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The frequency of HIV-1 infection and surveillance drug-resistant mutations determination among Iranians with high-risk behaviors, during 2014 to 2020

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ABSTRACT

Background and Objectives: Human immunodeficiency virus (HIV) has various transmission routes. Instant antiretroviral therapy (ART) is the recommended treatment for HIV infection. Highly active antiretroviral therapy (HAART) significantly decreases the acquired immunodeficiency syndrome (AIDS) and AIDS-related co-morbidities. Notwithstanding the suitability of HAART, the antiretrovirals (ARVs) have adverse effects and antiretroviral drug resistance mutations are reported among those who receive ARVs. In this survey, the abundance of HIV-1 infection in Iranians with high-risk behaviors, and detection of the surveillance drug-resistant mutations (SDRMs) were evaluated.

Materials and Methods: This cross-sectional study was conducted on 250 individuals with high-risk behaviors from September 2014 to February 2020. HIV-1 Ag/Ab in plasma samples was detected using enzyme immunoassay (EIA) kits. The conserved region of HIV-1 was detected in the plasma samples by real-time polymerase chain reaction (PCR) assay. Furthermore, in individuals with positive HIV-1 RNA, HIV-1 viral load testing was performed. After amplification and sequencing of the HIV-1 protease, reverse transcriptase, and integrase genes, surveillance drug resistance mutation (SDRM) and phylogenetic analysis were determined.

Results: Out of the 250 participants with high-risk behaviors, six (2.4%) were infected with HIV-1. According to the phylogenetic analysis, the CRF35_AD (83.3% or 5/6) was the dominant subtype, followed by CRF01_AE (16.7% or 1/6). In this research, in none of the HIV-1 infected patients, SDRM for protease inhibitors (PIs), nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), and integrase inhibitors (INs) were observed. Nevertheless, in one of the patients, V179L mutation was detected which is a rare non-polymorphic mutation and is listed as a rilpivirine (RPV) -associated resistance mutation.

Conclusion: The results of the current survey revealed that 2.4% of people with high-risk behaviors are infected with HIV and the level of drug resistance mutations (DRMs) in these people is very low.

Keywords: Human immunodeficiency virus-1; High-risk; Subtype; Infection; Iran

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INTRODUCTION

Human immunodeficiency virus (HIV) causes HIV infection and acquired immunodeficiency syndrome (AIDS). HIV belongs to the *Lentivirus* (*Retroviridae* family and *Orthoretrovirinae* subfamily). Based on the genetic specification and diversity of viral antigens, HIV is classified into two groups: HIV-1 and HIV-2. Currently, there is no beneficial vaccine to prevent HIV infection and the only option is the administration of antiretroviral drug use (1). According to the latest statistics reported by the World Health Organization (WHO), an estimated 37.7 million individuals were living with HIV in 2020 worldwide, and 27.5 million individuals were accessing antiretroviral therapy (ART) by end-2020. In other words, 73% of HIV infected individuals had access to ART in 2020 (2).

Various risk factors affect the prevalence of HIV infection among high-risk populations (3). High-risk behaviors for HIV transmission include unprotected sexual acts, infection with sexually transmitted disease (STDs) such as herpes simplex virus (HSV) and bacterial vaginosis increase the transmission probability, shared use of contaminated syringes/needles among intravenous drug users (IDUs), contaminated blood infusion, organ and tissue transplantation, and needle stick injuries among health care workers. It is noteworthy that pre-exposure prophylaxis (PrEP) is recommended for preventing HIV infection among people who are at increased risk of HIV transmission (3).

Sexual transmission is the main route of HIV-1 transmission all around the world. Approximately 80% of infections are associated with exposure to mucosal surfaces, and therefore AIDS is often a sexually transmitted syndrome. To prevent HIV spread to the general population, permanent HIV-1 prevention strategies are essential. The administration of ART significantly reduces the probability of HIV transmission (4). Hence, immediate ART is recommended for all HIV infected individuals. These people receive a complex HIV therapeutic regimen every day. It is noteworthy that, to inhibit various stages of the virus replication cycle, different drugs are approved by the U.S. Food and Drug Administration (FDA). These HIV medications include nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs), fusion inhibitors, CCR5 antagonists, and integrase inhibitors (INIs) (5).

