The First Characterization of HIV-1 Subtypes and Drug Resistance Mutations among Antiretrovirally Treated Patients in Kermanshah, Iran

Reza Golmohammadi^a Kazem Baesi^b Abdolvahab Moradi^a Molood Farrokhi^b
Willi McFarland^c Shahryar Parsamajd^d

^aDepartment of Microbiology, School of Medicine, Golestan University of Medical Sciences, Gorgan, ^bDepartment of Hepatitis and AIDS, Pasteur Institute of Iran, Tehran, and ^cDepartment of Epidemiology and Biostatistics, University of California, San Francisco, CA, USA; ^dCenter of Counseling and Behavioral Disorders, Kermanshah University of Medical Sciences, Kermanshah, Iran

Keywords

HIV-1 · Reverse transcriptase · Drug resistance · Kermanshah · Iran

Abstract

Background: Insufficient therapy during HIV-1 replication can promote the emergence of drug-resistant strains, reduce the effectiveness of antiretroviral treatment (ART), and increase the likelihood of the onward transmission of drug-resistant viruses. We characterized, for the first time, the prevalence of HIV-1 subtypes and drug resistance mutations in a western region of Iran. **Methods:** This study was conducted among 122 patients on ART at a major referral center in Kermanshah, Iran. Nested PCR was performed using RT gene-specific primers from the *pol* gene. Sequencing was followed by amplification and purification of the desired sequence. Subtypes and mutations were determined using the Stanford HIV Drug Resistance Database. **Results:** Most patients (92.6%) had subtype CRF 35-AD; 7.4% had subtype B. In total, 36.1% of the patients had at least 1 mutation associated with resistance RT inhibitors. The greatest rates of high-level resistance were observed for nevirapine (21.3%) and efavirenz (19.7%). **Conclusions:** Our results showed a high prevalence of drug resistance mutations in strains isolated

from patients on treatment. At our center, we therefore recommend that genotyping be performed. This would allow the physician to prescribe appropriate drugs, reduce treatment costs, and increase the longevity and quality of life of patients.

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Introduction

The introduction of antiretroviral therapy (ART) has greatly improved clinical status and length of life for HIV-1-infected patients worldwide [1–3]. However, continued benefit could become elusive with the development of drug resistance. Resistance mutations have developed in the viral proteins targeted by antiretroviral drugs [4, 5] and are selected for when the drug fails to block virus replication [6]. In conjunction with a rapid rate of mutation, there is great genetic variation in the subtypes of HIV. Knowledge of the subtypes prevalent in particular areas may mark different patterns of transmission of the virus and correlate with the emergence of drug resistance mu-

Kazem Baesi, PhD
Department of Hepatitis and AIDS, Pasteur Institute of Iran
Pasteur Ave, Tehran 1316943551 (Iran)
E-Mail kbaesi@gmail.com

Abdolvahab Moradi, PhD
Department of Microbiology, School of Medicine,
Golestan University of Medical Sciences, Falsafi Educational Campus
Shaskalate, 5th km Gorgan – Tehran Rd., Gorgan (Iran)
E-Mail abmoradi@yahoo.com

tations [7, 8]. Monitoring clinical isolates for these mutations is needed to guide initial therapy as well as the choice of drugs following treatment failure [9].

HIV-1 reverse transcriptase (RT) involves the enhancement of resistance to all anti-AIDS drugs by causing mutations into the viral genome. At the molecular level, mutations in RT result in resistance to RT inhibitors [10].

By 2013, a recorded 27,041 individuals had been infected with HIV-1 in Iran. ART was first introduced in Iran in 1997; since then, zidovudine, lamivudine, and indinavir are most commonly prescribed for HIV patients [11]. To date, 3,250 persons diagnosed with HIV were reported in Kermanshah province, the western border of the county and the location of our study [12]. Many patients in Kermanshah have been under treatment with ART for several years, yet we have no data on the prevalence of drug resistance. This is the first study in Kermanshah to determine the profiles of drug resistance mutations in RT and the subtypes of HIV-1 in circulation.

