INVESTIGATION OF DRUG-RESISTANCE SUBSTITUTIONS IN HIV-1 RT PROTEIN IN IRANIAN HIV INFECTED PATIE

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ИССЛЕДОВАНИЕ ЗАМЕН, РЕЗИСТЕНТНЫХ К ЛЕКАРСТВАМ, В ОБРАТНОЙ ТРАНСКРИПТАЗЕ ОТ ИРАНСКИХ ПАЦИЕНТОВ С ВИЧ-1

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Abstract:

Background:

Currently, more than 37 million people are living with human immunodeficiency virus type 1. Reverse transcription (RT) is a main part in the life cycle of retroviruses which is responsible for synthesis of DNA complementary to an RNA or DNA template. Recently several inhibitors have been introduced to target RT protein; however, drug resistance is one of the greatest challenges in the improvement of effective treatment for human immunodeficiency virus (HIV) infection. Here, we determined the resistance mutations in the RT gene in treatment failure patients and searched for the dominant subtype among them.

Methods:

HIV viral load and a reverse transcriptase nested polymerase chain (RT-nested PCR) reactions were performed in 15 patients with treatment failure to amplify the RT gene. Drug resistance mutations, as well as the viral subtypes, were analyzed by using several bioinformatics software and online tools.

Results:

The frequency of RT related drug-resistance mutations in patients was 33.3%, among which the major mutation comprised 20% of them occurring in codon 184. Moreover, the results showed that 6.6% and 26.6% of patients were resistant to Non-Nucleoside RT Inhibitor (NNRTIs) and Nucleoside RT Inhibitors (NRTIs), respectively. In addition, the vast majority of samples (12 patients of 15) belonged to subtype CRF35-AD.

Conclusions:

The present study reports updates on the mutations related to RT resistance in Iranian HIV patients receiving treatment, showing that 20% of the samples

contained a high-level of resistance to Lamivudine, and Emtricitabine which should be confirmed for further antiretroviral (AVR) regimens for HIV infected patients. In addition, two new mutations related to resistance to Nevirapine, Doravirine, Zidovudine, and Stavudine were introduced in this investigation.

The present results could be used as predictors on the response to anti-RT, and also highlight the importance of considering the periodic monitoring of HIV resistance test in HIV infected patients.

Keyword: HIV, RT, Drug-resistance

Резюме:

История вопроса:

В настоящее время более 37 миллионов человек живут с вирусом иммунодефицита человека типа 1. Обратная транскрипция (ОТ) является основной частью жизненного цикла ретровирусов, которая отвечает за синтез ДНК, комплементарной РНК или матричной ДНК. Недавно было введено несколько ингибиторов белка-мишени ОТ; однако лекарственная устойчивость является одной из самых серьезных проблем в улучшении эффективного лечения инфекции, вызванной вирусом иммунодефицита человека (ВИЧ). В настоящей работе мы определили мутации устойчивости в гене RT у пациентов с неудачным лечением и провели у них поиск доминантного подтипа.

Методы:

Вирусная нагрузка ВИЧ и реакции вложенной полимеразной цепной реакции с обратной транскрипцией (вложенная ПЦР с обратной транскрипцией) были выполнены у 15 пациентов, у которых лечение не привело к амплификации

гена RT. Мутации устойчивости к лекарствам, а также подтипы вирусов были проанализированы с помощью нескольких программ биоинформатики и онлайн-инструментов.

Полученные результаты:

Частота мутаций лекарственной устойчивости, связанных с ОТ, у пациентов составила 33,3%, среди которых основная мутация составила 20%, происходящих в кодоне 184. Более того, результаты показали, что 6,6% и 26,6% пациентов были устойчивы к ненуклеозидной ОТ. Ингибитор (ННИОТ) и Нуклеозидные ингибиторы ОТ (НИОТ) соответственно. Кроме того, подавляющее большинство образцов (12 пациентов из 15) относились к подтипу CRF35-AD.

