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AU1 ▶

Prevalence of Antiretroviral Drug Resistance Mutations Among Pretreatment and Antiretroviral Therapy-Failure HIV Patients in Uzbekistan

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AU3 ▶ **Abstract**

AU4 ▶ To evaluate the national prevalence of antiretroviral therapy (ART)-resistant HIV-1 viruses among both ART-initiators (pretreatment drug resistance, PDR) and ART-failure HIV patients in Uzbekistan. A nation-wide, cross-sectional active HIV-1 PDR surveillance was conducted in Uzbekistan from 2015 to 2016. In total, 713 blood plasma samples from adults were collected, including samples from ART-naive patients initiating ART and ART-failure HIV patients. HIV-1 genome *pol*region viral sequences were obtained from 309 patients, of those 106 on ART and 203 on ART-initiators. Analysis of HIV-1 subtypes and drug resistance mutations (DRMs) to HIV protease and reverse transcriptase inhibitors was performed. Among all the viruses studied, HIV-1 CRF 02_AG recombinant was the most common—57% (176/309). The second major group was represented by A1—40.5% (125/309). Two viruses were found to be recombinants formed by subtypes A1 and CRF02_AG sequences. ART-naive cohort I (PDR) included six samples that contained at least one surveillance drug resistance mutation (SDRM) (2.96%), with the most common being K103N mutation (4/6). In ART-experienced patients, cohort II, 77.4% (82/106) of viruses contained at least one mutation against PIs, NRTIs, or NNRTIs, with the most common mutations of M184V/I (49.1%; 52/106), K65R (18.9%; 20/106), K103N (23.6%; 25/106), and G190S (22.6%; 24/106). The significant difference in frequency of mutations was found between two dominant subtypes, A1 and CRF02_AG. The molecular epidemiological profile of HIV infection in Uzbekistan has changed toward a predominance of CRF02_AG viruses. In the first national-scale study of the PDR prevalence, it was found to be relatively low (2.96%). The DR mutations in failure patients correspond to the main treatment regimens (NRTI/NNRTI) adopted in the country. The observations provide new evidence for differences in ART efficacy and resistance profiles for different subtypes.

AU5 ▶ **Keywords:** resistance, mutations, HIV, subtype, Uzbekistan

Introduction

Formerly part of the USSR, and now an independent member of the Commonwealth of Independent States (CIS), the Republic of Uzbekistan is the largest Central Asian country with a population of over 30 million residents. The first cases of HIV infection had been observed in Uzbekistan in the late 80s. While in the early years of the epidemic, it was concentrated almost entirely in the intravenous drug user (IDU) group. Since then, the state of the epidemic has changed, and around 2010, sexual transmission began to dominate over the parenteral route, and the epidemic progressed from IDUs toward the general population.[1]

According to the National progress report of 2015, there were totally 30,315 HIV-positive individuals registered in Uzbekistan, and in 2018, this number had increased to 52,000[2] with more than 30% of them being women. The epidemic continued to grow, and the percentage increase in

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new HIV infections in 2018 since 2010 was one of the highest in the world, amounting to 32%.

The national antiretroviral treatment (ART) program was introduced in Uzbekistan in April 2006 with the financial support of the Global Fund to fight AIDS, tuberculosis, and malaria, and currently over 27,000 HIV patients (51% of people living with HIV) throughout the country received ART free of charge.[2] A network of AIDS centers has been established in the country to organize epidemiological monitoring and treatment of HIV infection, and the national ART protocols were developed.[3]

One of the most difficult barriers to ART program success is HIV drug resistance (HIVDR).[4,5] The first WHO recommendations on HIVDR surveillance was published in the year 2014[6,7] and in the same year the Ministry of Health of Uzbekistan (MoHUz) established the HIV drug resistance working group whose goal was to introduce WHO HIVDR prevention strategies at the national level. With financial and technical support from UNAIDS (www.unaids.org), Rospotrebnadzor (Russian Federation), and MoHUz, the methodology of HIVDR mutation analysis (HIV genotyping) became available.

In 2015, the national HIVDR surveillance program was developed jointly by the Research Institute of Virology and Republican AIDS center in accordance with the WHO recommendations. The first task of this program was the assessment of HIVDR mutation prevalence among HIV-positive people initiating ART (pretreatment drug resistance, PDR).[6,7]

The first financial tranche was received in the year 2015, and it was decided that a part of the funds should be spent conducting the first round of PDR (cohort I), and the other part should be used for the analysis of HIVDR in patients who experienced ART failure at that time (cohort II).

