Pretreatment HIV Drug Resistance and Virologic Outcomes to First-Line Antiretroviral Therapy in Peru

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Abstract

Access to nucleoside reverse transcriptase inhibitor (NRTI) and non-nucleoside reverse transcriptase inhibitor (NNRTI) first-line antiretroviral therapy (ART) for HIV has been increasing in Peru since a national ART program was initiated in 2004. Between 2007 and 2009, we found a 1% prevalence of pre-ART HIV drug resistance (PDR) among antiretroviral (ARV)-naïve Peruvians. Given that PDR has been associated with virologic failure (VF) of ART, in 2014–2015 we enrolled a follow-up cohort at the same institution to determine whether the rate of transmitted resistance had increased and compared virologic outcomes of those with and without PDR. Blood specimens from ARV-naïve individuals were assessed for PDR to NNRTI-based ART by an oligonucleotide ligation assay (OLA) sensitive to 2% mutant within an individual's HIV quasiespecies at reverse transcriptase codons M41L, K65R, K103N, Y181C, M184V, and G190A, and by Sanger consensus sequencing (CS). Rates of VF (plasma HIV RNA >200 copies/mL) were compared between those with and without PDR. Among 122 ARV-naïve adults, PDR was detected by OLA in 17 (13.9%) adults. Compared with the 2007–2009 cohort, the proportion with PDR at OLA codons was significantly increased ($p < .001$). A total of 11 of 19 OLA mutations conferring high-level drug resistance were also detected by CS, and 8 additional participants had mutations encoding low-level resistance detected by CS for a total of 25 participants (20.5%). VF at month 6 of NNRTI-ART appeared greater in participants with versus without PDR [4/18 (22.2%) vs. 3/71 (4.2%); $p = .03$]. An increasing prevalence of PDR was detected among ARV-naïve Peruvians. Studies are needed to determine risks of specific PDR mutations.

Keywords: HIV, pre-ART HIV drug resistant, oligonucleotide ligation assay, antiretroviral-naïve, Peru

Introduction

NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITOR (NRTI) and non-nucleoside reverse transcriptase inhibitor (NNRTI) combination therapy remains the first-line antiretroviral (ARV) therapy in Peru, where a national antiretroviral therapy (ART) program was initiated in 2004. Lima has the largest number of HIV-infected patients in Peru, where ART is primarily distributed through the Ministry of Health.¹ In 2017, 45,359 of 70,000 (64.7%) estimated people living with HIV/AIDS in Peru received ART, of whom 78.0% received regimens with NNRTI.^{2,3} Access to newer agents is uncommon due to high costs, as Peru recognizes

patent laws governing ARV. Given that dolutegravir-based ART and similar regimens with high genetic barriers to resistance are costly, public ART programs in Peru continue to rely on NNRTI as the basis for first-line and protease inhibitors (PIs) for second-line ART regimens.

Incomplete suppression of HIV replication due to low ARV levels can lead to the emergence of HIV drug resistance to ARV. When individuals with drug resistance experience virologic failure (VF), the rate of transmitted resistance (TDR) can increase.⁴ Although TDR was initially detected in developed countries, the prevalence of TDR, particularly to NNRTI,⁵ is increasing globally,^{6,7} and has been associated with VF of ART.^{8,9}

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Although testing for pre-ART HIV drug resistance (PDR) is recommended in high-resource communities,¹⁰ guidelines in Peru¹¹ do not recommend PDR testing due to the high cost of conventional tests such as Sanger consensus sequencing (CS). Instead, surveillance for TDR and PDR is recommended in specific populations with higher likelihoods of recent HIV infection, in whom drug resistance would likely increase first,¹² preferably by use of simple and inexpensive PDR tests.¹³

The oligonucleotide ligation assay (OLA) is a sensitive and economical point mutation assay optimized to detect HIV drug resistance mutations in HIV *pol*,¹⁴ with a sensitivity of 2% within an individual's HIV quasispecies.¹⁵ In a previous study of ART-naïve Peruvians conducted between 2007 and 2009, the prevalence of PDR detected by OLA at codons K103N, G190A, M184V, and Y181C was 1%.¹⁶ In 2014–2015, we enrolled a follow-up cohort at the same institution to determine whether the rate of PDR had increased. In addition, we compared the rates of VF between participants with PDR versus participants with wild-type (WT) viruses in pre-ART specimens; the detection of PDR by CS and OLA; and the associations of PDR detected by OLA and CS with VF.

