AIDS RESEARCH AND HUMAN RETROVIRUSES
Volume 30, Number 9, 2014

DOI: 10.1089/aid.2014.0089

Short Communication: Prevalence of HIV-1 Transmitted Drug Resistance in Liberia

Paul Loubet,[1,2,*] Charlotte Charpentier,[2–4,*] Benoit Visseaux,[2–4] Cecilia Nuta,[5] Eric Adu,[5] Jean-Marc Chapplain,[6] Maima Baysah,[7] Sylvia Walters-Doe,[5] Pierre Tattevin,[6] Gilles Peytavin,[2,3,8] Yazdan Yazdanpanah,[2,3,9] and Diane Descamps[2,3,4]

Abstract

No data on HIV-transmitted drug resistance (TDR) are available in Liberia in which the HIV prevalence in the general population is estimated at 1.5%. The aim of the study was to assess the prevalence of TDR in HIV-1 from recently diagnosed and untreated patients living in Monrovia, Liberia. The study was performed in the John F. Kennedy Medical Center and in the Redemption Hospital, both located in Monrovia. All newly HIV-1 diagnosed patients attending voluntary counseling testing centers and antiretroviral therapy naive were consecutively included. Protease and reverse transcriptase (RT) regions sequencing was performed using the ANRS procedures (www.hivfrenchresistance.org). Drug resistance mutations (DRM) were identified according to the 2009 updated WHO surveillance DRM list. Among the 116 HIV-1-infected patients enrolled in the study, 85 (73%) were women. Protease and RT sequencing was successful in 109 (94%) and 102 (88%) samples, respectively. Seventy-five (66%) patients were infected with CRF02_AG. One DRM was observed in six samples, leading to a TDR prevalence of 5.9% (CI 95% = 1.7–10.1). DRM were observed in two patients (2.0%; CI 95% = 0.0–4.7), four patients (3.9%; CI 95% = 0.1–7.7), and one patient (0.9%; CI 95% = 0.0–2.7) for nucleoside RT inhibitors (NRTI), non-NRTI (NNRTI), and protease inhibitors, respectively. Overall, one patient exhibited dual class-resistant viruses, harboring NRTI and NNRTI resistance mutations (1.0%; CI 95% = 0.0–2.9). This first survey study in Liberia reported a TDR prevalence of 5.9%, classified as moderate according to the WHO criteria, indicating that further surveillance is warranted to follow the level and evolution of TDR prevalence in recently HIV-1 diagnosed patients.

In 2012, 9.7 million people in low- and middle-income countries received antiretroviral therapy (ART), representing 61% of all who were eligible under the 2010 World Health Organization (WHO) HIV treatment guidelines.[1] Antiretroviral drugs have now been introduced for more than 5 years in most resource-limited settings, leading to a 40-fold increase in the number of adults and children who received ART between 2002 and 2012.[1] WHO recommends HIV drug resistance surveillance among ART-naive patients for all countries involved in ART access programs.

Previous studies reporting ART transmission drug resistance (TDR) from chronically infected, ART-naive, patients in different countries—Burkina Faso,[2] Côte d'Ivoire,[3] Uganda,[4] and Zambia[5]—showed a low prevalence of ART resistance, about or below 5%. Others survey studies in Guinea-Conakry, Niger, and Mali showed that TDR prevalence comprised between 5% and 10%.[6,7] A recent large meta-analysis showed, in West and Central Africa, an estimated prevalence of HIV-1 TDR of 5.7% at 3 to 4 years after ART rollout.[8]

[1]Ensemble pour une Solidarité Thérapeutique en Réseau (GIP-ESTHER), Paris, France.
[2]INSERM, IAME, UMR 1137, Paris, France.
[3]Université Paris Diderot, IAME, UMR 1137, Paris, France.
[4]AP-HP, Hôpital Bichat-Claude Bernard, Laboratoire de Virologie, Paris, France.
[5]John F. Kennedy Medical Center, Infectious Diseases Clinic, Monrovia, Liberia.
[6]Centre Hospitalo-Universitaire de Rennes, Service de Maladies Infectieuses et Tropicales, Rennes, France.
[7]Redemption Hospital, Infectious Diseases Clinic, Monrovia, Liberia.
[8]AP-HP, Hôpital Bichat-Claude Bernard, Laboratoire de Pharmacologie, Paris, France.
[9]AP-HP, Hôpital Bichat-Claude Bernard, Service de Maladies Infectieuses et Tropicales, Paris, France.
*These authors contributed equally to this work.

