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HIV-1 Drug Resistance and Virologic Outcomes Among Tanzanian Youth living with HIV

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Abstract

Adolescents living with HIV tend to have poor adherence that often breeds HIV resistance mutations and virologic failure. This study evaluated risk behavior, virologic outcomes, and HIV resistance mutations in Tanzanian youth living with HIV. Participants were primarily perinatally infected, mean age 16.7 years and 41.5% had virologic failure. Those receiving first-line therapy demonstrated over 90% resistance to their current therapy.

Introduction

An estimated 2.1 million adolescents (age 10 – 19 years of age) were living with HIV in 2016 and numbers continue to rise.¹ Between 2000 and 2015, AIDS related deaths decreased in all age groups except adolescents, where AIDS related deaths have more than doubled making AIDS the leading cause of death among adolescents in Africa.² Many of these adolescents acquired HIV perinatally and have been receiving antiretroviral therapy (ART) for more than a decade.

As children age into adolescence, the adherence burden of taking medication daily, coupled with the stigma of living with a potentially fatal, sexually transmitted disease, often leads to worse ART adherence and virologic outcomes. Adolescents living with HIV have the lowest rate of ART adherence and the highest rates of treatment failure when compared to children and adults.^{2–4} Inadequate ART adherence may lead to HIV mutations that compromise current and future therapeutic options.⁵

Implementation of HIV-1 RNA testing in Tanzania began in 2016; thus, there has been delayed recognition of virologic failure (VF) and ART regimen switch.³ VF results in

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increased risk of HIV transmission to sexual partners due to unsuppressed virus and continuing treatment on a failing regimen can lead to accumulation of resistance mutations that compromise future treatment options.⁶ The objective of this study was to describe virologic outcomes, HIV transmission risk factors, and HIV drug resistance mutations among a cohort of young people living with HIV (YLWH) in Tanzania.

Methods

This was a two site, prospective, cross-sectional study conducted in Moshi, Tanzania. Sites were chosen based on being the regional referral hospitals within Moshi Municipal. Kilimanjaro Christian Medical Centre (KCMC) has supported an adolescent HIV program since 2007 whereas Mawenzi Regional Referral Hospital (MRRH) only began an adolescent HIV clinic in 2015. There was no difference in adherence interventions for these clinics, adherence counseling occurred as per National guidelines standard of care. Participants 11 to 24 years of age who attended a monthly HIV adolescent clinic were enrolled at either KCMC (December 2013 – December 2014) or MRRH (February – July 2015). CD4 cell counts were routinely measured approximately every six months; however, HIV-1 RNA testing was not standard of care during this time.

Enrolled YLWH had a prospective HIV-1 RNA with additional plasma stored for resistance testing. Youth also participated in a structured interview the same day at MRRH, or within approximately 6 months at KCMC. The structured interview included demographic, mental health, stigma, disclosure, and adherence questions, which have been previously reported.^{7–9} Participants also reported behaviors such as sexual debut, number of sexual partners, condom use, and substance use that may increase risk of HIV transmission. Clinical files were retrospectively reviewed for the most recent CD4, current ART regimen, and when the participant was first diagnosed with HIV and started ART. Youth were considered to have perinatal HIV infection if they had clinical documentation of HIV infection or ART start at or less than ten years of age, a mother who died due to HIV, or had a mother living with HIV; otherwise mode of HIV acquisition was categorized as unknown.

HIV-1 RNA analysis was performed at the Kilimanjaro Clinical Research Institute (KCRI) Biotechnology Laboratory, using the Abbott m2000 (Des Plaines, IL) and participates in international external quality assurance programs. Virologic failure was defined as plasma HIV-1 RNA > 400 copies/mL. Samples from participants with virologic failure were sent to the Duke Human Vaccine Institute (DHVI) for resistance testing. Reverse transcription was performed using SuperScript® III Reverse Transcriptase (Invitrogen; Carlsbad, CA); Reverse Transcription Primer: Rev8 5' CCCTATTAGCTGCCCCATCTACATA 3'. The Gag-pol Gene was then amplified using a nested PCR with Platinum Taq High Fidelity Kit (includes 10X HiFi buffer, MgSO₄, Platinum Taq) (Invitrogen; Carlsbad, CA) as has been previously described in an infant cohort.¹⁰

Second round PCR products were preceded by Illumina MiSeq sequencer (Illumina Inc. San Diego, CA). Sequencing results were edited using Geneious R8 (Auckland 1010, New Zealand). Sequences were aligned and phylogenetic tree was draw by using SeaView.^{11,12} Genotypic sequence interpretation was performed by Stanford University HIVdb program.