Materials and Methods

This study was conducted at Hospital Nacional “Dos de Mayo” (HNDM), a public center for HIV care with the largest cohort of HIV-infected patients in treatment in Peru. Between 2014 and 2015, ARV-naïve patients who qualified to start first-line NNRTI-based ART following Peruvian guidelines¹⁷ were invited to enroll into this study, which was approved by the Ethics Committee of the HNDM and Seattle Children's Research Institute (SCRI), Washington. Blood was collected before ART initiation for retrospective testing of PDR using OLA and CS. Plasma HIV RNA loads and CD4 levels were obtained from the participants' medical records.

Whole blood (8–10 mL) was collected in EDTA tubes, separated into plasma and peripheral blood mononuclear cells (PBMCs) using Ficoll (Histopaque®-1077; Sigma Aldrich, St. Louis, MO), and frozen. DNA was extracted from PBMCs using the QIAamp DNA Mini Kit (Qiagen, Germany), following the manufacturer's instructions and frozen. Frozen DNA and plasma aliquots were shipped to SCRI.

Participants' PBMC samples that yielded ≥ 1 μ g of DNA were amplified by polymerase chain reaction (PCR). If DNA was insufficient, RNA was extracted from plasma using the QIAamp Viral RNA Mini Kit (Qiagen, Germany), and amplified by reverse transcription PCR (Primescript One-Step RT-PCR kit; Takara). A minimum of 1 μ g of PBMC DNA or an estimated 100 copies of plasma HIV RNA (based on plasma HIV RNA level) were subjected to PCR of HIV *pol*, using primers NEF10-NEF10 for first round and NEF11-NEF11 for second round.¹⁸ The correct size of the amplicon was verified by agarose gel electrophoresis.

The genotype of the PCR product from each sample was assessed by the OLA and CS. OLA tested for point mutations was associated with high-level resistance to NNRTI (K103N, Y181C, and G190A) and NRTI (M41L, K65R, and M184V) as previously described.¹⁸ Each OLA plate included a standard curve of mutant and WT plasmid mixtures at 0%, 2%, 5%, 10%, 25%, 50%, 75%, and 100% mutant concentration,

used to calculate the proportion of mutant virus in each participant's viral population. Samples and controls were run in duplicate wells. The optical density of the 2% mutant control was used as a cutoff for detection of mutant. All amplicons also underwent CS (BigDye Terminator v3.1 Cycle Sequencing Kit; Applied Biosystems, Foster City, CA), and the FASTA files were submitted to the Stanford HIV Drug Resistance Database¹⁹ for identification of mutations and generation of a genotypic score; and to GenBank, accession number (pending).

Fisher's exact and Mann-Whitney tests compared pre-ART demographic characteristics and clinical parameters by resistance genotypes. In addition, Fisher's exact and, when noted, a family Poisson generalized linear model (GLM) and risk ratio (RR) were used to compare rates of VF, as defined by Peruvian guidelines as plasma HIV RNA >200 copies/mL at 6 months of ART.¹¹ Statistical analyses were performed using STATA version 12.0 (College Station, TX).

Results

A total of 122 HIV-infected ARV-naïve adults qualifying for first-line ART were enrolled and tested for PDR, including 28 women and 94 men with a median age of 28 years (range 16–64). Most participants (84.0%) were diagnosed with HIV infection within the previous year, all had detectable viral load at ART initiation, the median plasma HIV RNA was 5.0 log₁₀ copies/mL (range 3.4–7.0), and the median CD4 count was 204 cells/ μ L (range 1–1996 cells/ μ L). No significant differences between subjects with versus without PDR were found in gender, age, sexual orientation, or number of sexual partners, except that the median age of sexual debut was somewhat lower among those with PDR (16 vs. 17; $p = .03$) (Table 1).