Table 1. Antiretroviral Drug Resistance Mutations in Virus Harbored by Recently Diagnosed Antiretroviral-Naive Patients in Liberia

Patient	*Sex*	*Age (years)*	*HIV-1 subtype*	*NRTI resistance mutations*	*NNRTI resistance mutations*	*PI resistance mutations*
30-RDH-3359	M	44	A	M41L		
30-RDH-3391	F	34	Unknown	M41L-M184V-T215F	Y188L	
30-RDH-3424	F	47	CRF02_AG			I47V-I84V
54-47-98	F	23	CRF36_cpx		K101E	
40-30-90	M	42	CRF02_AG		K103N	
30-RDH-3333	F	32	CRF02_AG		K103N	

F, female; M, male; NNRTI, nonnucleoside reverse transcriptase inhibitors; NRTI, nucleoside reverse transcriptase inhibitors; PI, protease inhibitors.

No data on TDR are available in Liberia in which the prevalence of HIV in the general population is estimated at 1.5% (about 40,000 people).[9] HIV prevalence is higher in urban areas, at 2.9% in Monrovia, the capital city. In Liberia, ART first became available in a postconflict setting in 2006, followed by a rapid and large-scale use of ART; in 2013, coverage reached 35% for HIV-infected patients needing ART and reached 48% in the prevention of mother-to-child transmission (PMTCT) in the population of pregnant women.

The aim of the study was to assess the prevalence of drug resistance mutations in HIV-1 from recently diagnosed and untreated patients living in Monrovia, Liberia.

The study was performed at the John F. Kennedy Medical Center and at the Redemption Hospital, both located in Monrovia. All newly HIV-1 diagnosed patients attending voluntary counseling testing centers and naive of ART, including PMTCT, were consecutively included during the month of June 2013. The study was approved by the National Aids Control Program, the National Aids Committee, and the Ministry of Health.

Protease and reverse transcriptase (RT) region sequencing was performed using the ANRS procedures (www.hiv-frenchresistance.org). Drug resistance mutations were identified according to the 2009 update surveillance drug resistance mutations list.[10]

Phylogenetic analyses were performed by estimating the relationships among RT sequences and reference sequences of HIV-1 genetic subtypes and circulating recombinant forms (CRF) obtained from the Los Alamos Database (http://hiv-web.lanl.gov). Phylogenetic reconstruction was performed using a Kimura two-parameter model and the neighbor-joining method. Protease and RT sequences were submitted to GenBank with the following accession numbers: KJ467818–KJ468028. Plasma concentrations of ART were systematically determined in samples exhibiting drug resistance mutations, using modified liquid chromatography coupled with tandem mass spectrometry (Acquity UPLC- TQD, Waters Corporation Milford, MA) as previously described.[11]

Among the 116 HIV-1-infected patients enrolled in the study, 85 (73%) were women; the median age of the patients was 35 years [interquartile range (IQR) = 29–42] and 30 (26%) patients had a WHO clinical stage of 3 or 4. The median CD4 cell count, available for 105 patients (91%), was 238/mm^3 (IQR = 85–415). The median plasma viral load was 5.84 $\log_{10}$ copies/ml (IQR = 5.34–6.48). Protease and RT sequencing was successful in 109 (94%) and 102 (88%) samples, respectively. Phylogenetic analyses showed that most of the patients, 75 (66%), were infected with CRF02_AG. The subtype distribution of the remaining patients showed a high diversity in HIV-1 subtypes as follows: 10 (9%) subtype G, four (3%) subtype C, four (3%) CRF36_cpx, two (2%) CRF43_02G, two (2%) subtype D, two (2%) CRF06_cpx, two (2%) CRF09_cpx, one (1%) subtype A, one (1%) CRF01_AE, one (1%) CRF22_01AE, and one (1%) CRF32_06A1. The HIV-1 subtype was undetermined in eight (7%) of cases.

Resistance analysis among the 102 samples with both protease and RT sequences showed that at least one drug resistance mutation was observed in six samples, leading to a prevalence of TDR of 5.9% [95% confidence interval (CI 95) = 1.7–10.1%] (Table 1). Nucleoside reverse transcriptase inhibitor (NRTI) resistance mutations were found in two samples (2.0%; CI 95 = 0.0–4.7%) exhibiting the M41L resistance mutation in one case and a virus harboring three resistance mutations (M41L–M184V–T215F) in the second case. Nonnucleoside reverse transcriptase inhibitor (NNRTI) resistance mutations were carried by four samples (3.9%; CI 95 = 0.1–7.7%). NNRTI-resistant viruses exhibited the K103N mutation in two cases, the K101E mutation in one case, and the Y188L mutation in the remaining case. Resistance analysis of protease showed the detection of two major protease inhibitor (PI) resistance mutations in one patient (0.9%; CI 95 = 0.0–2.7%): I47V and I84V. Overall, one patient exhibited dual class-resistant viruses, harboring NRTI and NNRTI resistance mutations (1.0%; CI 95 = 0.0–2.9%).

