



A Surveillance on Protease Inhibitor Resistance-Associated Mutations Among Iranian HIV-1 Patients

Zeynab Nasiri-Tajabadi¹, Farah Bokharaei Salim², Mohammad-Javad Najafzadeh^{3,4}, Saeed Kalantari⁵, Saba Garshasbi⁶, Saeid Amel Jamehdar^{1,7}, Hadi Farsiani^{1,7}, Zahra Mazaheri¹, Mojtaba Sankian⁸ and Masoud Youssefi^{1,7,*}

¹Department of Microbiology and Virology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

²Department of Virology, Iran University of Medical Sciences, Tehran, Iran

³Department of Parasitology and Mycology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

⁴Cancer Molecular Pathology Research Center, Ghaem Hospital, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

⁵Departments of Infectious Diseases and Tropical Medicine, Iran University of Medical Sciences, Tehran, Iran

⁶HIV Laboratory of National Center, Deputy of Health, Iran University of Medical Sciences, Tehran, Iran

⁷Antimicrobial Resistance Research Center, Mashhad University of Medical Sciences, Mashhad, Iran

⁸Immunology Research Center, Avicenna Research Institute, Mashhad University of Medical Sciences, Mashhad, Iran

*Corresponding author: Masoud Youssefi, MD, PhD, Central Lab., Imam Reza University Hospital, Imam Reza Sq., Mashhad, Iran. Tel: +98-5138022205, E-mail: youssefim@mums.ac.ir

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Abstract

Background: Drug resistance is emerging as one of the greatest challenges in the development of effective treatment for HIV infection. The importance of clinical studies in this field stems from the world wide growing of treatment drug-resistant mutations.

Objectives: This study was performed to determine the HIV subtype and the resistance mutations to the protease inhibitors in both untreated HIV patients and patients under treatment with protease inhibitors (PIs) in Iran.

Methods: The study was conducted on two groups of participants. The first group consisted of 25 HIV patients who did not receive any antiretroviral treatment. The second group included 25 HIV patients who have being treated with a combination of protease inhibitors. After genome extraction, a nested polymerase chain reaction was performed to amplify the protease gene. Upon confirmation using electrophoresis, the amplicons or PCR products were sequenced and analyzed to determine the drug resistance mutations as well as the viral subtypes.

Results: No mutations were found in the first group; however, 32% of the samples in the second group had PI related drug-resistance mutations. The major mutations were V82A and M46I, which were seen in 12% of the samples, while the minor mutation F53L was seen in 16% of the samples. The subtype analysis showed that 94% of the samples were subtype CRF35_AD, and 6% were of defined as subtype A.

Conclusions: The present study reports updates on the mutations related to protease resistance in Iranian HIV patients receiving treatment. Our data, as well as existing reports, support the need for the optimization of treatment to prevent emergence of resistant viruses and a search for new antiretroviral drug candidates for HIV patients.

Keywords: Mutation, Drug Resistance, HIV, Protease Inhibitor

1. Background

HIV-related death rates have been reduced significantly due to highly active anti-retroviral therapy regimen (HAART) (1). However, the efficiency of antiretroviral therapy (ART) is often limited due to the emergence of drug resistant HIV strains (2, 3). HIV-1 resistance to antiretroviral drugs is a common problem that limits the effectiveness of ART (4). Drug selection pressure (acquired resistance) or person-to-person transmission (transmission resistance) are two pathways leading to drug resistant HIV-1 patient

(5). In developing countries, the main reasons for the occurrence of drug resistance are financial constraints; a lack of testing to detect HIV drug resistance mutations (DRMs) before treatment; an interruption of ART because of disruptions in the supply of anti-HIV drugs; and the inappropriate administration of antiretroviral regimens (6). Currently, it is highly recommended that a drug resistance test be used to detect resistance to anti retrovirals when monitoring HIV treatment and to make the appropriate changes in the medication regimen (7). The current methods for testing HIV drug resistance include phenotypic drug sus-

ceptibility tests for measuring in vitro HIV-1 drug inhibition and genetic tests for identifying the mutations that lead to drug resistance (8).