Drug resistance mutations were detected by OLA in 17 of 122 (13.9%) participants (7/50 PBMCs and 10/72 plasma), including 11 with majority frequency variants ($\geq 20\%$) and 6 with minority frequency variants (Table 2). K103N (11/122, 9.0%) was the most prevalent mutation, followed by M184V (4/122, 3.3%), Y181C (2/122, 1.6%), and G190A (2/122, 1.6%). M41L and K65R were not detected. Indeterminate OLA reactions occurred at 15 of 732 (2%) codons, most frequently at codon 103 (7/122; 5.8%), followed by 190 (4/122; 3.2%), 184 (2/122; 1.7%), and 65 (2/122; 1.7%) codons. Most indeterminate OLA results at codon 103 were due to a mutation encoding K103R found at 16% prevalence in this cohort, sometimes coupled with a K102Q mutation; neither are associated with drug resistance.

Viral sequences obtained by CS were all HIV subtype B and showed mutations in HIV reverse transcriptase associated with ART resistance in 20 of 122 (16.4%) participants. These included the mutations conferring high-level resistance to ART also detected by OLA in 11 of 122 (9.01%) participants, and mutations associated with low-level resistance to NNRTI or zidovudine (ZDV) (score ≤ 10 in Stanford HIV database) or accessory mutations in 9 of 122 (7.4%) participants; 8 of these 9 did not have any OLA mutations and 1 had 2% K103N (Table 2). Combining OLA and CS results, PDR was detected in 25 of 122 (20.5%) participants.

After completion of enrollment, 109 of 122 participants started treatment following Peruvian guidelines. After 6 months of ART, 21 participants were lost to follow-up (LTFU)

TABLE 1. PRE-ANTIRETROVIRAL THERAPY DEMOGRAPHIC AND CLINICAL CHARACTERISTICS OF ANTIRETROVIRAL-NAIVE PERUVIANS WITH AND WITHOUT PRE-ANTIRETROVIRAL THERAPY DRUG RESISTANCE

Characteristics	Total (n = 122)	PDR status by OLA and/or CS		p ^a
		Mutations detected (n = 25)	Wild type (n = 97)	
Age in years, median (range)	28 (16–64)	32 (17–64)	28 (16–62)	.51
Male gender, N (%)	94 (77.1)	16 (64.0)	78 (80.4)	.07
CD4 cell count <200 cells/ μ L, N (%)	46/94 (48.9)	7/19 (36.8)	39/75 (52.0)	.30
Plasma HIV RNA log ₁₀ copies/mL, median (range)	5.0 (3.4–7.0)	4.9 (4.0–6.3)	5.0 (3.4–7.0)	.77
HIV risk factor, N (%)				.19
Heterosexual	50 (48.5)	11 (52.4)	39 (47.6)	
MSM	40 (38.8)	5 (23.8)	35 (42.7)	
Bisexual	13 (12.6)	5 (23.8)	8 (9.8)	
Age in years at sexual debut, median (range)	17 (10–31)	16 (11–20)	17 (10–31)	.03
No. of sexual partners in lifetime, median (range)	7 (1–200)	6 (2–30)	9 (1–200)	.42

^aFisher's Exact and Mann–Whitney.