Materials and Methods

This study was a case series comprising patients on ART who were recruited at the Behavioral Disease Consulting Center, the main referral clinic for HIV care in Kermanshah province. A total of 122 consecutive patients on ART were selected in 2013. They were on treatment and had been admitted to the Behavioral Disease Consulting Center for taking ART. As well as this, they were on ART for >1 year and had all provided written informed consent to participate in the study. Blood samples were taken from each patient and stored at -80°C .

RNA extraction from plasma was performed by using the High Pure RNA isolation kit (Roche, Germany) according to the manufacturer's instructions. PCR was performed on the reverse transcriptase (RT) gene of HIV-1 using the One Step RT-PCR kit (Qiagen, Germany). The first round of RT-PCR used the following materials and primers: 10 μL RNA, 1 $\times$ PCR buffer from 10 $\times$, 200 μM of dNTP10 mM, 1.5 units of Taq DNA polymerase (5 units/ μL), and 10 pM of each specific primer. To amplify the 803 bp of the RT gene, RT-PCR was performed with the outer forward (5'-GTGACTCAGATTGGTTGCAC-3') and reverse (5'-GTATGTCATTGACAGTCCAGC-3') primers under the following cycling conditions: 50 $^{\circ}\text{C}$ for 45 min (cDNA synthesis), initial denaturation at 94 $^{\circ}\text{C}$ for 15 min, and 35 cycles for synthesis of DNA from cDNA with denaturation at 94 $^{\circ}\text{C}$ in 30 s, annealing at 57 $^{\circ}\text{C}$ in 30 s, extension at 72 $^{\circ}\text{C}$ in 50 s, and the final heating step at 72 $^{\circ}\text{C}$ for 5 min. In the second round of RT PCR, 4 μL of DNA was used as the template with the same temperature programs as in the first round for the nested PCR by using inner forward primer (5'-GGATGGCCCCAAAAGTTAAAC-3') and reverse inner primer (5'-TATCAGGATGGAGTTTCATAAC-3'). PCR products (665 bp) were detected on 2% agarose gel.

The PCR products were purified using QIAquick gel extraction kit (Qiagen, Germany) according to the manufacturer's instructions, and sequenced in an automated DNA sequencer (ABI

Table 1. Transmission risks of antiretrovirally treated patients ($n = 122$)

Transmission risk	Female patients	Male patients	Total
Intravenous drug use	1 (2.4%)	61 (76.3%)	62 (50.8%)
Spouse infected	25 (59.5%)	4 (5.0%)	29 (23.8%)
Sexual	11 (26.2%)	4 (5.0%)	15 (12.3%)
Mother-to-child	3 (7.1%)	2 (2.5%)	5 (4.1%)
Unknown	2 (4.8%)	9 (11.3%)	11 (9.0%)
Total	42 (34.4%)	80 (65.6%)	122 (100%)

PRISM 3730 v3.0, Applied Biosystems, USA). The sequences were edited using Bioedit software. Subtypes and mutations were identified by the Stanford HIV Drug Resistance Database (<http://hivdb.stanford.edu>) [13].

The study protocol was reviewed and approved by the Ethics Committee of Golestan University of Medical Sciences.

Results

Of the 122 patients, 80 (65.6%) were male and 42 (34.4%) were female. Over three-quarters (76.3 %) of the male patients were intravenous drug users, whereas the majority of female patients (59.5%) were likely infected via their husbands (Table 1). The mean age was 38 ± 9 years. The average CD4 count was 250 ± 19 per mL of blood.

One hundred patients (82.0%) were taking the first-line regimen of lamivudine (3TC), zidovudine (AZT), and efavirenz (EFV). Thirteen (10.7%) were taking 3TC, AZT, and nevirapine (NVP); 9 (7.37%) were taking 3TC, tenofovir (TDF), and EFV.

The most common HIV-1 subtype was CRF35_AD, detected in 113 patients (92.6%). Nine patients (7.4%) had subtype B.

