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Phylogeny and drug resistance of HIV PR gene among HIV patients receiving RT inhibitors in Iran

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ABSTRACT

Objective: To survey the level and patterns of reverse transcriptase-based drug resistance and subtype distribution among antiretroviral-treated HIV-infected patients receiving only reverse transcriptase inhibitors in Iran.

Methods: A total of 25 samples of antiretroviral therapy experienced patients with no history of using protease inhibitors were collected. After RNA extraction, reverse transcriptase-nested PCR was performed. The final products were sequenced and then analysed for drug-resistant mutations and subtypes.

Results: No drug resistant mutations were observed among the 25 subjects. The results showed the following subtypes among patients: CRF 35_AD (88%), CRF 28_BF (8%), and CRF 29_BF (4%).

Conclusions: A significant increase in drug resistance has been noted in recently-infected patients worldwide. Subtype distributions are needed to perform properly-designed surveillance studies to continuously monitor rates and patterns of transmitted drug resistance and subtypes to help guide therapeutic approaches and limit transmission of these variants.

1. Introduction

The use of combinations of three or more antiretroviral drugs from two drug classes has proven remarkably effective in controlling the progression of HIV and has reduced mortality in HIV-infected individuals [1]. These benefits can be compromised by the development of transmitted HIV-1 drug resistance. This type of resistance is the consequence of mutations that emerge in the viral proteins targeted by antiretroviral agents [2–9].

The implementation of antiretroviral therapy (ART) more than a decade ago has improved the quality and life expectancy of HIV-infected patients. Factors such as low-adherence rates by patients, inappropriate ART regimens, incorrectly-prescribed drugs, extensive genetic variation, toxicity of medication, high pill burden, and HIV genomic mutations as the main cause of virological and treatment failure have led to the emergence and subsequent spread of antiviral drug resistance in HIV-infected patients [5,10–15]. Surveillance studies have shown that approximately 10% of new HIV-1 infections involve drug-resistant strains, indicating that treated individuals are involved in the spread of new infections. It has also been shown that individuals infected *de novo* with drug-resistant viruses can serve as a source of subsequent infection and contribute to the spread of drug-resistant HIV [16].

The increase in the prevalence of transmitted drug resistance in low- and middle-income countries varies by area according to the nature of the ART programs implemented. This justifies the need for HIV-1 genotyping resistance testing before the initiation of ART to monitor antiretroviral treatment of HIV-1 patients as a major factor for therapeutic regimens selection [3,10,17–19]. Sequence analysis of the HIV-1 *pol* gene provides important information about antiretroviral drug resistance-associated mutations affecting the susceptibility of HIV-1

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The study protocol was performed according to the Helsinki Declaration and approved by Tarbiat Modares University ethical committee (Reg No. 1389-9). Informed written consent was obtained from all patients.

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strains to protease (PR) and reverse transcriptase (RT) inhibitors and on subtype diversity [20]. The present study surveyed the level and patterns of PR-based drug resistance and subtype distribution among antiretroviral-treated HIV-infected patients who receive only RT inhibitors in Iran.

2. Materials and methods

2.1. Sample collection

This cross-sectional study examined 25 HIV-infected patients undergoing ART at the Iranian Research Centre for HIV/AIDS of Imam Khomeini Hospital in Tehran, Iran. All patients received a combination of ART apart from PR inhibitors. A total of 25 individuals were enrolled in the study (100% participation). The study protocol was approved by the Medical Research Ethics Committee of Tarbiat Modares University in Tehran. All participants provided written informed consent prior to sample collection.

Initially, 5 mL blood samples were collected in ethylenediaminetetraacetic acid blood collection tubes. The plasma was separated by centrifugation at 3000 r/min and frozen at −70 °C until use for RNA extraction. The HIV pol regions, which include viral protease genes, were amplified and sequenced to determine the subtype and antiretroviral-resistant mutations.

