

Genotyping and antiretroviral drug resistance of human immunodeficiency Virus-1 in Jazan, Saudi Arabia

Eitezaz A. Zaki, MSc^{a,b}, Mai M. El-Daly, PhD^{a,c,d}, Ahmed Abdulhaq, PhD^e, Tagreed L. Al-Subhi, MSc^c, Ahmed M. Hassan, MSc^c, Sherif A. El-Kafrawy, PhD^{a,c,d}, Mohammad M. Alhazmi, MD^f, Majid A. Darraj, MD^{g,h}, Esam I. Azhar, PhD^{a,c,*} 

Abstract

Determination of human immunodeficiency virus-1 (HIV-1) genotypes and identification of antiretroviral drug-resistant mutations. Among treatment naïve HIV patients in Jazan, Saudi Arabia. HIV is a major public health problem. HIV genotyping and antiretroviral resistance testing is an important guide for better management of treatment-naïve. Antiretroviral resistance testing before starting of treatment regimen leads to a better virological response. A total of 57 samples of treatment-naïve patients were collected from King Fahd Central Hospital in Jazan, Saudi Arabia. Samples were tested for HIV-1 antibodies, western blot, viral load, HIV-1 genotypes through direct sequencing, and antiretroviral resistance testing. The HIV-1 Genotypes were as follow; C: 66.6%, D: 10.5%, G: 8.8%, B: 7.0%, CRF01_AE: 3.5%, A and CRF02_AG: 1.8% each. 77.2% of cases showed susceptibility to the 3 major classes of antiretroviral drugs; Protease inhibitor (PI), Nucleoside reverse transcriptase inhibitor (NRTI), and non-nucleoside reverse transcriptase inhibitors (NNRTI); while 8.8% had mutations conferring resistance to NRTI. Mutations conferring resistance to PI were detected in 7.0% of cases, and 1.8% of cases had mutations conferring resistance to both NRTI and PI. Mutations conferring resistance to NNRTI were detected in 5.3% of cases. Mutations associated with antiretroviral drugs include (V82A+I84I), (L10F+Q58E), (L10F+V82Y), L10FV, L33LF, L89LMV, M184V, E138A, V106I, and V179VD. The prevalence of HIV-1 antiretroviral resistance mutations is 22.8% in the studied population, which may warrant antiretroviral drug resistance testing as a pretreatment to help and guide physicians for the proper HIV treatment.

Abbreviations: AIDS = acquired immunodeficiency syndrome, ART = antiretroviral therapy, ARV = antiretroviral, CCR5 = chemokine co-receptors, FDA = Food and Drug Administration, HAART = highly active antiretroviral therapy, HIV = human immunodeficiency virus, NNRTI = non-nucleoside reverse transcriptase inhibitors, NRTI = nucleoside reverse transcriptase inhibitor, PI = protease inhibitor, WHO = World Health Organization.

Keywords: HIV-1 genotyping, antiretroviral drug resistance, Saudi Arabia

1. Introduction

Human immunodeficiency virus (HIV) is a major health problem globally.^[1] According to the Global AIDS update by the World Health Organization (WHO) and the Joint United Nations

Program on HIV/AIDS,^[2] in 2017, 36.9 million people are infected with HIV worldwide, 1.8 million are newly infected with HIV, and about 940,000 people died of AIDS.^[3] In Saudi Arabia, there are around 1.5 HIV positive people per 100,000 people

Editor: Akhilanand Chaurasia.

Recommendations: HIV-1 genotyping and antiretroviral drug resistance analysis for chronic HIV infection is recommended prior to the initiation of treatment as it is a useful tool for the management of HIV treatment. Further studies on molecular epidemiology and monitoring drug resistance are needed to fill the gap in knowledge in this area and to help and guide the management of HIV-1 patients.

This study was funded by King Abdulaziz City for Science and Technology (KACST), King Abdulaziz University, Jeddah, Saudi Arabia under grant no. (1728-37-AT). Recipient: Eitezaz A. ZAKI.

Competing interests: The authors declare no conflicts of interest.

The authors have no conflicts of interest to disclose.

