GENETIC DIVERSITY AND HIV-1 DRUG RESISTANCE MUTATIONS IN LENINGRAD REGION

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Keywords: HIV drug resistance, HIV genotyping, ART, molecular epidemiology.
Resume: In the examined group, the A6 subtype prevailed (97.4%), however, in isolated cases, subtype B and a recombinant between the circulating recombinant form CRF_03AB and subsubtype A1 were found. According to the results of the analysis, 95.79% of patients had at least one significant mutation associated with drug resistance for the corresponding virus subtype. A total of 105 different drug resistance mutations were identified at 35 positions in the virus genome.

Relevance. HIV infection belongs to the so-called socially significant diseases that are dangerous for others, and is also recognized as a threat to national security [9, 10]. In the first half of 2020, 38,126 people with antibodies to HIV were identified in the Russian Federation, and by the end of the first half of 2020, 1,094,050 Russians with laboratory-diagnosed HIV infection lived in the country [1]. Also, according to the Federal AIDS Center, drug resistance in patients who did not take ARP occurs in 5.5% of cases [2].

The Leningrad Region is a constituent entity of the Russian Federation located in the northwest of the European part of the country. The regional center is the city of federal significance - St. Petersburg, which has a significant impact on the region under study.

In St. Petersburg, the HIV epidemic began in the late 1980s and was sluggish, as it spread mainly in risk groups through sexual contact, the group of cases was small and subtype B dominated. Then the size of the risk group increased significantly due to the penetration of the virus into population of injecting drug users (IDUs). Since the mid-1990s, an avalanche-like epidemic spread of HIV has begun in Russia [3, 4]. In the IDU group, subsubtype A6 has become widespread, and injecting has become the main route of HIV transmission [5].

Despite the fact that the incidence in St. Petersburg and the Leningrad Region in the first half of 2020 did not exceed the national average, these regions are among the most affected by HIV infection. At the same time, heterosexual contacts are currently the dominant route of transmission of the virus [1]. According to the literature data, the study region is characterized by the predominance of HIV-1 subsubtype A6, however, other subtypes circulate together with it [2, 6].

The latest data on the prevalence of HIV drug resistance among patients with virologically ineffective ART in St. Petersburg date back to 2012 [5], and there is practically no information reflecting the current situation with the secondary drug resistance of the virus. Similar data on patients in the Leningrad region are not found in the literature.

In St. Petersburg, as a major transport, tourist, cultural, industrial and border center, there is a high migration activity of the population. This may contribute to the introduction and spread of new genetic variants of the virus and recombination processes in the viral population of the region.

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Aim: characterization of the modern subtypic profile and mutations of HIV-1 drug resistance among patients with virological failure of antiretroviral therapy in the Leningrad region.

Objective: 1. to study the genetic diversity of HIV in the territory of the Leningrad region; 2. draw conclusions about the prevalence of primary drug resistance in the Leningrad region.

Materials and methods. In the course of work in 2016-2018. studied clinical material from 138 patients from the Leningrad region with confirmed virological ineffectiveness of ART. The blood plasma was sent to the North-Western Federal Center for the Prevention and Control of AIDS (NWF AIDS Center) based at the St. Petersburg Pasteur Research Institute of Epidemiology and Microbiology to determine HIV drug resistance.

In the obtained blood plasma, the viral load (VL) was detected using the AmpliSens® HIV-Monitor-FRT commercial kit with a sensitivity threshold of 500 copies/ml, and samples with detectable viral load (VL) were subsequently subjected to RT-PCR and Sanger sequencing. For reverse transcription and amplification of HIV, commercial kits "RT-PCR-kit-Pro/Rev" and "PCR-kit-Pro/Rev" were used, the sequencing reaction was carried out according to the instructions for the kit "AmpliSense® HIVResist-Seq". HIV genotyping was performed based on the analysis of the nucleotide sequences of the 1302 nt polymerase (pol) gene region encoding the protease (PR) and part of the reverse transcriptase (RT/RT) in the 2253–3554 nt region, the coordinates are given for the one presented in the international database GenBank HIV HXB2 (K03455.1). The sequencing reaction products were analyzed using an ABI Prism 3500 genetic analyzer (Applied Biosystems, USA).

The primary analysis of nucleotide sequences was performed using the NCBI Blast program in comparison with the nucleotide sequences presented in the international GenBank database. Nucleotide sequences were aligned using the MEGAv.7.0 program using the ClustalW algorithm [7]. For the construction of phylogenetic trees and subsequent phylogenetic analysis, the Neighbor-joining algorithm was used, which allows optimization of trees in accordance with the criterion of "balanced minimum evolution", when assessing the reliability of phylogenetic relationships, multiple generation of samples using the Bootstrap method for 1000 independent constructions of each phylogenetic tree was used.

Genotyping of the studied isolates was carried out in parallel using the REGA HIV-1 Subtyping Tool 3.0 program and based on the analysis of their phylogenetic relationships with reference sequences from the international GenBank database. Analysis of HIV genetic sequences for the presence of drug resistance mutations was performed using the Stanford HIV DB https://hivdb.stanford.edu/hivdb/by-mutations/)

Results and their discussions. Of the 138 patients, more than half (58.5%) were male. The study group was dominated by the age category of 35-44 years (59.6%), the median age was 36 years. All patients had an HIV viral load greater than 1000 copies/ml, which made it possible to obtain viral genome sequences encoding a protease and a reverse transcriptase site.

