

Setup and optimization of primer for protease gene of human immunodeficiency virus-1 as evaluation drug resistance manner in Iranian patients

Abstract

Human immunodeficiency virus (HIV-1) is a problem of global health. After the development of anti-retroviral therapy, the mortality and morbidity rate was reduced in whom was infected with HIV-1. However, because of many reasons such as virologic, immunologic, HAART regimen has a different result in HIV-1 infected patients. CD4 T Lymphocyte count and viral load test confirmed and showed that in some patients have treatment failure and need to analysis HIV-1 drugs target sequence and review in the administration of HAART regimen. There is some commercial kit for HIV-1 genotyping. Beside of these commercial kits, ANRS designed and released primer sets for amplification and sequencing of the HIV-1 genes such as Pol, Env. In the present study, protease sequence in the blood samples of 20 patients was amplified and investigated with in-house developed primer. These protease sequences were not amplified by ANRS primers.

Keywords: hiv-1, primer, PCR, ANRS, drug resistance

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Introduction

In the early 1980s, a new disease emerged among Men who have sex with men (MSM). This unknown origin infection was followed by the unusual opportunistic illnesses along with rare malignancies such as Kaposi sarcoma, lymphoma.^{1,2} Human immunodeficiency virus type 1 (HIV-1) was known as a predisposing factor for opportunistic infection in homosexual patients.^{3,4} By medical management of HIV-1 infected patients with antiretroviral drugs, replication of HIV-1 significantly can receive to the undetectable levels.^{5,6} There is a different kind of approved antiretroviral medicines available to prescribe in the world.⁷ About 10⁴ to 10⁵ copy/ml virus present in plasma samples derived from naïve/untreated patients.⁸ Reverse transcriptase (RT) of HIV-1, RNA virus, has no proofreading ability. This error-prone transcription activity can generate one to 10 mutations in each viral replication cycle.⁹ Antiretroviral therapy considerably decreases selection of virus clones.¹⁰ Increasing the viral load up to 1000 copy/ml is a sign of treatment failure and evaluation of drug resistance pattern is essential in the patients who are under HAART treatment.

There are many commercial HIV genotyping kits for evaluation of drug resistance.¹¹⁻¹³ However, these kind of commercial kits are expensive, less sensitive to non-B subtypes and they are not accessible in some countries. For these reasons, some reports have suggested setup and optimization in-house kit for evaluation mutation that occurs in the genome of virus.¹⁴⁻¹⁶

Material and methods

Primer design

The HIV-1 Reference sequences were downloaded from HIV database. A set of primer were designed and validated within the region of the protease gene of HIV-1 (from codon 1-95) by oligo7 and primer-primer 6 softwares Table 1. At first, the sequence of primer

bioinformatically analyzed in NCBI database in order to evaluation of specifying of primers. Finally, the designed primers were synthesized by Macrogen Company (South Korea).

Table 1 Sequence of forward and revers primer

Forward primer sequence	CTCAAATCACTCTTTGGCAACG
Reverse primer sequence	CATTCTGGCTTTAAYBTACTGG

Sample collection

A blood sample was taken from HIV each patients who were referred to Iranian research center for HIV and AIDS (IRCHA) for drug resistance test. A plasma sample from each patient was collected into the defined tube and stored in -70c until extraction HIV RNA.

RNA extraction

RNA extraction performed with QIAamp® Viral RNA kit (Qiagen, Germany according to the protocol of kit). After RNA extracted, RNA kept in -70c till PCR reaction.

Polymerase chain reaction

Target sequence amplified with Qiagen one-step RT-PCR kit according to the following gradient cycle, 50°C 50min (cDNA synthesis), and 95°C 15min. PCR reaction performed with 95°C 30sec, 60°C 45sec, and 72°C 1min for 40 cycles with Bio-Rad PCR machine Table 1. The PCR product analyzed with 1% gel agarose along with 100bp ladder (thermo life technology) and then sequenced via Sanger Sequencing method (Macrogen Company, South Korea). The results of sequencing analyzed in Stanford HIV drug resistant database for evaluation of drug resistance pattern.

Results

Analysis of primer sequence in 23 reference sequence confirmed that this primer know all this sequence especially CRF-AD35 which

is dominant subtype among Iranian patients (Figure 1 & Figure 2). 18/20 patients who have viral load above 1000copy/ml that they couldn't amplify with ANRS protease primer was amplified with

this set primer and PCR products analyzed on 1% gel agarose and sequenced by Sanger sequencing method (Figure 3 & Figure 4).

DNA Sequences	Translated Protein Sequences
Species/Abbrev	Group Name
1. Ref. A1. AU. 03. PS1044 Day0. DQ676872	
2. Ref. A1. RW. 92. 92RW008. AB253421	
3. Ref. B. TH. 90. BK132. AY173951	
4. Ref. C. ET. 86. ETH2220. U46016	
5. Ref. D. CD. 83. ELI. K03454	
6. Ref. F2. CM. 97. CM53657. AF377956	
7. Ref. G. BE. 96. DRCBL. AF084936	
8. Ref. 01. AE. AF. 07. 569M. GQ477441	
9. Ref. 01. AE. TH. 90. CM240. U54771	
10. Ref. 12. BF. AR. 99. ARMA159. AF385936	
11. Ref. 15. 01B. TH. 99. 99TH. R2399. AF530576	
12. Ref. 16. A2D. KR. 97. 97KR004. AF286239	
13. Ref. 17. BF. PE. 02. PE02. PCR0155. EU581828	
14. Ref. 21. A2D. KE. 91. KNH1254. AY945737	
15. Ref. 28. BF. BR. 99. BREPM12609. DQ085873	
16. Ref. 29. BF. BR. 02. BREPM119. AY771590	
17. Ref. 29. BF. BR. 99. BREPM11948. DQ085871	
18. Ref. 35. AD. AF. 05. 05AF026. EF158043	
19. Ref. 35. AD. AF. 05. 05AF094. EF158040	
20. Ref. 35. AD. AF. 05. 05AF095. EF158041	
21. Ref. 38. BF1. UY. 05. UY05. 4752. FJ213780	
22. Ref. 40. BF. BR. 04. 04BRRJ115. EU735538	
23. Ref. 44. BF. CL. 00. CH80. FJ358521	

Figure 1 HIV-I forward primer location.

