Naturally Occurring Mutations in HIV-1 Protease Gene Among People Living With HIV

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Abstract- The emergence of resistance to antiretroviral drugs is the main problem in their long-term efficacy and by considering the wide use of protease inhibitors (PIs), monitoring drug resistance mutations is necessary. Therefore, this study aimed to investigate the PIs drug resistance mutations in Iranian patients as well as subtyping using bioinformatics analysis. Fifteen Iranian patients living with Human Immunodeficiency Virus (HIV) (PLWH) were examined. RNA was used to amplify and sequence the HIV protease gene; also, HIV viral load was determined for all samples. The sequencing results were analyzed by several strong bioinformatics tools to determine the drug-resistance mutations and HIV subtypes. Some polymorphisms in the protease gene were recognized; however, there was no significant rate of major or minor drug resistance mutations in our studied patients. Subtyping analysis revealed the new subtype (D) and the previously reported ones, A and CRF-AD 35, in patients. This study confirmed that the resistance mutations and genetic polymorphisms of the protease region are rare in Iranian-infected patients that can be concluded that prescribing protease inhibitor class in HIV-infected patients is promising in controlling HIV in Iran. In addition, conducting periodic studies to determine the new mutations and the rate of drug resistance to PIs in Iranian individuals highlights the importance of WHO guidelines that recommends monitoring of genotypic-resistance testing and investigation of mutations in HIV-related genes.

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Keywords: Human immunodeficiency viruses (HIV); Protease; Drug resistance

Introduction

Acquired immune deficiency syndrome (HIV/AIDS) is a very complex and complicated disease that often requires intensive care support; however, the life expectancy of HIV-infected individuals has improved with the expansion of antiretroviral therapy (ART) (1). Retroviruses like HIV can respond effectively to selective pressures such as drug treatment by several mutations which occur rapidly since the conversion from the RNA genome to DNA is error-prone (2).

The availability and accessibility of ART have profoundly reduced the mortality and morbidity of HIV-related infections; however, the treatments cannot eradicate the virus (3). While about 25 drugs that belong to seven classes targeting different stages in the life cycle of HIV have been introduced, there is no permanent cure or vaccine to control AIDS. The introduction of antiviral treatment could improve the quality and life expectancy of HIV-infected patients. However, low drug adherence, toxicity, high pill burden, and the error-prone mechanism of HIV reverse transcriptase have caused the rise of drug resistance in HIV-infected patients (4).

Protease has always been one of the main therapeutic targets for developing antiviral drugs against HIV-AIDS and nine FDA-approved protease inhibitors have been improved, including Saquinavir (SVQ), Indinavir (IDV), Ritonavir (RTV), Nelfinavir (NFV), Amprenavir (APV), Lopinavir (LPV), Atazanavir (ATV), Tipranavir (TPV), and Darunavir (DRV). Due to the great anti-AIDS potential of protease inhibitors, they are essential components of antiretroviral therapy (ART) (5).

Bioinformatics tools have been established during the last few years and have been the main means to analyze

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different virus genes (6-8). Several studies have investigated mutations related to drug resistance and determined different rates of drug resistance mutations in HIV patients globally as well as in Iran. This study aimed to define the PIs-resistance mutations in the Iranian HIV-infected patients as well as HIV subtyping by using bioinformatics software and databases.

Materials and Methods

Study population

The sera of 15 naïve-treatment patients enrolled in this study from a clinic affiliated with Shiraz University of Medical Sciences were studied. All the subjects provided informed consent and agreed that their samples be used for research. The patients' codes were used instead of names in the study databases for patient privacy and the study was approved by the university ethics committee (Reference Number: IR.SUMS.REC.1399.698).

RNA extraction and real-time PCR assay

"Artus kit" (QIAGEN) according to the manufacturer's instructions was used for both viral RNA extraction and real-time PCR viral load (seme kit). Extracted RNA was followed by cDNA synthesis using MMLV reverse transcriptase and random hexamer primers.

PCR and sequencing

The protease gene primers listed and the thermal-cycling conditions in the first round of PCR are shown in Table 1. The PCR products were examined on 2% agarose gel and subsequently sequenced.