Combination therapy with antiretroviral (ARVs) inhibits various stages of the replication cycle of the virus (6). Highly active antiretroviral therapy (HAART) significantly reduces the progress toward AIDS and AIDS-related mortality. Since the HAART increases the life expectancy of patients, the therapeutic regimen should be available for nearly the entire lifespan of a HIV-infected person. After several months of using at least three combinations of ARV drugs, the virus replication will be reduced (a viral load lower than 50 copies/mL) (7). Despite the advantages of HAART, a series of adverse effects are reported for certain ARVs and antiretroviral drug resistance mutations are also reported. Viral susceptibility to the ART over time determines the efficiency of treatment (5).

Because of high-speed replication of the virus and the extremely error-prone of reverse transcriptase, the major problem for PrEP and treating HIV patients is the genetic variation of the virus (8). The major cause of first-line treatment failure in HIV infected individuals is drug resistance mutations (DRMs). Besides, among HIV-1 infected people who are receiving ART, primary HIV-DRM diminishes the efficiency of ART (5). Among individuals who never received ART and likely are infected with a resistant HIV strain, primary HIV-DRMs and resistance of HIV-1 to ARTs are common (9). Before administration of antiretroviral therapy, the HIV-DRMs test is recommended for naïve HIV-infected individuals. Genotypic and phenotypic resistance assays are used to determine and detect the HIV-DRMs. Generally, in ARV- naïve individuals, the HIV-DRMs test should be simultaneous with using the genotypic assay in the reverse transcriptase (RT), protease (PR), and integrase (IN) genes (5). Genotypic assays determine the HIV-DRMs in the viral genome. It should be noted that because phenotypic methods are expensive and time-consuming, it is preferable to use genotyping assays to determine drug resistance (10).

It is reported that the high transmission rate of HIV infection among Americans roots is their sexual behaviors, but in Eastern Europe the situation is different, and IDUs are at increased risk of HIV infection (11). Also, in developed countries, such as the US and UK, homosexual behaviors are reported as the major route of HIV transmission, but in Southeastern and Eastern countries the main route of HIV transmission is shared injection. However, in African coun-

tries unprotected sex act with high-risk individuals, and polygamy are reported as the most important factors (12). In Iran, the most important factor for HIV transmission is shared injection among IDUs, but heterosexual is the second transmission route of HIV infection (13). The current survey aimed to determine the frequency of HIV-1 infection and surveillance drug-resistant mutation among Iranian with HIV-1-infection who are ART- naïve individuals with a history of high-risk behaviors.

MATERIALS AND METHODS

Ethical approval. The project was approved by the ethics committee of Iran University of Medical Sciences (IUMS), Tehran, Iran, that is accordance with Helsinki declaration (ethical code: IR.IUMS.FMD.REC.1399.306). All of the volunteers participating in this study were informed about this research prior to their enrollment. No animals were used in this survey, and all humans research procedures were followed in accordance with the standards set forth in the Declaration of Helsinki principles of 1975, as revised in 2013.

Study population and collection of the specimens. This cross-sectional research was conducted on 250 consecutive individuals with high-risk behaviors who were referred to hospitals and clinics related to Iran University of Medical Sciences (IUMS), Tehran, Iran between September 2014 and February 2020.

About four mL of peripheral blood was taken from each subject into a sterile EDTA-containing vacutainer tube. The plasma of the samples were separated by centrifugation (3500 RPM for 3 minutes), and then plasma was kept at -80°C until use.

Detection of HIV Ag/Ab using Enzyme immunoassay (EIA). HIV-1 Ag/Ab in plasma samples was detected using two fourth-generation Enzyme Immunoassay (EIA) kits: (1) a fourth-generation ELISA apDia HIV Ag/Ab kit (apDia bvba, Raadsherenstraat 3, B-2300 Turnhout, Belgium) (Sensitivity: 100%, Specificity: 99.6%), 2) a fourth-generation ELISA Dia.Pro HIV Ab/Ag kit (DIA.PRO, Diagnostic Bio-Probes Srl. Milano, Italy) (Sensitivity: 100%, Specificity: 100.0%), according to the manufacturer's instructions protocols.

Viral RNA extraction, detection of HIV-1 RNA by Real-Time PCR, and viral load assessment. The viral RNA was isolated from 500 µl of the plasma samples by a QIAamp DSP Virus Kit (Qiagen GmbH, Hilden, Germany), according to the manufacturer's instructions procedures, and then the quality and quantity of the extracted RNA was determined using a NanoDrop spectrophotometer (Thermo Scientific, Wilmington, USA), and preserved at -70°C for detection of HIV-1 RNA.