All data generated or analyzed during this study are included in this published article [and its supplementary information files]

^a Department of Medical Laboratory Technology, Faculty of Applied Medical Sciences, King Abdulaziz University, ^b Department of Virology, Jeddah Regional Lab, Ministry of Health, ^c Special Infectious Agents Unit, King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia, ^d Clinical Pathology Department, National Liver Institute, Menoufia University, Shebin El-Kom, Egypt, ^e Deanship of Scientific Affairs and Research, Jazan University, ^f Department of Medicine, ^g Department of Infectious Diseases, King Fahd Central Hospital, ^h Department of Medicine, College of Medicine, Jazan University, Jazan, Saudi Arabia.

* Correspondence: Esam I. Azhar, King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia (e-mail: eazhar@kau.edu.sa).

Copyright © 2020 the Author(s). Published by Wolters Kluwer Health, Inc.

This is an open access article distributed under the terms of the Creative Commons Attribution-Non Commercial License 4.0 (CCBY-NC), where it is permissible to download, share, remix, transform, and buildup the work provided it is properly cited. The work cannot be used commercially without permission from the journal.

How to cite this article: Zaki EA, El-Daly MM, Abdulhaq A, Al-Subhi TL, Hassan AM, El-Kafrawy SA, Alhazmi MM, Darraj MA, Azhar EI. Genotyping and antiretroviral drug resistance of human immunodeficiency Virus-1 in Jazan, Saudi Arabia. *Medicine* 2020;99:49(e23274).

Received: 2 June 2020 / Received in final form: 13 September 2020 / Accepted: 22 October 2020

<http://dx.doi.org/10.1097/MD.00000000000023274>

annually among Saudi citizens and 1.2 per 10,000 among non-Saudis. Saudi Arabia remains a low-prevalence country in the face of HIV according to the Global AIDS Report.^[4]

HIV is classified into 2 different types: type 1 (HIV-1) and type 2 (HIV-2).^[5] HIV-1 is divided into 4 groups: main (M), outlier (O), neither (non-M/non-O), or new (N), and P.^[6,7] The M group is subdivided into 9 subtypes (A, B, C, D, F, G, H, J, and K) and circulating recombinant forms (CRFs).^[8] Subtype B is the most common epidemic subtype infecting people in America, Europe, Asia, and Australia, while the subtypes concentrated in Africa are A, C, and D.^[5]

The Food and Drug Administration (FDA) has approved >25 Antiretroviral (ARV) drugs into 6 categories.^[9] These categories include protease inhibitors (PIs), nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), CCR5 antagonists, fusion inhibitors (FI), and integrase strand transfer inhibitors (INSTIs). Treatment of HIV patients mandates the use of a mixture of ARVs from ≥ 2 classes targeting different steps in the HIV life cycle.^[10] The most common cause of failure for HIV treatment is the appearance of drug resistance.^[11] Genotype assays can detect drug resistance mutations in HIV genes if present.^[12] The HIV-1 genotypic resistance is an important test used to guide the physicians to select the most appropriate initial ART for naive patients and posterior treatment in patients with virological failure.^[13]

In Saudi Arabia, little is known about the profile of HIV antiretroviral resistance. In 2010, Jamjoom et al,^[14] described the genotyping and antiretroviral resistance profile (RT and protease) of HIV-1 in cases on antiretroviral therapy from Jeddah, Saudi Arabia. They reported that 52% of cases were susceptible to all 3 major categories of antiretroviral drugs used, 41% had mutations known to confer high-level resistance to one or more of NRTI, and 16% had mutations known to confer high-level resistance to NNRTI, 13% had mutations known to confer high-level resistance to one or more of protease inhibitors (PI). Recently, Al-Mozaini et al,^[15] reported the resistance profiles in the HIV cohort on treated patients from Riyadh and found the highest frequency for drug resistance in the studied cohort to be for NRTI (68%) followed by resistance mutations for both NNRTI and PRI (47%).

The objective of this study was to determine the HIV-1 genotypes as well as to identify the antiretroviral drug resistance mutations among the treatment-naïve HIV-infected patients in Jazan, Saudi Arabia.

2. Subjects and methods

2.1. Samples collection

A total of 57 blood samples from treatment-naïve patients were recruited at the HIV clinic, King Fahd Central Hospital, Jazan, Saudi Arabia, between 2016 and 2017. Samples were transferred to the Special Infectious Agents Unit, King Fahd Medical Research Center, King Abdulaziz University, Jeddah for laboratory investigations. Ethical approval for this study was provided by the Unit of Biomedical Ethics, King Abdulaziz University Hospital.