The anti-HIV-1 drugs are available in six approved classes, including nucleoside and nucleotide reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs), fusion inhibitor, CC chemokine receptor 5 antagonist and integrase inhibitor (5). The protease, enzyme code 3.4.23.16, is a homodimer aspartyl enzyme whose subunits have 99 residues separately. During viral maturation in HIV-1 replication cycle, the protease enzyme is responsible for breaking selectively and proficiently the peptide bonds in the viral polyproteins of *gag* and *pol* genes (9). Data on the structure and function of the HIV protease enzyme and the experimental settings to check the actions of other aspartyl protease inhibitors have been fundamental in designing new inhibitors for HIV-1 protease enzyme (10). As has been mentioned extensively in clinical trials and cohort studies (11), the use of PIs in combination with retroviral drugs leads to an increased suppression of the HIV-1 virus (12, 13). Therefore, HIV-1 protease (PR) is a key target in management of HIV-1 infected patients. As one of the most important drug targets, the PR is exposed to strong selective pressure during ART treatment. The resistance to protease occurs by the accumulation of certain major or minor DRMs (14). Mutations are classified into major mutations, which develop extreme resistance to PIs, and minor mutations, which cause low-level resistance to PIs (15).

2. Objectives

The present study was conducted to obtain the complete sequence of the protease gene and to detect the associated DRMs in treated and untreated Iranian patients infected with HIV.

3. Methods

3.1. Study Population

This cross-sectional study was conducted on the blood samples of 50 HIV-1 positive patients sampling was performed by Mashhad and Tehran health centers. Patients were divided into 2 groups. The first group consisted of 25 HIV patients who did not receive any treatment. The second group included 25 HIV patients who had received a PI regimen, specifically Kaletra (Lopinavir and Ritonavir), Atazanavir, Ritonavir, or Darunavir for at least 1 year; the CD4 count had been determined by flow cytometry.

3.2. Measurement of Laboratory Parameters

Peripheral blood mononuclear cells (PBMCs) were collected from blood samples using the Ficoll-Paque method. Proviral DNA was extracted using the Genet Bio kit (Seoul, South Korea) according to the manufacturer's instructions. A nested polymerase chain reaction (Nested PCR) was performed in two rounds. In the first round, 2.5 μ L of 10X PCR buffer, 2 μ L of 25 mmol/L $MgCl_2$, 0.5 μ L of 2.5 mmol/L dNTP, 1 mmol/L of 10 pmol/ μ L outer primers, 0.3 μ g of Taq DNA polymerase (Genet Bio, Seoul, South Korea), and 3 μ L of extracted DNA provirus were mixed together, and distilled water was added to a final volume of 25 μ L. Tubes were placed in a thermocycler machine (Applied Biosystems, Foster City, CA, USA) with the following cycling program: denaturation at 95°C for 3 min, 30 cycles of denaturation at 94°C for 30 s, annealing at 56°C for 40 s, extension at 72°C for 35 s, and the final extension of strands at 72°C for 3 min. The second amplification using inner primers was similar to the first one except that only 1 μ L of PCR product was used and the following cycling program was used: denaturation at 94°C for 4 min, 30 cycles of denaturation at 94°C for 30 s, annealing at 58°C for 40 s, extension at 72°C for 40 s, and 3 min final extension step of at 72°C. We used previously published primers to amplify PR gene (16). The sequence of the primers used in this study is presented in Table 1. The length of the final product was 424 nucleotides. Paired-end sequencing was performed using the genetic analyzer ABI 3130xl (Applied Biosystems, USA).

Table 1. List of Primers Used in HIV-1 Drug-Resistance Testing (16)

Name	Primer Direction	Nucleotide Sequence (5' to 3')
DRPRO5	Forward (external)	AGA CAG GYT AAT TTT TTA GGG A
DRPRO2L	Reverse (external)	TAT GGA TTT TCA GGC CCA AIT TTT GA
DRPRO1M	Forward (internal)	AGA GCC AAC AGC CCC ACC AG
DRPRO6	Reverse (internal)	ACT TTT GGG CCA TCC ATT CC

3.3. Drug Resistance Analysis

Chromas 2.6.2 software was used to read sequenced nucleotides. The sequences were analyzed and interpreted by the Stanford University HIV Drug Resistance Database (HIVdb) [http://hivdb.stanford.edu] (17) to determine DRMs and viral subtypes.

