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Prevalence and factors associated with HIV-1 drug resistance mutations in treatment-experienced patients in Nairobi, Kenya

A cross-sectional study

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Abstract

An estimated 1.5 million Kenyans are HIV-seropositive, with 1.1 million on antiretroviral therapy (ART), with the majority of them unaware of their drug resistance status. In this study, we assessed the prevalence of drug resistance to nucleoside reverse transcriptase inhibitors (NRTIs), nucleoside reverse transcriptase inhibitors (NNRTIs), and protease inhibitors, and the variables associated with drug resistance in patients failing treatment in Nairobi, Kenya.

This cross-sectional study utilized 128 HIV-positive plasma samples obtained from patients enrolled for routine viral monitoring in Nairobi clinics between 2015 and 2017. The primary outcome was human immunodeficiency virus type 1 (HIV-1) drug resistance mutation counts determined by Sanger sequencing of the polymerase *(pol)* gene followed by interpretation using Stanford's HIV Drug Resistance Database. Poisson regression was used to determine the effects of sex, viral load, age, HIV-subtype, treatment duration, and ART-regimen on the primary outcome.

HIV-1 drug resistance mutations were found in 82.3% of the subjects, with 15.3% of subjects having triple-class ART resistance and 45.2% having dual-class resistance. NRTI primary mutations M184 V/I and K65R/E/N were found in 28.8% and 8.9% of subjects respectively, while NNRTI primary mutations K103N/S, G190A, and Y181C were found in 21.0%, 14.6%, and 10.9% of subjects. We found statistically significant evidence ($P = .013$) that the association between treatment duration and drug resistance mutations differed by sex. An increase of one natural-log transformed viral load unit was associated with 11% increase in drug resistance mutation counts (incidence rate ratio [IRR] 1.11; 95% CI 1.06–1.16; $P < .001$) after adjusting for age, HIV-1 subtype, and the sex-treatment duration interaction. Subjects who had been on treatment for 31 to 60 months had 63% higher resistance mutation counts (IRR 1.63; 95% CI 1.12–2.43; $P = .013$) compared to the reference group (<30 months). Similarly, patients on ART for 61 to 90 months were associated with 133% higher mutation counts than the reference group (IRR 2.33; 95% CI 1.59–3.49; $P < .001$). HIV-1 subtype, age, or ART-regimen were not associated with resistance mutation counts.

Drug resistance mutations were found in alarmingly high numbers, and they were associated with viral load and treatment time. This finding emphasizes the importance of targeted resistance monitoring as a tool for addressing the problem.

Abbreviations: 3TC = lamivudine, ABC = abacavir, ART = antiretroviral therapy, ATV/r = atazanavir/ritonavir, AZT = zidovudine, DTG = dolutegravir, EFV = efavirenz, HIV-1 = human immunodeficiency virus type 1, HIVDRM = human immunodeficiency virus drug resistance mutations, IQR = interquartile range, IRR = incidence rate ratio, NNRTIs = non-nucleoside reverse transcriptase inhibitors, NRTIs = nucleoside reverse transcriptase inhibitors, NVP = nevirapine, *pol* = polymerase gene, TAM = thymidine analog mutations, TDF = tenofovir disoproxil fumarate.

Keywords: antiretroviral therapy, drug resistance mutations, human immunodeficiency virus type 1 genotyping, Kenya, plasma human immunodeficiency virus type 1 viral load, poisson regression model, treatment duration

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The datasets generated during and/or analyzed during the current study are publicly available.

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1. Introduction

In 2019, 38 million people worldwide were living with HIV, with 25.4 million receiving antiretroviral therapy (ART).[1] In Kenya, an estimated 1.5 million people are infected with HIV, with 1.1 million receiving ART, up from 54,093 in 2005.[1] The use of ART has been shown to significantly reduce HIV-related mortality, especially if viral suppression is achieved.[2] Conversely, the increase in human immunodeficiency virus type 1 (HIV-1) drug resistance (HIVDR) contributes to the accumulation of HIV variants unresponsive to existing treatment regimens.[3,4] If not addressed, it could result in millions of deaths, a rise in new, difficult-to-treat variants, and higher healthcare costs. Consequently, close monitoring of HIVDR emergence and timely response at the population level is critical.

Since 2014, Kenya's Ministry of Health has advised that all people with HIV infection, regardless of CD4 count, WHO clinical stage, age, pregnancy or breastfeeding status, or risk category, are eligible for ART if they are willing to take the drug as recommended.[5] The current first-line ART for adults is tenofovir disoproxil fumarate (TDF) + lamivudine (3TC) + dolutegravir (DTG) or TDF+3TC+ efavirenz (EFV), while patients who are unable to use TDF due to impaired renal function are put on abacavir (ABC). Those who cannot tolerate DTG are put on atazanavir/ritonavir (ATV/r). Patients with virological failure on zidovudine (AZT), lopinavir/ritonavir, nevirapine (NVP), and EFV are to be switched to DTG. ATV/r is an alternative to DTG when the patient cannot tolerate it.[5] Patients are switched to second-line ART (AZT+3TC+ATV/r, TDF+3TC+ATV/r, or DTG-based 2nd line ART) based on viral load results. However, as a baseline investigation, HIV drug resistance testing is not currently recommended. A drug resistance test is only recommended if a PI-based first-line regimen has failed or if a second-line regimen has failed and switching to a third-line regimen is the only option.

