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BRIEF REPORT

A Case Series of Acquired Drug Resistance-Associated Mutations in Human Immunodeficiency Virus-Infected Children: An Emerging Public Health Concern in Rural Africa

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The acquisition of drug-resistance mutations among African children living with in human immunodeficiency virus on antiretroviral treatment has been scarcely reported. This threatens the overall success of antiretroviral programs and the clinical outcomes of children in care. We present a well characterized series of children from rural Tanzania with acquired drug-resistance mutations to contribute to the better understanding of this emerging public health concern.

Keywords. Africa; antiretroviral treatment; children; HIV; resistance mutations.

There are few data on the acquisition of drug-resistance mutations among African children living with human immunodeficiency virus (HIV) on antiretroviral treatment (ART). Overall, in resource-limited settings, HIV-1 treatment failure in children is estimated to be 40% [1]. Virologic suppression and long-term treatment success are harder to achieve in children than in adults [2]. This is mostly due to high pre-ART viral loads (VLs), poorer virologic response, and risk of subtherapeutic drug concentrations caused by limited pediatric drug formulations, variable pharmacokinetics, and rapid changes in body weight [1, 3–7]. These factors, often associated with suboptimal adherence, may promote the emergence of drug-resistance mutations. Only 1 study from Kenya has described the pattern of acquired drug-resistance mutations in African children presenting ART failure [5, 8]. In Tanzania, a small study found a virologic failure (VF) rate of 58%, 100% with drug-resistance mutations [9].

The emergence of acquired drug-resistance mutations in children threatens ART programs in sub-Saharan Africa and needs to be studied further. We present a well characterized series of children from a rural Tanzanian setting with treatment failure due to the acquisition of drug-resistance mutations.

MATERIAL AND METHODS

Study Setting and Population

The children in this study attended the Chronic Diseases Clinic of Ifakara (CDCI), in the Saint Francis Referral Hospital. The CDCI works in cooperation with the Ifakara Health Institute, the Swiss Tropical and Public Health Institute, and the University Hospitals of Basel and Bern. Patients attending the CDCI are offered informed consent to be enrolled in the Kilombero and Ulanga Antiretroviral Cohort (KIULARCO) [10]. The KIULARCO study received ethical clearance from the corresponding ethical review boards in Tanzania and Switzerland. This is the largest peripheral HIV cohort in Tanzania with almost 8000 patients. In January 2013, a pediatric and prevention of mother-to-child transmission (PMTCT) unit, named "The One Stop Clinic of Ifakara", was established within the CDCI. By March 2015, 340 children and adolescents were under active follow-up. Care and treatment for patients were provided according to the National AIDS Control Program. CD4 counts were used to routinely monitor the ART response. Viral load was requested by clinicians after treatment failure was suspected due to poor immunological or clinical evolution. In case VL was detected, HIV drug-resistance genotyping was performed.

Clinical Data

At each visit, clinical, laboratory, and pharmacy data were collected electronically. Adherence was estimated using self-reported adherence and pill counting and considered "suboptimal" if <95% of the prescribed pills had been taken. The individual prescriptions were reviewed to assess their adequacy. For children transferred from other facilities, ART dosage was checked by direct observation of their drugs at the enrollment visit.

Viral Load and Genotypic Resistance Testing

Blood samples were collected in 8 mL BD Vacutainer EDTA collection tubes. Plasma HIV RNA VL and HIV drug-resistance genotypes were determined at the Ifakara Health Institute laboratory. Cell-free plasma was collected by centrifugation at 956

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relative centrifugal force for 5 minutes and frozen at −80°C until testing for VL or drug-resistance genotyping. Human immunodeficiency virus RNA from plasma was extracted using the QIAamp Viral RNA Mini Kit (QIAGEN, Hilden, Germany), following the manufacturer's protocol. Viral RNA quantification was performed with the TaqMan RNA-to-CT 1-Step Kit (Life Technologies) using the StepOne Real-Time PCR system (Life Technologies), with a detection limit of 200 viral RNA copies/mL. Human immunodeficiency virus drug-resistance genotyping was performed by Sanger sequencing on an ABI Genetic Analyzer (4-capillary model 3130) using a validated in-house polymerase chain reaction protocol [11]. Human immunodeficiency virus-1 drug resistance was predicted according to the Stanford University's HIV Drug Resistance Database Program version 6.2.0 (http://hivdb.stanford.edu).