3.4. GenBank Accession Numbers of PR Sequences

The nucleotide sequences of the PR sequences of HIV received from this study (424 bp) were submitted to the Gen Bank database and are accessible under the accession numbers MG971405 to MG971420.

3.5. Ethical Considerations

The research was performed totally in adherence with the principles of the Helsinki declaration on human research. Prior to the study, the Medical Ethics Committee of Mashhad University of Medical Sciences reviewed and approved study protocol (IR.MUMS.fm.REC.1395.105). The study objectives were presented to all participating patients, who were then complete a written consent form.

3.6. Statistical Analysis

Descriptive analysis (frequency, percentage, mean, standard deviation) were performed with Statistical Package for Social Science version 11.5.

4. Results

Patients who participated in this study aged ranging from 11 - 57 years, (mean age of 40.1 years \pm 10.5 years, median 41 years). 33 out of the 50 participants, (66%) were male and 17 (34%) were female. The mean count of CD4 cells in the samples was 335.6 ± 304.3 cells/mm³ ranging from 50 - 1324 cells/mm³ (median of 284 cells/mm³). Based on the data recorded in the patient files, 52% of the HIV patients were infected by drug abuse, 32% by sexual contact, 4% by infected blood products, 6% by mother-to-child transmission, and 6% by an undisclosed mechanism (Table 2).

In the group that had received treatment, 68% (17) of the participants did not have any resistance mutations and, thus, were sensitive to PIs. On the other hand, 32% (8) had at least one resistance mutations to PI drugs. Three individuals had high-level resistance, 2 had moderate-level resistance, 6 had low-level resistance and 4 had potential level resistance. This adds up to 15 because some individuals had multiple resistance level to various drugs. All participants who were not under treatment were susceptible to PIs.

The highest resistance was related to nelfinavir (28%) and the lowest resistance was to both darunavir and lopinavir (12%). The significant major mutations observed in the treated patients that were found to induce drug resistance were M46I and V82A (12%), I50V and I54V (8%), V32I, L54IFV, L76V, and L90M (4%). The minor mutations in these patients that caused resistance were F53L (16%), L33F and L10IV (12%), and Q58E, T74P, and L10F (4%) (Figure 1). The subtype analysis that was performed simultaneously with the analysis of the mutations showed that 94% of the samples were of subtype CRF35_AD, and 6% were of subtype A. The frequency of the resistance levels to PIs is shown in (Figure 2).

5. Discussion

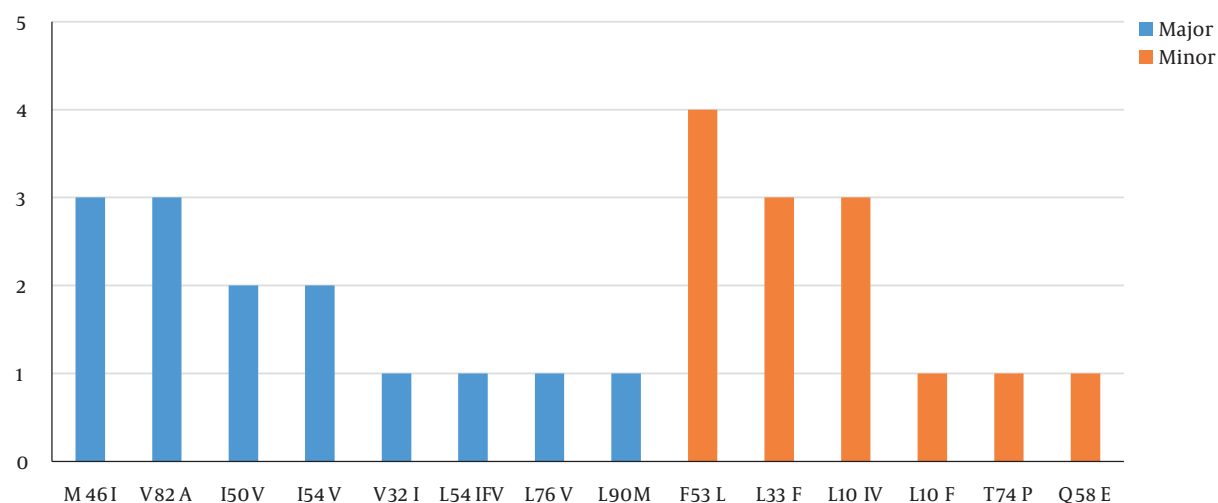
To prevent the transmission of drug-resistant HIV strains, the rapid detection of resistant strains in among patients and the development of strategies to cope with resistant strains circulating in the community are important issues that should be addressed in the public health system. In this study, samples from patients infected with HIV who did not receive any ART were tested for the presence or absence of protease gene mutations associated with drug resistance; the results showed no resistance mutations among these patients. Whereas in the samples from patients infected with HIV who had received PIs, 32% had drug resistance mutations to PI drugs that are available in Iran, including Kaletra (lopinavir and ritonavir), Atazanavir, Ritonavir, and Darunavir. Baesi and Hamkar in Tehran, Iran found that 40% and 45% of samples from HIV patients had resistance mutations to PIs, respectively (18, 19); these results were consistent with the percentage of resistance mutations seen in the present study. However, Naziri in Gorgan, Iran reported that 5% of the samples had PI resistance mutations (20). In this study, the most common PI resistance mutations were at codons 82 and 46 (23%), while the most common PI mutations reported by Baesi and Hamkar were at codon 90 (18, 19). Globally, studies have reported the prevalence of PI-resistant HIV as follows: North America up to 12.9%, Europe (10.9%), Latin America (6.3%), Africa (4.7%) and (4.2%) in Asia (21).