Выводы:

В настоящем исследовании представлены обновленные данные о мутациях, связанных с устойчивостью к ОТ у иранских пациентов с ВИЧ, получающих лечение, и показано, что 20% образцов содержат высокий уровень устойчивости к ламивудину и эмтрицитабину, что необходимо подтвердить для дальнейших схем антиретровирусной терапии (АРТ) для ВИЧ-инфицированных пациентов. Кроме того, в это исследование были внесены две новые мутации, связанные с устойчивостью к невирапину, доравирину, зидовудину и ставудину.

Настоящие результаты могут использоваться в качестве предикторов ответа на анти-ОТ, а также подчеркивают важность рассмотрения периодического мониторинга теста на устойчивость к ВИЧ у ВИЧ-инфицированных пациентов.

Ключевое слово: ВИЧ, ОТ, лекарственная устойчивость

Introduction:

Acquired immune deficiency syndrome (HIV/AIDS) has started to spread since 1970s rapidly and became a mysterious pandemic in the 1980s; it was revealed that HIV caused AIDS[1, 2]. HIV genome encodes several structural and non-structural proteins, among which a viral DNA polymerase or reverse transcriptase (RT) enzyme is responsible for the synthesis of DNA complementary to an RNA or DNA template[3]. RT plays a critical role in HIV replication immediately, and is considered as a prime HIV inhibitor target; as the first anti-AIDS drug analog, AZT (zidovudine, ZDV) was approved [4]. However, clinical outcomes revealed a single drug treatment was not effective. So far, more than 26 HIV inhibitors have been approved, of which 13 target RT[4]. The availability of antiretroviral therapy (ART) has meaningfully decreased the mortality and morbidity of HIV infection, this treatment cannot eradicate the virus completely[5]. HIV steadily shows a high genetic variability and has a tremendous potential to develop resistance to the existing drugs which make a big concern for health care ; also, drug resistance valuation can be useful to clinicians in their decision about switching ARV regimens when treatment failure is suspected[6].

Bioinformatics tools have been developed during the last decades and have been the main means to analyze different virus genes [7-10].

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. In spite of previous studies on HIV drug resistance mutations in Iran, defining more effective treatment methods always requires updates to the mutations rate in Iranian population and this study aimed to define the drug resistance mutations in the RT gene in Iranian HIV infected patients as well as

HIV subtyping among them by using bioinformatics software and databases. Bioinformatics tools have been developed during the last decades and have been the main means to analyze different virus genes.

2-Material and methods:

Study population

The sera of 15 patients enrolled in this study from 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.

Extraction and Real time

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

PCR and Sequencing

The primers listed in Table 1 were employed to amplify the RT gene. Thermal-cycling conditions in the first round PCR are shown in Table 2. 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, the subtypes of the sequences were identified.

Results:

Demographic data

A total of 15 patients were enrolled in the present study, including 6 (82.8%) males and 9 (17.2%) females, with a mean age of 42.3 ± 10.7 years (range, 19-53 year). The mean of viral load among the enrolled patients was 430000.

Amino acid Changes

In comparison with the reference sequence (CAA12685), several mutations were found listed in Table 3; the most prevalent mutations occurred in positions 170, 180, and 195 with 33%. Stanford University HIV Drug Resistance Database showed low-level to high-level resistance to different HIV inhibitors which summarized in table4. Subtyping results showed that 12 of 15 samples belonged to CRF35-AD and only 3 samples were A1 (Table 5).

Discussion:

The results of the present investigation showed the high-level resistance to HIV inhibitors in 4 samples. Sample 135 harbored a mutation in 227 which showed high-level resistance to Nevirapine and Doravirine and sample 154 contained mutations in positions 184, and 215 which led to high resistance to Lamivudine, Emtricitabine, Zidovudine, Stavudine. In addition, Samples 84 and 20 showed a high-level resistance to Lamivudine, Emtricitabine and low-level resistance to Abacavir, and Didanosine. Results showed that around 33.3% of the samples had at least one drug resistance mutation, and mutation in codon 184 was the most prevalent (20%) drug resistance mutation among the samples, which is the most important NRTI resistance mutation.

Similar to our study, Sadeghi et al. have examined 15 Iranian infected patients and they found a major drug resistance mutation in codons 184(73%)[11]. In contrast to the present result, Sadeghi et al. found the mutation in codon 103 as well. By considering this fact that both studies enrolled the same number of patients, the different geographical regions may be the reason for the different results.