RESULTS

We present a series of children with acquired drug-resistance mutations: 10 of 12 enrolled in the CDCI at HIV diagnosis and 2 of 12 (#11, #12) transferred to our clinic after ART initiation. Six patients (#1–#6) were identified through a previous cross-sectional analysis within KIULARCO. The remaining 6 children were identified prospectively after presenting unsatisfactory evolution: patients #7, #8, and #9 presented poor CD4 increase, although they did not meet the World Health Organization (WHO) criteria of immunological failure; patient #10 presented clinical failure; patient #11 had been exposed to low doses of antiretrovirals; and patient #12 presented with both immunological and clinical failure.

Clinical Characteristics

The clinical characteristics of the patients are summarized in Table 1. Median age at ART initiation was 6.4 years (interquartile range [IQR], 5.3–9.4). Ten children were orphans. None was diagnosed through a PMTCT program or had documented exposure to PMTCT. Remarkably, 10 of 12 children were born before 2006, the year in which the first PMTCT intervention was scaled-up to our district. All children were initiated on a nonnucleoside reverse-transcriptase inhibitor (NNRTI)-based regimen. During follow-up, 7 of 12 had been exposed to more than 1 first-line regimen, mostly due to unavailability of drugs. In 3 of 7 cases, children were switched back and forth more than once. Only 1 child (#12) was treated for tuberculosis before presenting treatment failure. Median time on ART at the time when VF was detected was 3.7 years (IQR, 3.2–5.3). Most of the patients (11 of 12) presented VL > 1000 copies/mL. Suboptimal adherence was common (7 of 12). Information about the correctness of the antiretroviral dosages prescribed was available for 10 of 12, and 7 of 10 had been exposed to subtherapeutic doses for periods ranging from 2 months to 3 years. All prescription errors were due to failure to adjust the doses to the current weight, affecting in most cases all the regimen drugs. Only 4 of 12 children met the WHO criteria for clinical and/or immunological failure: patients #6 and #12 presented both immunological and clinical failure with persistent CD4 levels below 100 cell/mm^3 and severe malnutrition; patient #4 had persistent CD4 levels below 100 cell/mm^3; and patient #10 presented clinical failure due to severe malnutrition, and although the CD4 evolution was unsatisfactory he did not met the WHO criteria for immunological failure.

Genotypic Resistance Profile

Ten children carried virus with double resistance to NNRTIs and nucleoside/nucleotide reverse-transcriptase inhibitors (NRTIs). None harbored major resistance to protease inhibitors (PIs) (Table 1).

Nucleotide reverse-transcriptase inhibitor mutations were seen in 11 of 12 patients, mostly M184V (10 of 11). Six children harbored thymidine analog mutations, the majority (5 of 6) carrying ≥2. Non-nucleoside reverse-transcriptase inhibitor mutations were observed in 11 of 12 children, and K103N was the most common (5 of 11).

Pre-ART plasma samples were available for 7 of 10 patients enrolled at HIV diagnosis. None of them presented pre-ART drug-resistance mutations, suggesting that they developed these mutations after treatment initiation. For another patient (#9), diagnosed together with her mother after cessation of breastfeeding, a pre-ART sample from the mother did not show resistance-associated mutations. The remaining 4 children presented history of poor adherence and/or underdosage of drugs, rendering the acquisition of resistance mutations likely.

Outcome

All patients were switched to a PI boosted with ritonavir (PI/r) + 2 NRTIs regimen. Based on the resistance testing, the regimens prescribed contained no predicted active NRTI in 5 of 12 cases, and 1 predicted active NRTI in 6 of 12 cases. Only 1 patient obtained a regimen with 2 active NRTIs.

By the time of the analysis, 6 of 12 children were under active follow-up and had completed 6 months of second-line ART with good self-reported adherence and compatible pill counting, 2 of 12 had not completed 6 months on the PI/r-based regimen, 1 of 12 had interrupted ART repeatedly, 2 of 12 had been transferred, and 1 had stopped ART (Table 1).

Viral load was performed in 4 of 6 children who had completed 6 months on second-line ART, and 3 of 4 presented satisfactory VL reduction. The remaining patient, aged 17, presented a VL 5-fold higher than the one when VF was detected, but HIV genotyping showed no drug-resistance mutations, suggesting low compliance with ART.