Amino acid changing

Sample sequences were analyzed by the CLC sequence viewer version Beta (QIAGEN); also, the Stanford University HIV Drug Resistance Database site was used for HIV subtyping and defining the resistance mutations.

Subtyping

Using three online software, COMET version 2, NCBI, and Stanford HIVdb version 6.0.10, we identified the subtypes of the sequences.

Results

Demographic data

A total of 15 patients were enrolled in the present study, including 10 (66.67%) males and 5 (33.34%) females, with a mean age of 40.3±10.7 years (range, 20-49 years). The mean viral load among the enrolled patients was 620000.

Amino acid changes

In comparison with the reference sequence (CAA09312), several mutations were found, as listed in Table 2; the most prevalent mutations occurred in positions 34, 35, 36, 56, 61, 68, 76, 88, and 92 which were not effective in drug resistance. Subtyping results revealed the CRF-AD and A subtypes and also the new subtype (D) (Table 3).

Table 1. The list of primers used in this study and thermal-cycling conditions of PCR

		Primers	PCR products length (bp)
Outer pair	**F**	TAATTTTTTAGGGAAGATCTGGCCTTCC	520
	R	GCAAATACTGGAGTATTGTATGGATTTTCAGG	
Inner pair	**F**	TCAGAGCAGACCAGAGCCAACAGCCCCA	450
	R	AATGCTTTTATTTTTTCTTCTGTCAATGGC	

Table 2. List of general mutations in comparison to reference sequence

Mutations	Prevalence	Mutations	Prevalence	Mutations	Prevalence
Q 1 K	1	A 21 L	1	S 62 V	1
V 2 I	9	T 30 S	1	S 62 L	8
V 2 S	1	E 34 D	10	E 64 D	1
T 3 L	1	E 34 N	1	C 66 H	1
L 4 F	3	E 34 H	1	H 68 K	13
Q 6 K	1	M 35 K	1	I 76 V	13
P 8 F	1	M 35 I	10	L 88 M	13
I 9 F	1	M 35 T	1	L 92 I	13
I 9 L	8	M 35 H	1	F 98 L	1
I 9 V	1	S 36 N	11		
V 10 L	1	S 36 F	1		
T 11 S	2	S 36 K	1		
T 11 P	2	L 37 A	1		
I 12 V	11	L 37 I	1		
I 14 V	2	K 40 R	2		
I 14 M	1	K 44 R	1		
G 15 E	2	I 53 F	1		
G 16 A	1	R 56 K	13		
Q 17 A	1	D 59 E	1		
Q 17 H	1	Q 60 E	1		
L 18 Q	8	V 61 I	11		
L 18 K	1	S 62 A	2		
K 19 R	12	S 62 F	1		
E 20 K	2	S 62 M	1		

Table 3. Drug resistance mutations and subtyping analysis by using three online software

Patients number	Mutations	Subtyping
1		CRF35_AD
2		CRF35_AD
3		CRF35_AD
4	I50L (high-level resistance to Atazanavir)	A
5		CRF35_AD
6		D
7		CRF35_AD
8		CRF35_AD
9		CRF35_AD
10		A
11		CRF35_AD
12		CRF35_AD
13		CRF35_AD
14		CRF35_AD
15		CRF35_AD

Discussion

The rapid and effective detection of resistant strains among HIV-infected patients is the main method to prevent the transmission of drug-resistant HIV strains as well as develop different strategies to cope with resistant strains circulating in the community which are vital. While in comparison with the reference sequence several mutations were found, a new amino acid substitution from isoleucine to leucine in codon 50 with a high level of resistance to Atazanavir was detected in one patient. Although the such position was previously reported (9), the type of amino acid substitution was different (Table 4).