In Kenya, viral load monitoring is performed before starting ART, 6 months after starting a new treatment regimen, and annually thereafter in an attempt to increase ART effectiveness and minimize the spread of drug resistance mutations.[5] In a nationwide study, 13.3% of adults, 43.1% of children, and 36.6% of adolescents were found to have virological failure (VL >1000 copies/mL).[6] Several studies have found an increase in the incidence of HIV drug resistance mutations (HIVDRM) in tandem with expanded ART coverage. In the country, the prevalence of acquired drug resistance was estimated at 52.7%,[4] while the prevalence of transmitted drug resistance was 9.2%.[7]

The prevalence of ART drug resistance among ART-exposed patients in Kenya is unknown. Furthermore, the current strategy of carrying out a genotypic test only after a patient has failed a second-line ART regimen predisposes certain patients to needless toxicity and pill burden that could be avoided if resistance testing was performed prior to starting therapy. Thus, in the current study, we examined treatment-experienced patients sourced from the general population who had virological failure and were enrolled in routine viral load testing to assess the frequency of drug resistance to nucleoside reverse transcriptase inhibitors (NRTIs), nucleoside reverse transcriptase inhibitors (NNRTIs), and protease inhibitors. In addition, using a Poisson regression model, we identified the variables associated with HIVDRM acquisition.

2. Methods

2.1. Research ethics

The use of HIV-positive plasma was approved by the Kenya Medical Research Institute-Scientific and Ethical Research Unit (Kenya Medical Research Institute/SERU/3935). This study's participants were all part of a larger ongoing project involving clinical and virological aspects of HIV, and they had all given their informed consent for their samples to be used in future HIV research. Given that the current study focussed on additional virological aspects of HIV infection on the same biological samples, a waiver of consent was sought as it was not deemed to adversely affect the rights and welfare of the subjects.

2.2. Study design and setting

This was a cross-sectional study that utilized 128 plasma samples from HIV-1 infected and treatment-experienced people with virological failure who lived in Nairobi - Kenya, between August 2015 and August 2017. The prevalence of HIV-1 infection among adults (15–49 years) in Nairobi is 8.4%, with an estimated 145,668 HIV seropositive patients by 2016.[8] The choice of 81 females versus 47 males reflects on the epidemic where young women carry a higher burden of the epidemic. Kenya, like many other resource-constrained countries, has taken a public health approach to providing ART. The laboratory experiments were carried out at Kenya Medical Research Institute's Laboratory for Molecular Biology.

2.3. Study participants

The study population consisted of HIV-infected individuals visiting Nairobi HIV clinics for routine care. Individuals who met the following criteria were included into the study: 15 years old; confirmed HIV-1 seropositive; receiving ART for at least 6 months; being diagnosed with virologic failure (Viral load >1000 copies/mL) despite being on ART; and residing in Nairobi county during the enrolment period (August 2015–August 2017). Exclusion criteria included viral load of <1000 copies/mL, treatment naivety, non-residence in Nairobi County, and being under the age of 15 during the enrolment period.

2.4. Human immunodeficiency virus type 1 genotypic drug resistance testing

2.4.1. Viral RNA extraction. HIV-1 RNA extraction was carried out using the Abbott M2000SP automated platform (Abbott Molecular Inc, Des Plaines, USA) in accordance with the manufacturer's instructions. Briefly 350 μL of plasma was lysed using 4.7 M guanidium isothiocyanate and 10% tween. The lysate was transferred to sterile spin column, washed twice with 500 μL of wash buffer and finally eluted in 50 μL of elution buffer. RNA was stored at −80°C.

2.4.2. The polymerase gene amplification and sequencing. Thermo Fisher Scientific™ HIV Genotyping workflow: Amplification Module-catalogue number A32317 (Thermo Fisher Scientific, San Francisco, USA) that amplifies a 1.1-kb fragment including protease (6–99) and reverse transcriptase (1–251) regions was performed as per manufacturer's instructions.[9] In brief, 10 μL of extracted RNA was incubated at 65°C for 10 minutes and mixed with 40 μL Reverse Transcription-Polymerase Chain Reaction Master Mix containing Superscript™ III

One-Step Reverse Transcription-Polymerase Chain Reaction with Platinum™ Taq High Fidelity Enzyme. The cycling conditions were as follows: Reverse transcription; 50°C for 45 minutes; enzyme inactivation at 94°C for 2 minutes; PCR initial denaturation at 94°C for 2 minutes and 40 cycles (94°C for 15 second, 50°C for 20 second, 72°C for 2 minutes) and final 10 min-extension at 72°C. For nested PCR, 2 μL of PCR products were amplified in a 50 μL reaction with AmpliTaq Gold LD DNA polymerase (Thermo Fisher Scientific, San Francisco, USA) as follows: initial denaturation 94°C for 4 minutes, 40 cycles (94°C for 15 second, 55°C for 20 second, 72°C for 2 minutes), and a final 10 minutes step at 72°C. Amplified PCR product (1.08 kb) was verified by 1% agarose gel electrophoresis. The amplified PCR fragment was purified using Clean Sweep PCR purification reagent (Thermo Fisher Scientific, San Francisco, USA) according to the manufacturer's instructions.