To some extent, all PIs have cross-resistance (22). This means that the resistance mutations that occur in the presence of one PI, reduce the effectiveness of other PIs. There is evidence that HIV strain that has reduced susceptibility to Kaletra (lopinavir and ritonavir) shows a high-level resistance to Indinavir (Crixivan) and Ritonavir, a moderate-level resistance to Amprenavir (Agenerase), while is susceptible to Saquinavir (Invirase) (22). The PI treatment regimen containing Kaletra (lopinavir/ritonavir) is widely used. The World Health Organization (WHO) has recommended the Kaletra regimen to be used as the first-line combination ART for children under three years old infected with HIV in the developing world; however in many cases the Kaletra regime is used as second-line ART (23). To date, decreased susceptibility to Kaletra has been mainly associated with mutations in 46I, 54V, 71V, 82A, and 84V codons (24-26). The Atazanavir resistance can occur due to numerous mutations with different effects; among these I50L, I84V, and N88S cause high level resistance. When ritonavir is used to boost atazanavir, increased number of mutations are required to cause impaired antiviral effect (27). Phenotypic studies on several PI resistance mutations showed that V32I, I50V, I54M, L76V, and V82F largely decreases the susceptibility to Darunavir (28). Ritonavir is

Table 2. Characteristics of HIV-Positive Patients According to Protease Inhibitor (PI)^a

	PI-Naïve (n = 25)	PI-Experienced (n = 25)	Total (n = 50)
Age (y)	41.04 ± 10.63	39.20 ± 10.53	40.1 ± 10.51
CD4 cells	279.3 ± 317.4	395.3 ± 280.0	335.68 ± 304.391
Gender			
Male	16 (64)	17 (68)	33 (66)
Female	9 (36)	8 (32)	17 (34)
Reported transmission route			
Intravenous drug use	11 (44)	15 (60)	26 (52)
Sexual contact	10 (40)	6 (24)	16 (32)
Infected blood products	2 (8)	0	2 (4)
Mother-to-child transmission	1 (4)	2 (8)	3 (6)
An undisclosed mechanism	1 (4)	2 (8)	3 (6)

^aData represented as mean ± SD or frequency (percentage).

**Figure 1.** Frequency of major and minor mutations related to protease inhibitor resistance

not currently used for its own antiviral effect, and instead is mainly used to boost the blood levels of other PIs (29). High-dose ritonavir monotherapy, which was used in the past, caused mutations similar to those associated with Indinavir (30). Our study showed that the mutations associated with Kaletra occurred most frequently at codons 46, 54 and 82. The mutations associated with Atazanavir occurred at codons 46 and 76 and for Darunavir, mutations occurred at codons 32, 50 and 76.

Our findings showed that a vast majority (94%) of studied patients had HIV-1 CRF35_AD subtype. This finding is consistent with previous reports, which recognized the recombinant subtype CRF35-AD as the most prevalent variant in Iran (6, 31-36). The prevalence of CRF35_AD

among HIV patients who were infected by syringe (injecting drugs) has been observed across Iran (6).