Detection of a conserved region of HIV-1 (long terminal repeat) in the plasma samples of all the participants was carried out by real-time polymerase chain reaction (PCR) method with TaqMan probe (LTR-Probe) and primers (LTR-F, LTR-R2), and as an internal control, TaqMan probe and special primers for human β-globin gene were used, as described previously in detail (14).

Moreover, the level of HIV-1 viral load in studied individuals with high-risk behaviors who were positive for HIV Ag/Ab test, and HIV-1 RNA, was assessed using an Artus HIV-1 RG RT-PCR (Qiagen GmbH, Hilden, Germany) kit, based on the manufacturer's procedures (the detection limit: 4.5 IU/µl). It is noteworthy that plasma specimens from 5 HIV-1 infected individuals and 5 healthy subjects were used as positive and negative controls, respectively.

Detection of HIV-1 drug-resistance mutations (DRMs) in HIV-1 infected individuals. Human immunodeficiency virus-1 DRMs were assessed in HIV-1-infected people with high-risk behaviors using genotyping assay, based on the standard protocols, which had been described in detail previously (15-17).

Sequences obtained from the amplified specimens (1015 bp of PR, and RT genes, and 915 bp of IN gene of HIV-1) were aligned with the HIV-1 reference sequence (HXB2 [accession number: K03455]) by the CLC Main Workbench software version 5.5 (CLC-bio, Boston, MA, USA), and then surveillance drug resistant mutations (SDRMs) were determined in the obtained sequences (16).

Determination HIV-1 subtypes and phylogenetic analysis. For determination of HIV-1 subtypes, the sequences obtained from this survey were aligned with various reference sequences of HIV-1 (subtypes and circulating recombinant forms), which retrieved from the Los Alamos Sequence Database (http://www.hiv.lanl.gov/) by MEGA software ver-

sion 7.0.21.

Also, two phylogenetic trees were constructed by MEGA software version 7.0.21 (Figs. 1 and 2) with the neighbor-joining method, and the statistical significance of the phylogenetic trees were evaluated using the bootstrap method (1,000 replicates).

Sequences obtained from the amplified samples of the HIV-1 infected individuals were submitted to GenBank with accession numbers, from MN882751 to MN882756 (1015 bp of protease and reverse transcriptase regions of HIV-1), and from MN882757 to MN882762 (915 bp of integrase region of HIV-1).

Statistical analysis. Statistical analysis of this study was carried out by SPSS software version 20 (SPSS Inc, Chicago, IL, USA). The quantitative variables 'normality was evaluated using the Kolmogorov–Smirnov test. The continuous variables were assessed by the Student's t-test or Mann-Whitney U-test. The statistical differences between various groups were analyzed using Fisher's exact test and chi-squared tests. A P-value less than 0.05 was considered statistically significant.

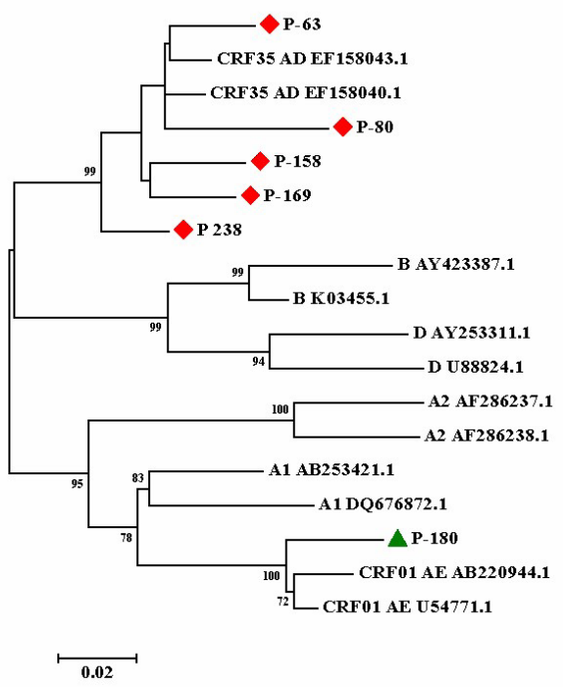

Fig. 1. The phylogenetic tree was drawn using MEGA7 software based on the nucleotide sequences (1015 bp) of HIV-1 protease and reverse transcriptase genes obtained from plasma samples of 6 Iranian HIV-1 infected naïve individuals with high-risk behaviors and those corresponding to various subtypes/CRFs of HIV-1 obtained from the GenBank HIV database. The virus subtypes detected from Iranian patients with HIV infection was CRF35_AD in five patients and CRF01_AE in one patient. The phylogenetic tree was conducted by the neighbour-joining method, and the bootstrap values ≥ 70% obtained after 1000 replicates are shown.