In addition to the WHO mutations list, we assessed the prevalence of natural polymorphism at position 138 of RT, associated with a significant decrease in the susceptibility to etravirine and rilpivirine, and a substitution at codon 138 was found in five cases (4.9%; CI 95 = 0.7–9.1%), with E138A ($n = 2$), E138K ($n = 1$), 1 E138G ($n = 1$), and E138Q ($n = 1$). The presence of the E138 natural polymorphism was not associated with a specific HIV-1 subtype.

No clustering of ART-resistant strains was evidenced by phylogenetic analysis (data not shown).

ART concentration measurements were performed in samples harboring drug resistance mutations ($n = 6$), showing undetectable antiretroviral plasma concentrations in all cases, suggesting the absence of ART for at least the past several weeks regarding the respective ART half-lives.

This first TDR survey performed in Monrovia, Liberia, showed a prevalence of primary resistance of 5.9%, indicating that drug-resistant viruses are circulating among

treatment-naive patients in this country. According to the WHO criteria, this TDR prevalence is classified as moderate.

Our findings are issued from patients followed-up in two centers of the capital city of Liberia, probably not reflecting TDR prevalence at a national level, since ART coverage in urban areas is likely to be substantially higher than the national average. Furthermore, HIV prevalence is 2-fold higher in Monrovia compared to the prevalence in the general population at the country level. Thus, we can hypothesize that exclusive urban recruitment may overestimate TDR prevalence.

In our study TDR was assessed by direct sequencing, so minority resistant variants were not detected. Of note, the use of more sensitive techniques has been shown to increase the detection of TDR by 2- to 3-fold.[12]

Some criteria differed between the survey method used in our study and the WHO HIV drug resistance (HIVDR) threshold survey method.[13] First, the median age of the patients in our study was 35 years, whereas WHO HIVDR criteria recommended performing studies on TDR in patients below the age of 25 years. In addition, WHO HIVDR criteria recommended ensuring that patients were HIV infected within the past 3 years, criteria that we could not include in our study. However, others WHO HIVDR survey method criteria were fulfilled in our study: (1) patients were consecutively included; (2) the number of samples was much higher than the threshold of 47; and (3) we excluded all patients who may have been previously exposed to ART, including PMTCT in pregnant women. Furthermore, the selected survey area of our study is a small geographic area and is based in the capital city where ART has been widely available for more than 3 years and is available to at least 20% of eligible individuals (coverage of 35% in Liberia). In addition, involved laboratories are part of the National Health System.

In our study, the highest rate of TDR was observed for the NNRTI drug class with 3.9%, compared to 2.0% and 0.9% for the NRTI and PI drug classes, respectively. These findings match the large use of NNRTI as first-line treatment in Liberia following WHO recommandations.[1] The NNRTI TDR rate of 3.9% we described in Liberia is similar to that reported in a recent multicenter study, performed in six African countries, showing an overall NNRTI TDR prevalence of 3.3%.[14] The NNRTI TDR rate of 3.9% we observed in Liberia corresponds to what could be expected after 7 years of ART rollout, based on the recent meta-analysis showing an increase of 36% per year in NNRTI TDR in East Africa, while there has been a nonsignificant increase of 15% per year in Central and West Africa.[8]

Once again, these findings emphasize the need for HIV viral load monitoring in resource-limited settings in order to assess the virological outcome of a first-line regimen, especially with the use of an NNRTI-based regimen. Thus, in Liberia all patients received an NNRTI-based regimen as the first-line regimen, mostly zidovudine/lamivudine/nevirapine, but also tenofovir/emtricitabine as the NRTI backbone in 20% of cases. In addition, at this time no monitoring of HIV-1 viral load is available in Liberia.

Our findings reported a threshold level of resistance in ART-naive patients of between 5% and 15% in Monrovia, Liberia, indicating that further surveillance in Monrovia, but also in other cities of the country, is warranted to follow the level and evolution of HIV-1 TDR in recently HIV-1-diagnosed patients.

Acknowledgments

We thank all the patients and all the physicians of the two clinical units who participated in this study. We thank Michelle Ricks, Teresa Cummings, Coco Vaneway, and Sam Doepoh for their help in conducting this study. We thank Pierre Mendiharat, Gilles Raguin, and Christophe Michon, from 'Ensemble pour une Solidarité Thérapeutique en Réseau' (GIP-ESTHER). We thank Suzon Drumard for her technical help.

This work was supported by the Ensemble pour une Solidarité Thérapeutique en Réseau (GIP-ESTHER).

Author Disclosure Statement

No competing financial interests exist.

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Address correspondence to:
Charlotte Charpentier
Hôpital Bichat-Claude Bernard
Laboratoire de Virologie
46 Rue Henri Huchard
75018 Paris
France

E-mail: charlotte.charpentier@bch.aphp.fr