Some limitations of the study should be noted. First like many other patients-based studies some lacking data might exist due to the incomplete patients' files. Second we checked genotypically resistant mutations, it would be helpful if we could add phenotypical assays however, we think the present study provides remarkable data on resistance mutations regardless of the applied method and finally larger sample size could definitely improve the study.

5.1. Conclusion

Remarkable treatment outcomes and long-term effectiveness of the antiretroviral drugs among HIV-1 patients

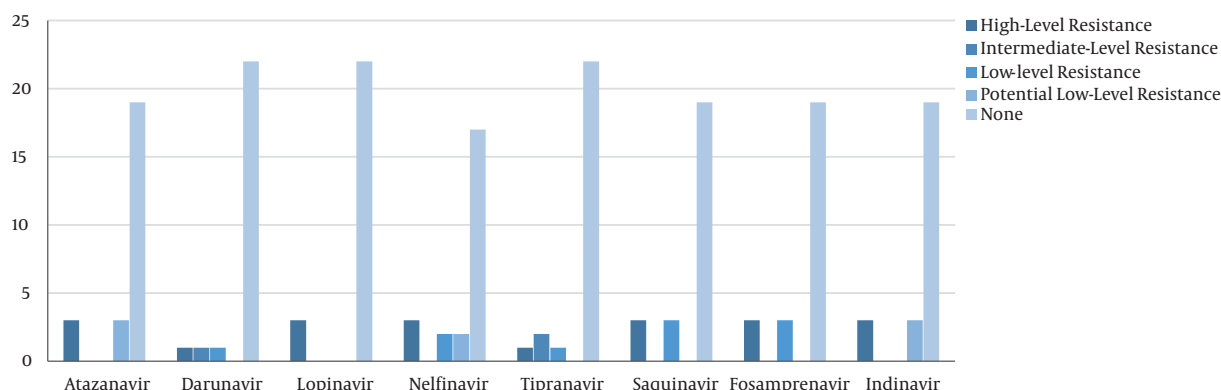


Figure 2. Frequencies of different resistance levels to protease inhibitors

could be achieved by analyzing HIV-1-drug resistance profile before PI treatment onset. High prevalence of drug resistance mutations was reported in our investigation in our HIV patients. According to the present results, it can be concluded that the proper direction of therapeutic plans and restrictions of transmission of resistant variants should be considered as strategies to control and prevent emerging resistant strains.

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Footnotes

Authors' Contributions: Zeynab Nasiri-Tajabadi and Farah Bokharaei Salim contributed equally in this work.

