

HIV drug resistance among naïve HIV-infected patients in Iran

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Background: Antiretroviral (ARV) therapy extends life for persons living with HIV. Antiretroviral treatment (ART) has been rapidly expanding coverage around the world, including in Iran. However, ART drug resistance also rapidly develops with expanding use and limits effectiveness and treatment options. The aim of this study was to monitor the appearance of new mutations conferring HIV pretreatment drug resistance in the treatment of naïve patients with HIV in Iran. **Materials and Methods:** Blood samples were obtained from ARV treatment-naïve patients from 8 different provinces in Iran in 2016 for genotyping for drug resistance mutations. **Results:** Sequences were successfully obtained from 90 specimens. Of these, 2 (2%) mutations conferring resistance to protease inhibitors, 2 (3%) conferring resistance to nucleoside reverse transcriptase inhibitors (NRTIs), and 9 (13%) conferring resistance to non-NRTI (NNRTI) were detected. Any ARV-resistant drug mutation was found in 11 patients (12%). **Conclusion:** Nearly one in 8 ARV-naïve patients had mutations associated with NNRTI resistance in diverse areas of Iran in 2016. Iranian ARV therapy guideline for HIV could consider non-NNRTI-based first-line therapies and expand routine drug resistance testing before treatment initiation as according to HIV drug resistance recommendations of the World Health Organization.

Key words: Drug resistance, HIV, Iran

How to cite this article: Farrokhi M, Gholami M, Mohraz M, McFarland W, Baesi K, Abbasian L. HIV drug resistance among naïve HIV-infected patients in Iran. *J Res Med Sci* 2019;24:31.

INTRODUCTION

Effective antiretroviral (ARV) drugs have remarkably increased survival and well-being of patients who are living with HIV.^[1-3] The benefits of combination antiretroviral treatment (ART) are occurring even in low- and middle-income countries including Iran.^[4,5] Unfortunately, the expansion of ART can be quickly accompanied by the emergence of drug resistance, and the transmission of HIV strains with mutations conferring drug resistance blunts the benefits of ART. Transmitted resistance can be particularly devastating to low- and middle-income countries because it puts pressure on using more expensive ARV for first-line therapies.

The limited use of routine HIV viral load monitoring and drug resistance testing in many resource-limited countries may miss the emergence and accumulation of transmitted ARV resistance^[6] which in turn play a role in treatment failure.^[7] On a population level, the detection of pretreatment drug resistance (PDR) in newly HIV-infected patients can warn program planners of the existence and circulation of drug-resistant viral mutations. On an individual patient level, treatment guidelines in industrialized nations recommend regular testing to detect drug resistance-associated mutations in patients before initiating ART for optimizing therapeutic regimens.^[8,9] The lack of routine resistance testing of patients before ART initiation has been hypothesized as a cause for the development of drug resistance

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Quick Response Code:



Website:

www.jmsjournal.net

DOI:

10.4103/jrms.JRMS_689_18

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Received: 12-09-2018; **Revised:** 18-12-2018; **Accepted:** 30-01-2019

in developing countries, along with poor adherence to treatment and financial constraints preventing changes to different regimens.^[10]

ART distribution in Iran started in 1997 with the first-line therapy of zidovudine, lamivudine, plus indinavir.^[11] At present, 6 different classes of HIV regimens now exist in Iran.^[12] The four most predominant classes are nucleoside and nucleotide analog reverse transcriptase inhibitors (NRTIs), non-NRTI (NNRTI), protease inhibitors (PIs), and integrase inhibitors. Since the initiation of national coverage of ART in Iran, several studies of HIV genotyping and detection of mutations related to drug resistance have been carried out.^[13,14] However, the situation for which drugs are in use, in which parts of the country, and the emergence of drug resistance requires frequent updates. We therefore conducted the present study to find the drug resistance mutations among ARV-naïve patients in several locations in Iran.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

