

Drug-selective pressure effect on HIV integrase mutations in antiretroviral naïve and experienced patients

Parya Basimi¹, Molood Farrokhi², Maryam Ghanbari³, Seyed Ali Dehghan Manshadi^{2,4}, Maryam Naghib⁵, Kazem Baesi¹

¹Hepatitis and AIDS Department, Pasteur Institute of Iran, Tehran, Iran

²Iranian Research Center for HIV/AIDS, Iranian Institute for Reduction of High Risk Behaviors, Tehran University of Medical Sciences, Tehran, Iran

³Department of Microbial Biotechnology, Faculty of Biological Science, Tehran North Branch, Islamic Azad University, Tehran, Iran

⁴Department of Infectious Diseases and Tropical Medicine, Tehran University of Medical Sciences, Tehran, Iran

⁵Department of Microbiology, Kerman Branch, Islamic Azad University, Kerman, Iran

Abstract

Introduction: Resistance to antiretroviral drugs is a serious problem often related to selective drug-induced pressure and sub-optimal drug dosing. This study aimed to investigate drug resistance-associated mutations in human immunodeficiency virus type 1 (HIV-1) integrase gene caused by the drug pressure of reverse transcriptase inhibitors (RTIs) and protease inhibitors (PIs).

Material and methods: For this purpose, RNA of 50 HIV-infected patients (25 drug-naïve patients and 25 patients under antiretroviral therapy [INI naïve]) was extracted and one step RT-nested PCR was carried out on HIV integrase (IN). Then, gene sequences were analyzed to determine sub-types and antiretroviral resistance-associated mutations (RAMs).

Results: Phylogenetic analysis revealed that recombinant sub-type CRF35-AD was the most prevalent in all patients (87.2%), followed by A1 sub-type (12.8%). Among the 25 ART-experienced patients, two mutations (N155I, G163R) associated with resistance to integrase inhibitors (INI) were found. Among the 25 naïve patients, several polymorphisms were observed, which was also lower in this group than in the ART group.

Conclusions: The results of this study indicated that the integrase mutations can be caused by the effect of selective pressure induced by antiviral agents, such as RTIs and PIs. Therefore, examination of the integrase drug resistance mutations is recommended before starting treatment in Iran.

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Key words: HIV, integrase drug resistance mutation, selective pressure, treatment failure, phylogeny.

Introduction

Combination of various antiretroviral drugs (ART) used against human immunodeficiency virus (HIV) has a significant effect on the reduction of morbidity and mortality rates caused by HIV replication. However, due to genetic diversity and im-

perfect viral suppression of the virus, it results in augmentation of resistant viruses, which are not only dangerous for individuals, but also put at risk the entire global population [1, 2].

HIV medicines are divided into several various drug classes based on their function and how they battle against HIV.

Address for correspondence: Kazem Baesi, Hepatitis and AIDS Department, Pasteur Institute of Iran, Tehran, Iran, e-mail: k_baesi@pasteur.ac.ir

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In Iran, most commonly used classes are nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs), and integrase inhibitors (INIs). A recommended preliminary ART regimen option is a combination of three HIV drugs. Due to mutations, genetic diversity occurs in gene of reverse transcriptase, protease, and integrase (IN) enzymes of HIV, along with elevated turnover of the virus under selective pressure of the drug and immune system [3]. A report on the effect of various HIV sub-types in contributing to disease progression and resistance to ART agents is controversial [4-6].

There are two ways of obtaining resistance to antiretroviral drugs: 1) transmitted drug resistance (TDR) that arises when a person receives a strain of HIV that is already resistant to certain antiretroviral (ARV) drugs, and in fact they transmit the virus; 2) acquired drug resistance (ADR), which develops when HIV mutations appear due to selective pressure of antiretrovirals in individuals receiving ARV drugs [7].

Individuals are resistant in both ways. They are at high-risk for treatment failure if a particular treatment regimen fails to be identified and not changed, and other therapeutic regimens could be due to cross-resistance with other antiretroviral compounds in the same drug class or other drug classes failure [8, 9]. In addition, selective pressure causing resistance mutations in the target of drug genes can also affect the entire HIV genome, and cause polymorphisms and drug resistance mutations in other genes, such as the integrase.

The most important problem in HIV treatment is the presence of human immunodeficiency virus type 1 (HIV-1) strains with a primary drug resistance that is defined as drug resistance in ART medicines from at least two different drug classes [5]. Primary drug resistance is due to drug selective pressure. For example, NRTIs and NNRTIs may cause drug resistance mutations in the integrase gene, while the patient did not receive any integrase inhibitors.

Various research on primary drug resistance in advanced and middle- and low-income countries have shown an estimated 7-17% and 7%, respectively [10, 11].

The aim of this study was to investigate the effect of selective pressure of NRTIs, NNRTIs, and PIs drugs on induction of ART resistance-associated mutations of IN gene among drug-naïve patients and those who have received ART (naïve INI) in Tehran. This study have been conducted to characterize IN genetic mutations in these individuals for the first time in Tehran, Iran. In fact, the results of current analyses could help in the administration of more efficient treatment based on drug resistance tests among HIV-1 positive patients.

