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Chapter 2

Transmitted antiretroviral drug resistance among newly HIV-1 diagnosed young individuals in Kampala

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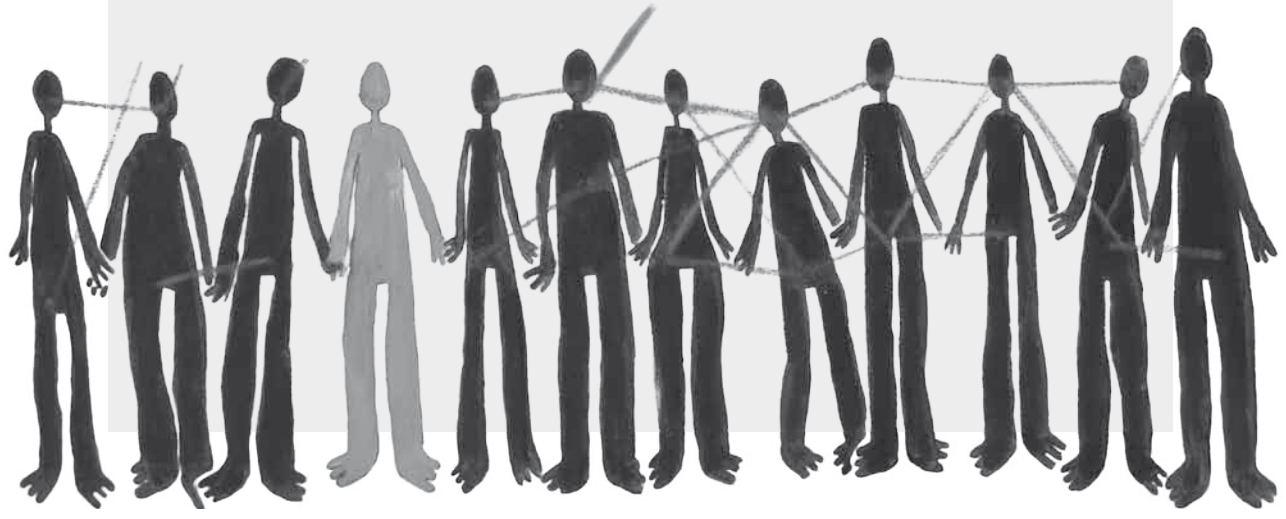

ABSTRACT

Objective

To assess the emergence of transmitted HIV-1 drug resistance (TDR) in Kampala, Uganda, ten years after the scale-up of antiretroviral treatment (ART), and to compare with a previous survey among antenatal clinic attendees in 2007 (reporting 0% TDR).

Design

A cross-sectional survey was conducted among newly HIV-1 diagnosed, antiretroviral-naive young adults attending two large voluntary counseling and testing centers within the geographic area of Kampala.

Methods

Proxy criteria for recent HIV-1 infection were used as defined by the World Health Organization. Population sequencing of the *pol* gene was performed on plasma samples with HIV-1 RNA ≥1000 copies/mL. Drug resistance mutations (SDRMs) were identified according to the 2009 World Health Organization list for surveillance of TDR. HIV-1 subtypes were designated using maximum likelihood phylogenetic reconstruction.

Results

Genotypic test results were obtained for 70 of 77 (90.9%) participants. SDRMs were identified in six samples yielding a prevalence of TDR of 8.6% (95% confidence interval 3.2% to 17.7%). Two had SDRMs to nucleoside reverse-transcriptase inhibitors (D67G, L201W), three had SDRMs to non-NNRTIs (G190A, G190S, K101E), and one had SDRMs to protease inhibitors (N88D). Frequencies of HIV-1 subtypes were: A (36/70, 51.4%), C (2/70; 2.9%), D (23/70, 32.9%) and unique recombinant forms (9/70, 12.9%).

Conclusions

This repeated survey suggests an increase of TDR in Kampala, compared with a previous survey. This finding justifies increased vigilance with respect to surveillance of TDR in areas in Africa where ART programs are rolled-out.