2.2. HIV RNA extraction and cDNA synthesis

HIV RNA was extracted from 140 μL of plasma using the column purification method (QIAamp Viral RNA Mini Kit, Qiagen, Germany) according to manufacturer instructions. Following RNA viral denaturation at 70 °C for 10 min, cDNA synthesis was performed at 42 °C for 1 h using 200 IU of M-MuLV reverse transcriptase (Fermentas) and 2 μL of antisense outer primers (Table 1) plus 2 μL dNTP and 0.5 μL RNAse inhibitor (Fermentas).

2.3. Nested PCR amplification

First-round PCR was performed using 5 μL cDNA, 1 × PCR buffer, 0.5 μL of 10 mmol/L dNTP, *Pfu* polymerase, 0.75 μL 50 mmol/L $MgCl_2$, 0.5 mmol/L of 10 pmol/μL primer solution containing outer primers (Table 1), and 15 μL of double-distilled water. Briefly, the amplification profile consisted of denaturation at 95 °C for 5 min, followed by 30 cycles of denaturation at 94 °C for 50 s, annealing at 58 °C for 40 s, extension at 72 °C for 50 s, followed by a final extension phase at 72 °C for 5 min.

Table 1
The sequence of primers.

Primer	Sequence	Product length
External reverse	TGCCCTATYTCTAARTCAGATCC	586 bp
External forward	TTAGYCCTATTGARACTGTACCAG	
Internal reverse	AATATTGCYGGTGAYCCTTTCCATC	324 bp
Internal forward	GCCTGAAAATCCATYCAAYACTCC	

An aliquot (about 2.5 μL) of the primary PCR product was used for 35 cycles of nested PCR as follows: initial denaturation at 95 °C for 5 min, 20 cycles of denaturation at 94 °C for 50 s, annealing at 58 °C for 40 s, and polymerization at 72 °C for 50 s, with a final elongation at 72 °C for 5 min. An Eppendorf gradient PCR system thermal cycler was used for all PCR reactions. The results were checked by electrophoresis of the nested PCR products on 1.5% agarose gel and visualized with ethidium bromide under UV light.

2.4. Purification and DNA sequencing

The PCR products were purified using a gel purification kit (Bioneer, Global Genomics Partner) according to manufacturer instructions and sequenced on both strands (bi-directionally) using the dideoxy chain termination method (ABI PRISM 3700 DNA Analyser Automated Sequencer, Applied Biosystems, USA).

3. Results

At the time of the study, the plasma samples were selected retrospectively from 25 ART patients attending the Iranian Research Centre for HIV/AIDS at Imam Khomeini Hospital in Tehran in Iran. Of these, 19 (76%) were males and 6 (24%) were females. The primary routes of HIV/AIDS transmission were intravenous drug use (80%) and sexual contact (20%).

All 25 patients received highly active antiretroviral therapy for at least 1 year. The therapy regimen contained nucleoside reverse transcriptase inhibitor and non-nucleoside reverse transcriptase inhibitor. None of the patients had experienced the use of protease inhibitors (PIs). The CD4 cell count was below 250–300 cells/m^3. In all isolates, the PR segment of the *pol* gene amplified and produced useful sequences in this region. Phylogenetic analysis of the PR sequences derived from all isolates revealed no drug resistant mutations associated with the PIs.

In this study, recombinant CRF 35_AD (88%) was found to be the predominant HIV subtype, followed by subtypes CRF 28_BF in 8% and CRF 29_BF in 4% of patients failing treatment in Tehran. It was found that subtype CRF 35_AD of HIV was the most prevalent among HIV-infected patients in Iran. This study also showed that the major route of HIV/AIDS transmission was through injection drug users in Iran.

4. Discussion

The number of adults living with HIV in Iran was estimated to be 26125 at the end of 2012 and was comprised of 89.8% males and 10.2% females [21]. Most participants were intravenous drug addicts, which is in accordance with results of recent studies from different areas of Iran [22].

This study focused on mutations of the PR gene that confer resistance to ART drugs in patients not previously treated with PIs. The prevalence of transmitted PR resistance-associated mutations in the sample of 25 patients receiving ART in Tehran was investigated and it was found that none of the ART patients had major resistance mutations. This is in agreement with global studies showing minimal prevalence of antiretroviral drug resistance in individuals experiencing ART [23–25].

No major mutations associated with drug resistance were observed in this study, although other studies performed in Iran