2.2. Methodology

2.2.1. Serological tests (EIA). Plasma samples were tested for HIV P24 Antigen and Antibodies using the Genscreen ULTRA HIV Ag-Ab Score kit (BIO-RAD, Germany), according to manufacturer's instructions.

2.2.2. Western blot assay. Positive samples for HIV-1 Ab were tested for Western Blot using INNO-LIA HIV /III score kit (Fujirebio, Belgium) according to the manufacturer's instructions.

2.2.3. Viral load measurement. The HIV-1 RNA viral load was performed using the Abbott RealTime HIV-1 Assay (Abbott, Germany) according to the manufacturer's instructions. The selection of the genotyping assay was based on samples that fulfilled the viral load ≥ 1000 copies per ml.

2.2.4. Reverse transcription PCR (RT-PCR). Total RNA was extracted using Roche MagNA pure compact Nucleic Acid Isolation Kit (Roche, NY) according to the manufacturer's instructions. The extracted RNA was subjected to nested RT-PCR amplification according to Zhou et al,^[16] generating a 1.1 kb fragment of the pol gene region of the HIV-1 genome spanning the entire PR region and approximately two-thirds of the RT region. PCR was performed using the One-Step RT-PCR Kit (QIAGEN, Germany), on a Veritti 96 well thermal Cycler (Applied BioSystems, Singapore). PCR products were visualized by electrophoresis on ethidium bromide-stained 1% agarose gel with the size of 1084 base pairs fragment.

2.2.5. Sequencing analysis. Nested PCR products were purified using the QIAquick PCR Purification kit (QIAGEN, Germany) according to the manufacturer's instructions. For DNA sequencing, purified DNA was subjected to DNA sequencing in 3500 DNA genetic analyzer (Applied Biosystems, USA). Six sequencing primers overlapping the entire amplicon were used.^[16] The sequencing raw data were assembled using Geneious 8.1.5 software (Biomatters, Auckland, New Zealand).^[17] The obtained sequences were aligned with HIV reference sequences available from the National Center for Biotechnology Information (NCBI) database (<https://blast.ncbi.nlm.nih.gov/Blast>), using ClustalW, to determine the HIV-1 genotypes.

2.2.6. Phylogenetic analysis. Phylogenetic analysis was performed using MEGA6 software with the neighbor-joining method and Maximum Composite Likelihood with 1000 bootstrap replicates.^[18] Sequences from this study were deposited in the Genbank with the accession numbers MN078747-MN078803.

2.2.7. Detection of HIV-1 genotypes and drug resistance. All the generated sequences were submitted to The Stanford Genotypic Resistance Interpretation Algorithm software to determine both HIV-1 genotyping and the resistance-associated mutations of HIV-1 in PR and RT regions (<http://hivdb.stanford.edu/pages/algs/HIVdb.html>).

2.3. Statistical analysis

Categorical data were reported as frequency and percentage (%). Data were analyzed using SPSS version 21 (IBM, NY, USA). Continuous data were reported as mean \pm SE.

3. Results

3.1. Patients characteristics

A total of fifty-seven treatment-naïve HIV-1 patients were recruited from King Fahd General Hospital in Jazan (South-

Table 1
The HIV-1 genotypes distribution among 57 patients.

Genotype	Number	%
C	38	66.6%
D	6	10.5%
G	5	8.8%
B	4	7.0%
CRF01_AE	2	3.5%
CRF02_AG	1	1.8%
A	1	1.8%

western Saudi Arabia). Samples were tested for the prevalence of HIV-1 genotypes and for the identification of antiretroviral drug resistance mutations in Jazan, Saudi Arabia. The mean age of the participating patients was 32.45 ± 1.68 years, the number of men was 46 (80.7%), and the women 11 (19.3%). The mean of plasma viral load was 183184.7 ± 338675.1 C/mL. The nationality of patients included Saudi 64.9% and non-Saudi 35.1%.

3.2. Genotypes distribution

The phylogenetic analysis of HIV-1 genotypes (Table 1 and Fig. 1) showed that the distribution of HIV-1 genotypes was as follows: genotype C was the major genotype (66.6%, 38/57), it is clustered with strains from North America, South America, Europe, Africa, and Asia, followed by genotype D (10.5%, 6/57) that was clustered with strains from Africa. Genotype G (8.8%, 5/57) was clustered with strains from Europe and Africa, genotype B (7.0%, 4/57) was clustered with strains from North America and East Asia, CRF01_AE (3.5%, 2/57) was clustered with strains from Asia, genotype A, and CRF02_AG were less frequently detected (1.8%, 1/57) each, they were clustered with strains from Africa.