INTRODUCTION

Expanded access to combination antiretroviral therapy (ART) in many countries in sub-Saharan Africa during the past decade[1] has remarkably improved the prognosis of HIV-1 infected individuals.[2] Important deficiencies in health systems, such as lack of virological monitoring and intermittent drug supply, have raised concerns about the rapid emergence and spread of drug resistant HIV-1 strains in Africa.[3,4] Increasing levels of transmitted drug resistant HIV-1 variants (TDR) could compromise the effectiveness of standard first-line ART regimens,[5,6] which has severe public health consequences in areas where treatment options are limited. With the wider use of ART in industrialized countries, TDR to non-nucleoside reverse transcriptase inhibitors (NNRTIs) in newly infected individuals steadily increased, in San Francisco from 0% in 1996-1997 to 13.2% in 2000-2001,[5] and in Europe from 2.3% in 1996–1998 to 9.2% in 2001–2002.[6] Genotypic resistance to two or more classes of antiretroviral (ARV) drugs increased from 2.5% to 13.2%.[5]

Uganda was among the first African countries to distribute life-saving ARV medication. By the end of September 2009, nationwide an estimated 200,413 patients were receiving ART, reaching 39% of those in need.[1] In the capital city of Kampala the massive scale-up of ART was initiated in the year 2000, following limited-scale distribution since the mid 1990s. A survey performed in Entebbe, situated in the greater Kampala area, in 2006-2007 did not detect any significant drug resistance mutations among 47 newly HIV-1 diagnosed pregnant women with CD4 count > 350 cells/μL attending an antenatal clinic.[7]

We report the results of a subsequent survey in 2009-2010 that evaluated the prevalence of TDR among newly HIV-1 diagnosed young individuals attending voluntary counseling and testing (VCT) sites in Kampala, Uganda.

METHODS

Study design and population

A cross-sectional survey was conducted among clients attending two large free-access, non-governmental VCT sites in Kampala, Uganda: AIDS Information Centre (AIC), situated in Mengo area, and Naguru Teenage Health Information Centre (NTC), situated in Bugolobi area. The institutional review boards at the Academic Medical Center and the Uganda Virus Research Institute approved the study. Mandatory eligibility criteria, as defined by the World Health Organization (WHO), were used to identify individuals who were likely to have been recently infected: newly diagnosed with HIV-1 and aged

between 18 and 25 years, or laboratory evidence of recent HIV-1 infection (defined as a confirmed HIV-1 positive antibody test with a negative HIV-1 antibody test within the past 12 months, or an indeterminate/negative HIV-1 antibody test with detectable HIV-1 RNA or positive p24 antigen).[9] Exclusion criteria were any previous ARV use (also for the prevention of mother-to-child transmission of HIV-1), documented WHO clinical stage 4 event and previous pregnancy (parity).[9] All participants provided written informed consent prior to study enrolment. During the enrolment period of maximum 12 months, the VCT clients were all screened and sequentially enrolled. A case report form was completed and a blood draw was performed in all participants.

Laboratory procedures

Plasma was separated within two hours from blood draw and stored immediately at –80°C. HIV-1 RNA was tested with the Amplicor MONITOR 1.5 (Roche, Roche Molecular Systems, NJ, USA). HIV-1 RNA was extracted from 140µl of blood plasma using the Qiamp viral RNA mini kit (Qiagen Inc, Chatsworth, CA). Polymerase gene-specific primers were used for reverse transcriptase, followed by nested PCR to amplify a 1030-base pair *pol* gene encompassing amino acids 1–99 of protease and 1–242 of reverse transcriptase. The PCR products were then purified with a QIA-quick PCR purification kit (Qiagen, Valencia, CA) and sequenced in the sense and antisense direction with a set of nested primers.[10] To ensure the quality of the data set, each sequence was checked before inclusion using ViroScore Suite v8.1 (ABL, France).

Genotypic Resistance and Phylogenetic Analysis

Samples were sequentially genotyped and TDR was analyzed. Drug resistance mutations (SDRMs) were identified according to the 2009 WHO list for surveillance of genotypic TDR updated in 2009, which excludes polymorphisms.[11] For SDRM analysis, the Stanford calibrated population resistance analysis tool version 5.0 beta was used.[12] *Pol* region subtype classification and recombinant patterns were determined using the REGA subtyping tool[13] and the SCUEAL application[14], further confirmed using phylogenetic analysis. We performed maximum likelihood phylogenetic reconstruction using PhyML based on the General Time-Reversible model with gamma distributed rate variation among nucleotide sites.

Statistical methods

The survey sample size was estimated from the hypothesis that the prevalence of TDR in the target population was initially low (estimated at 2%) and increased with time (estimated at 10%). To detect such increase with 80% power using a two-sided significance level of 0.05, the required number of HIV-1 sequences per geographic area was 78. Assuming 10% amplification failure, the target sample was 85 individuals. The proportions

of sequences containing ≥1 SDRM were calculated overall and by each of the three main drug classes, i.e. protease inhibitors (PIs), nucleoside reverse transcriptase inhibitors (NRTIs) and NNRTIs. TDR prevalence was estimated with a 95% confidence interval (CI) based on the binomial distribution. As a secondary analysis, the WHO-recommended truncated sampling technique was used to categorize TDR prevalence as low (<5%), moderate (5–15%), or high (>15%) for each of the three drug classes, based on the testing of the first £ 47 sequences.[15] Categorical data were compared using Chi-square test. Continuous data were investigated using Kruskal-Wallis or Student t-test. All analyses were performed using Stata version 10 (StataCorp LP, TX).