In this cross-sectional study, a total of 50 HIV-infected patients, with an average age of 35 to 40, were enrolled. Sample inclusion criteria were determined based on the WHO recommendations and surveillance on HIV drug resistance [12]. From these, 25 patients were placed in a drug-naïve group (ART-naïve), and 25 patients were under ART regimens that consisted of two NRTI plus one NNRTI, and two NRTI plus one PI for at least one year. These two groups were naïve to INI, and their CD4+ T cell counts were ranging between 350 and 500 cells/mm³. Main objectives of the study were explained to patients, and written informed consent were obtained. Patients were recruited from the infectious Disease Division of Imam Khomeini Hospital (a referral hospital located in the capital city of Tehran) for 10 months. Samples were collected between 2018 and 2019. This study was approved by ethics committee of Biomedical Research in Pasteur Institute of Iran (No., ID: IR.PIL.REC.1395.2).

RNA extraction, cDNA synthesis, and RT nested-PCR were obtained. Blood samples were collected in sterile EDTA-containing tubes, and purified viral RNA was extracted from plasma according to manufacturer's protocol (QIAamp viral RNA mini kit; Qiagen, Hilden, Germany), and stored at -70°C to screen for antiretroviral drug resistance mutations.

Material and methods

First round of cDNA synthesis

PCR was carried out using Qiagen one step RT-PCR kit (Qiagen, Hilden, Germany) as indicated by the manufacturer. Two parts of HIV IN gene were amplified by nested RT-PCR following standard procedures. Briefly, the HIV IN gene was amplified using the following reaction mixture: 10 µl RNA, 1× PCR buffer from 10×, 200 µ Mof dNTP10 mM, 1.5 units of enzyme (5 units/µl), and 10 pM of each specific primer. RT-PCR was conducted using the following cycling conditions: 50°C for 45 min (cDNA synthesis), initial denaturation at 94°C for 15 min, and 35 cycles for the synthesis of DNA from cDNA, with denaturation at 94°C in 30 s, annealing at 57°C in 30 s, extension at 72°C in 50 s, and the final heating step at 72°C for 5 min. Second round of RT-PCR was carried out with the same temperature programs as the first round.

RT-nested PCR amplification was made to yield a 734-base-pair (bp) product from the viral IN region using two sets of primers (Table 1), which was designed by oligo7 and gene runner Oligo analyzer software from HIV reference sequence (accession No.: NC-001802.1).

Table 1. IN primers

Name	Sequence	Position	Product
F IN 1	5'CAGCACAYAARGGRATTGGAG3'	3705	922
R IN 1	5'CTACYTGCCACACAATCATCAC3'	4605	
F IN 2	5'TAGTAGCYARCTGTGATAAATGTC3'	3882	734
R IN 2	5'ACAATCATCACCTGCCATCTG3'	4595	

A second PCR product was separated on a 1% agarose gel, and electrophoresis of IN gene was done with SYBR Green staining. Finally, these products were excised and decontaminated by a QIAamp gel purification kit (Qiagen, Hilden, Germany), according to manufacturer's instructions.

Sub-type and drug resistance analysis

Samples were loaded onto a sequencer, and sequencing was performed as per manufacturer's instructions and sequenced using Sanger method (ABI PRISM 3700 DNA analyzer automated sequencer; Applied Biosystem, Foster City, CA, USA). IN sequences were analyzed with BioEdit software, version 5.0.6, and the Stanford University HIV Drug Resistance Database was used for drug resistance interpretation. The Stanford and Comet databases were used to predict the sub-type of the viruses (<http://hivdb.stanford.edu/>)(<https://comet.lil.lu/>) [13, 14]. Integrase nucleotide sequences reported in this study have been deposited in the GenBank database under accession numbers of MT360315 and MT360361.

Results

Fifty HIV-positive patients participated in this study, of whom 47 samples were appropriate for sequencing. Most of the patients were males (80%) and between 35 and 40 years old. Reported transmission routes were 66% injection drug users, 24% sex partners, 2% infected through blood products (hemophilia patients), and 8% were of unknown origin (Table 2).

The results – of sub-types of analysis of IN gene indicated that the most common sub-type was CRF 35-AD (87.2%), followed by A1 sub-type (12.8%) [15].

In this study, the prevalence of CRF 35-AD among injecting drug users was 61%, multiple sex partners 29%, and 10% unknown. In the A1-subtype, injecting drug users and hemophilia patients were 83.4% and 16.6%, respectively (Table 2).

Two types of ART regimen were studied in the treatment group, when CD4+ count of an HIV-positive patient was between 350 and 500 cells/ μ l. Most of them (82%) used combined doses of zidovudine (AZT), lamivudine (3TC), and efavirenz (EFV) as the first-line therapy for at least one year. The remainders were using AZT, 3TC, and kaletra (lopinavir/ritonavir). From these groups, nine and five samples were negative for PCR testing, respectively. In the first treatment group, two out of 22 positive patients (9%) were resistant to INI. The N155I mutation was unusual major mutation observed. Generally, the N155H mutation is one of the most important IN gene mutations that cause drug resistance, but in our study, the N155I mutation was revealed as an unusual mutation. This mutation alone cannot result in resistance. Another accessory mutation, G163R, was observed in this group. This mutation is associated with susceptibility to bicitgravir sodium (BIC), dolutegravir sodium (DTG), and low-level resistance to elvitegravir (EVG) and raltegravir (RAL). Additionally, there are multiple polymorphisms in the ART-experienced patients compared to ART-naïve patients.