The polymerase (*pol*) gene sequencing was performed using Thermo Fisher Scientific™ HIV Genotyping kit: Cycle Sequencing module-catalogue number A32318 which utilized 6 overlapping primers (F1, F2, F3, R1, R2, and R3). 2 μL of PCR product was added to 18 μL of sequencing mix. Cycle sequencing conditions were as follows: 25 cycles of 96°C for 10 seconds, 50°C for 5 seconds, and 60°C for 4 minutes. Sanger sequencing was performed using BigDye XTerminator kit (Thermo Fisher Scientific, San Francisco, USA) on Applied Biosystems 3500xL DX genetic analyzer (Applied Biosystems, Foster City, USA). Sequencing files were automatically interpreted by Recall,[10] and drug resistance mutations identified using Stanford human immunodeficiency virus database genotyping algorithm. The *pol* sequences were achieved in the EMBL Nucleotide Database with the following accession numbers, [MW165068-MW165069, MW178208-MW178236, and MW995389-MW995470].

2.5. Statistical analyzes

HIVDRM counts were the primary outcome of concern. The predictor variables included age, sex, viral load, treatment period, ART regimen, and HIV-1 subtype. The HIV-1 subtype was determined from the sequenced *pol* gene using the REGA subtyping tool. Clinical data (viral load, treatment-duration, and ART-regimen) were obtained from medical records, while demographic information (age and sex) was collected at enrolment. Only patients who were taking an ART from a drug class as part of their prescription medication were included in the denominators when determining the percentage of patients with resistance to that drug class. Fisher exact test and Wilcoxon rank sum test were used to compare categorical variables, and Welch two sample *t*-test was used to compare continuous variables, with 2-sided *P* values reported in all cases. Before fitting our model to predict HIVDRM, Spearman's correlation was used to examine the relationship between predictor and outcome variables. Poisson regression model was used to calculate incidence rate ratios (IRR) and associated 95% confidence intervals (CIs). Prior to fitting our model, we hypothesized that treatment duration influences HIVDRM acquisition, and that this is dependent on the individual's sex and therefore treated it as effect modifier. The deviance χ^2 test and likelihood ratio tests of fitting alternate distributions were used to assess model fit. Age, sex, and HIV-1 subtype were included in the fitted models as possible confounders, regardless of statistical significance. For all other variables, only those with a $P < .25$ at univariate analysis were considered to be influencing the outcome and were included in the full model. There was missing data for age (14 subjects), viral load (3 subjects) and duration of treatment (3 subjects) which were excluded from statistical analyses. All statistical analyses were conducted with the R statistical package (R version 4.0.3).

3. Results

3.1. Patient demographics, viral load, human immunodeficiency virus type 1 subtype, and antiretroviral therapy- regimens

Table 1 summarizes the study participants' demographic and clinical characteristics. The median age was 35 years (interquartile range [IQR], 29–42), with 81 (63.3%) females and 47 (36.7%) males. When the age was stratified into 10-year strata, significant differences between the strata were found ($P = .005$). The median HIVDRM per subject was 5 (IQR, 1.75–7), with no statistically significant difference between females 5 (IQR, 1–7)

Table 1

Demographic, clinical and virological characteristics of study subjects.

Characteristic	Female, N = 81*	Male, N = 47*	*P* value†
ln (viral load)	9.09 (8.05–10.72)	10.54 (8.77–12.13)	.047
HIV-1 subtype			.013
A	58 (71.6)	23 (48.9)	
A2	0 (0.0)	1 (2.1)	
B	0 (0.0)	1 (2.1)	
C	4 (4.9)	5 (10.6)	
CRF01_AE	8 (9.9)	5 (10.6)	
D	7 (8.6)	10 (21.3)	
G	0 (0.0)	1 (2.1)	
J	0 (0.0)	1 (2.1)	
K	4 (4.9)	0 (0.0)	
Age strata (Yr)			.005
15–25	12 (16.0)	6 (15.4)	
26–35	35 (46.7)	6 (15.4)	
36–45	19 (25.3)	20 (51.3)	
46+	9 (12.0)	7 (17.9)	
Unknown	6	8	
Treatment duration (Mo)			.028
0–30	14 (17.7)	14 (30.4)	
31–60	27 (34.2)	9 (19.6)	
61–90	30 (38.0)	12 (26.1)	
91–150	8 (10.1)	11 (23.9)	
Unknown	2	1	
ART regimen			.093
ABC+3TC+EFV	2 (2.5)	1 (2.1)	
ABC+3TC+LPV/r	0 (0.0)	2 (4.3)	
ABC+3TC+NVP	1 (1.2)	2 (4.3)	
AZT+3TC+ATV/r	1 (1.2)	0 (0.0)	
AZT+3TC+EFV	3 (3.8)	2 (4.3)	
AZT+3TC+LPV/r	0 (0.0)	2 (4.3)	
AZT+3TC+NVP	20 (25.0)	8 (17.0)	
TDF+3TC+ATV/r	2 (2.5)	0 (0.0)	
TDF+3TC+EFV	44 (55)	20 (42.6)	
TDF+3TC+NVP	5 (6.2)	8 (17.0)	
Unknown	3 (3.8)	2 (4.3)	

ln (VL) = natural log transformed viral load, 3TC = lamivudine, ABC = abacavir, ART = antiretroviral therapy, ATVr = atazanavir/ritonavir, AZT = zaidovudine, EFV = efavirenz, LPVr = lopinavir/ritonavir, NVP = nevirapine, TDF = tenofovir disoproxil fumarate.

* Median (IQR); n (%).