According to the data from Republican AIDS Center MoHUz, at the beginning of the blood sample collection in 2015, the main treatment regimens used as first-line therapy in Uzbekistan were as follows: tenofovir (TDF)/zidovudine (AZT)/abacavir (ABC)+emtricitabine (FTC)/lamivudine (3TC) as the NRTI backbone; efavirenz (EFV)/nevirapine (NVP) as the third NNRTI drug were administered to 100% of the patients. The PI-based ART regimens were used as second-line only and were mostly limited to ritonavir-boosted lopinavir (LPV/rtv; 96.0%); in 4.0% of the patients, INSTI (RAL or DTG) drugs were used.

The genotyping analysis of the samples collected in 2015–2016 was performed in subsequent years (2017–2019) and its completion was delayed due to financial and technical reasons. In this article, the results of the final analysis of all the sequences obtained in this HIVDR study are presented.

Materials and Methods

Sample size and study participants

Plasma specimens from 713 HIV-infected patients were collected between May 2015 and January 2016 with informed consent. The study protocol was reviewed and approved by the Research Ethics Committee of the Ministry of Health of the Republic of Uzbekistan.

During that period, there were 19 ART providing facilities (AIDS centers) in Uzbekistan; all of them were included into the PDR survey (cohort I) according to the WHO recommendation for the countries with few ART clinics.[6]

The information about the number of HIV patients in each clinic initiating ART in the previous year was unknown. The only information available was the total number of patients on ART at the end of a previous calendar year by clinics, and so, the probability proportional to proxy size sampling method was used. For countries with few ART facilities, the standard required sample size recommended for PDR surveillance was 346 samples.

The number of sampled patients at each clinic was calculated proportionally to the size of the clinic (i.e., the total number of patients on ART at the end of a year).

The enrollment criteria for the cohort I (PDR) included that the patients provide informed consent, ≥18 years, and with viral load ≥1,000 copies/mL. Patients with a history of receiving ART (reinitiating ART or having mother-to-child transmission prevention) were not excluded from enrollment; nevertheless, such patients were not identified during the recruitment.

Recruitment of patients with ART failure (cohort II) was carried out in the same clinics as PDR patients. The number of samples was not precalculated for monitoring purposes and depended on the number of patients meeting the following criteria: ≥18 years, viral load ≥1,000 copies/mL at the time of enrollment (considered ART failure), and being on ART for more than 12 months and still on ART at the time of enrollment.

A simple questionnaire with essential data, including the date of HIV diagnosis, sociodemographic information (i.e., age, gender, presumptive transmission route), laboratory data (i.e., CD4 count and HIV viral load), and antiretroviral treatment history for the ADR cohort, was applied to each case before blood specimen withdrawal. ◀AU6

Genotype analysis

HIV-1pol genome region sequencing was performed at the Laboratory of Molecular Genetic Analysis of the Research Institute of Virology. The extraction of viral RNA and further amplification were performed using the AmpliSense HIV Genotype EPh kit (InterLabService™). Sequencing of obtained HIV genome pol region-amplified products was further performed with the Applied Biosystems 3500xL Genetic Analyzer using six primers. For subtyping, the HIV-1 pol sequences obtained were analyzed with the Stanford University HIV Drug Resistance Database (https://hivdb.stanford.edu) and REGA HIV-1 Subtyping Tool—Version 3.0.[8]

HIV drug resistance mutation prevalence analysis

The HIVdb program tool of the Stanford database was used to assess HIV drug resistance mutations (DRMs) among the ART-experienced populations.[9] HIVDR was defined as the presence of the penalty score of ≥15 for any antiretroviral drug. The Calibrated Population Resistance (CPR) tool was used to assess the PDR prevalence.[10]

Results

Study population

Of totally enrolled 713 patients, there were 344 HIV-positive adults enrolled into the PDR study (cohort I) and 369 into the ADR study (cohort II).

Table 1. Study Population Demographic Characteristics

	PDR cohort	*ADR cohort*	*Total*
Obs	**344**	**369**	**713**
Age, median (IQR)	36 (30–42)	36 (31–41)	35 (30–41)
Gender, *n* (%)			
Female	168 (48.8)	212 (57.5)	380 (53.3)
Male	176 (51.2)	156 (42.3)	332 (46.6)
Transgender	0 (0)	1 (0.3)	1 (0.1)
Transmission route			
Parenteral	92 (2.7)	150 (40.7)	421 (59.0)
Sexual	146 (42.4)	159 (43.1)	305 (42.8)
Unknown	106 (30.8)	60 (16.3)	166 (23.3)
Viral load (log_{10} copies/mL), median (IQR)	4.6 (4.0–5.3)	3.9 (3.3–4.9)	4.3 (3.5–5.1)
CD4 count (cells/mm^3), median (IQR)	264 (151–371)	—	264 (151–371)

IQR, interquartile range; PDR, pretreatment drug resistance.