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References

- Bakhouch K, Oulad-Lahcen A, Bensghir R, Blaghen M, Elfilali KM, Ezzikouri S, et al. The prevalence of resistance-associated mutations to protease and reverse transcriptase inhibitors in treatment-naive (HIV1)-infected individuals in Casablanca, Morocco. *J Infect Dev Ctries*. 2009;3(5):380-91. [PubMed: 19759509].
- Pillay D, Green H, Matthias R, Dunn D, Phillips A, Sabin C, et al. Estimating HIV-1 drug resistance in antiretroviral-treated individuals in the United Kingdom. *J Infect Dis*. 2005;192(6):967-73. doi: 10.1086/432763. [PubMed: 16107948].
- Tamalet C, Fantini J, Tourres C, Yahi N. Resistance of HIV-1 to multiple antiretroviral drugs in France: A 6-year survey (1997-2002) based on an analysis of over 7000 genotypes. *AIDS*. 2003;17(16):2383-8. doi: 10.1097/01.aids.0000076341.42412.59. [PubMed: 14571191].
- DiazGranados CA, Mantilla M, Lenis W. Antiretroviral drug resistance in HIV-infected patients in Colombia. *Int J Infect Dis*. 2010;14(4):e298-303. doi: 10.1016/j.ijid.2009.05.006. [PubMed: 19665910].
- Tang MW, Shafer RW. HIV-1 antiretroviral resistance: Scientific principles and clinical applications. *Drugs*. 2012;72(9):e1-25. doi: 10.2165/11633630-000000000-00000. [PubMed: 22686620]. [PubMed Central: PMC3689909].
- Vahabpour R, Bokharaei-Salim F, Kalantari S, Garshasbi S, Monavari SH, Esghaei M, et al. HIV-1 genetic diversity and transmitted drug resistance frequency among Iranian treatment-naive, sexually infected individuals. *Arch Virol*. 2017;162(6):1477-85. doi: 10.1007/s00705-017-3228-1. [PubMed: 28181034].
- Hirsch MS. HIV drug resistance—a chink in the armor. *N Engl J Med*. 2002;347(6):438-9. doi: 10.1056/NEJMe020072. [PubMed: 12167687].
- Shafer RW, Kantor R, Gonzales MJ. The genetic basis of HIV-1 resistance to reverse transcriptase and protease inhibitors. *AIDS Rev*. 2000;2(4):211-28. [PubMed: 19096725]. [PubMed Central: PMC2604813].
- Dayer MR, Dayer MS. Whiskers-less HIV-protease: A possible way for HIV-1 deactivation. *J Biomed Sci*. 2013;20:67. doi: 10.1186/1423-0127-20-67. [PubMed: 24024748]. [PubMed Central: PMC3847613].
- Brik A, Wong CH. HIV-1 protease: Mechanism and drug discovery. *Org Biomol Chem*. 2003;1(1):5-14. [PubMed: 12929379].
- Lima VD, Hogg RS, Harrigan PR, Moore D, Yip B, Wood E, et al. Continued improvement in survival among HIV-infected individuals with newer forms of highly active antiretroviral therapy. *AIDS*. 2007;21(6):685-92. doi: 10.1097/QAD.0b013e32802e330c. [PubMed: 17413689].
- Nachman SA, Stanley K, Yogev R, Pelton S, Wiznia A, Lee S, et al. Nucleoside analogs plus ritonavir in stable antiretroviral therapy-experienced HIV-infected children: A randomized controlled trial. Pediatric AIDS Clinical Trials Group 338 Study Team. *JAMA*. 2000;283(4):492-8. [PubMed: 10659875].
- Eron J Jr, Yeni P, Gathe J Jr, Estrada V, DeJesus E, Staszewski S, et al. The KLEAN study of fosamprenavir-ritonavir versus lopinavir-ritonavir, each in combination with abacavir-lamivudine, for initial treatment of HIV infection over 48 weeks: A randomised non-inferiority trial. *Lancet*. 2006;368(9534):476-82. doi: 10.1016/S0140-6736(06)69155-1. [PubMed: 16890834].