† Wilcoxon rank sum test; Welch two sample *t*-test; Fisher's exact test; Pearson's Chi-Squared test.

and males 5 (IQR, 3–7), $P > .99$. There was a significant difference in natural-log-transformed viral load between females 9.09 (IQR, 8.05–10.72) and males 10.54 (IQR, 8.77–12.13), $P = .047$. The median time between ART-regimen prescription and genotypic test was 59.8 months (IQR, 34.5–76.8). Stratifying treatment-duration into 30-month intervals revealed sex-specific differences ($P = .028$). TDF+3TC+EFV (50.4%), AZT+3TC+NVP (22.0%), and TDF +3TC+NVP (10.2%) were the 3 most commonly prescribed ART-regimens. There was no significant difference in the prescription of ART-regimens between males and females, $P = .093$. HIV-1 subtypes were found to have a wide range of distribution. HIV-Subtype A accounted for 63.3% of all subtypes while subtype D accounted for 13.3% and CRF01_AE 10.2%. A2, B, G, J, and K accounted for 6.2% of the total. HIV-1 subtypes did not differ significantly between males and females ($P = .081$).

3.2. Distribution of antiretroviral drug resistance mutations

The HIVDRM counts followed a poisson distribution, with a range of 0 to 15 counts and a median of 5 (IQR, 1.75–7) (Fig. 1). M184 V/I 87 (28.8%), K65R/E/N 27 (8.9%), L74 V 3 (1.0%), and Y115F 12 (4.0%) were the mutations conferring resistance to NRTIs, along with thymidine analog mutations (TAMs) M41L 14 (4.6%), D67N 17 (5.6%), K70R 20 (6.6%), L210W 10 (3.3%), T215Y/F 23 (7.6%), K219Q/E 32 (10.6%), and K65R/E/N 27 (8.9%). M184 V/I confers resistance to 3TC and ABC in the presence of 2 or 3 TAMs. K65R/E/N and K70E mutations conferred resistance to TDF and M41L, D67N, K70R, L210W, T215Y/F, and K219Q/E conferred resistance to AZT. K103N/S 56 (21.0%), G190S/A 39 (14.6%), Y181C/I 29 (10.9%), V108I 13 (4.9%), P225H 12 (4.5%), Y188L 7 (2.6%), M230L 7 (2.6%), L100I 5 (1.9%), V106 M 5 (1.9%), and K101P 2 (0.7%) were the mutations conferring resistance to NNRTIs. Low genetic resistance is a feature of NNRTIs. That is, a single NNRTI-related mutation confers high-level resistance to all 3 NNRTIs (NVP, EFV, and ETV). PI-resistant virus strains were found in a minority of subjects. M46I, which was found in 3 patients, confers Darunavir resistance. There were no other mutations at L76 V, I47 V, I50 V, I54 M/L, or I84 V. Lopinavir was the only PI class that demonstrated significant HIVDRM with mutations at V32I (2 patients), I47 V/A (2 patients), and V82A/F/T/S (3 patients). This study found no atazanavir resistance mutations (I50L, I84 V, or N88S).

Overall, NVP 132 (81.0%), EFV 116 (71.2%), 3TC 108 (66.3%), and FTC 108 (66.3%) were found to have disproportionately high levels of resistance. As shown in (Table 2), 19/124 (15.32%) of the subjects had a high level of resistance to all of the drugs in their regimen. Notably, none of those participants were on a boosted PI regimen. High levels of resistance to 2 of the drugs in the prescribed ART-regimen was observed in 56 subjects, of these; 32 subjects were on TDF+3TC+EFV, 16 were on AZT +3TC+NVP, and 5 were on TDF+3TC+NVP (Table 2). One subject had high levels of resistance to 2 drugs in the ABC+3TC +EFV, ABC+3TC+NVP, and AZT+3TC+EFV regimens. In 25 subjects, high levels of resistance to 1 drug in the regimen were observed. Thirteen of these subjects were on TDF+3TC+EFV, 3 on TDF+3TC+NVP, and 2 each on ABC+3TC+EFV, AZT+3TC +NVP, and TDF+3TC+ATVr. Twenty two (22/124) of the subjects were susceptible to all of the drugs in their treatment plan (Fig. 2).