Generally, the previous reports have shown the vast variety in the rates of PIs resistance-associated mutations in Iranian HIV-infected patients. This rate was 6.6 in the present study which was similar to Memarnejadian's study (10). In contrast to our result, Baesi *et al*., did not report any PIs resistance-associated mutations (11); also, in Davarpanah's study (12) 14% of the patients had a mutation in codon L10L/V and the results of the analysis of protease by Farrokhi *et al*., (13) revealed substitutions in G73SC and I47VA with the rates of 13.8% and 6.9%, respectively. Furthermore, Memarnejadian' study mentioned three major mutations with the high

prevalence substitutions in codons 32 and 47 by a percentage of 4.45%. In comparison with the mentioned studies, 2 investigations by Gholami *et al*., (14) and Jahanbakhsh *et al*., (15) determined the high rate of resistance mutations in a variety of codons. The high prevalence of PI mutations in Gholami's study belonged to substitution in codon L10F/I of 12%, while the highest prevalence of PI-resistant mutations was reported in Jahanbakhsh's study in which all samples had a mutation in codon 36, and 97.9 percent in codon 20. Atazanavir resistance was found in our report which was similar to Farrokhi, Gholami, and Gholami's studies, while DavarPanah *et al*., Baesi *et al*., and Jahanbakhsh did not mention any resistance to any PIs.

Table 4. The comparison results of the present study and previous studies in terms of drug resistance mutations, resistance to PIs, and subtyping

Studies	Present study	Farrokhi	DavarPanah	Nasiri-Tajabadi	Baesi	Gholami	Memarnejadian	Jahanbakhsh
Mutations	I50L	G73SC, I47VA	L10L/V	V32I, I50V, I54M, L76V, V82F		L10F/I, V32I, L33F/I, M46I, I47M/A, I54V, Q58E, A71V, T74S, V82A, L89V	V32I, I47A, V82M	L10I/V, V11I, G16E, K20R, L33V, M36I, D60E, I62V, L63P, I64L/V, H69K, L89M, I93L
Resistance to PIs	Atazanavir	Tipranavir Fosamprenavir Darunavir Atazanavir Nelfinavir		Atazanavir lopinavir nelfinavir Darunavir Ritonavir		Lopinavir Nelfinavir Atazanavir Indinavir Fosamprenavir	Nelfinavir Lopinavir Indinavir Fosamprenavir	
Subtyping	CRF35AD(80%) A(6.6%) D(13.4%)	CRF 35AD (82.75%) B (13.8%) C (3.45%)	CRF35AD (100%)	CRF35AD (94%) subtype A (6%)	CRF 35AD (88%) CRF 28BF (8%) CRF 29BF (4%)	CRF35AD(68%) AE and C(28%) A(4%)	CRF35AD (93%) C (2.3%) B(2.3%) A1(2.3%)	CRF35AD(95.7%) B (2.1%) AE (2.1%)

For Iranian HIV-infected patients, Kaletra (lopinavir/ritonavir) has been the major PIs prescribed (9); the present study did not show any resistance to such PIs. However, resistance to the mentioned PIs in Iranian patients is described previously (9,10,14). The different drug resistance results may be attributed to the enrolment of different numbers of patients, different geographical regions, and different subtypes.

Subtyping analysis by reliable online software was done for the first time; the presence of subtype D was reported in one Iranian patient and this subtype is dominant in Uganda and Tanzania. Similar to the other studies, subtypes were reported in two samples that have been reported previously (9,10,14), while the majority of sequences belonged to CRF35-AD subtypes which were in agreement with all studies in Table 4.

Generally, it can be concluded that the dominant subtype among Iranian patients by considering protease

sequences is CRF35-AD, which has been reported in several studies from Iran. However, investigations have shown several subtypes in Iranian patients with lower prevalence, including subtypes B, C, CRF01-AE, and CRF-BF.

The difference in the subtyping findings may result in different numbers of enrolled patients, and different genomes and geographical regions which were used in different subtyping tools.

Altogether, the results of this study revealed the lack of presence of either major or minor PIs drug resistance mutations in patients that suggest the current PIs can be effective in People Living with HIV (PLWH). However, this data should be combined with the results of previous studies to make a better decision for the planning of the treatment strategy. The necessity of PIs-resistance mutation monitoring in Iranian HIV-infected patients before determining the treatment regimen can be useful. Furthermore, the present finding showed the new subtype (D) in Iranian HIV-infected patients, indicating the importance of HIV subtyping among Iranian patients periodically. This can help us predict the transmission route and the plausible level of virus pathogenicity as well as define subtype-dependent resistance in Iranian society.

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