We did not find mutation in codons 103, 225, and 138, and prevalence of mutations in codon 184 was 20%, which showed higher prevalence than Gol Mohammadi's study[12]. The observed difference between the present and Gol Mohammadi's research may be related to the number of patients enrolled in each study which was significantly higher in their study.

In another study, Farrokhi et al. conducted another study on 90 naïve HIV-infected patients in Iran and they could define several drug resistance mutations in the RT gene[13]. The most prevalent mutations were in three positions, K103N, E138A, and M184V/I. Mutation in codon 184 was related to high resistance to Amivudine and Emtricitabine and mutations in position 103 was related to a high level of drug resistance to Efavirenz and Nevirapine. Similar to our findings, in just one sample mutation in codon 215 was found. In addition, in our study the prevalence of substitution in codon 184 was 20 (3 samples) and this mutation has a prevalence around 3% (2 of 90 patients). Different results between the two studies may be related to the difference in the number of patients and the fact that we studied on treatment failure patients and in Farrokhi's study the patients were selected from naïve HIV-infected patients. In comparison with the present study, another study by Farrokhi showed the higher prevalence of mutation in codons 184, 215[14]. By considering that in Farrokhi's study the number of enrolled patients was 51 and it

was significantly higher than the present study, the difference in results may be rooted in this fact.

Similar to the present study, Gol Mohamadi et al. showed similar results of resistance of the mutations related to NRTIs, but higher resistance was shown found to NNRTIs[12]. In the present study, 26.6% of the samples showed the low to intermediate resistance to Abacavir which was higher than Gol Mohammadi's study; also, in our study, 20% showed resistance to Lamivudine, and Emtricitabine which was nearly similar to Gol Mohammadi's study. In Gol Mohammadi's study, they found Abacavir resistance in one sample and resistance to Lamivudine and Emtricitabine was around 15.5%.

By considering the present results, the findings of the mentioned studies as well as the results of two studies conducted by Mohraz et al. in 2018 [15] and Davarpanah et al. in 2017 [16], it would be logical to conclude that mutation in codon 184 is the most prevalent mutation in Iranian patients which frequently showed the high level of resistance to important HIV inhibitors Lamivudine, and Emtricitabine.

Subtyping results by using three reliable software showed that the dominant subtype among the samples was CRF35-AD (80%) which was similar to several previous studies on Iranian patients[17, 18]. Furthermore, Rolland et al. enrolled 3840 patients from the Middle East and North Africa in 2015, and their results suggested the subtype CRF35 AD was dominated in Iran and Afghanistan[19].

However, in some studies (Gol Mohammadi et al., Naderi et al., and also Baesi et.al), A1 was introduced as the dominant subtype in Iran[12, 20-22].

In addition, in a few studies, B was suggested as the main subtype[22]. The difference in results can be due to several factors, such as different genome regions

and different subtyping tools used by researchers, which may result in moderate diversity in Iranian HIV subtypes in recent years.

Our findings showed a high rate of resistance to two important HIV inhibitors, Lamivudine, and Emtricitabine, in Iranian patients, as indicated in previous studies, and it can be suggested that the mutation in codon 184 needs to be checked before suggesting AVR regimen for Iranian HIV infected patients. Furthermore, our findings presented new mutations in Iranian patients (in Codons 115, and 227) which caused a high-level resistance to Nevirapine, Doravirine, Zidovudine, and Stavudine though the rate of the mentioned mutations did show the high prevalence.

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Compliance with Ethical Standards

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Conflict of Interest: Author Behzad Dehghani declares that he has no conflict of interest. Author Tayebeh Hashempour declares that she has no conflict of interest. Author Zahra Hasanshahi declares that she has no conflict of interest. Author Esmaeil Rezaei declares that he has no conflict of interest. Author Javad Moayedi declares that he has no conflict of interest. Author Zahra Mousavi declares that she

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Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

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ТАБЛИЦЫ/ TABLES

Tables:

Table 1: The list of primers used in this study

		Primers	PCR products length
Outer pair	Forward	ACACCTGTCAACATAATTGG	
	Reverse	CTATTAACTCTTTTGATGGGTC	810
Inner pair	Forward	GTTAAAGCCAGGAATGGATGG	
	Reverse	TTCTGTATATCATTGACAGTCCAG	750

Table 2: Thermal-cycling conditions of PCR

	Temperature (C°)	Time	Cycles
Initial denaturation	94	5 Min	1
Denaturation	94	30 Sec	
Annealing	50	30 Sec	
Extension	72	50 Sec	30

Table 3: List of mutation found in samples sequences in comparisons with reference sequence.