Species/Abbrev	Group Name
1. Ref. A1. AU. 03. PS1044 Day0. DQ676872	
2. Ref. A1. RW. 92. 92RW008. AB253421	
3. Ref. B. TH. 90. BK132. AY173951	
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21. Ref. 38. BF1. UY. 05. UY05. 4752. FJ213780	
22. Ref. 40. BF. BR. 04. 04BRRJ115. EU735538	
23. Ref. 44. BF. CL. 00. CH80. FJ358521	

Figure 2 HIV-I revers primer location.

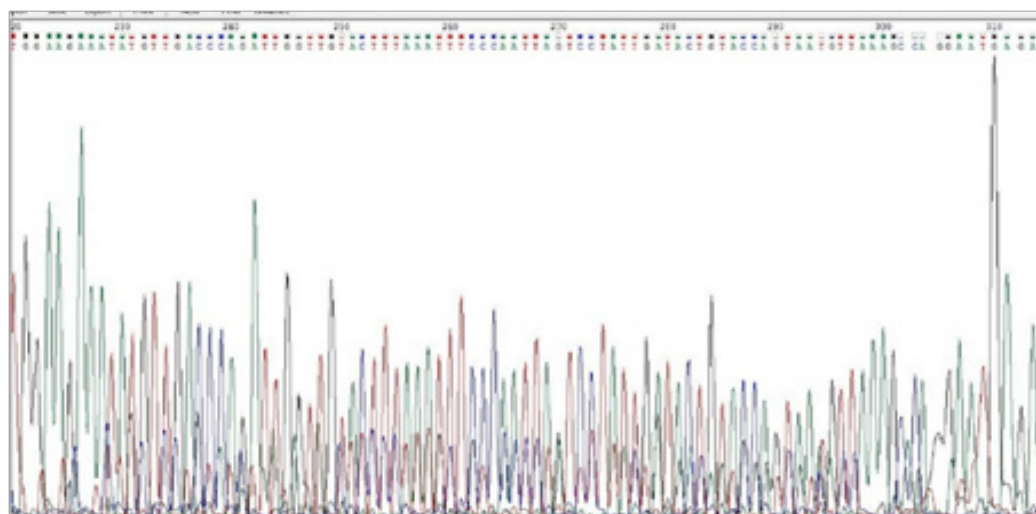


Figure 3 Sequencing result.

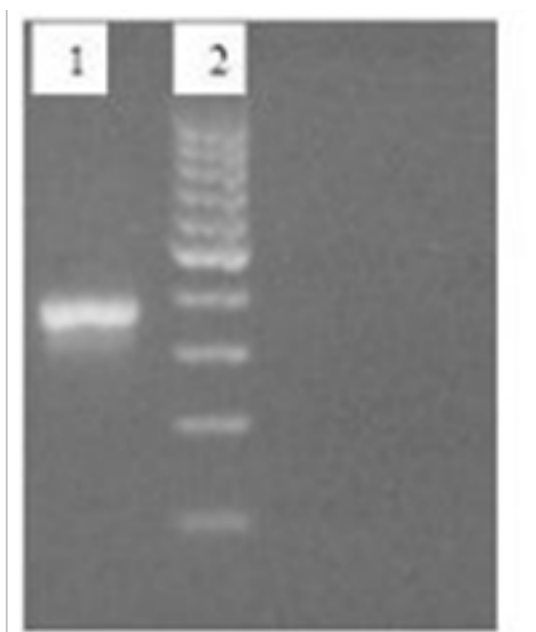


Figure 4 Gel agarose result, lane 1, 344bp specific band and lane 2, 100pb ladder.

Discussion

When the first HIV-1 pandemic occurred (group M) nearly 60 million people were infected and more than twenty-five million of infected patients were died.¹⁶ The combination of retroviral therapy, HAART, had significantly reduced morbidity and mortality rate that was associated with HIV-1 infection^{17,18} However, for many reasons such as virologic, immunologic and clinical responses patients are at risk for treatment failure.¹⁹ Virology failure may be occur because of lack of response to HAART or mutation that occur in the present of drug pressure. Evaluation of drug resistance is important for management and follow-up of infected patients.²⁰ There is some in-house developed method for amplification and analysis of HIV sequence in the patients who have treatment failure.¹⁵ In our study, 20 patients have treatment failure and did not amplify by ANRS protease primer.¹⁵ Based on our results, 18 of 20 were amplified with our in-house designed set primer by one round PCR reaction instead of nested PCR. Finally, Sensitivity of this primers is more than ANRS protease primer. It may be because of special Iranian dominant subtype i.e. CRF-AD35²¹ and our primer specially designed for this kind of subtype.

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None.

Conflict of interest

Author declares that there is no conflict of interest.

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