3.3. Antiretroviral drug resistance mutations

Results of antiretroviral drug resistance mutations at the pol gene region (Table 2) showed that among 57 patients, there were no mutations conferring resistance to each of PI, NRTI, and NNRTI separately in 91.2%, 89.5%, and 94.7% of the patients respectively. In the recruited cases, 77.2% of the subjects were clear from all mutations that might confer antiretroviral resistance to all the 3 categories of anti-HIV drugs (PI, NRTI, and NNRTI). Among the observed resistance mutations, 4/57 (7.0%) had conferring resistance to PI, other detected mutations were (L10F+V82Y), L10FV, L33LF, and L89LMV. Only one resistance mutation to NRTI was detected (M184V) in 5/57 (8.8%). Resistance mutations to NNRTI were detected in 3/57 (5.3%), these mutations were V179VD, V106I, and E138A. One patient had multi-drug mutation; the mutations (V82A+I84IV) and (L10F+Q58E) were found in the protease region while mutation M184V was found to confer resistance to NRTI drugs.

Among the treatment-naïve patients studied, 13 (22.8%) conferred resistance mutations to different antiretroviral drugs (Table 3). Four patients had mutations in the protease region with no association to drug resistance (L33LF, L89LMV, L10FV, and [L10F+V82Y]), these patients were genotype C. Five patients had the same resistance mutations to NRTI (M184V), these patients had different genotypes (B, C, and G), and this mutation is associated with resistance to the antiretroviral drugs:

Lamivudine (3TC), Abacavir (ABC), and Emtricitabine (FTC). Three patients had resistance mutations to NNRTI (V179VD, V106I, and E138A). The patient with V179VD mutation was genotype C and had resistance to the antiretroviral drugs: Efavirenz (EFV), Etravirine (ETR), Nevirapine (NVP), and Rilpivirine (RPV). The patient with V106I mutation was genotype CRF01-AE, and had resistance to the antiretroviral drugs, ETR, NVP, and RPV, the patient with E138A mutation, was genotype B and had resistance to two antiretroviral drugs: ETR, and RPV. One patient had multiple resistance mutations to PI (V82A+I84IV, L01F+Q58E), and M184V mutation to NRTI, he was genotype C and had resistance to the antiretroviral drugs: Atazanavir (ATV), Darunavir (DRV), Lopinavir (LPV), 3TC, ABC, and FTC.

4. Discussion

The ARV drug resistance testing is recommended for the acute and early infection before initiation of treatment, during treatment of chronic HIV infection, in cases of treatment failure in patients on ARV therapy, and during pregnancy. It is important to reduce drug resistance to highly active antiretroviral therapy (HAART) as it increases the chance of second-line success and subsequent treatments after viral recovery during first-line treatment. Resistance testing should help physicians avoid unnecessary drug exchanging, eliminate compliance problems, and perform well-directed keys rather than experimental drug changes. The use of effective drugs for extended time periods save costs associated with drug conversion and avoids unnecessary toxins from inactive drugs. ARV therapies with PI and RTI and recently with integrase inhibitors, in addition to combination drugs, have been available for several years in the Kingdom of Saudi Arabia.

The results of this study report on HIV-1 genotyping and the prevalence of antiretroviral drug-resistant mutations in 57 treatment-naïve patients in Jazan, Saudi Arabia, recruited from 2016 to 2017. To our knowledge, this is the first study performed in naïve chronic HIV cases from Jazan, Saudi Arabia.^[14,19]

In the current study, among the treatment-naïve patients, the average age was 32.45 ± 1.68 with a male to female ratio of 4:1. Our results showed that the most prevalent genotype was genotype C (66.6%) as reported by earlier studies from Saudi Arabia,^[14] this genotype is also most prevalent in the Horn of Africa, Djibouti,^[20] and the most frequent genotype in North-eastern South Africa, India, and parts of China.^[21] Genotype D was identified in 10.5% of the recruited subjects which is reported to be more prevalent in North Africa and the Middle East, genotype G in 8.8%, while the other genotypes (B, CRF01_AE, CRF02_AG, and A) collectively constitute 14.1% of the recruited subjects. Genotype B is more prevalent in West and Central Europe, the Americas, Australia, and several Southeast Asian countries, as well as northern Africa and the Middle East,^[22,23] genotype CRF01_AE is predominant in Asia,^[24] while genotypes G and CRF02_AG are more prevalent in West Africa.^[25] Genotype A is more prevalent in Central and East Africa as well as East European countries.