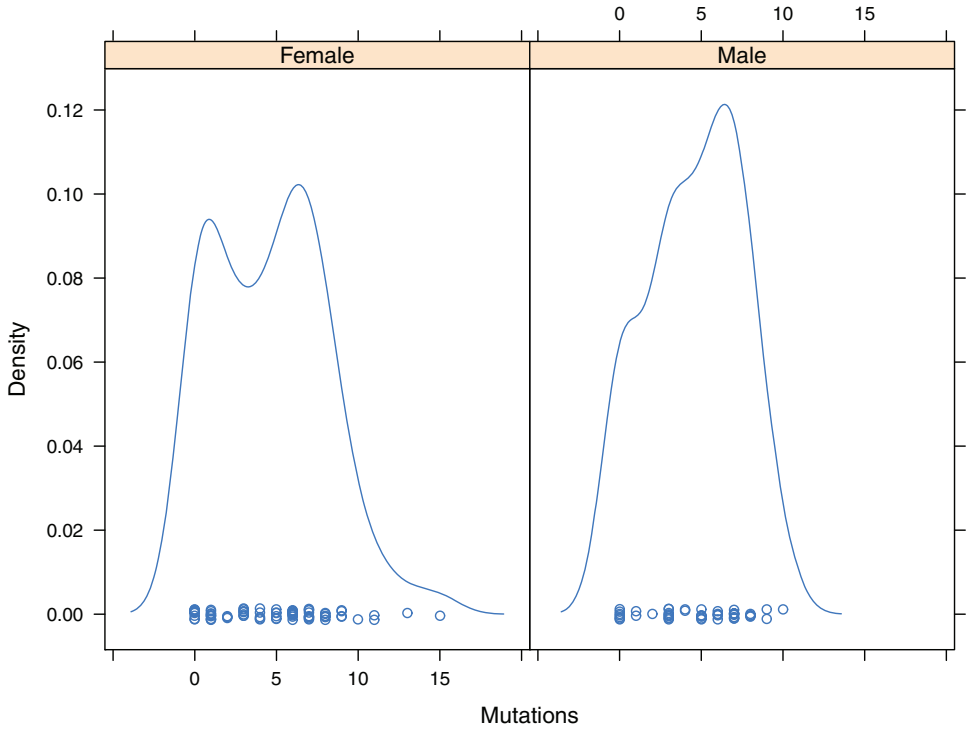

Figure 1. Distribution of HIV-1 Drug Resistance Mutations in the study subjects stratified by Sex. The blue circles depict the location of the mutations in relation to the curve. This density plot shows a non-normal right skewed distribution with a wider range in females (0–15) than males (0–10). The overall median and mean were 5 (IQR, 1.75–7) and 4.5 (IQR, 1.75–7) respectively. The primary outcome (number of drug resistance mutations) did not differ significantly between sexes ($P = .78$).

Table 2

Resistance patterns associated with ARV regimen in 124 patients.

ART Regimen	High-level of resistance to 3 drugs	High-level of resistance to 2 drugs	High-level of resistance to 1 drug	Intermediate resistance to 1 drug	Susceptible to all drugs
ABC+3TC+EFV	2	1	0	0	0
ABC+3TC+LPVr	0	0	1	0	1
ABC+3TC+NVP	2	1	0	0	0
AZT+3TC+ATVr	0	0	1	0	0
AZT+3TC+EFV	2	1	2	0	0
AZT+3TC+LPVr	0	0	1	1	0
AZT+3TC+NVP	4	16	2	0	6
TDF+3TC+ATVr	0	0	2	0	0
TDF+3TC+EFV	6	32	13	1	13
TDF+3TC+NVP	3	5	3	0	2
total	19	56	25	2	22
proportion (95%CI)	0.153 (0.095–0.229)	0.452 (0.362–0.543)	0.202 (0.135–0.283)	0.016 (0.002–0.057)	0.177 (0.115–0.256)

3.3. Poisson regression analysis

An analysis of the interaction between sex and treatment-duration revealed that sex had an effect on treatment duration and outcome, with longer treatment duration being more strongly associated with lower HIVDRM counts in males (interaction $P = .002$), (see Table 3). As a result, the IRR cannot be interpreted without taking into account the sex and treatment-duration interaction. The significance of the interaction's contribution to this model was confirmed by a drop-in-deviance test. We have statistically significant evidence ($\chi^2 = 10.72$, degrees of freedom [df] = 3, $P = .013$) that the difference in treatment duration and HIVDRM differs by sex. The residual deviance (278.70 with 96 df) indicates a lack of fit in the interaction model, implying that the difference is due to a sex-specific variable not included in our model. To visualize evidence of interaction, we created scatter plots. As seen in Figure 3, the interaction of the sex and duration of treatment is evident. The lines would be parallel if there was no interaction.