ART, antiretroviral therapy; CS, consensus sequencing; PDR, pre-ART HIV drug resistance; MSM, men who have sex with men; OLA, oligonucleotide ligation assay.

and 88, including 1 taking PI-ART and 87 on NNRTI-ART, had virologic outcome. Among those taking NNRTI-based ART, VF was observed in 7 of 87 (8.1%) participants; including 4 of 18 (22.2%) participants with any PDR by OLA and/or CS versus 3 of 69 (4.3%) participants without PDR ($p = .03$, and $RR = 5.1$ by GLM; $p = .02$). However, among participants with PDR detected by OLA, 2 of 12 (16.7%) participants had VF (including 1 of 6 participants with majority ($\geq 20\%$) and 1 of 6 participants with minority ($<20\%$) frequency variants) compared with 5 of 75 participants (6.7%) with WT virus ($p = .25$). Among participants with any drug resistance mutation (DRM) by CS, 3 of 13 (23.1%) participants had VF compared with 5 of 75 (6.7%) participants without PDR ($p = .08$). Among the 6 individuals with mutations detected only by CS, 2 (33.3%) experienced VF (these had V179D and F77L; Table 2), which was similar ($p = .06$) to VF observed in 5 of 75 individuals without detectable mutations.

Discussion

This study of PDR found that (1) the relatively simple and inexpensive OLA detected an increased prevalence of PDR in 2014 compared with 2007–2009; (2) PDR by OLA and CS combined was associated with increased rates of VF during NNRTI-based ART; however, (3) neither OLA nor CS alone detected PDR associated with VF.

An increased prevalence of PDR was detected in the largest ART program in Peru by OLA and CS between 2007–2009 and 2014–2015,¹⁶ and was higher than studies at other institutions in Peru.^{16,20} These mutations primarily conferred high-level resistance to NNRTI-based ART regimens. The increased prevalence of PDR has been observed in other countries in the region using similar drugs in their ART programs.⁵ PDR has been linked to increases in acquired HIV drug resistance in individuals on ART, and in Peru PDR has been associated with inadequate adherence and an increased treatment dropout.²¹

Mutations evaluated by OLA were selected due to their high prevalence in previous studies of resistance,¹² and their strong association with VF to NNRTI-based ART that primarily included nevirapine (NVP).¹⁹ Similar to other coun-

tries, Peru's national ART program switched from NVP to EFV-based ART some years ago. Findings in our population, although weakened due to LTFU, suggest that OLA mutations are not strongly associated with VF. In our study, CS did not detect additional participants with high-level resistance compared with OLA alone. However, the low-level NRTI and NNRTI mutations and polymorphisms detected by CS appear to have detected risk, as resistance by the two assays was associated with VF. Therefore, this study suggests that majority frequency major mutations did not confer a high risk of VF. The association of resistance by OLA combined with CS with VF may signal a potential complex interaction, or a chance effect in this small study population.

Others studies have shown that several mutations account for most cases of TDR, including the two major NRTI-associated DRMs (M184V and K65R) and four major NNRTI-associated DRMs (K103N, Y181C, G190A, and V106M).²² The most frequent mutation detected in this cohort was K103N, which is strongly associated with failure against NVP,²³ and even when present at minority frequencies in an individual's quasispecies, K103N has been associated with a higher risk of NNRTI treatment failure.²⁴ However, in this study population, K103N was not associated with a high risk of VF.

The K65R mutation is strongly associated with resistance to tenofovir, which has recently been added to the Peruvian national ART program and is currently one of the core components of first-line ART in Peru. We did not detect K65R in our study population, but given the current widespread use of tenofovir, it is advisable to include surveillance of this mutation in the coming years. Future options in Peru include new drugs such as dolutegravir, but further research is required to discern the best approach to prevention and management of HIV DR.

Limitations of this study include the relatively small study population that had OLA drug resistance testing, that not all subjects initiated NNRTI-based ART, and that a moderate proportion of subjects did not return for viral load monitoring: all of which may have reduced our power to detect associations between PDR and virologic outcome. The observational nature of this study likely contributed to some subjects' failure to initiate ART during the timeframe of the

TABLE 2. PRE-ANTIRETROVIRAL THERAPY HIV DRUG RESISTANCE MUTATIONS DETECTED IN 25 OF 122 PARTICIPANTS BY OLIGONUCLEOTIDE LIGATION ASSAY AND CONSENSUS SEQUENCING, AND VIROLOGIC OUTCOME AT MONTH 6 OF ANTIRETROVIRAL THERAPY