The current study shows that HIV resistance to the major categories of drugs used in Saudi Arabia is important and should be taken into account in the treatment of patients with chronic HIV. The most frequent mutations detected in this study were V82A+I84IV which are associated with PI conferring resistance to Atazanavir, Lopinavir, Lamivudine, Emtricitabine, Darunavir,

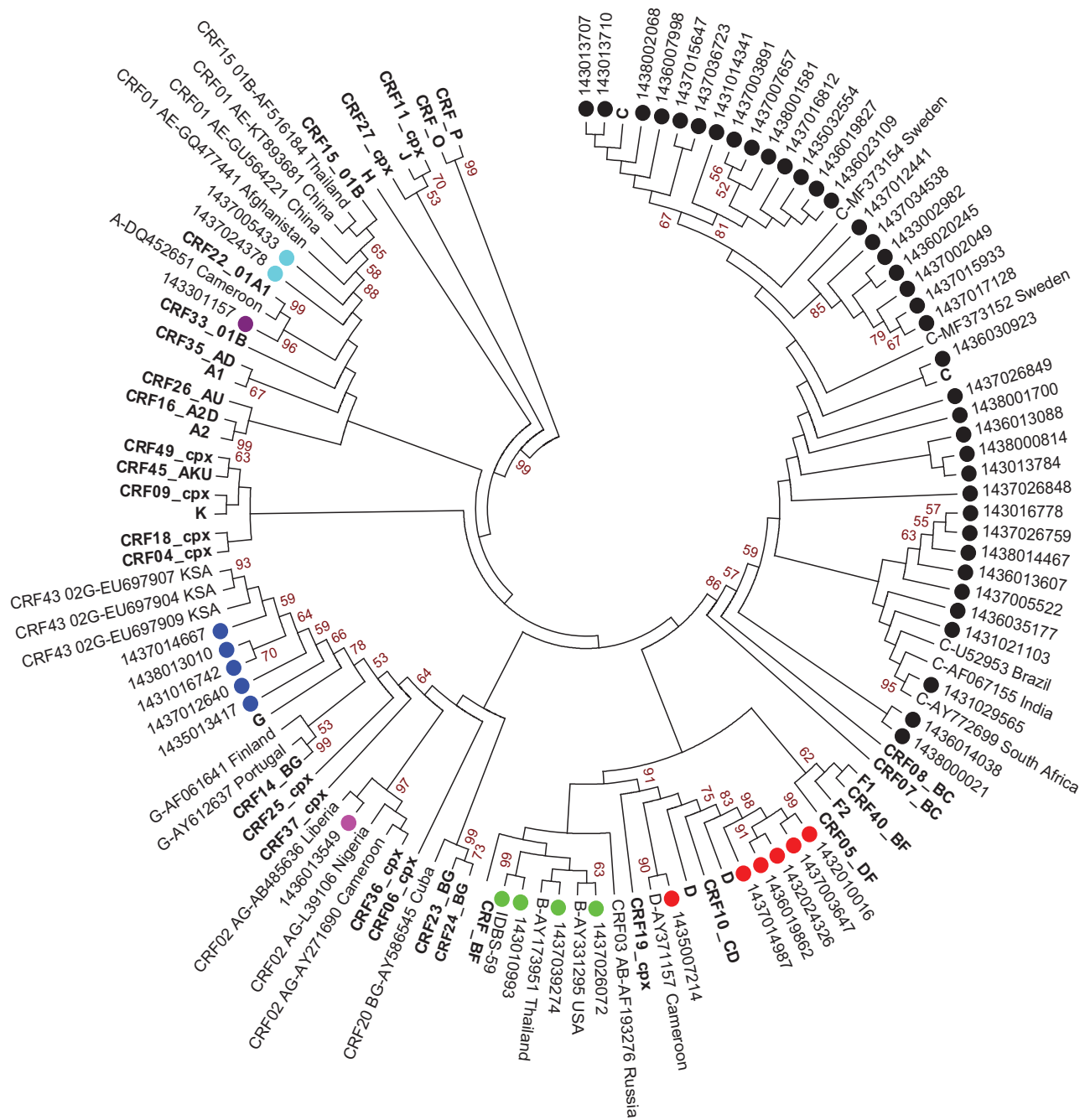


Figure 1. Phylogenetic analysis for HIV-1 genotypes. HIV=human immunodeficiency virus.