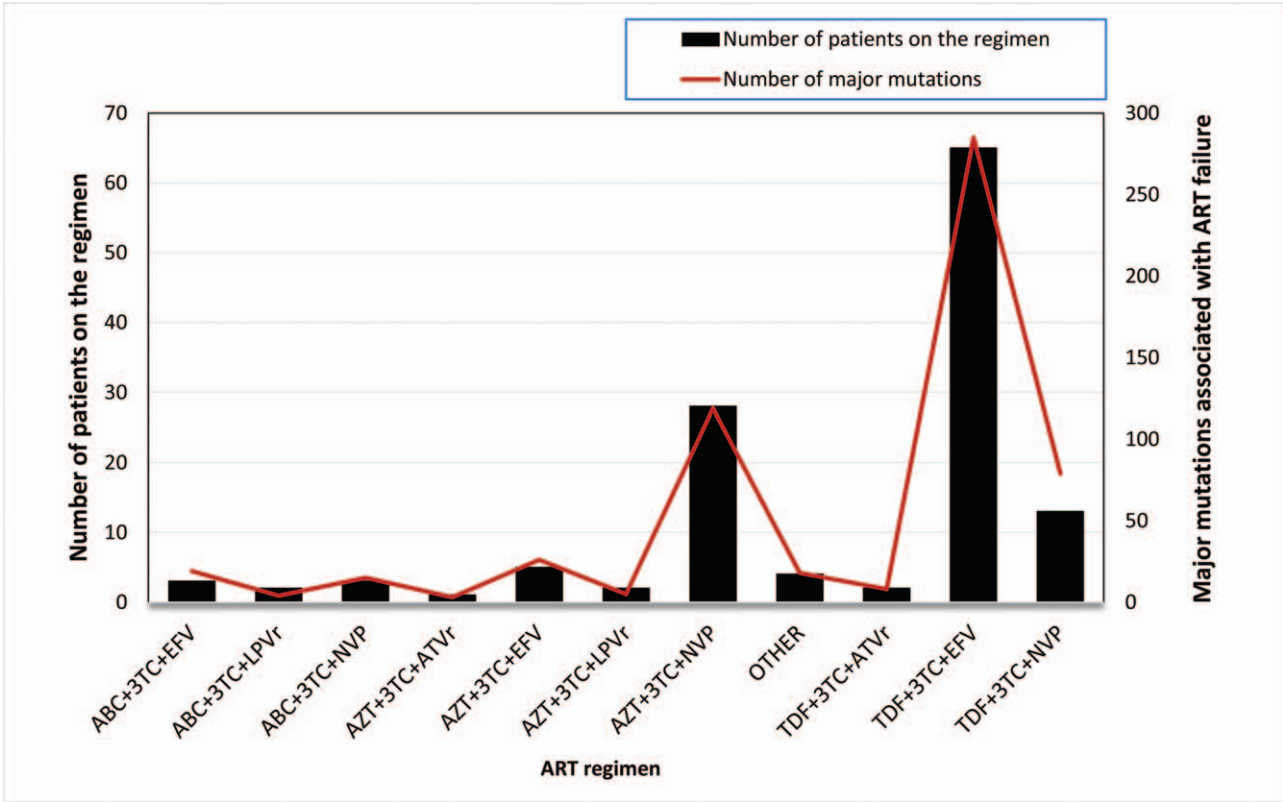

Figure 2. Resistance mutations to prescribed ART regimen in treatment experienced patients. The black bars indicate the subjects prescribed a particular regimen and the mutations (red line) discovered in the 124 subjects. TDF+3TC+EFV triple combination was prescribed to 64% of the study subjects and subsequently, accounted for 49.05% (285/581) profiled in the study. 3TC = lamivudine, ABC = abacavir, ATVr = atazanavir/ritonavir, AZT = zidovudine, EFV = efavirenz, LPVr = lopinavir/ritonavir, NVP = nevirapine, TDF = tenofovir disoproxil fumarate.

Table 3

Univariate and multivariate Poisson regression analysis of HIV-1 drug resistance mutations.

	Univariate			Multivariate		
Characteristic	**IRR[1]**	**95% CI[1]**	***P value**	**IRR[2]**	**95% CI[2]**	***P value**
ln (VL)	1.09	0.05, 1.13	<.001	1.11	1.06, 1.16	<.001
Sex						
Female	—	—		—	—	
Male	1.00	0.84, 1.18	.99	1.64	1.06, 2.53	.025
Age strata (Yr)						
15–25	—	—		—	—	
26–35	1.10	0.85, 1.43	.48	1.00	0.76, 1.33	.98
36–45	0.89	0.69, 1.17	.41	0.84	0.63, 1.12	.23
46+	0.81	0.58, 1.13	.22	0.74	0.51, 1.07	.11
Duration strata (Mo)						
0–30	—	—		—	—	
31–60	1.03	0.81, 1.32	.80	1.63	1.12, 2.43	.013
61–90	1.41	1.13, 1.77	.003	2.33	1.59, 3.49	<.001
91–150	0.94	0.70, 1.26	.68	1.56	0.93, 2.59	.088
HIV subtype						
A	—	—		—	—	
C	0.86	0.60, 1.18	.37	0.85	0.59, 1.20	.38
CRF01_AE	0.78	0.58, 1.05	.11	0.89	0.60, 1.29	.56
D	0.91	0.70, 1.15	.44	1.03	0.77, 1.36	.82
Minority (A2,B,G,J,K)	0.96	0.68, 1.133	.83	1.14	0.77, 1.63	.51
ART Regimen						
ABC+3TC+EFV	—	—				
ABC+3TC+LPVr	0.32	0.09, 0.84	.036			
ABC+3TC+NVP	0.79	0.39, 1.55	.50			
AZT+3TC+ATVr	0.47	0.11, 1.39	.20			
AZT+3TC+EFV	0.82	0.46, 1.50	.50			
AZT+3TC+LPVr	0.39	0.13, 0.98	.064			
AZT+3TC+NVP	0.67	0.42, 1.12	.11			
TDF+3TC+ATVr	0.63	0.26, 1.39	.30			
TDF+3TC+EFV	0.71	0.46, 1.16	.14			
TDF+3TC+NVP	0.96	0.59, 1.63	.90			
Sex* Duration strata						
Male* 31-60	0.47	0.26, 0.81	.008	0.34	0.16, 0.67	.002
Male* 61-90	0.78	0.49, 1.24	.30	0.55	0.32, 0.94	.03
Male* 91-150	0.77	0.42, 1.41	0.40	0.67	0.34, 1.29	.23

CI = adjusted confidence interval, CI = confidence interval, IRR[1] = incidence rate ratio, IRR[2] = adjusted incidence rate ratio.

* *P* values from χ^2 test for categorical variables or Wilcoxon test for continuous variables.

ln (VL)) = natural log transformed viral load, 3TC = lamivudine, ABC = abacavir, ART = antiretroviral therapy, ATVr = atazanavir/ritonavir, AZT = zidovudine, EFV = efavirenz, LPVr = lopinavir/ritonavir, NVP = nevirapine, TDF = tenofovir disoproxil fumarate.

The level of viremia expressed as natural log-transformed viral load units was the strongest predictor of HIVDRM, (see Table 3). After adjusting for age, HIV-1 subtype, and sex-treatment duration interaction, 1 natural log increase in viremia natural log transformed viral load was associated with an 11% increase in HIVDRM counts (IRR 1.11; 95% CI 1.06–1.16; $P < .001$). Treatment-duration was the second most significant predictor of HIVDRM. Subjects on ART-regimen for 31 to 60 months had a 63% higher HIVDRM counts compared to those on treatment for < 30 months (reference group), IRR, 1.63; 95% CI 1.12–2.43, $P = .013$. Similarly, compared to the control group, there was a 133% increase in HIVDRM counts in subjects on treatment for 61 to 90 months (IRR, 2.33 95% CI 1.59–3.49, $P < .001$). There was no statistically significant association between HIVDRM counts and subjects who had been on treatment for more than 90 months ($P = .088$). Males had a 64% increase in HIVDRM counts in our multivariate model compared to females (IRR 1.64, 95% CI 1.06–2.53, $P = .025$). This was not observed in the univariate model ($P = .99$), highlighting the importance of the sex-duration of treatment interaction and significantly higher level of viremia in males compared to females ($P = .047$). Age, HIV-1 subtype and ART-regimen were not significantly associated with HIVDRM counts.

