-1 viral load >1000 copies/mL, 3 (17.6%) had a plasma HIV-1 viral load of between 500 and 1000 copies/mL and 1 (5.9%) had a plasma HIV-1 viral load of <500 copies/mL.

The most frequent mutations were M184V (12/21, 57%), causing resistance to lamivudine and emtricitabine; K103N (8/21, 38%), which causes resistance to NNRTIs; V108I (5/21, 24%), which reduces susceptibility to nevirapine and efavirenz by about 2-fold;[15] H221Y (4/21, 19%); and Y188L (3/21, 14%), which causes high-level resistance to nevirapine and efavirenz. Thymidine analogue mutations (TAMs), known to cause clinical resistance to zidovudine and stavudine, and cross-resistance to abacavir, didanosine and tenofovir were found in 16/21 participants (76%), 2 of whom had ≥3 TAMs.

HIV-1 subtypes were A1 ($n = 7$), C ($n = 6$), D ($n = 7$) and CRF01_AE ($n = 1$).

Factors associated with virological failure and HIV-DRM

In the multivariable logistic regression analysis adjusted by age, gender, education, WHO stage, BMI, years since HIV diagnosis, time on ART and distance to the clinic, only having CD4 <200 cells/mm^3 at the time of recruitment into this study was independently associated with virological failure [adjusted OR (aOR) = 7.71, 95% CI = 2.86–20.78, $P < 0.001$] and presence of HIV-DRM (aOR = 8.46, 95% CI = 2.48–28.93, $P = 0.001$) (Table 3).

Discussion

In this cross-sectional study, 9 out of 10 adults on first-line ART for at least 6 months had suppressed viraemia. Only severe immune suppression was independently associated with virological failure and with emergence of HIV-DRM. A similar observation has been documented by Seyler *et al.*[16] while studying an HIV cohort in Abdjan, western Africa. The observed high rates of viral suppression were superior to the WHO target for viral suppression of ≥70% after at least 1 year of first-line ART,[17,18] and reached the 90% viral suppression goal set by UNAIDS for 2020.[19] A systematic review of virological efficacy of adult patients in ART programmes in sub-Saharan Africa has reported 76% viral suppression after 12 months on ART and 67% after 24 months.[20]

Of note, most virological data in Tanzania currently come from studies with generally small numbers of participants, limited follow-up and varying in study population (e.g. paediatrics). Hawkins *et al.*[21] reported a prevalence of 14.9% and 75.7% for virological failure and HIV-DRM, respectively, while studying a population in north-west Tanzania. A study conducted by Ngarina *et al.*[22] in the city of Dar es Salaam, Tanzania, among women for 24 months post-partum, documented a proportion of virological failure of 61% at 12 months on ART, with drug resistance demonstrated in 34%. Overall, studies conducted in Tanzania reported a prevalence of virological failure ranging from 19% to 61% and an over 75% prevalence of HIV-DRM.[10,22–25]

Several factors may have contributed to the observed virological efficacy of ART in our setting. First, unlike many other HIV treatment programmes in resource-limited settings, the CDCI is reasonably well staffed with physicians specifically dedicated to

Table 2. Genotypic resistance results for 21 patients on ART with detectable HIV-1 viral load