DISCUSSION

This case series raises concern about a scarcely reported emerging public health concern in sub-Saharan Africa. To our knowledge, only 1 study from Kenya has described the pattern of

Table 1. Clinical Characteristics and Antiretroviral Drug-Resistance Mutations of 12 Children Living With HIV in Rural Tanzania

Child	Age at ART Initiation (Years)	Orphan Status	Baseline HIV Genotyping	CD4 Count in Cell/mm^3 (and %) at ART Initiation	1st-Line ART Regimens Prescribed	Number of 1st-Line ART Switches	History of Suboptimal Adherence	Under Dosage of Drugs	Time on ART When DRM Detected (Years)	VL at the Time of Treatment Failure Detection (Copies/mL)	NNRTI Resistance Mutations	NRTI Resistance Mutations	2nd-Line Regimens	VL ≥ 6 Months After Switched to 2nd-Line (Copies/mL)
1	4.5	Maternal orphan	No DRM	238 (7%)	d4T/3TC/NVP	N/A	Yes	Yes	4.6	5396	A98G, K103N, V108I, K238T	M41L, V75M, M184V, L210W, T215Y	TDF/FTC/ LPV/r	Not done[a]
2	4.9	Double orphan	No DRM	425 (13%)	d4T/3TC/NVP AZT/3TC/NVP TDF/3TC/EFV	3	No	Yes	3.4	330	Y181C	M184V	TDF/FTC/ LPV/r	Undetectable
3	13.2	Non-orphan	No DRM	317 (10%)	AZT/3TC/EFV TDF/3TC/EFV	1	Yes	No	2.7	22 071	V90IV, K103N, P225H	Y115FY, M184MV	AZT/3TC/ ATV/r	N/A[b]
4	6.1	Paternal orphan	No DRM	152 (12%)	d4T/3TC/NVP	N/A	No	Yes	1.2	174 546	A98G, K103N, V108I, K 238T	M41L, V75M, M184V, L210W, T215Y	TDF/FTC/ LPV/r	345
5	11.7	Paternal orphan	No DRM	124 (12%)	d4T/3TC/NVP AZT/3TC/NVP	1	Yes	No	3.3	1340	K101E, Y181C, H221Y	–	TDF/FTC/ ATV/r TDF/FTC/ LPV/r	N/A[c]
6	11.5	Paternal orphan	No DRM	16 (1%)	AZT/3TC/EFV AZT/3TC/NVP	2	Yes	Yes	4.0	22 623	K103KN	M184IMV, L210LW	TDF/FTC/ LPV/r ABC/3TC/ LPV/r	124 276
7	8.7	Double orphan	Not done[d]	375 (12%)	AZT/3TC/EFV	N/A	No	Yes	5.8	1478	–	M184V	TDF/FTC/ ATV/r TDF/FTC/ LPV/r	N/A[e]
8	6.6	Maternal orphan	No DRM	331 (10%)	AZT/3TC/EFV	N/A	Yes	N/A	3.3	31 000	L100I, Y188L	M184V	TDF/FTC/ LPV/r	N/A[c,f]
9	0.8	Non-orphan	Not done[d,g]	66 (2%)	d4T/3TC/NVP AZT/3TC/NVP	2	No	No	5.1	5648	K101E, G190A	M41L, D67N, T69L, V75M, M184V, L210W, T215Y	TDF/FTC/ LPV/r	Undetectable
10	5.4	Maternal orphan	Not done[d]	201 (3%)	d4T/3TC/NVP AZT/3TC/EFV	1	Yes	N/A	7.0	3775	V90I, K103N, V108I, K238T	M41L, D67G, K70R, V75M, M184V, L210W, T215F, K219E	TDF/FTC/ LPV/r	N/A[h]
11	5.8	Double orphan	N/A[i]	158 (no % information)	AZT/3TC/EFV	N/A	Yes	Yes	1.8	Not done[a]	A98AG, K101P, K103N, E138AE	M184V, T215F	TDF/FTC/ LPV/r	Not done[a]

Table 1 continued.

Child	Age at ART Initiation (Years)	Orphan Status	Baseline HIV Genotyping	CD4 Count in Cell/mm^3 (and %) at ART Initiation	1st-Line ART Regimens Prescribed	Number of 1st-Line ART Switches	History of Suboptimal Adherence	Under Dosage of Drugs	Time on ART When DRM Detected (Years)	VL at the Time of Treatment Failure Detection (Copies/mL)	NNRTI Resistance Mutations	NRTI Resistance Mutations	2nd-Line Regimens	VL ≥ 6 Months After Switched to 2nd-Line (Copies/mL)
12	7.0	Double orphan	N/A[i]	No information	AZT/3TC/EFV ABC/3TC/EFV	1	No	Yes	7.3	386 400	A98G, K103S, G190A, Y318F	M41L, D67N, L74I, M184V, L210W, T215Y	TDF/FTC/ LPV/r	N/A[e]