4. Discussion

Antiretroviral drugs suppress but do not eliminate HIV infection.[11] In some cases, mutations occur, reducing suppression of HIV-1 replication with currently available antiretroviral drugs.[12–14] The efficacy of an ART-regimen depends on the activity of the regimen's individual ARV drugs in the regimen. If two ARV drugs in a triple drug combination have mutations conferring resistance to the individual ARVs, the ART regimen may be ineffective in suppressing HIV-1 replication, resulting in poor clinical outcomes, including death.[15]

The prevalence of HIVDRM conferring resistance to various ART regimens was found to be high (82.3%). Notably, a high proportion of dual and triple-class antiretroviral drug resistance

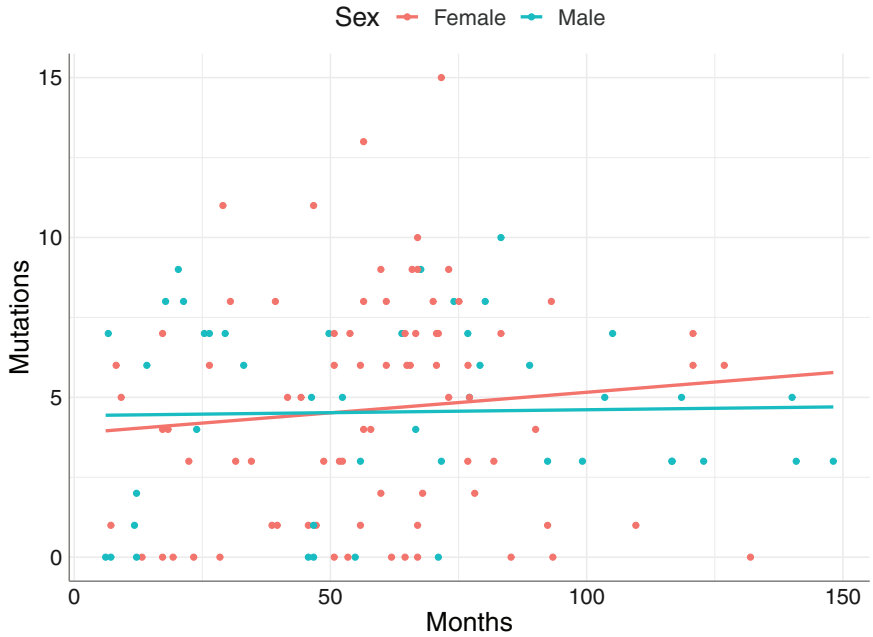

Figure 3. The interaction effect of sex on duration of treatment (months) and drug resistance mutations. There is evidence of a sex interaction (female vs male) on the primary outcome (drug resistance mutation counts, with female having a larger effect on accumulating mutations over a longer period of time. If no evidence of interactions existed, the lines would have been parallel.

(60.5%) necessitates an immediate switch to an alternate regimen. The high levels of resistance are due to the K103N, G190A, and Y181C mutations, which confer resistance to NVP (81.0%) and EFV (71.2%). Since these NNRTIs have a low genetic barrier to resistance, a single mutation confers resistance to all of them.[16] This is consistent with results from other studies conducted in similar settings, such as South Africa's 86%,[17] Uganda's 84.6%,[18] Malawi's 95%,[19] and Nigeria's 90%.[20] According to WHO guidelines, in countries where resistance to these drugs exceeds 10%, an alternative first-line regimen that does not contain EFV or NVP should be used.[21] To comply with the WHO guidelines, Kenya has changed its treatment of adults and children with virologic failure from NVP to DTG-based regimens. EFV, unlike NVP, has distinct clinical features and is still recommended in Kenya as an alternative to DTG to women of childbearing age due to its high potency and high viral suppression rates in treatment-naive patients.[22] Furthermore, since EFV has a longer plasma half-life (40–55 hour) than NVP (25–30 hour), a delayed or missed EFV dose is less likely to result in drug levels dipping below the inhibitory concentration.[23] M184V/I was the most common major NRTI resistance mutation. It was found in 28.8% of the sequences and confers high-level resistance to 3TC and ABC while also increasing susceptibility to AZT and TDF.[24,25] The fact that 3TC is a backbone for most Kenyan ART-regimens explains this predominance of M184V/I.[5] Similar M184V/I predominance trends (81.2%) have been reported in Uganda.[26] The K65R mutation was found in 32 sequences on 3TC, ABC, and TDF regimens, increasing viral resistance to TDF and ABC by 2-fold and 3TC and FTC by 5 to 10-fold, respectively.[27] M184V and K65R together are sufficient to inhibit the activity of a TDF and ABC-containing regimen while also increasing sensitivity to AZT.[28,29] Twenty eight samples with high AZT resistance all had ≥3 TAMs, meaning that the presence of TAMs reduces viral AZT susceptibility. In contrast to type II TAMs, type I TAMs reduce the virus's resistance to ABC and TDF-containing regimens.[30] The PI-resistant virus was found in a small percentage of subjects with virological failure. This finding is consistent with previous research from LMICs.[31] The high genetic barrier to resistance of boosted PI-based ART could explain the low prevalence of PI-resistance.[32] This is reassuring, given that the country's third-line regimen includes PI.