Table 2. Demographic characters and sub-type profiles of patients

Factor		Sub-types	
		CRF 35-AD	B
Age, years	35-40		
Sex, n (%)			
Male	40 (80)		
Female	10 (20)		
Infection risk factor, n (%)			
Intravenous drug user	33 (66)	25 (61)	5 (83.4)
Sexual contact	12 (24)	12 (29)	–
Blood transfusion	1 (2)	–	1 (16.6)
Unknown	4 (8)	4 (10%)	–
CD4+ cell count, cells/ mm^3 , median (range)	425 (350-500)		

There were no drug resistance mutations in the ART-naïve group in the present study. However, several polymorphisms were observed in different samples, as shown in Table 3. The most common polymorphisms in the IN gene of the ART-experienced group were V126F, T124A, T125A, I113V, I72V, 160M/V, K136Q, and V201I, while in the naïve group, there were D167E, E198D, and Q216H. Polymorphisms information of the IN gene in the study participants are shown in Table 3.

Discussion

The emergence of resistance to antiretroviral drugs remains a critical contributor to the failure of HIV-1 therapy [16, 17]. Due to high mutation rates, mono-therapy and pharmaco-therapy problems, poor drug absorption or insufficient drugs' doses, selective drug-induced pressure, and ultimately, drug-resistant strains, all lead to treatments' failure [18, 19]. Antiretroviral treatment, as a result of selective pressure, leads to decreasing population of susceptible viruses and survival viruses, which have a potential for mutation and recombination. Thus far, few studies have been investigating the role of drug pressure in the appearance of mutations in IN gene globally, and this is the first report on this issue in Iran.

Mantovani *et al.* reported that mutations due to selective pressure on IN were about 10% to 20% in ART-treated groups (without INI), and less than 1% in untreated group [20]. The results of the present study are similar to a previous one, with 10% drug resistance in ART-treated groups (without INI) and less than 1% in untreated group. It showed that the prevalence of resistance in naïve patients was rare [21].

Any resistance is a potential threat, because reduced susceptibility to a specific ARV affects patient care and trans-

Table 3. Different prevalence of IN polymorphisms in HIV-1-infected drug naïve and ARV experience patients (INI-naïve)

Mutation	ARV experience	Naïve
L45I	1	0
A49S	3	0
D55G	1	0
I60M/V	7	3
I72V	7	4
E96D	1	0
I113V	8	4
T124A	8	3
T125A	7	4
V126F	8	3
G134S	4	0
K136Q	7	4
D167E	1	2
E198D	0	1
V201I	7	4
K211R	1	0
Q216H	0	2
T218S	2	0
D270F/V	3	0
S283G	2	0

mission of infection within a population. Resistant patients have a higher risk of mortality and weaker immunological response than other HIV/AIDS patients [22, 23]. In our study, two mutations, N155I and G163R (related to the IN-drug resistance) were detected in the treatment group. In the naïve group, only polymorphisms were observed, which were lower in comparison to the treatment group. It may be concluded that drug resistance mutations in the treated group occurred because of selective pressure of antiretroviral drugs. In this regard, Mantovani *et al.* in 2012 showed selective pressure of drug on raltegravir resistance mutations in patients with failure of treatment, which showed no mutation associated with raltegravir resistance [20]. Silberstein and colleagues examined mutations of IN in two groups of patients (antiretroviral and naïve). Their results showed that M154I and V165I IN polymorphisms occurred in 21.3% and 13.4% of antiretroviral-treated patients, respectively [24].

V126F, T124A, T125A, I113V, I72V, I60M/V, K136Q, and V201I polymorphisms were frequent in the ART-treated group than the control group. As well as this, D167E, E198D, and Q216H polymorphisms were more common in the control group. There is a negative relationship between naïve and ART groups mutations. The presence of mutations in the IN gene in people who are naïve for INI is probably due to indirect selective pressure of RT and protease inhibitors, which target RT and proteases [20].

While viruses are encountered by a drug selective pressure or immune system, drug resistance mutations are naturally occurring. These resistant viruses can spread to community and affect ART treatment in countries, in which ART is used extensively [12].

The limitation of this work was a low number of samples. Moreover, due to the fact that this study was conducted for the first time in Iran, we used a pilot method for sampling, which included another limitation: the lack of access to all clinical data of patients.

Conclusions

The study results indicated that IN mutations may cause the effect of selective pressure induced by antiviral agents, such as RTI and PI. Therefore, examination of the IN-drug resistance mutations is recommended before starting treatment in Iran, which help to select the right therapeutic strategy for Iranian patients.

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Conflict of interest

The authors have no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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