MATERIALS AND METHODS

Patients

We sought a cross-sectional sample of patients meeting eligibility criteria according to WHO recommendations for the observation of transmitted HIV drug resistance in countries scaling up ART.^[15] Because of hard to access of HIV-infected patients, we used convenient sampling in this study according to inclusion criteria. Criteria included having a blood sample obtained before the initiation of ART and limiting the inclusion of chronically infected persons (>3 years) to maximize the likelihood of recruitment of newly infected persons. We initially identified 105 patients diagnosed from March to September 2016 through the AIDS/STI control office, ministry of health and medical education-Iran, originating from 8 different provinces, Tehran (30 samples), Kermanshah (20 samples), Esfahan (10 samples), Fars (10 samples), Razavi Khorasan (10 samples), Gilan (10 samples), Hormozgan (5 samples), and Khuzestan (10 samples). These patients were approached through their clinic providers requesting a specimen for the purpose of drug resistance testing. The study protocol was approved by the Tehran University of Medical Sciences ethical review board (ID #22407). Informed consent was obtained from each patient prior blood collection.

RNA isolation and amplification

A specimen of 5 ml blood was collected in a sterile EDTA tube and labeled with the patient's name and/or identification number and the collection date. The whole blood was centrifuged for 10 min to separate the serum. Serum was stored at 4°C–8°C until shipment. The samples were shipped to our HIV laboratory in Tehran for genotyping.

Viral RNA was extracted by QIAamp viral RNA mini-kit, and real time-polymerase chain reaction (RT-PCR) was performed by the Qiagen One-Step RT-PCR Kit (Qiagen, Hilden, Germany). The RT and protease genes were amplified using the following methods. For obtaining RT PCR product, 2 series of primer was used, introduced in last studies^[16] [Table 1]. Nested PCR was done with inner primers of last studies with separate material of PCR belonging to Fermentas/Thermo Fisher Scientific Company.^[16,17] Nested PCR products were visualized on a 1.5% agarose gel with Fermentase DNA stain dye. The PCR products were decontaminated by a gel purification kit Qiagen (QIAquick PCR Purification Kit) and sequenced on both genes was sequenced by Sanger method.

Data analysis

HIV drug resistance analyses were performed using Stanford HIV Resistance Database (<http://hivdb.stanford.edu/>) and the WHO mutation list.^[18]

RESULTS

HIV-1 drug resistance mutations

Blood samples were obtained from 105 patients. Of these, 90 were successfully amplified. Losses occurred through mishandling of specimens in the processing and shipment chain. Twenty-seven patients (30%) were female. A slight majority (51%) were infected through presumed intravenous drug use, 33% through sexual contact, and the remainder with other or unknown modes of transmission [Table 2].

Overall, 90 patients' sequences were related to protease (PR) and 70 patients had sequences related to RT (i.e., 20 patients did not have RT sequences).

Table 1: Sequences of primers used

Title of primer	Sequence of primer
PrF1	G AGC CAA CAG CCC CAC CAG
PrR1	GCCATTGTTAAACGGCCATCC
PrF2	CT ATC ACT CTT TGG CAA CG
PrR2	CTG GTA CAA TAG GRC TAA T
RTF1	GTTGACTCACVATGGTTGTAC
RTR1	GTA TRT CAT TGA CAG TCC AGC
RTF2	GGATGGCCAGGTGTTAAAC
RTR2	TTRTCAGGATCCGGTTCATAACC

Only 1 major mutation-associated PIs (I50V) was detected in these ART-naïve patients [Table 3]. Minor mutations associated with PIs included G73A, L24F, and L23I with 1 each.

Several mutations associated with NRTI and NNRTI resistance were detected. The most common mutation related to NRTI was M184V/I present in 2 (3%) of 70 patients, with K65R, M41 L, D67N, V75M, L210W, and T215Y each detected in 1 (1%) patient. The most prevalent mutations related to NNRTI were K103N detected in 4 (6%) patients and E138A detected in 3 (4%) patients.