Sample ID	ART regimen	Viral load (HIV RNA copies/mL)	NRTI-DRM	NNRTI-DRM	HIV subtype	Years after HIV diagnosis
10001	TDF/FTC/EFV	37803	M184V, L210L/W	K103K/N	D	7.59
10002	TDF/FTC/EFV	1296	M41L, T69D, V75M	K101E	C	4.07
10003	TDF/FTC/EFV	6144	K70R, M184V	K103N, V108I, H221Y, E138A	A1	3.76
10004	TDF/FTC/EFV	984	M184V, L210W, T215Y	K103N, M230L	C	3.59
10005	TDF/FTC/EFV	10028	none	K103N, E138G	D	2.22
10006	TDF/FTC/EFV	1385	none	none	D	5.66
10007	TDF/FTC/EFV	105	none	none	A1	3.94
10008	TDF/FTC/EFV	102	none	none	D	3.37
10009	TDF/FTC/EFV	42088	M41L, D67G, K70R, M184V, M210W, T215F	V90I, K103N, V108I, K238T	A1	1.06
10010	ZDV/3TC/NVP	59466	M184V	V179D, Y188L	C	7.89
10011	ZDV/3TC/NVP	8141	M41L, M184V, T215F	A98G, K101E, V108I, G190A	A1	7.82
10012	ZDV/3TC/NVP	637	M184V	L100I, Y188L	D	5.68
10013	ZDV/3TC/NVP	98	M184V	K103N	A1	5.52
10014	ZDV/3TC/NVP	17854	none	Y181C, H221Y	A1	5.57
10015	ZDV/3TC/NVP	39904	M41L, V75M, M184V, L210W, T215F	V108I, Y181C, H221Y	D	6.4
10016	ZDV/3TC/NVP	1009	M41L, M184V, T215Y	V106A, V179D, Y188F, Y188H	D	3.16
10017	ZDV/3TC/EFV	8989	K70R, M184V	K103N, V108I, H221Y, E138A	A1	5.7
10018	ZDV/3TC/EFV	1721	M184V	K103N, E138A, Y188L	C	3.23
10019	ZDV/3TC/EFV	1334	D67N, K70R, M184V, T215Y, K219E	K101E, Y188C	C	2.63
10020	ZDV/3TC/EFV	921	D67K, K70A, L74E	L100V	CRF01_AE	1.61
10021	ZDV/3TC/EFV	185	none	none	C	4.04

TDF, tenofovir disoproxil fumarate; FTC, emtricitabine; EFV, efavirenz; ZDV, zidovudine; 3TC, lamivudine; NVP, nevirapine.

Table 3. Factors associated with virological failure and development of HIV-DRM among adults attending the CDCI using logistic regression analysis

	Virological failure				Development of HIV-DRM			
	univariate		multivariate		univariate		multivariate	
Variable	OR (95% CI)	*P*	aOR (95% CI)	*P*	OR (95% CI)	*P*	aOR (95% CI)	*P*
Male	0.73 (0.26–2.05)	0.260	0.64 (0.19–2.14)	0.470	0.78 (0.24–2.489)	0.675	0.66 (0.15–2.89)	0.581
CD4 <200 cells/mm^3	6.36 (2.52–16.07)	0.000	7.71 (2.86–20.78)	<0.001	5.13 (1.80–14.61)	0.002	8.46 (2.48–28.93)	0.001
WHO clinical stage III–IV	0.93 (0.38–2.26)	0.869	0.83 (0.31–2.21)	0.414	1.46 (0.51–4.13)	0.476	2.04 (0.53–7.89)	0.303
BMI <18.5 kg/m^2	1.24 (0.27–5.69)	0.781	1.01 (0.20–5.21)	0.987	1.73 (0.37–8.12)	0.490	1.65 (0.28–9.69)	0.579
Age estimate for every 5 years	0.93 (0.74–1.16)	0.515	1.00 (0.77–1.28)	0.975	0.870 (0.67–1.13)	0.298	0.97 (0.70–1.32)	0.831
Time on ART (years)	1.13 (0.92–1.38)	0.255	1.14 (0.94–1.39)	0.18	1.10 (0.86–1.42)	0.441	1.17 (0.83–1.66)	0.377

HIV treatment and care, including HIV specialists who continuously provide mentorship for capacity building to local medical practitioners. Secondly, the programme has a well-equipped laboratory for HIV treatment monitoring and an on-site electronic data collection and management system, which together enhance effectiveness of HIV treatment and care.[12] Thirdly, the programme has a team of counsellors connected to a network of community health workers who help to reduce stigma and improve adherence. Counsellors provide routine pre- and post-HIV testing, pre-ART counselling and routine on-ART counselling for those identified to have problems with adherence. In addition, the clinic has a tracking team detecting loss-to-follow-up and forging links to the community health workers to assist patients with resuming treatment. Observations from rural HIV clinics in various resource-limited countries have shown that inadequately managed HIV treatment and care programmes may result in high levels of virological failure and widespread drug resistance.[26,27]