RESULTS

Patient characteristics

Study enrolment took place from February 2009 to February 2010 at AIC and from May 2009 to May 2010 at NTC. A total of 884 individuals were screened, of whom 81 (9.2%) met the eligibility criteria. Excluding four individuals due to protocol violations (i.e. 3 did not meet the age criterion, 1 had a previous pregnancy), 77 participants (43 from AIC and 34 from NTC) were included in the analysis (table 1). Seventy-six participants qualified based on the age criterion and one participant had a new confirmed HIV-1 diagnosis after a recent negative test. The mean age was 21.6 years (standard deviation, SD 2.1). Females comprised 70.1% (n=54). The mean age was lower for females (21.1 years, SD 2.0), compared to males (22.7 years, SD 1.9, p=0.0017). The median CD4 count was 417 cells/μL (interquartile range (IQR): 318.5-551.5 cells/μL) and the median HIV-1 RNA load was 4.49 $\log_{10}$ copies/ml (IQR: 3.96-5.28 $\log_{10}$ copies/ml). 94.8% (n=73) of participants were Ugandan nationals. Nearly all (73, 94.8%) participants reported sexual encounters with the opposite sex, whereas other exposures were uncommon. The median age at sexual debut was 18 years, with a range between 14 and 27 years. During the three years prior, 71 (92.2%) participants reported to have engaged in unprotected sex, with an average of 2.1 (SD 1.8) sexual partners, and 23 (29.9%) reported a first episode of a sexually transmitted infection. Among participants who had a steady sexual partner, 68.8% was unaware of their partner's HIV-1 status. Baseline characteristics, except for mean age, did not differ between sites (table 1).

Genotypic profiles

70 samples were successfully genotyped and seven samples failed to amplify or had no valid genotype. One or more SDRMs were identified in six of the 70 valid sequences, yielding an estimated TDR prevalence of 8.6% with a 95% CI 3.2% to 17.7%. The proportion of sequences with SDRMs associated with NRTIs, NNRTIs and PIs was 2.9% (2/70),

Table 1. Patient characteristics, by study site.

	Total	Aids Information Centre (AIC)	Naguru Teenage Health Information Centre (NTC)	p-value
Patients	77	43	34	
Sex				0.085
Female	54 (70.1)	27 (65.9)	27 (79.4)	
Male	23 (29.9)	16 (39.0)	7 (20.6)	
Age – mean yrs (sd)	21.6 (2.1)	22.4 (1.9)	20.7 (1.9)	<0.001
Ugandan nationality	73 (94.8)	41 (95.4)	32 (94.1)	0.809
Marital status				0.075
Now married/cohabiting	14 (18.2)	5 (11.6)	9 (26.5)	
Divorced/separated	0	0	0	
Widowed	4 (5.2)	4 (9.3)	0	
Never married/single	58 (75.3)	34 (79.1)	24 (70.6)	
Other	1 (1.3)	0	1 (2.9)	
Education level				0.134
None/illiterate	2 (2.6)	1 (2.3)	1 (2.9)	
Primary school	24 (31.2)	9 (20.9)	15 (44.1)	
Secondary school	30 (39.0)	18 (41.9)	12 (35.3)	
Higher education	21 (27.3)	15 (34.9)	6 (17.7)	
Main occupation				0.014
None/at home	24 (31.2)	7 (16.3)	17 (50.0)	
Student	16 (20.8)	9 (20.9)	7 (20.6)	
Employed	35 (46.7)	25 (61.0)	10 (29.4)	
Hemoglobin – median g/dL (IQR) [a]	12.6 (11.3-14.3)	13.1 (11.4-14.8)	12.4 (10.8-14.0)	0.1284
CD4 cell count –- median cells/µL (IQR) [b]	417 (318.5-551.5)	377.5 (236-519)	418.5 (264-573)	0.1159
HIV RNA – median $\log_{10}$ c/ml (IQR)	4.49 (3.96- 5.28)	4.47 (3.90-5.03)	4.55 (3.83-5.27)	0.4447

Data represent n (%) unless otherwise specified; [a] Data available for n=73; [b] Data available for n=76

4.3% (3/70) and 1.4% (1/70), respectively. We observed six different SDRMs: D67G, K101E, G190S, G190A and L210W in reverse-transcriptase and N88D in protease. TDR was confined to a single drug-class in all six sequences. Table 2 summarizes the demographic and virological characteristics of the six participants who harbored an SDRM.