Abbreviations: ABC, abacavir; ART, antiretroviral treatment; ATV/r, ritonavir-boosted atazanavir; AZT, zidovudine; d4T, stavudine; DRM, drug-resistance mutation; EFV, efavirenz; FTC, emtricitabine; HIV, human immunodeficiency virus; LPV/r, ritonavir-boosted lopinavir; N/A, not applicable; NNRTI, non-nucleoside reverse-transcriptase inhibitor; NRTI, nucleoside/nucleotide reverse-transcriptase inhibitor; NVP, nevirapine; TDF, tenofovir disoproxil fumarate; VL, viral load; 3TC, lamivudine.

[a] Viral load not done due to a technical problem.

[b] The patient opted to stop ART.

[c] At the time of the manuscript writing, the patient had not yet completed 6 months on second-line ART.

[d] Pre-ART sample was not available.

[e] The patient was transferred to another facility before completing 6 months on second-line ART.

[f] Viral load was done 6 weeks after having been switched to second-line and was 260 copies/mL.

[g] The mother's pre-ART sample genotype did not show DRMs.

[h] The patient interrupted ART 3 times since switched to second-line ART.

[i] The patient initiated ART in another facility and pre-ART sample was not available.

acquired drug-resistance mutations in children failing ART in Africa [8]. In this cohort study, 34% (34 of 100) of patients presented VF, of whom 68% (23 of 34) had drug-resistance mutations, 14 of 23 harboring multiclass mutations. Similar to our findings, the common mutations were M184V and K103N. Factors related to VF in children, such as suboptimal adherence, nonparental caregiver, and ART regimen switches [1, 12, 13], were common among our patients. Furthermore, we have identified a frequently overlooked factor: the prescription of inadequate doses of antiretrovirals. Awareness needs to be raised among health workers, and tools to facilitate the prescription of pediatric formulations need to be widely disseminated and routinely used [14].

Importantly, 8 of 12 children did not meet the WHO criteria of clinical or immunological failure [15] despite presenting with multiclass drug-resistance mutations, thus emphasizing the urgent need for routine VL monitoring in children.

Four patients had VL monitored 6 months after switching. One teenager had a very high VL but no evidence of drug-resistance mutations, suggesting that both self-reported and pill-counting adherence were inaccurate. This case is a reminder of the difficulties among some adolescents to adhere to ART. Strategies to improve disclosure of infection status and adherence need to be further developed in partnership with teenagers.

Most patients (11 of 12) could not receive 3 active second-line drugs. Recent studies show that the rates of virologic suppression after switching to a PI/r + 2NRTIs regimen remain high despite resistance to both NRTI and NNRTI [8, 16]. However, these findings require further confirmation in children, who present a higher rate of acquired drug-resistance mutations and face the challenges of longer exposure to ART than adults. Yet, in most African settings, few pediatric regimens are available, and advocating for child-friendly formulations is necessary. The inclusion of integrase inhibitors such as dolutegravir in pediatric ART programs in resource-limited settings needs to be explored urgently. The upcoming clinical trial PENTA 20 (www.clinicaltrials.gov) may provide key information to support its rollout in Africa.

This study has limitations. First, it presents a small number of patients. However, given the scarcity of published data on acquired drug-resistance mutations in children in Africa, it provides a valuable insight into this public health concern. Second, we are unable to assess the prevalence of acquired drug-resistance mutations. However, preliminary results of an ongoing study in our cohort identified 25% prevalence of VF among children on ART (L. M., personal communication). Finally, it is possible that the mutations in the pre-ART plasma samples dropped below the level of detection of the genotypic assay used and we were not able to detect them.

CONCLUSIONS

In conclusion, after a decade of successful rollout of ART in Africa, children and adolescents still represent an underprivileged

population. Antiretroviral treatment coverage is lower and failure rates are higher than in adults. Children living with HIV have peculiarities and needs that must be acknowledged by the often overwhelmed health workers, and specific policies targeting the pediatric population should be implemented. Moreover, VL is not available in most African treatment centers, leading invariably to a late recognition of failure and development of multiclass drug-resistance mutations. New classes of antiretrovirals and their adequate pediatric formulation are urgently needed to ensure the long-term survival of millions of children living with HIV in sub-Saharan Africa.

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