We found a strong association between viral load and increased HIVDRM counts ($P < .001$), which is in concordance with previous studies that found viremia to be a surrogate for increased viral replication rate and a predictor of HIVDRM.[33–35] Furthermore, each one $\log_{10}$ copies/mL increase in VL has been found to more than double the per coital-act probability of HIV transmission.[36] Longer treatment duration was associated with increased HIVDRM. This result was consistent with previous findings that longer durations of unchanged treatment due to insufficient access to VL surveillance or delayed transitions to second-line treatment were correlated with enhanced drug resistance,[37,38] but it differed from the findings of Napravnik et al, who found no association.[39] Our discovery that a subject's sex and duration of treatment independently influenced the rate of HIVDRM acquisition was unique to this study. For instance, being male was associated with 66% less HIVDRM counts compared to a female who had been on the same treatment for 30 to 60 months while all other factors remained constant ($P = .002$). This finding suggests a unique opportunity to learn how the subject's gender affected the outcome variable. In an attempt to identify the influence of this interaction, we examined the IRR for sex and the outcome variable. When we compared the adjusted ($P = .025$) versus unadjusted ($P = .99$) models we found a significant association between HIVDRM counts and sex. It is unclear why the duration of treatment would influence HIVDRM between sexes. This lack of clarity on the cause is backed up by

the residual deviance (278.70 with 96 df), which indicated that there are some significant covariates that may be used to explain the variations but were not used in our model. We speculate, with caution, that pharmacokinetic differences between the sexes may account for these findings,[40,41] and this possibility warrants further investigation.

The influence of the HIV subtype on drug resistance has received increased attention as a result of the global intensification of ART. According to our findings, the most common HIV-1 subtype was genotype A (63.3%), followed by subtype D (13.3%), which is consistent with previous research.[42–47] Subtype CRF01_AE has increased significantly to 10.2%, up from <5% recorded in 2008.[46] The significant increase in genotype CRF01_AE, which is prevalent in Asia, and the decrease in subtype C (7.0%) from >10%,[42,46] could be due to immigration and emigration into the East African region. We also found subtypes A2, B, G, J, and K, which cumulatively accounted for 6.2% of the total HIV-1 subtypes. Antiretroviral drugs have generally proven to be effective against a wide range of HIV subtypes, despite being primarily tested in people with subtype B.[48,49] As a result, most studies indicate that individual ARVs and standard ART regimens are equally effective, regardless of HIV subtype.[50–52] In this study, the HIV-1-subtype and acquisition of HIVDRM were not significantly associated. In contrast, some studies showed that women and infants infected with HIV-subtype C were more likely to develop NVP resistance than other HIV subtypes.[53,54]

The HIV epidemic in sub-Saharan Africa is disproportionately infecting adolescent girls and young women aged 15 to 24 who seroconvert 5 to 7 years earlier than their male peers.[55] This vulnerability is due in part to socio-economic factors such as intergenerational transactional sex between young women who have well-off older men as lovers in an environment of severe poverty and restricted resources.[56–58] Such a transaction predisposes young women to HIV infection since the older age of sexual partner is associated with increased risk of HIV-1 infection and the young woman is less likely to negotiate condom use given the gender-power dynamics.[59] Contrary to expectations, age was not associated with DRMs although being male was associated with increased DRM counts ($P = .025$). This warrants further investigation to ascertain whether an increase of DRMs is associated with reduced fitness.

Our study has some limitations. First, there was lack of adherence data for the study subjects. It is well known that adherence is the most critical non-virological factor influencing the development of HIVDRM.[60] Moreover, inadequate adherence to the prescribed ART regimen results in HIV replication in the presence of the drug, which invariably results in the selection of mutations within the virus that confer antiretroviral drug resistance. In non-adherent patients, mutations have been shown to evolve rapidly for older generation NNRTIs and NRTIs, but more slowly for many newer NRTIs but rarely for boosted protease inhibitors.[61] Intriguingly, in resource limited settings, women are more likely than men to be non-adherent to ART due to Intimate partner violence,[62] or lack of money for travel to ART centre and hunger.[63] Knowledge of the factors associated with medication adherence could help HIV clinicians to target persons in need of intervention and design interventions that will ensure adherence. Second, there was no data on treatment interruption by the patients which has an effect on adherence and is associated with viral rebound and a shift to a resistant genotype.[64] Third, DRMs data prior to current ART-regimen initiation was not available. Thus, we can not rule out the possibility of transmitted drug resistance that existed prior to prescription of the current ART-regimen.

5. Conclusions

To summarize, we found an alarmingly high prevalence of drug resistance mutations. Importantly, patients with triple and dual-class drug resistance should alter ART-regimens immediately to avoid the possibility of transmitting multidrug-resistant HIV-1 strains, which would have fewer treatment options. The most significant predictors of HIVDRM were viral load and treatment duration. The most striking finding was that a subject's sex and treatment-duration independently influenced HIVDRM counts, emphasizing the importance of targeted resistance monitoring and switching ART regimens while taking into account the risk of exhausting future treatment options. More research is needed to determine the variables that contributed to the finding that a subject's sex and treatment time independently influenced HIV-1 drug resistance mutations.

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