The demographic characteristics of all patients are presented in Table 1. The median age of population evaluated was 35, and the majority (53.3%) were women. The parenteral route of transmission (59.0%) prevailed over sexual route (42.8%); in a significant proportion of patients, the route of transmission remained unknown (23.3%).

The median viral load in ART-naive patients was 39,729 copies/mL, which is several times higher than those on ART with recorded ART failure at enrollment (≥1,000 copies/mL)—8,789 copies/mL.

Among adults from cohort II with ART failure, only 54 (14.7%) received the second-line treatment regimens that included 2NRTI+LPV/rtv. The rest of the patients failed on first-line ART regimens with majority of them including 3TC (255/369, 69.1%) and EFV/NVP (100%) (Table 2).

Distribution of HIV-1 subtypes

In total, 309 nucleotide sequence consensuses with a length of 911–1,238 bp had been obtained for mutation and subtype analysis in both cohorts; among them, 203 in PDR cohort I and 106 in ADR cohort II. According to the REGA interpretation, most of the viruses were recognized as circulating recombinant form CRF02_AG (176/309, 57.0%). There were four additional subtypes identified: 125 (40.5%) viruses belonged to subtype A1, 3—to subtype B, 2 were defined as recombinant of A1, G, and one to subtype C. Two sequences were preliminarily considered unique recombinant forms (URFs) between subtype A1 and CRF02_AG. There were no significant differences in the transmission of different subtypes depending on the route of infection (Table 3). A more detailed molecular and bioinformation analysis was not included in the objective of this article and will be discussed in detail in a specially dedicated work.

Table 2. Distribution of Regimens in the Study

Treatment regimen in ADR cohort (n = *369*)	N *(%)*
TDF +3TC+EFV	79 (21.4)
AZT +3TC+EFV	76 (20.6)
TDF+FTC+EFV	46 (12.5)
AZT +3TC+NVP	43 (11.7)
ABC +3TC+LPV	42 (11.4)
TDF +3TC+NVP	26 (7.0)
ABC +3TC+EFV	20 (5.4)
TDF+FTC+LPV	12 (3.3)
ABC +3TC+NVP	11 (3.0)
Other	14 (3.8)

3TC, lamivudine; ABC, abacavir; AZT, zidovudine; EFV, efavirenz; FTC, emtricitabine; LPV, lopinavir; NVP, nevirapine; TDF, tenofovir.

Prevalence of HIVDR in ART-naive cohort I (PDR)

Of 344 samples analyzed, 203 cohort I sequences of RT and PR HIV-1 pol regions have been obtained and further evaluated for DRMs. The main PDR outcomes according to the WHO recommendations[6] are presented in Table 4.

Among the 203 sequences, 6 samples were found, each of which contained 1 mutation from the list of surveillance mutations (2.96%).[11] According to REGA analysis results,[8] three of the patients were infected with CRF 02_AG viruses and three with subtype A1 HIV-1 viruses. The most common mutation was K103N (4/6) associated with NNRTI resistance. The remaining two sequences carried M41L mutation in RT pol region (NRTI resistance), and I85V mutation in PR pol region.

Among the nonsurveillance mutations, the most prevalent were A62V (NRTI, 16.3%; 33/203) and E138A (NNRTI, 2.5%; 5/203).

Prevalence of HIVDR mutations in ART-experienced cohort II

Of the 369 cohort II samples analyzed, 106 sequences were obtained. Of them, 82 contained at least one mutation against PI, NRTI, or NNRTI drugs (77.4%, 82/106). The analysis for HIVDRMs in cohort II was carried out according to the Stanford penalty scores to identify the following levels of resistance: high-level resistance (≥60), intermediate (30–59), low (15–29), potential low level (10–14), and susceptible (0–9). The high-level resistance was mostly spread against NVP (64.2%; 68/106), EFV (58.5%; 62/106), FTC (50.0%; 53/106), 3TC (50.0%; 53/106), and ABC (27.4%; 29/106). Only one patient revealed resistance to LPV/rtv (0.9%; 1/106).

The most common NRTI resistance mutations were M184V/I (49.1%; 52/106), A62V (36.8%; 39/106), and K65R (18.9%; 20/106) mutations followed by Y115F (6.6%; 7/106), L74I (6.7%; 6/106), and M41L (5.7%; 6/106).