^{13,14} Descriptive statistics were used to summarize demographic and virologic outcomes. Statistical analyses were performed using Chi-Square and Wilcoxon rank sum tests. All statistics were generated using Stata/SE 13.1 (StataCorp, College Station, Texas). Resistance sequences have been accepted in GenBank (accession numbers [MG867377](#)–[MG867448](#)).

Ethical review and informed consent

Written informed consent was provided by participants 18 years or older. A parent or guardian provided consent for youth younger than 18 years of age and youth provided assent. The Duke University Medical Center Institutional Review Board, the KCMC Research Ethics Committee, and the Tanzanian National Institute of Medical Research approved the study protocol.

Results

The study included 280 participants of whom 248 (88.6%) were receiving ART and had samples available for HIV-1 RNA measurement. Of those, 103 (41.5%) met the definition of virologic failure (HIV-1 RNA > 400 copies/mL); 71 (68.9%) had plasma remaining for resistance testing. There were no demographic differences between the YLWH with or without resistance testing completed (Table 1).

Mean age of the cohort was 16.7 years, 54% were female, and approximately 83% were known to be perinatally HIV-infected. Of those with virologic failure, 32% were failing a second-line regimen (17 females; 16 males). Nineteen youth (18.5%) with virologic failure reported sexual activity, of whom approximately two-thirds (63%) reported condom use during the most recent sexual encounter.

Resistance mutations were common, as detailed in Figure 1. The most common nucleoside reverse transcriptase inhibitor (NRTI) mutations included M184V, most often present in those receiving zidovudine/lamivudine (AZT/3TC) drug combination. One or more thymidine analog mutations (TAMs) were present in 38% (27/71). The most common non-nucleoside reverse transcriptase inhibitor (NNRTI) mutation was K103N, conferring high-level resistance to both nevirapine (NVP) and efavirenz (EFV). Youth receiving a first-line ART regimen with virologic failure had greater than 90% predicted phenotypic resistance (93% receiving EFV; 94% receiving NVP) to their NNRTI-backbone; 28% had predicted resistance to all three ART drugs. Of the youth receiving a second-line regimen, one individual had predicted intermediate resistance to lopinavir boosted-ritonavir (LPV/r). This sequence had four protease inhibitor (PI) mutations including M46I and L76V. No major PI resistance was noted for the eight participants receiving atazanavir boosted-ritonavir (ATV/r). Subtypes were diverse: Subtype A was present in over half (53.5%, N=38); Subtype C (21.1%, N=15); Subtype D (19.7%, N=14); and mixed A/C, A/D, C/D (5.6% N=4). There were no subtype B sequences.

Discussion

To our knowledge, this is the first study to describe acquired drug resistance among an adolescent cohort in Africa. Other pediatric or adult cohorts may include the adolescent age

range,^{3,15} but understanding resistance in a solely adolescent cohort is important given the surge of adolescents living with and at risk for acquiring HIV.¹⁶ This cohort demonstrated a very high frequency of virologic failure (41.5%) and high levels of resistance to first-line NNRTI based therapy with predicted susceptibility to second-line PI based therapy. As HIV-1 RNA testing was implemented in 2016, clinicians now have the ability to recognize virologic failure and intervene. Realizing switch therapy and improving durability of antiretroviral therapy are key to the success of the 90-90-90 goals.¹⁷