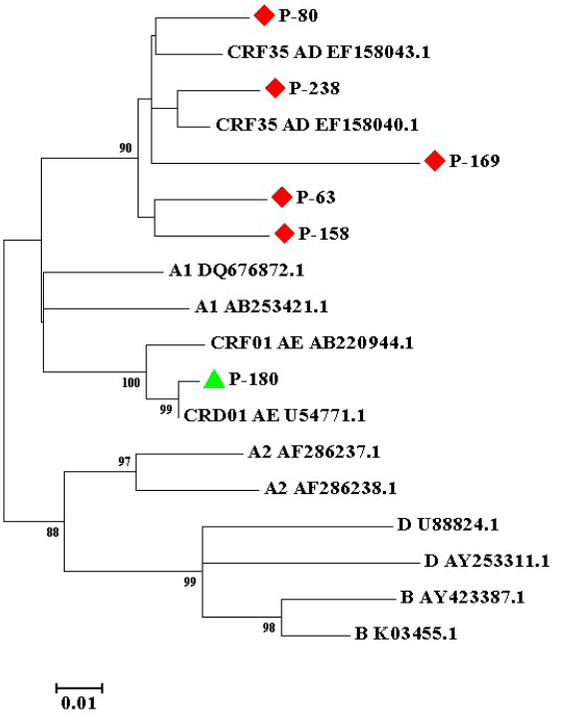

Fig. 2. The phylogenetic tree was drawn using MEGA7 software based on the nucleotide sequences (915 bp) of HIV-1 integrase gene obtained from plasma samples of 6 Iranian HIV-1 infected naïve individuals with high-risk behaviors and those corresponding to various subtypes/CRFs of HIV-1 obtained from the GenBank HIV database. The virus subtypes detected from Iranian patients with HIV infection was CRF35_AD in five patients and CRF01_AE in one patient. The phylogenetic tree was conducted by the neighbour-joining method, and the bootstrap values ≥ 70% obtained after 1000 replicates are shown.

RESULTS

Characteristics of the participants. The present survey was carried out on 250 Iranian people with high-risk behaviors who were referred to clinics and hospitals in Tehran, Iran. Among these individuals, 6 (2.4%) individuals were infected with HIV. The median viral load of HIV-1 in plasma samples of

HIV-infected participants was high-risk behaviors was 222,382 (range 65,891 to 1,231,564), and the mean of CD4+ T cell count was 665 ± 84 /μL (range 561 to 784 /μL). Out of the 250 evaluated subjects, 164 (65.6%) were male. The mean age of the studied participants was 29.5 ± 8.9 (range, 5-62 years). The demographic and epidemiological parameters of the participants are shown in Table 1. Also, complete information about Iranian HIV-1 infected individuals with high-risk behaviors are summarized in Table 2.

HIV-1 viral load in these 6 (2.4%) HIV-1 infected patients 1231564, 262782, 326289, 181982, 98011 and 65891 copies /mL, were detected, respectively. Also, CD4+ T cell count in these 6 (2.4%) patients 561, 698, 576, 698, 671 and 784 /μL, were observed respectively.

Significant relationship was not observed between the HIV-1 infection intravenous drug use (P = 0.050), as shown in Table 1, no significant association was found between HIV infection and various risk factor for this infection.

Determination of HIV-1 subtypes and surveillance drug-resistance mutations (SDRMs) in the HIV-1 pol gene. Among the 6 HIV-1 positive individuals, SDRMs in the protease, reverse transcriptase, and integrase genes of HIV-1 were determined by analyzing the nucleotide sequences that obtained from the amplification of pol gene. The sequences were aligned with the pol gene sequences of various subtypes and circulating recombinant forms (CRFs) of HIV-1 which were retrieved from the Los Alamos Sequence Database, and two phylogenetic trees were constructed using Molecular Evolutionary Genetics Analysis (MEGA) software (version 7.0.21) by neighbor-joining method (Figs. 1 and 2).