and Abacavir. The other mutations associated with PI were L10F +Q58E which confer resistance to Atazanavir, Lopinavir, Lamivudine, Emtricitabine, Darunavir, and Abacavir. These results are inconsistent with the previous study of Jamjoom et al,^[14] who reported that the most prevalent PI mutations were I54V, L90M, V82A, M46I, I50V, and D30N. This discrepancy may be due to the difference in recruited subjects, where our study recruited treatment naïve patients while the Jamjoom study recruited patients who received antiretroviral therapy and some of them had treatment failure.

The only mutation associated with NRTI resistance was M184V, which confers resistance to Atazanavir, Lopinavir, Lamivudine,

Emtricitabine, Darunavir, and Abacavir. The mutations associated with NNRTI resistance were V179VD, V106I, and E138A. The E138A, conferring resistance to Rilpivirine, and Etravirine, V179VD confers resistance to Efavirenz, Etravirine, Nevirapine, and Rilpivirine, while V106I confers resistance to Etravirine, Nevirapine, Rilpivirine, and Doravirine. The mutation patterns detected in this study are discordant with results from a previous study reported from Saudi Arabia^[14] which might be mainly due to the exposure of the patients in the earlier study to antiretroviral treatment.

In the current study, the frequency mutation associated with drug-resistance to NRTI was M184V 10.5%, which is the major

Table 3**Summary of genotypes and profile of the resistance mutation in naïve treatment patients.**

Sample code	Antiretroviral drugs	Genotype	PI resistance mutations	NRTI resistance mutations	NNRTI resistance mutations
HIV008_KSA	Susceptible	C	L33LF*	None	None
HIV018_KSA	Susceptible	C	L89LMV*	None	None
HIV046_KSA	Susceptible	C	L10FV*	None	None
HIV048_KSA	Susceptible	C	(L10F, V82Y)*	None	None
HIV027_KSA	ATV, DRV, LPV, 3TC, ABC, FTC	C	(V82A, I84IV), (L10F, Q58E)	M184V	None
HIV001_KSA	3TC, ABC, FTC	B	None	M184V	None
HIV002_KSA	3TC, ABC, FTC	C	None	M184V	None
HIV005_KSA	3TC, ABC, FTC	C	None	M184V	None
HIV007_KSA	3TC, ABC, FTC	G	None	M184V	None
HIV015_KSA	3TC, ABC, FTC	G	None	M184V	None
HIV017_KSA	EFV, ETR, NVP, RPV	C	None	None	V179DV
HIV031_KSA	ETR, NVP, RPV	CRF01_AE	None	None	V106I
HIV049_KSA	ETR, RPV	B	None	None	E138A

Abacavir (ABC), Atazanavir (ATV), Darunavir (DRV), Doravirine (DOR), Efavirenz (EFV), Emtricitabine (FTC), Etravirine (ETR), Lamivudine (3TC), Lopinavir (LPV), Nevirapine (NVP), Rilpivirine (RPV).

NRTI=nucleoside reverse transcriptase inhibitor; NNRTI=non-nucleoside reverse transcriptase inhibitors; PI=protease inhibitor.

* These mutations do not cause PI resistance.

mutation observed in most of the previous studies.^[14,19,20,22–26]

The agreement with our study only in resistance to Lamivudine but disagree in resistance to Atazanavir/r, Lopinavir/r, and Emtricitabine. While the mutations associated with resistance to NNRTI were V179DV (1.8%), V106I (1.8%), and E138A (1.8%). The mutations associated with resistance to PI were V82A+I84IV 1.8%, and L10F+Q58E 1.8%.