Only one participant receiving LPV based second-line therapy had a virus with resistance. The L76V mutation reduces activity of LPV, but may actually increase susceptibility to ATV.¹⁴ Given the lower pill burden of ATV/r (one pill daily) versus LPV/r (2 pills twice daily), more youth are being switched to this newer available regimen. Though no youth demonstrated resistance to ATV, a single mutation (I50L) would predict resistance to the drug. Data from an adult cohort in neighboring Kenya showed that approximately one-quarter of adults receiving second-line (PI-based) therapy could not be suppressed on currently available therapy.¹⁸ As more YLWH receive second line therapy, resistance is inevitable, and integrase inhibitors are not yet available in Tanzania. Though less than 20% of youth with virologic failure reported sexual activity; one-third reported not using a condom during their last sexual encounter. Urgent attention must be paid to this key population to address the dangers of unprotected sex, especially with the risk of transmitting resistant virus.

There are several limitations to this study. First, this was a cross-sectional study and follow up of any ART switch and resulting outcomes are not known. Second, it was presumed that resistance mutations were acquired due to incomplete ART adherence. It is not possible to know rates of transmitted resistance prior to youth starting ART. Based on mathematical modeling, it has been predicted that if levels of pre-treatment resistance exceed 10% in sub-Saharan Africa, global targets to end AIDS as a public threat by 2030 will fail.^{5,19} These data suggest that with >90% of YLWH having NNRTI resistant virus, this demographic could pose a major risk of transmitting NNRTI resistant HIV.

In conclusion, NRTI and NNRTI resistance mutations in this primarily perinatally HIV infected youth cohort were common. With the exception of one participant, major PI mutations were uncommon, though resistance will likely continue to accumulate for those receiving second-line therapy with poor adherence.

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Panel A

Panel B

Panel C

Figure 1:
HIV Resistance Mutations Detected across Antiretroviral Therapy Classes (N=71)

Table 1:**Youth Characteristics Based on Virologic Results**

		Total (N=248)	Total with VF (N=103)	Total with VS (N=145)	P value
Mean Age, (sd)		16.7, (2.8)	16.6, (2.5)	16.8, (2.9)	0.89
Female sex N, (%)		135 (54.4%)	60 (58.3%)	75 (51.7%)	0.31
PHIV		206 (83.1%)	85 (82.5%)	121 (83.4%)	0.85
Substance use * during the last 6 months		13 (5.2%)	6 (5.8%)	7 (4.8%)	0.73
Sexual activity		48 (19.4%)	19 (18.5%)	29 (20.0%)	0.77
Condom used during most recent sexual encounter **		33 (68.8%)	12 (63.2%)	21 (72.4%)	0.42
Site	KCMC	155 (62.5%)	67 (65.0%)	88 (60.7%)	0.49
	Mawenzi	93 (37.5%)	36 (35.0%)	57 (39.3%)	
ART Regimen ***	1 st line	182 (73.4%)	70 (68.0%)	112 (77.2%)	0.10
	2 nd line	66 (26.6%)	33 (32.0%)	33 (22.8%)	
Duration receiving ART years, (sd)		5.9 (2.8)	5.6 (2.7)	6.0 (3.0)	0.25
HIV-1 RNA copies/mL, Log scale, (sd)		2.1, (2.0)	4.3, (0.8)	0.50, (0.8)	<0.01
CD4 cells/mL ³		565 (347)	401 (259)	681 (355)	<0.01

VF: Virologic Failure (HIV-1 RNA >400 copies/mL); VS: Virologic Suppression (HIV-1 RNA ≤ 400 copies/mL); Standard deviation (sd); Perinatally HIV infected (PHIV); Antiretroviral therapy (ART)

* Substance use includes reporting use of alcohol, glue sniffing, marijuana, cocaine, heroin use.

** Condom use percent reported with positive sexual activity as the denominator.

*** 1st line is a non-nucleoside reverse transcriptase inhibitor (NNRTI)-based regimen: nevirapine (200 mg twice daily) or efavirenz (600mg daily); 2nd line is a protease inhibitor based regimen: lopinavir boosted ritonavir or atazanavir boosted ritonavir-based regimen;