Phylogenetic analyses of protease, reverse transcriptase, and integrase fragments showed that CRF35_AD accounted for 83.3% (5/6) of the HIV-infected participants, followed by CRF01_AE with 16.7% (1/6). Analysis of the nucleotide sequences revealed that none of the HIV-1 infected individuals carried HIV-1 variants with SDRMs for PIs, NRTIs, NNRTIs, and also INIs, however, one of the patients had V179L mutation which is a rare non-polymorphic mutation and is listed as a rilpivirine (RPV) -associated resistance mutation.

DISCUSSION

In the current study, HIV-1 infection and SDRMs of Iranians with a history of high-risk behaviors were determined. Prevalence rate of HIV-1 infection 6 (2.4%) was observed among these participants and a rare non-polymorphic mutation and is listed as a RPV-associated resistance mutation in one of the patients was detected in the current study. In this study, the highest viral load was detected in one of HIV-1 infected patient with a history of injecting drug use. Four out of six HIV-1 infected patients had unprotected sex (Heterosexual) and two patients had unprotected sex (Homosexual).

This is the one of study to express the prevalence of mutations allows resistance to newly approved second-generation NNRTI, including RPV, in HIV-1 subtype CRF35_AD and CRF01_AE -infected individuals in Iran. Subtype CRF35_AD was observed in most of the patients (83.3%), even a greater percentage was found between high-risk behavior individuals that followed by CRF01_AE with 16.7%. It seems that the findings of the current study are consistent with other studies conducted in Iran (15, 18).

According to recent reports on the epidemiology of HIV, approximately 90% of all identified cases were infected through sexual transmission. Therefore, sexual transmission is the first cause of HIV transmission in people with high-risk sexual behaviors and is the second leading cause of infection transmission in society. Reportedly, partners' behaviors, such as

Table 1. Demographic and epidemiological characteristics of Iranian with high-risk behaviors, during 2014 to 2020

Parameters		HIV-1 positive	HIV-1 negative	Total	*P.* value
Number (%)		6 (2.4%)	244 (97.6%)	250 (100.0%)	
Age (Year) ± SD		35.5 ± 16.2 (18-61)	29.3 ± 8.7 (5-62)	29.5 ± 8.9 (5-62)	0.395
Gender	Male	4 (66.7%)	160 (65.5%)	164 (65.6%)	1.000
	Female	2 (33.3%)	84 (34.4%)	86 (34.4%)	
Epidemiological parameters					
Intravenous drug user		1 (16.7%)	10 (4.1%)	11 (4.4%)	0.239

Table 2. Complete information about Iranian HIV-1 infected individuals with high-risk behaviors

Patients	Age	Gender	Marital status	HIV-1 Viral load (copies/mL)	CD4 count /µL	IDUs[3] +	Unprotected sex (Heterosexual)	Unprotected sex (Homosexual)
P- 63	30	F1	Married	1231564	561	_	_	_
P- 80	20	M2	Single	262782	698	_	+	+
P- 158	44	M	Married	326289	576	_	+	_
P- 169	18	M	Single	181982	698	_	+	+
P- 180	40	F	Single	98011	671	_	+	_
P- 238	61	M	Married	65891	784		_	_

1. Female

2. Male

3. Injection drug users

multiply sexual partners, is an important factor for HIV transmission. Various studies showed that some men who have sex with men (MSM) are again exposing their high-risk behaviors (19). The findings of the current study are consistent with the findings of previous studies recently conducted on HIV-1 infection in Iran (13, 20). However, the findings of the current study are not similar to some of the previous studies (21, 22). These differences can be explained in future studies.

Also, the frequency of SDRMs in HIV-1 infected patients was determined. Using genotyping assay, for protease, reverse transcriptase, and integrase genes of HIV-1 between six HIV-1-infected individuals with a history of high-risk behaviors, HIV-DRMs tests were evaluated according to the standard protocols. None of the six HIV-1-infected patients demonstrated HIV-DRMs. However, the findings of the current study indicated that one of these patients had V179L mutation which is a rare non-polymorphic mutation and is listed as a RPV-associated resistance mutation. Generally, one of the appropriate ARVs for the initial combines therapeutic regimen for treatment- naïve patients is RPV (23). The prevalence of NNRTI DRMs in recently diagnosed HIV-1-infected patients has remained stable since 2003 (24).