Using the WHO-recommended truncated sequential sampling technique, four of the first 47 sequences harbored an SDRM (moderate prevalence category), of which two were NRTI-associated (low prevalence category), one PI-associated (low prevalence category); and one NNRTI-associated. HIV-1 subtype frequencies were: A (36/70, 51.4%), C (2/70; 2.9%), D (23/70, 32.8%), A1/D recombinants (9/70, 12.9%).

Table 2. Demographic and virological characteristics of the six participants who harboured a drug resistance mutation.

#	Date of enrolment	ID	Age (years)	Sex	Estimated year of infection	Estimated country of infection	CD4 count (cells/µl)	HIV-1 RNA load (log_{10} c/ml)	Viral subtype	Surveillance Drug Resistance Mutations		
										NRTI	NNRTI	PI
1	19-Mar-09	33	23	Female	Unknown	Unknown	615	3.84	D		G190S	
2	21-May-09	702	20	Female	2008	Uganda	348	5.88	A (A1)	L210W		
3	05-Jun-09	705	20	Male	2008	Uganda	706	4.60	A	D67G		
4	27-Jul-09	248	23	Male	Unknown	Uganda	457	5.55	A1D			N88D
5	06-Jan-10	438	24	Male	Unknown	Uganda	270	5.47	A (A1)		G190A	
6	25-Mar-10	728	24	Male	2009	Uganda	495	5.88	D		K101E	

NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non-NRTI; PI, protease inhibitor

DISCUSSION

This survey among 70 newly HIV-1 diagnosed young VCT clients in Kampala demonstrated an estimated prevalence of TDR of 8.6%, which is likely to represent an increase compared to the previous survey in 2006-2007 that did not detect any SDRMs among 47 pregnant women from the greater Kampala area.[7] Identified SDRMs were associated with NNRTIs (3), NRTIs (2) and PIs (1), but in each sequence TDR was confined to a single drug class. This study is among the first to suggest an increase of TDR between repeated surveys within the same geographic area in Africa, although the subsequent surveys targeted different subpopulations.

Most studies from Africa that were conducted during the early scale-up of ART have reported low levels of TDR.[7-9] In Botswana, the 2007 threshold survey indicated that five years following the countrywide ART roll-out, TDR was still less than 5%.[16] The IAVI cohort, however, of newly HIV-1 infected individuals in east and southern Africa reported a 5% overall prevalence of TDR, with an increase from 3% (4/157) in 2005-2006 to 7% (12/169) in 2007-2008.[17] The proportions of participants who harbored TDR was particularly high in Entebbe (4/17, 23.5%) and Kigali (8/68, 11.8%).[17] Consistent with this report, our study supports the hypothesis that increasing ARV drug exposure in African populations, following the roll-out of ARVs for treatment and prevention of mother-to-child transmission, may cause a rise in TDR and thereby new public health challenges.

In this study the categorization of TDR using prevalence (6/70) corresponded with the WHO-recommended truncated sequential sampling technique (4/47), i.e. "moderate" overall and "low" for each drug class separately. It should, however, be noted that the small sample sizes resulted in a wide confidence interval, warranting caution in interpreting and extrapolating the results.

This study has several limitations. Given the challenges, especially in resource-limited settings, in identifying individuals during acute or recent HIV-1 infection, WHO recommends the use of proxy criteria for the surveillance of TDR. A recent study in Botswana, however, found poor agreement between the WHO criteria and two laboratory-based methods to detect new infection.[16] The WHO approach could therefore lead to the inclusion of individuals with established infection, during which drug resistant mutants may have reverted to wild-type virus,[20, 21] thereby possibly underestimating the true current prevalence of resistance transmission. Although the study specifically selected newly diagnosed, ARV-naïve individuals, it cannot be completely ruled out that some participants had unknown or undisclosed prior exposure to ARV therapy and/or prophylaxis.

In conclusion, ten years following the ART scale-up in Kampala, Uganda, this repeated survey demonstrated that 8.6% of newly HIV-1 diagnosed youth harbored TDR, which is likely to represent an increase compared to the previous survey. The study findings should trigger public health action in performing additional surveys in the upcoming years to evaluate the evolution of TDR in the country and can provide guidance to drug resistance prevention strategies. This is especially urgent since current options for first-line therapy in Uganda are limited and access to second-line therapy is not widely available.

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