In total, 44 patients (36.1%) had mutations associated with resistance to at least one RT inhibitor drug and 22 patients (18.0%) had mutations associated with resistance to both nucleoside reverse transcriptase inhibitor (NRTI) and non-nucleoside reverse transcriptase inhibitor (NNRTI) class drugs. Table 2 lists the frequency of clinically significant mutations identified in the patient population in both these drug classes. The most common RT gene mutations associated with drug resistance in the NRTI class were M184V, M41L, K70R, T69P, D67N, K219E, and V75M. Common RT gene mutations associated with drug resistance in the NNRTI class were K103N,

Table 2. Frequency of clinically significant ART drug resistance mutations among ART-treated patients ($n = 122$)

NRTI mutations	<i>n</i> (%)	NNRTI mutations	<i>n</i> (%)
M184V	17 (13.9)	K103N	18 (14.8)
M41L	6 (4.9)	E138A	10 (8.2)
K70R	5 (4.1)	P225H	6 (4.9)
T69P	5 (4.1)	G190A	6 (4.9)
D67N	4 (3.3)	V179F	3 (2.5)
K219E	4 (3.3)	Y181S	3 (2.5)
V75M	4 (3.3)	V108I	3 (2.5)
M184I	2 (1.6)	K238N	3 (2.5)
T215Y	2 (1.6)	V106I	3 (2.5)
L210W	2 (1.6)	A98G	2 (1.6)
L74I	2 (1.6)	H221Y	2 (1.6)
K219E	2 (1.6)	F227L	2 (1.6)
V75L	2 (1.6)	K101E	2 (1.6)
Y115F	2 (1.6)	V90I	2 (1.6)
D67G	2 (1.6)	Y188C	2 (1.6)
D67F	1 (0.8)	V179T	2 (1.6)
F77L	1 (0.8)	V179L	1 (0.8)
K65R	1 (0.8)	G190R	1 (0.8)
K70G	1 (0.8)	E138K	1 (0.8)
M184G	1 (0.8)	P236L	1 (0.8)
M184R	1 (0.8)	M230L	1 (0.8)
L74V	1 (0.8)	L100F	1 (0.8)
T215P	1 (0.8)	G190I	1 (0.8)
V75G	1 (0.8)	G190T	1 (0.8)
A62V	1 (0.8)	Y188F	1 (0.8)
T215S	1 (0.8)	Y181T	1 (0.8)
T215A	1 (0.8)	Y181F	1 (0.8)
T215F	1 (0.8)	Y181C	1 (0.8)

ART, antiretroviral therapy; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor.

E138A, P225H, G190A, V179F, Y181S, V108I, K238N, and V106I. The most common mutation associated with resistance to NRTIs was M184V, which occurred in 17 patients (13.9%). The most common mutation associated with resistance to NNRTIs was K103N, and it occurred in 18 patients (14.8%).

The most common mutations detected among the 100 patients on the 3TC/AZT/EFV regimen were: K103N ($n = 14$), M184V ($n = 13$), E138A ($n = 9$), P225H ($n = 4$), G190A ($n = 3$), M41L ($n = 2$), and K70R ($n = 2$). The mutations detected in the 13 patients on the 3TC/AZT/NVP regimen included: M41L ($n = 2$), E138A ($n = 1$), G190A ($n = 1$), and K70R ($n = 1$). The mutations detected in the 9 patients on the 3TC/TDF/EFV regimen were: K103N

($n = 4$), M184V ($n = 4$), M41L ($n = 2$), G190A ($n = 2$), P225H ($n = 2$), and K70R ($n = 2$).

Table 3 shows the levels of susceptibility and resistance based on the different mutations. Most patients were susceptible to the NRTI regimens that included 3TC (82.0%), AZT (91.0%), and TDF (91.8%), and to the NNRTI regimens that included EFV (75.4%) and NVP (74.6%). Mutations associated with high-level resistance to 3TC and emtricitabine (FTC) were present in the isolates of 19 patients (15.5%). Although EFV was part of the first-line therapy for 10 patients, mutations associated with high-level resistance to EFV were detected in the isolates of 24 patients (19.7%). Mutations associated with high-level resistance to NVP were detected in the isolates of 26 patients (21.3%) [14, 15].

Discussion

For the first time in the western Iranian province of Kermanshah, we reported the HIV-1 strain subtypes and resistance mutations among patients on ART. The HIV-1 subtype CRF35_AD predominated, accounting for >90% of isolates. Subtype B was also found. The most important mutation associated with NRTI drug resistance was M184V and the most important mutation associated with NNRT drug resistance was K103N. Both mutations were detected in approximately 1/7 patients treated with these drugs. Mutations associated with high-level resistance to 3TC, EFV, and NVP were detected in approximately 1/5 patients taking these ART drugs.