In this survey, the prevalence of DRM in patients with HIV infection has been compared with the results of similar reports conducted in Iran. Surveillance drug resistance mutations to NRTIs (10%) including M41L and M184V, to NNRTIs (5%) including K103N and to PIs (0%) were reported in Iran (25). Non-nucleoside reverse transcriptase inhibitor-resistance mutations were observed that including K101KE, K103KN, G190G, and K103N mutations. Furthermore, in RT genes, K101R (11.5%), K101Q (3.8%), V106I/VI (3.8%), V179I (79%), and P225A (1.9%) were observed. In addition, V118VI DRMs to NRTI was observed. In the PR region, DRMs including M36I, H69K, L89M, K20R/KR, and L63P/T/M/A/V/LP were detected (26). Among the participants of this study, mutations rates of the patients showed a low level of drug resistance. However, it must be noted that some previous studies reported a higher prevalence of SDRMs among the Iranian population compared to this research (27).

It is known that between HIV-1 infected individuals, DRMs to NNRTIs occur more than PIs, NRTIs and INIs. Drug resistance mutations related to NNRTIs may have significant effects on viral fitness, and causes virus resistance and transmission of HIV infection (28). In this survey analyzing the nucleotide sequences of six HIV-1 positive individuals showed a low level of drug resistance. No significant association was found between HIV infection and various risk factors of HIV infection.

Rilpivirine is classified as a NNRTI ARVs that formerly, demonstrated in naïve HIV infected patients who had HIV viral load <100,000 copies/mL. Since 2013 it is using for those who receive ART and no NNRTI-resistance mutations is observed. There is no document due to the utilization of RPV in HIV infected individuals with NNRTI resistance mutations. So far, fifteen mutations have been identified that reduce sensitivity to the RPV, such as K101E/P, E138A/G/K/Q/R, V179L, Y181C/I/V, H221Y, F227C, and M230I/L (29). In the current study, one of the patients had V179L mutation to RPV. V179L mutation is an uncommon nonpolymorphic mutation that rarely occurs in HIV infected patients NVP, EFV, and RPV (30).

Moreover, various types of HIV subtypes are de-

tected in different societies. In China and Southeast Asia, the transmission of CRF_01AE was reported among those with heterosexual relationships (31). In Kuwait, subtype CRF01_AE (34.3%), subtype C (24.5%), and subtype B (17.6%) were reported (32). In Slovenia, Subtype B is reported as dominant HIV subtypes among MSM, with a higher proportion to those with heterosexual activities or IDUs (33). In western European and different central countries, the distribution of non-B subtypes was reported, such as subtype G, subtype A, and subtype C in Portugal, Greece, and the UK, respectively. In the United States, subtype B (97%), subtype C (1.3%), subtype G (0.55%), subtype A (0.45%), subtype D (0.1%), and subtype F (0.03%) were reported (34). In Cameroon, CRF02_AG (56%) was a dominant subtype, followed by CRF36_cpx (9%) and different subtypes. In South Africa, subtype C (94.2%) and subtype B (1.4%) were reported (35). In Taiwan, HIV-1 subtype B (90%) was observed (36). In Brazil, HIV subtype B (60.5%), subtypes C (23.3%), and F (7%) were reported (37). In the Iranian population, previous studies reported subtype CRF35_AD as the most prevalent (17, 18, 25, 38, 39). Furthermore, some investigations have demonstrated the presence of different subtypes of HIV-1, such as subtype B, C, and CRF01_AE among Iranians (18, 38, 39). In the current study, subtype CRF35_AD was the most prevalent (83.3%), followed by CRF01_AE (16.7%).

Identifying and screening high-risk behaviour individuals was one of the limitations of our study. DRMs tests were performed quickly after awareness of HIV-1 diagnosis; nevertheless, the infection may have occurred any time in the past.

CONCLUSION

In conclusion, the prevalence of SDRMs was estimated among individuals diagnosed with HIV-1 in 2014–2020 in Iran. The results demonstrated a low prevalence of SDRMs in HIV-1 strains isolated from individuals with high-risk behaviors. At this research center, we recommend the rapid performance of genotypic testing, because it prevents the circulation of drug-resistant HIV-1 strains in the community. As a result, it provides physicians with the opportunity to prescribe appropriate and effective ARVs. It also reduces treatment costs and, on the other hand, helps to extend the life and quality of life of HIV-1 infected patients.

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