Table 3. Distribution of HIV-1 Subtypes by Transmission Routes

Transmission	*02_AG*	*%*	*A6*	*%*	*6302_A1*	*%*	*B*	*%*	*C*	*%*	*URF*	*%*	*Total*
Parenteral	59	33.5	40	32.0	—	—	—	—	—	—	1	50.0	100
Sexual	69	39.2	58	46.4	—	—	3	100.0	—	—	—	—	130
Unknown	48	27.3	27	21.6	2	100.0	—	—	1	100.0	1	50.0	79
Total	176	100.0	125	100.0	2	100.0	3	100.0	1	100.0	2	100.00	309

URF, unique recombinant form.

Among the thymidine analogue mutations (TAMs), the highest prevalence was attributed to T215YF (8.5%; 9/106), M41L (5.7%; 6/106), D67N (3.8%; 4/106), and K70R (3.8%; 4/106). In total, TAMs accounted for 18.9% (20/106 of all samples).

Among the NNRTI mutations, the most frequently identified were nonpolymorphic mutations K103N, 23.6% of samples (25/106), G190S (22.6%; 24/106), K101E (15.1%; 16/106), and Y181C (17.0%; 18/106).

Of note, the significant difference in frequency of mutations was found between two dominant subtypes, A1 and CRF02_AG (Table 5). T5 ▶

In general, mutations in A1 were much more common at failure than in CRF02_AG (97.8% vs. 62.5%, $p < .001$).

The major G190S NNRTI mutation was much more prevalent in A1 subtype samples than in CRF02_AG viruses (22/46 vs. 1/56, $p < .001$); a similar situation was observed for K65R (16/46 vs. 2/56, $p < .001$) and K101E mutations (14/46 vs. 1/56, $p < .001$); a less pronounced difference was noted for Y115F. In contrary, K103N—the most common NNRTI mutation, was significantly more prevalent among CRF02_AG recombinant viruses (5/46 vs. 19/56, $p = .001$). The A62V mutation (37/46, vs. 1/56, $p < .001$) was much more widely present in A1 subtype vs. CRF02_AG. In 7 samples, A62V was present as the only mutation (6.6%; 7/103) and all seven viruses were qualified by the REGA tool as the HIV-1 A1 subtype. In 31 cases, A62V was found in combination with K65R or M184V (29.2%; 31/103).

The rare PI mutations were found in two ART-experienced patients—M46I, I47A, I84V, and L76V.

Discussion

The major goal of this study was to assess the prevalence of surveillance of DRMs among HIV-infected ART-naive patients based on the WHO recommendations in a prospective study at a national scale in Uzbekistan in 2015–2016 (cohort I).[6] In addition, the cross-sectional analysis of HIVDR was carried out in ART-failure patients in the same clinics (cohort II).

There was very little information about the distribution of HIV-1 subtypes in Uzbekistan at the time the study began. In 2002, most of 142 patients studied were infected with subtype A strain (88.0%) emerging from Russia and Ukraine and common for all the former Soviet Union countries.[12] Thirteen of the viruses studied (9.2%) clustered with CRF02_AG, an HIV strain common in West Africa, probably descended from a single ancestor. Soon, the same virus was discovered in Kazakhstan.[13]

The cocirculation of these two dominant strains in Central Asia had resulted in new recombinant formations; among them, the recombinant CRF63_02A1 was subsequently widespread in Russia.[14]

As was later shown by molecular clock analysis, the time to the most recent common ancestor (tMRCA) of the CRF63_02A1 epidemic was in 1996,[15] which indicated that the CRF02_AG virus came to Uzbekistan earlier than was generally believed.

This study is the most representative of all HIV subtype studies conducted in Uzbekistan and shows that CRF02_AG currently occupies a dominant position in the country (57.2%) and continues to form the new recombinants. These data will be given detailed consideration in the forthcoming publication.

The overall prevalence of HIVDR surveillance mutations[11] in cohort I was found to be 2.96% (3/203). The first-line antiretroviral treatment in 2015 was entirely represented by 2NRTI+NNRTI regimens containing evenly EFV and NVP, and the second-line regimen included LPV/rtv. The mutations detected in this study were fully consistent with the ART regimens adopted in Uzbekistan during the sampling period and included mostly the K103N mutation associated with NNRTI resistance, as well as M41L TAM mutation in reverse transcriptase, M46L mutation providing broad resistance to PIs, and weak I85V surveillance in PI mutation.