ID	PDR mutations detected by OLA					PDR mutations detected by CS ^a	Antiretroviral treatment initiated	Virologic status at month 6 of ART; VF (≥200 copies/mL)(+), (−) or LTFU	
	NRTI resistance mutations			NNRTI resistance mutations					
	M41L	K65R	M184V	K103N	Y181C				G190A
PDR detected at codons included in OLA conferring high-level resistance									
1	WT	WT	WT	WT	WT	100%	G190A	AZT/3TC+EFV	−
2	WT	WT	WT	WT	2%	ind	none	AZT/3TC/NVP	−
3	WT	WT	WT	100%	WT	WT	K103N	AZT/3TC+EFV	+
4	WT	WT	WT	70%	WT	WT	K103N	AZT/3TC+EFV	−
5	WT	WT	ind	100%	WT	WT	K101P, K103N	Did not start	LTFU
6	WT	WT	5%	5.5%	WT	WT	none	AZT/3TC+EFV	−
7	WT	WT	WT	72%	WT	WT	K103KN	AZT/3TC+EFV	−
8	WT	WT	2%	WT	WT	WT	none	DDI +3TC+EFV ^b	+
9	WT	WT	WT	2%	WT	WT	V179D	DDI +3TC+EFV	−
10	WT	WT	WT	100%	WT	WT	K103N, E138G, P225HP	DDI +3TC+ATV/r ^c	+
11	WT	WT	WT	98%	WT	WT	K103N	TDF +3TC+EFV	−
12	WT	WT	WT	92%	WT	WT	K103N	AZT/3TC+EFV	LTFU
13	WT	WT	WT	99%	WT	WT	K103N	DDI +3TC+NVP	LTFU
14	WT	WT	WT	9%	WT	29%	V179D, Y188CY, G190AG	DDI +3TC+EFV	LTFU
15	WT	WT	3%	WT	WT	WT	none	AZT/3TC/NVP	−
16	WT	WT	WT	WT	20%	WT	Y181CY	DDI +3TC+EFV	−
17	WT	WT	2%	ind	WT	WT	none	DDI +3TC+EFV	−
PDR detected only by CS at codons not included in OLA possibly conferring resistance									
18	WT	WT	WT	WT	WT	WT	D67DN	AZT/3TC/NVP	−
19	WT	WT	WT	WT	WT	WT	E138G, V179D	Did not start	LTFU
20	WT	WT	WT	WT	WT	WT	F77FL	AZT/3TC+EFV	+
21	WT	WT	WT	WT	WT	WT	V179DE	DDI +3TC+EFV	−
22	WT	ind	WT	WT	WT	WT	V179D	D4T+3TC+EFV	LTFU
23	WT	WT	WT	WT	WT	WT	V179D	AZT/3TC+EFV	−
24	WT	WT	WT	WT	WT	WT	V179D	AZT/3TC+EFV	−
25	WT	WT	WT	WT	WT	WT	V179D	AZT/3TC+EFV	+

Numbers indicate percentage mutant in each participant's viral quasiespecies.

^aStanford HIV Drug Resistance Database.

^bParticipant switched to PI-based ART 12 days after starting NNRTI-ART.

^cParticipant initiated a PI-based ART regimen and was excluded from virologic outcome analyses.

ind, indeterminate OLA result; LTFU, lost-to-follow-up; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; NVP, nevirapine; PIs, protease inhibitors; VF, virologic failure; WT, wild type.

study and to the varied ART regimens. In addition, the advanced HIV disease and impoverished state of some participants likely compromised their ongoing linkage to medical care and survival.

In conclusion, the prevalence of PDR is increasing among ARV-naïve Peruvians. PDR was associated with increased rates of VF in participants taking NNRTI-ART that included EFV, although to a lesser degree compared with past studies of PDR in participants who initiated NVP-based ART, suggesting that not all NNRTI resistance is high risk but may identify people who need close monitoring of VL.

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Author Disclosure Statement

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