Additionally, the rapidly changing dynamics of HIV resistance require a continuous update of resistance data. Unfortunately, this is constrained by the comparatively high cost and technical requirements for testing. Update of resistance data should be considered in patients receiving treatment for consideration in future studies. The database available to compare new mutations used in this study was based on the dominant genotype B strains in Europe and the United States.^[27] This might cause significant differences in the drug susceptibility of other genetic patterns. Reports indicate that different HIV-1 genotypes can be more susceptible to PI or NNRTIs than genotype B, including genotype C, which is quite dominant in our population.^[27] Therefore, the decision in the adoption of therapeutic regimens based on genotype B pattern of sensitivity should be exercised, and more extensive studies might be needed to investigate the role of other mutations in antiretroviral treatment resistance in different genotypes.

Table 2**Patterns of antiretroviral drug resistance mutations.**

Category	No. (%)	Drug-resistance mutations
Susceptible to PI	52 (91.2%)	None
Susceptible to NRTI	51 (89.5%)	None
Susceptible to NNRTI	54 (94.7%)	None
Susceptible to PI+NRTI+NNRTI	44 (77.2%)	None
Resistance to PI	4 (7.0%)	(L10F+V82Y), L10FV, L33LF, L89LMV
Resistance to NRTI	5 (8.8%)	M184V
Resistance to NNRTI	3 (5.3%)	V179DV, V106I, E138A
Resistance to PI+NRTI	1 (1.8%)	PI: (V82A+I84IV), (L10F+Q58E) NRTI: M184V

NRTI=nucleoside reverse transcriptase inhibitor; NNRTI=non-nucleoside reverse transcriptase inhibitors; PI=protease inhibitor.

5. Conclusions

This study showed that the prevalence of HIV-1 antiretroviral resistance mutations is 22.8% in the studied population of naïve chronic HIV-1 patients, which justifies the need for antiretroviral drug resistance testing prior to treatment to aid and guide the physician with regard to the proper HIV treatment regimen.

Author contributions

Conceptualization: Esam I. Azhar.

Data curation: Mai M. El-Daly, Tagreed L. Al-Subhi, Ahmed M. Hassan, Majid A. Darraj.

Formal analysis: Eitezaz A. Zaki.

Investigation: Ahmed Abdulhaq.

Methodology: Eitezaz A. Zaki, Tagreed L. Al-Subhi.

Resources: Mohammad M. Alhazmi.

Software: Mai M. El-Daly.

Supervision: Mai M. El-Daly, Sherif A. El-Kafrawy, Esam I. Azhar.

Writing – original draft: Eitezaz A. Zaki.

Writing – review & editing: Mai M. EL-Daly, Sherif A. El-Kafrawy.

References

- [1] Ottria L, Lauritano D, Oberti L, et al. Prevalence of HIV-related oral manifestations and their association with HAART and CD4+ T cell count: a review. *J Biol Regul Homeost Agents* 2018;32(2 suppl 1):51–9.
- [2] UNAIDS. AIDS epidemic update: December 2007. “UNAIDS/07.27E / JC1322E”;1-41.
- [3] UNAIDS. Fact Sheet – Latest statistics on the status of the AIDS epidemic. Available at: <http://www.unaids.org/en/resources/fact-sheet>. Accessed September 4, 2018.
- [4] Ministry of Health, Saudi Arabia. Global aids response progress report. Country progress report. Kingdom of Saudi Arabia. Available at: http://www.unaids.org/sites/default/files/country/documents/SAU_narrative_report_2015.Pdf. Accessed December 15, 2017.
- [5] Teixeira C, Gomes JR, Gomes P, et al. Viral surface glycoproteins, gp120 and gp41, as potential drug targets against HIV-1: brief overview one-quarter of a century past the approval of zidovudine, the first antiretroviral drug. *Eur J Med Chem* 2011;46:979–92.
- [6] Palanisamy N, Osman N, Ohnona F, et al. Does antiretroviral treatment change HIV-1 codon usage patterns in its genes: a preliminary bioinformatics study. *AIDS Res Ther* 2017;14:2. doi 10.1186/s12981-016-0130-y.