Mutations		Mutations	Prevalence	Mutations	Prevalence
M 9 T	1	S 156 I	1	F 207 L	1
I 24 L	1	M 157 K	1	Y 208 T	3
T 28 M	1	K 159 R	1	Y 208 P	1
A 32 T	1	K 159 N	1	P 210 R	1
I 43 T	1	K 166 T	1	D 211 E	1
V 53 I	2	D 170 E	5	D 211 A	1
N 60 D	2	V 172 I	4	D 211 Q	1
T 62 N	1	Q 175 H	1	Q 212 K	3
G 63 K	2	Y 176 F	1	Q 212 H	1
I 85 L	1	M 177 V	1	Q 212 R	1
I 85 V	1	D 179 F	1	K 213 S	1
A 91 P	1	I 180 L	5	K 213 L	1
K 95 Q	1	V 182 A	1	H 214 I	1
K 96 N	1	G 183 R	1	H 214 R	1
D 106 E	1	M 184 T	1	Q 215 R	1
A 107 G	1	T 193 I	2	T 215 Y	1
P 112 S	1	T 193 R	1	K 216 N	1
D 114 Y	3	V 195 I	5	E 217 N	1
Y 115 F	2	I 198 L	4	E 217 L	1
E 120 K	1	I 198 M	1	P 218 H	1
T 121 N	3	Q 200 A	3	P 218 L	2
I 125 L	1	Q 200 D	1	P 219 Q	1
I 128 T	2	Q 200 N	1	P 219 F	1

I 128 R	1	I 202 L	2	F 220 I	1
I 135 N	1	I 202 R	1	I 221 L	4
Y 137 D	1	I 203 L	4	I 221 H	1
Q 138 H	1	I 203 V	1	M 223 D	1
Y 139 N	1	R 204 K	4	Y 225 F	1
I 142 L	4	R 204 S	1	F 227 L	1
W 146 R	1	W 205 G	1	W 232 L	1
K 147 N	1	G 206 D	1	T 233 Q	1
V 234 G	1				
V 234 Y	1				
Q 235 S	1				

Table 4: The subtyping results for 15 samples by using 3 reliable software.

Samples	**Subtype**
8	CRF35_AD
9	CRF35_AD
11	CRF35_AD,
14	A1
16	CRF35_AD
Rn	A1
150	CRF35_AD
135	A1
136	CRF35_AD
154	CRF35_AD
40	CRF35_AD
56	CRF35_AD
84	CRF35_AD
17	CRF35_AD

20	CRF35_AD

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Table 5: The list of drug resistance mutations in 15 sample sequences by using

Samples	NVP (Nevirapine) NNRTI	DOR (Doravirine) NNRTI	ABC (Abacavir) NRTIs	TDF (Tenofovir) NRTIs	3TC (Lamivudine) NRTIs	FTC (Emtricitabine) NRTIs	AZT (Zidovudine) NRTIs	d4T (Stavudine) NRTIs	ddI (Didanosine) NRTIs
135	high-level (F227L)	high- level (F227L)							
136									
150			intermediate resistance (Y115F)	low-level (Y115F)					
154			low-level (T215Y)	low-level (Y115F)	high-level (M184V/I)	high-level (M184V/I)	high-level (T215Y)	high-level (T215Y)	low-level (T215Y)
11									
14									
16									
8									
9									

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RN									
40									
56									
84			low-level (M184V/I)		high-level(M184V/I	high-level (M184V/I)			low-level (M184V/I)
17									
20			low-level (M184V/I)		high-level (M184V/I)	high-level (M184V/I)			low-level (M184V/I)

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