14. Mueller SM, Spriewald BM, Bergmann S, Eismann K, Leykauf M, Korn K, et al. Influence of major HIV-1 protease inhibitor resistance mutations on CTL recognition. *J Acquir Immune Defic Syndr*. 2011;**56**(2):109-17. doi: [10.1097/QAI.0b013e318fe9446e](https://doi.org/10.1097/QAI.0b013e318fe9446e). [PubMed: [21107269](https://pubmed.ncbi.nlm.nih.gov/21107269/)].
15. Rhee SY, Taylor J, Wadhwa G, Ben-Hur A, Brutlag DL, Shafer RW. Genotypic predictors of human immunodeficiency virus type 1 drug resistance. *Proc Natl Acad Sci U S A*. 2006;**103**(46):17355-60. doi: [10.1073/pnas.0607274103](https://doi.org/10.1073/pnas.0607274103). [PubMed: [17065321](https://pubmed.ncbi.nlm.nih.gov/17065321/)]. [PubMed Central: [PMC1622926](https://pubmed.ncbi.nlm.nih.gov/PMC1622926/)].
16. Nii-Trebi NI, Ibe S, Barnor JS, Ishikawa K, Brandful JA, Ofori SB, et al. HIV-1 drug-resistance surveillance among treatment-experienced and -naive patients after the implementation of antiretroviral therapy in Ghana. *PLoS One*. 2013;**8**(8). e71972. doi: [10.1371/journal.pone.0071972](https://doi.org/10.1371/journal.pone.0071972). [PubMed: [23977189](https://pubmed.ncbi.nlm.nih.gov/23977189/)]. [PubMed Central: [PMC3747072](https://pubmed.ncbi.nlm.nih.gov/PMC3747072/)].
17. Liu TF, Shafer RW. Web resources for HIV type 1 genotypic-resistance test interpretation. *Clin Infect Dis*. 2006;**42**(11):1608-18. doi: [10.1086/503914](https://doi.org/10.1086/503914). [PubMed: [16652319](https://pubmed.ncbi.nlm.nih.gov/16652319/)]. [PubMed Central: [PMC2547473](https://pubmed.ncbi.nlm.nih.gov/PMC2547473/)].
18. Baesi K, Ravanshad M, Ghanbari Safari M, Saberfar E, Hajjabdolbaghi M. [Survey of human immunodeficiency virus-1 protease gene drug resistance among HIV-infected patients in an Iranian research center for HIV/AIDS]. *Pathobiol Res*. 2012;**14**(4):13-20. Persian.
19. Hamkar R, Mohraz M, Lorestani S, Aghakhani A, Truong HM, McFarland W, et al. Assessing subtype and drug-resistance-associated mutations among antiretroviral-treated HIV-infected patients. *AIDS*. 2010;**24 Suppl 2**:S85-91. doi: [10.1097/01.aids.0000386738.32919.67](https://doi.org/10.1097/01.aids.0000386738.32919.67). [PubMed: [20610954](https://pubmed.ncbi.nlm.nih.gov/20610954/)].
20. Naziri H, Tabarraei A, Ghaemi A, Davarpanah MA, Javid N, Moradi A. [Drug-Resistance-Associated Mutations and HIV Sub-Type Determination in Drug-Naive and HIV-Positive Patients under Treatment with Antiretroviral Drugs]. *Med Lab J*. 2013;**7**(3):1-8. Persian.
21. Frentz D, Boucher CA, van de Vijver DA. Temporal changes in the epidemiology of transmission of drug-resistant HIV-1 across the world. *AIDS Rev*. 2012;**14**(1):17-27. [PubMed: [22297501](https://pubmed.ncbi.nlm.nih.gov/22297501/)].
22. Friend J, Parkin N, Liegler T, Martin JN, Deeks SG. Isolated lopinavir resistance after virological rebound of a ritonavir/lopinavir-based regimen. *AIDS*. 2004;**18**(14):1965-6. [PubMed: [15353986](https://pubmed.ncbi.nlm.nih.gov/15353986/)].
23. Grossman Z, Schapiro JM, Levy I, Elbirt D, Chowers M, Riesenber K, et al. Comparable long-term efficacy of Lopinavir/Ritonavir and similar drug-resistance profiles in different HIV-1 subtypes. *PLoS One*. 2014;**9**(1). e86239. doi: [10.1371/journal.pone.0086239](https://doi.org/10.1371/journal.pone.0086239). [PubMed: [24475093](https://pubmed.ncbi.nlm.nih.gov/24475093/)]. [PubMed Central: [PMC3903498](https://pubmed.ncbi.nlm.nih.gov/PMC3903498/)].
24. Mo H, King MS, King K, Molla A, Brun S, Kempf DJ. Selection of resistance in protease inhibitor-experienced, human immunodeficiency virus type 1-infected subjects failing lopinavir- and ritonavir-based therapy: Mutation patterns and baseline correlates. *J Virol*. 2005;**79**(6):3329-38. doi: [10.1128/JVI.79.6.3329-3338.2005](https://doi.org/10.1128/JVI.79.6.3329-3338.2005). [PubMed: [15731227](https://pubmed.ncbi.nlm.nih.gov/15731227/)]. [PubMed Central: [PMC1075714](https://pubmed.ncbi.nlm.nih.gov/PMC1075714/)].