Some studies have been performed on drug resistance mutations and HIV genotyping in Iran, most of them in Tehran and at the national referral center in the capital. In 2010, Hamkar et al. [16] found subtype CRF 35_AD in approximately half (48%) of their patients on ART (in Tehran). Our data were more similar to 2 other studies by Baesi et al. [17, 18] (in 2015) which found a frequency of 80 and 88% CRF 35_AD. In 2016, a third study by Baesi's group (Farrokhi et al. [19]) found the frequency of CRF35_AD to be 96.1%. These findings are also consistent with an earlier national pilot study by Mousavi et al. [20] (in 2010) which found subtype CRF35_AD to be the most common in Iran.

The prevalence of drug resistance mutations in this study, where 36% of patients had at least 1 mutation, was lower than the 58% reported in Iran in 2014 [21]. We found that the most prevalent mutation associated with NRTI drug resistance was M184V, which causes resistance to 3TC and FTC. However, our prevalence of 13.9%

Table 3. Levels of resistance to drugs in antiretrovirally treated patients ($n = 122$)

Level of resistance	NRTIs, n (%)				NNRTIs, n (%)	
	3TC	AZT	TDF	FTC	EFV	NVP
Sensitive	100 (82.0)	111 (91.0)	112 (91.8)	100 (82.0)	92 (75.4)	91 (74.6)
Low	2 (1.6)	3 (2.5)	4 (3.2)	2 (1.6)	5 (4.1)	4 (3.2)
Intermediate	1 (0.8)	5 (4.1)	3 (2.5)	1 (0.8)	1 (0.8)	1 (0.8)
High	19 (15.6)	3 (2.5)	3 (2.5)	19 (15.9)	24 (19.7)	26 (21.3)

NRTIs, nucleoside reverse transcriptase inhibitors; NNRTIs, non-nucleoside reverse transcriptase inhibitors; 3TC, lamivudine; AZT, zidovudine; TDF, tenofovir; FTC, emtricitabine; EFV, efavirenz; NVP, nevirapine.

is lower than the previously reported range of 19–60% in patients throughout Iran over the last several years [16, 22, 23]. The most prevalent mutation associated with NNRTI drug resistance in our study was K103N (in 14.8%), also lower than the 29% reported in a prior study of patients treated in Tehran in 2010 [16]. Nevertheless, the frequency of K103 N in our study was greater than in a study of ART-naïve and ART-experienced patients in Shiraz in 2014 [22]. We found high-level resistance to NVP and EFV at 20.3 and 19.7%, respectively, similar to in a previous study of patients on ART in Tehran in 2012 [23].

We recognize the limitations of our study. First, although our series included a large proportion of all patients seen at the Kermanshah referral clinic, they do not represent all HIV patients in the province. Second, all participants were on ART, but we do not have the distinct prevalences of primary and acquired ART resistance mutations. Third, the sample size was small, making statistical inferences difficult, especially compared to other studies in Iran which also suffer from small sample size.

Despite these limitations, the results from the major referral clinic of Kermanshah province are valuable for understanding the epidemiology of HIV in the west of Iran and in our part of the Middle East. Data may also help guide clinical practice for the use of ART regionally. Up to the present, drug resistance genotyping tests have not been routinely performed in our center. Given the high prevalence of drug resistance mutations, we recommended that patients should be tested for drug resistance prior to the initiation of and during the course of treatment, especially when first-line regimens fail. This will allow physicians to prescribe appropriate initial drugs, reduce treatment costs in the long term, and improve the longevity and quality of life of patients.

Acknowledgements

We are sincerely appreciative to the laboratory staff of the Department of Microbiology, Golestan University of Medical Sciences, the Center for Behavioral Diseases Consulting in Kermanshah Province, as well as the central office staff who helped in this project. Funding for this study was provided by the Department of Technology and Research, Golestan University of Medical Sciences.

Statement of Ethics

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. Informed consent was obtained from all participants included in the study.

Disclosure Statement

The corresponding author declares no conflict of interest.

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