To date, only a few Tanzanian studies have investigated the virological effectiveness of ART and the emergence of HIV-DRM among ART-experienced HIV patients, typically under stavudine-based

regimens.[24,28] To our knowledge, this is the first well-powered study addressing virological effectiveness and the emergence of HIV-DRM among patients on the current WHO-recommended ART regimen in Tanzania under programmatic conditions. Our findings provide further evidence from rural African settings that higher virological suppression rates can be achieved when HIV treatment and care programmes are continuous and effectively managed.[28]

Furthermore, the study enabled us to estimate the prevalence of HIV-DRM in HIV-infected patients receiving ART for at least 6 months. Our findings show that approximately 80% of the individuals who experienced virological failure harboured at least one clinically relevant resistance mutation in the reverse transcriptase gene. Of those, about 80% had >1000 HIV RNA copies/mL. Of note, most individuals who had low-level viraemia, notably <500 RNA copies/mL, had no detectable ART resistance mutations.[29] Although this observation should be interpreted cautiously owing to our reported few cases with viral load <500 copies/mL, these findings are consistent with previous studies in sub-Saharan Africa and support the WHO threshold used for defining virological failure.[30] Our findings are in agreement with the WHO recommendations for HIV viral load testing in resource-limited settings, which suggest that patients with low viraemia should undergo intensified adherence interventions to regain HIV-1 RNA suppression without switching ART,[31] and those with repeated high viral load measures should be switched to second-line ART without delay.[32,33]

Interestingly, we did not detect the K65R mutation, which reduces the susceptibility to tenofovir disoproxil fumarate, despite the fact that one-third of patients with detectable HIV-DRM had been on a tenofovir-based regimen for a minimum of 6 months. K65R is the most important discriminatory mutation, causing intermediate resistance to tenofovir, while increasing the susceptibility to zidovudine, and is mostly selected in patients with HIV subtype C.[34] Our observation provides relevant preliminary information on the mutational patterns among patients under the current WHO guideline recommendations for tenofovir-based regimens. However, this observation warrants further in-depth studies on the emergence of HIV-DRM among patients on tenofovir-based regimens in these settings. We observed a high prevalence of TAMs, perhaps explained by the fact that >50% of the study patients were on a zidovudine-based regimen, known to select TAMs in patients failing treatment.

Our study has several limitations, the main one being the fact that we analysed only those under care during this cross-sectional study. Non-adherence to ART is strongly associated with lower compliance with medical visits, loss to follow-up and death, and these patients were not included by design. Similarly, the design of the study excludes those patients who did not complete 6 months on treatment, leading to survivorship bias and hence overestimating the rates of viral suppression. On the other hand, the availability of single timepoint HIV-1 RNA determinations might have overestimated the cases of persistently detectable viraemia, particularly among those with HIV-1 RNA <1000 copies/mL. Furthermore, patients did not receive HIV-DMR testing prior to ART initiation and, thus, some observed drug resistance mutations might have been transmitted during primary infection. Finally, all genotypic data were obtained through standard Sanger sequencing and detection rates of HIV-DRM would be higher if ultrasensitive HIV-1 drug resistance testing by next-generation sequencing had been used.[35]

In summary, this study provides information that improves our understanding of virological failure and HIV-DRM emergence under programmatic conditions in rural sub-Saharan Africa. Our findings show that high levels of virological suppression with a first-line ART regimen can be achieved in rural African settings when HIV treatment and care programmes are well managed.

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Transparency declarations

None to declare.

Author contributions

Conceived and designed the experiments: A. J. N., E. L. and T. K. Performed the experiments: A. J. N. and L. M. Analysed the data: A. J. N., E. L. and T. R G. Wrote the paper: A. J. N., L. M., T. R. G., S. E., M. B., M. T., H. F., C. H., I. F., T. K. and E. L.

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