25. Monno L, Saracino A, Scudeller L, Pastore G, Bonora S, Cargnel A, et al. HIV-1 phenotypic susceptibility to lopinavir (LPV) and genotypic analysis in LPV/r-naive subjects with prior protease inhibitor experience. *J Acquir Immune Defic Syndr*. 2003;**33**(4):439-47. [PubMed: [12869832](https://pubmed.ncbi.nlm.nih.gov/12869832/)].
26. Luis Jimenez J, Resino S, Martinez-Colom A, Bellon JM, Angeles Munoz-Fernandez M; Spanish Group of Paediatric H.I.V. Infection. Mutations at codons 54 and 82 of HIV protease predict virological response of HIV-infected children on salvage lopinavir/ritonavir therapy. *J Antimicrob Chemother*. 2005;**56**(6):1081-6. doi: [10.1093/jac/dki356](https://doi.org/10.1093/jac/dki356). [PubMed: [16195257](https://pubmed.ncbi.nlm.nih.gov/16195257/)].
27. Wensing AM, Calvez V, Gunthard HF, Johnson VA, Paredes R, Pillay D, et al. 2017 update of the drug resistance mutations in HIV-1. *Top Antivir Med*. 2017;**24**(4):132-3. [PubMed: [28208121](https://pubmed.ncbi.nlm.nih.gov/28208121/)]. [PubMed Central: [PMC5677049](https://pubmed.ncbi.nlm.nih.gov/PMC5677049/)].
28. Van Marck H, Dierynck I, Kraus G, Hallenberger S, Pattery T, Muyllderms G, et al. Unravelling the complex resistance pathways of darunavir using bioinformatics resistance determination (BIRD). *Antivir Ther*. 2007;**12**. S141.
29. Johnson VA, Calvez V, Gunthard HF, Paredes R, Pillay D, Shafer RW, et al. Update of the drug resistance mutations in HIV-1: March 2013. *Top Antivir Med*. 2013;**21**(1):6-14. [PubMed: [23596273](https://pubmed.ncbi.nlm.nih.gov/23596273/)].
30. Johnson VA, Brun-Vezinet F, Clotet B, Gunthard HF, Kuritzkes DR, Pillay D, et al. Update of the drug resistance mutations in HIV-1: Spring 2008. *Top HIV Med*. 2008;**16**(1):62-8. [PubMed: [18441382](https://pubmed.ncbi.nlm.nih.gov/18441382/)].
31. Jahnbakhsh F, Hattori J, Matsuda M, Ibe S, Monavari SH, Memarnejadian A, et al. Prevalence of transmitted HIV drug resistance in Iran between 2010 and 2011. *PLoS One*. 2013;**8**(4). e61864. doi: [10.1371/journal.pone.0061864](https://doi.org/10.1371/journal.pone.0061864). [PubMed: [23626742](https://pubmed.ncbi.nlm.nih.gov/23626742/)]. [PubMed Central: [PMC3633940](https://pubmed.ncbi.nlm.nih.gov/PMC3633940/)].
32. Baesi K, Moallemi S, Farrokhi M, Alinaghi SA, Truong HM. Sub-type classification of Iranian HIV-1 sequences registered in the HIV databases, 2006-2013. *PLoS One*. 2014;**9**(9). e105098. doi: [10.1371/journal.pone.0105098](https://doi.org/10.1371/journal.pone.0105098). [PubMed: [25188443](https://pubmed.ncbi.nlm.nih.gov/25188443/)]. [PubMed Central: [PMC4154867](https://pubmed.ncbi.nlm.nih.gov/PMC4154867/)].
33. Soheilli ZS, Ataiee Z, Tootian S, Zadsar M, Amini S, Abadi K, et al. Presence of HIV-1 CRF35_AD in Iran. *AIDS Res Hum Retroviruses*. 2009;**25**(1):123-4. doi: [10.1089/aid.2008.0199](https://doi.org/10.1089/aid.2008.0199). [PubMed: [19182925](https://pubmed.ncbi.nlm.nih.gov/19182925/)].
34. Baesi K, Moradbeigi M, Ravanshad M, Baghban A. Phylogeny and drug resistance of HIV PR gene among HIV patients receiving RT inhibitors in Iran. *Asian Pac J Trop Biomed*. 2016;**6**(5):451-4. doi: [10.1016/j.apjtb.2015.12.020](https://doi.org/10.1016/j.apjtb.2015.12.020).
35. Gholami M, Sadeghi L, Baesi K, Rouzbahani NH, Mohraz M. Survey of antiretroviral drug resistance pattern among HIV-infected patients with treatment failure in Iran. *J Hum Virol Retrovir*. 2015;**3**(1):1-6.
36. Naziri H, Baesi K, Moradi A, Aghasadeghi MR, Tabarraei A, McFarland W, et al. Antiretroviral drug resistance mutations in naive and experienced patients in Shiraz, Iran, 2014. *Arch Virol*. 2016;**161**(9):2503-9. doi: [10.1007/s00705-016-2955-z](https://doi.org/10.1007/s00705-016-2955-z). [PubMed: [27368990](https://pubmed.ncbi.nlm.nih.gov/27368990/)].