In the total 90 individuals, 11 (12%) patients had any PDR mutation. Of these, 2 (2%) patients from Tehran province carried virus with mutations related to PI resistance. In total, 9 (13%) patients carried virus with mutations related to NNRTI resistance: 4 (13%), 2 (10%), 1 (10%), 1 (10%), and 1 (10%) from Tehran, Kermanshah, Fars, Khuzestan, and Gilan, respectively, and 2 (3%) patients from Tehran carried virus associated with NRTI resistance, with overlap of multiple class mutations in 2 patients. One patient had a strain with 2 mutations related to NNRTI (K101E and G190A) and 6 mutations related to NRTI (M184V/I, L210W, T215Y, M41L, V75A, and D67N). The other patient's strain had K103N (associated with NNRTI resistance) and K65R and M184I (associated with NRTI resistance).

Table 2: Demographic characters of patients

	n (%)
Patients	
Female	27 (30)
Male	63 (70)
Epidemiological parameters	
Age (years)±SD	36±9
Intravenous drug user	46 (51)
Sexual	30 (33)
Other	14 (16)

SD=Standard deviation

Table 3: Frequencies of mutations associated with pretreatment HIV drug resistance, Iran, 2016 (n=90 drug-naïve patients)

	Protease		Reverse transcriptase				
	Major	Minor	NNRTI	NRTI			
I50V	1	G73A	1	K103N	4	M184V/I	2
		L24F	1	E138A	3	K65R	1
		L23I	1	K238N	1	M41L	1
				V179T	1	D67N	1
				Y318F	1	V75A	1
				K101E	1	L210W	1
				G190A	1	T215Y	1

NNRTI=Nonnucleoside reverse transcriptase inhibitors; NRTI=Nucleoside reverse transcriptase inhibitors

DISCUSSION

Our study presents an update of pretreatment HIV drug resistance mutations among treatment-naïve patients from 8 major provinces of Iran in 2016, several years into the national scale-up of HIV care. As a cross-sectional study of patients recently diagnosed, our data suggest a prevalence of PDR of 2% for PIs, 3% for NRTI, and 13% for NNRTI. According to the WHO, our findings place Iran in the low (<5%) to moderate (5%–15%) category for transmitted resistance.^[15,19]

Although low to moderate for the present, our results are higher than previous local studies,^[13,14] especially for NNRTI resistance. By comparison, Vahabpour *et al.* recently found a lower percentage of NNRTI resistance (7%).^[14] In addition, Farrokhi *et al.* detected no mutation associated with NNRTI resistance in their study of ART-naïve patients.^[16] However, we note that there were differences between our study and these other recent efforts in terms of patient populations, sampling methods, and survey sites, namely our patients were from HIV care clinics (before ART initiation) whereas these other studies drew from voluntary counseling and testing sites.

Particular mutations detected among our patients are worth noting. Two patients had HIV strains with the M184V/I mutation, which conveys a high level of resistance to lamivudine and emtricitabine.^[12] Four patients had HIV strains with the K103N mutation, which causes a high level of drug resistance to efavirenz and nevirapine. The finding is consistent with a previous study in Iran and is worrisome as these drugs are part of the first-line therapy nationally. Unlike a previous study in Iran,^[16] we did find the major mutation I50V related to PI resistance in one patient. I50V is a nonpolymorphic substrate-cleft mutation associated with fosamprenavir, lopinavir, and darunavir.^[20,21] In addition, in contrast to prior studies in Iran,^[22,23] we detected the minor mutations – G73A, L24F, and L23I related to PI resistance.

We recognize limitations to our study. First, the sample size is small. As such, there is a need to interpret findings cautiously. For the present, we take our data to be a recent snapshot for the detection of PDR with an eye to previous and future studies. A second limitation is the inability to sequence specimens from all the original 105 eligible patients identified, mostly due to issues in transportation. Finally, we also lack details on the clinical characteristics of patients, including viral load, CD4 counts, and more precise timing of acquisition of infection. We therefore assume that their recent diagnosis and ARV-naïve status point to their relatively recent infection.

Despite limitations, our study does give warning to the potential increase in transmitted NNRTI resistance at a level

of concern according to the recommendations of the WHO.^[5] We recommend considering the introduction of non-NNRTI first-line therapy in conjunction with expanding routine viral load and HIV drug resistance testing before treatment initiation in Iran.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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