The A62V and E138A mutations were found in 16.3% (33/203) and 2.5% (5/203) of PDR patients, corresponding with A62V associated exclusively with A1 subtype and most likely reflected the genome polymorphism in A1 HIV-1 subtype. This work does not provide the data of phylogenetic analysis, however, according to previously published data,[16–18] there is every reason to believe that the same genetic variant of subtype A HIV-1 circulates in Uzbekistan as

Table 4. Pretreatment Drug Resistance Survey Main Outcomes

1a	Prevalence of HIVDR among all ART initiators, regardless of prior exposure to ARVs	3%
1b	Prevalence of HIVDR among ART initiators without prior exposure to ARVs	3%
1c	Prevalence of HIVDR among individuals initiating ART with NNRTI-based regimens without prior exposure to ARVs	3%
2a	Proportion of all ART initiators without prior exposure to ARVs	100%
2b	Proportion of all ART initiators with prior exposure to ARVs	0%
2c	Proportion of all ART initiators with unknown prior exposure to ARVs	—

Table 5. A6 and CRF02_AG Subtype-Specific Distribution of NNRTI and NRTI Mutations in ADR Cohort

Mutations	*A6 (n = 46)*	*%*	*O2_AG (n = 56)*	*%*	p
At least one mutation	45	97.8	35	62.5	<.0001
NRTI					
A62V	37	80.4	1	1.8	<.0001
K65R	16	34.8	2	3.6	<.0001
M184V	26	56.5	20	35.7	.0324
NNRTI					
Y181C	13	28.3	4	7.1	.0049
G190S	22	47.8	1	1.8	<.0002
K101E	14	30.4	1	1.8	<.0003
Y115F	6	13.0	1	1.8	.0266
K103N	5	10.9	19	33.9	.0070

in other countries of the former USSR, there is sub-subtype A6. The A62V and E138A mutations are well known to be polymorphic in these viruses.[19,20] The only PI mutation found was M46L associated with resistance to most PIs, including LPV/rtv used as the only third drug of the second regimen.

In ART-experienced patients, DRMs for NRTI and NNRTI were highly prevalent with the most widespread being M184V/I mutation—49.1% of all ART-failure cases (49.1%; 52/106) were evenly distributed between two dominant subtypes A1 (sub-subtype A6) and CRF02_AG. The most interesting finding was the uneven distribution of NRTI and NNRTI mutations between A1 and CRF02_AG.

First of all, attention was drawn to the more rarely encountered resistance in the CRF02_AG viruses than in A1 (35/56 vs. 45/46, $p < .001$).

Subtype A1 (likely A6), which is common for Eastern Europe and supposedly invaded from Russia through labor migration routes, had contained significantly more mutations than CRF02_AG (97.8%, 45/46). If the viruses in which only the A62V mutation was detected are disregarded and considered to be the result of polymorphism rather than resistance in ART patients, then the proportion of A1 ART patients with drug resistance is reduced (83.6%), but remains statistically significant ($p = .029$).

The nonpolymorphic mutations prevailing in A6 included K65R, G190S, K101E, and Y181C. The frequent occurrence of G190S mutation and the relative ease with which it occurs in sub-subtype A6 had already been documented in Russia.[21] The other three mutations in A6, more frequent when compared with CRF02_AG, were described for the first time, and this finding needs more careful and prolonged observation. The same is true with regard to K103N mutation, which was more prevalent in CRF02_AG. These observations provide new evidence for differences in ART efficacy and resistance profiles for different subtypes.

Thus, the prevalence of HIVDR surveillance mutations in 2015 was relatively low and did not require revision of treatment protocols, however, the proportion of resistance to NNRTIs in the treatment of failure patients draws attention to the need for systematic national-scale surveillance of HIVDR both in naive and treatment-experienced HIV-infected patients in Uzbekistan.

Conclusion

The molecular epidemiological profile of HIV infection in Uzbekistan has changed toward a predominance of CRF02_AG viruses. In the first national-scale study of PDR prevalence, it was found to be relatively low (2.96%). The DR mutations in failure patients correspond to the main treatment regimens (NRTI/NNRTI) adopted in the country. The observations provide new evidence for differences in ART efficacy and resistance profiles for different subtypes.

Sequence data/accession number(s)

The obtained nucleotide sequences were deposited in GenBank and are available under accession numbers: MF401457–MF401512, MF431622–MF431713, MF431717, MF431719, MF497081, MF497088, MF497090–MF497219, MF497220, MF497222–MF497262, and MF497263.

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Author Disclosure Statement

The authors declare no conflicts of interest.

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AU15: Please mention the significance of bold values in Table 1.
AU16: Please define ''ARVs.''