- [7] Klimas N, Koneru AO, Fletcher MA. Overview of HIV. *Psychosom Med* 2008;70:523–30.
- [8] Toledo PVM, Carvalho DSd, Rossi SGd, et al. Genetic diversity of human immunodeficiency virus-1 isolates in Paraná, Brazil. *Braz J Infect Dis* 2010;14:230–6.
- [9] AIDSinfo . Guidelines for the Use of Antiretroviral Agents in HIV-1 Infected Adults and Adolescents. 2017; Available at: <https://aidsinfo.nih.gov/ContentFiles/AdultandAdolescentGL.pdf>. Accessed 08 November
- [10] Toussi SS, Rosenberg M. Antiretroviral therapy for HIV-infected infants, children, and adolescents in resource-rich settings. In: Hope T., Stevenson M., Richman D. (eds) *Encyclopedia of AIDS*. Springer, New York, NY. https://doi.org/10.1007/978-1-4614-9610-6_448-1.
- [11] Simon V, Ho DD, Karim QA. HIV/AIDS epidemiology, pathogenesis, prevention, and treatment. *Lancet* 2006;368:489–504.
- [12] Patarca R, Isava A, Campo R, et al. Human immunodeficiency virus type 1 pharmacogenomics in clinical practice: relevance of HIV-1 drug resistance testing (Part 2). *J Environ Pathol Toxicol Oncol* 2003;22:235–79.
- [13] Rhee S-Y, Jordan MR, Raizes E, et al. HIV-1 drug resistance mutations: potential applications for point-of-care genotypic resistance testing. *PLoS One* 2015;10:e0145772doi:10.1371/journal.pone.0145772.
- [14] Jamjoom GA, Azhar EI, Madani TA, et al. Genotype and antiretroviral drug resistance of human immunodeficiency virus-1 in Saudi Arabia. *Saudi Med J* 2010;31:987–92.
- [15] Al-Mozaini M, Alrahbani T, Al-Mograbi R, et al. Antiretroviral resistance in HIV-1 patients at a Tertiary Medical Institute in Saudi Arabia: a retrospective study and analysis. *BMC Infect Dis* 2018;18:425doi.org/10.1186/s12879-018-3339-7.
- [16] Zhou Z, Wagar N, DeVos JR, et al. Optimization of a low cost and broadly sensitive genotyping assay for HIV-1 drug resistance surveillance and monitoring in resource-limited settings. *PLoS One* 2011;6:e28184doi: 10.1371/journal.pone.0028184.
- [17] Kearse M, Moir R, Wilson A, et al. Geneious basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 2012;28:1647–9.
- [18] Tamura K, Stecher G, Peterson D, et al. MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol Biol Evol* 2013;30:2725–9.
- [19] Wali G. Editor Analysis of the Primary Antiretroviral Drug Resistance among HIV-1 Naive Patients in a Tertiary Hospital in Saudi Arabia. *IDWeek* 2014; 2014: Idsa.
- [20] Elmi Abar A, Jlizi A, Darar HY, et al. HIV-1 drug resistance genotyping from antiretroviral therapy (ART) naive and first-line treatment failures in Djiboutian patients. *Diagn Pathol* 2012;7:1–7.
- [21] Nwobegahay JM, Bessong PO, Masebe TM, et al. Prevalence of antiretroviral drug resistance mutations and HIV-1 subtypes among newly-diagnosed drug-naïve persons visiting a voluntary testing and counselling centre in Northeastern South Africa. *J Health Popul Nutr* 2011;29:303–9.
- [22] Toledo PVM, de Carvalho DS, de Rossi SG, et al. Genetic diversity of human immunodeficiency virus-1 isolates in Paraná, Brazil. *Braz J Infect Dis* 2010;14:230–6.
- [23] Chen JH, Wong KH, Li PC, et al. In-house human immunodeficiency virus-1 genotype resistance testing to determine highly active antiretroviral therapy resistance mutations in Hong Kong. *Hong Kong Med J* 2012;18:20–4.
- [24] Sungkanuparph S, Oyomopito R, Sirivichayakul S, et al. HIV-1 drug resistance mutations among antiretroviral-naïve HIV-1-infected patients in asia: results from the TREAT Asia studies to evaluate resistance-monitoring study. *Clin Infect Dis* 2011;52:1053–7.
- [25] Negedu-Momoh OR, Olonitola OS, Odama LE, et al. Antiretroviral-drug resistant mutations and genetic diversity in HIV-1 infected individuals in Nigeria. *World J AIDS* 2014;4:187–97.
- [26] Martínez-Cajas JL, Pai NP, Klein MB, et al. Differences in resistance mutations among HIV-1 non-subtype B infections: a systematic review of evidence (1996-2008). *J Int AIDS Soc* 2009;12:11doi:10.1186/1758-2652-12-11.