Based on two typing methods, phylogenetic analysis and sequence analysis using the REGA HIV-1 Subtyping Tool 3.0 program, which made it possible to more accurately

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assess the distribution of HIV-1 subtypes, the following ratio of HIV-1 subtypes was shown: the A6 subtype typical for Russia prevails (97, 4%), in isolated cases subtype B and a recombinant between the circulating recombinant form CRF_03AB and subsubtype A1 were encountered.

According to the results of the analysis, 95.8% of patients had at least one significant mutation associated with drug resistance for the corresponding virus subtype. In total, we encountered 105 different mutations of drug resistance in 35 positions of the virus genome. The mutations encountered in the majority (89.5%) cause resistance to nucleoside (NRTI) (42.9%) and non-nucleoside (NNRTI) (46.7%) reverse transcriptase inhibitors. The smallest proportion among the encountered mutations (10.5%) is represented by mutations associated with resistance to protease inhibitors (PIs).

In the overwhelming majority of detected cases of drug resistance (93.68%), two or more mutations occurred in the analyzed isolates. At the same time, in such patients, the virus is most often resistant to two (73.21%) classes of drugs, but sometimes to three (8.16%) classes of ARP at once.

The obtained and analyzed nucleotide sequences of the HIV polymerase gene region were deposited in the international GeneBank database under the numbers OL505461 - OL505538.

The genetic diversity of HIV in the examined group correlates with the characteristic for the territory of the Russian Federation - the absolute predominance of subsubtype A6 [2]. It is important to note that when genotyping with the REGA HIV-1 Subtyping Tool 3.0, all isolates were assigned to subsubtype A1, however, our own phylogenetic analysis allows us to assign them to subsubtype A6 with full confidence. This discrepancy can be explained by the fact that the latest versions of the software used do not take into account data confirming the need to distinguish the A6 sub-subtype separately from the A1 sub-subtype. In addition, in a single case, a complex recombinant between CRF_03AB and subtype A was encountered, the recombination analysis of which, in the framework of this work, we carried out within the pol gene.

In comparison with the data of 2012 related to St. Petersburg, the frequency of occurrence of HIV drug resistance mutations increased by more than three times: from 30% to 95.8% [5]. Such an increase in the number of resistant variants of the virus can be explained by a change in the population of HIV-infected people in Russia: during this period, the epidemic left vulnerable groups of the population, and as a result, the social status of PLHIV increased. Among patients taking ARP, adherence has increased, and at the same time, the number of patients with suboptimal adherence to treatment, which is the most dangerous for the formation of drug resistance, has increased [8]. At the same time, the incidence of transmissible primary resistance is growing in the region, which undoubtedly contributes to the prevalence of drug resistance among people taking ARP, since at the moment there is no testing of patients before starting therapy for the presence of drug resistance mutations [6].

In addition to the occurrence of mutations, their structure also changed. M184V is still in first place in terms of occurrence (77%). In second place is the Q151M mutation (51%), associated with drug resistance to several nucleoside reverse transcriptase inhibitors at once, while it was not noted in 2012 [5]. Mutations G190S (47%) and K103N

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(13%), associated with resistance of the virus to non-nucleoside inhibitors, were in third place in terms of frequency of occurrence; in this study, mutations in position 101 (47%), also associated with resistance to NNRTIs, took third place. Mutations of resistance to NRTIs and NNRTIs have occurred with equal frequency, in many cases together, causing resistance to most reverse transcriptase inhibitors.

Conclusions: HIV A6 (IDUA) remains the predominant HIV genovariant in the Leningrad Region among patients with virologically ineffective ART. A significant increase in the frequency of HIV drug resistance mutations in the region compared to 2012 was shown. Among samples with drug resistance mutations, in all cases, mutations of pharmacoresistance to two or three groups of drugs were found. Given the high incidence of drug resistance mutations in patients with virologically ineffective ART, surveillance of HIV drug resistance in both ART recipients and ART-naive individuals appears to be necessary. Lack of control may lead to the spread of primary ART-resistant HIV.

Literature

1. Fact sheet. HIV infection in the Russian Federation as of December 31, 2020 (In Russian) http://www.hivrussia.info/wp-content/uploads/2021/03/VICH-infektsiya-v-Rossijskoj-Federatsii-na-31.12.2020-..pdf

2. Level and structure of HIV drug resistance among naive patients in the Russian Federation (In Russian) http://www.hivrussia.info/wp-content/uploads/2020/12/2020-Rossijskaya-baza-dannyh-LU-VICH-u-naivnyh-patsientov.pdf

3. Onishchenko G.G. HIV infection is a problem of humanity. HIV infection and immunosuppression. 2009; V. 1, No. 1. P. 5–9. (In Russian)

4. Belyakov N. A. The brain as a target for HIV: act speech. St. Petersburg: Baltic Medical Education Center. 2011. P. 48. (In Russian)

5. Dement'yeva N. Ye, Sizova N. V., Lisitsyna Z. N., N. A. Belyakov Molecular and epidemiologic characteristic of HIV infection in saint-petersburg. Medical academic journal 2012; 12(2). P. 97–104. (In Russian)

6. Ingabire T., Semenov A.V., Esaulenko E.V., Zueva E.B., Schemelev A.N., Bushmanova A.D. Primary HIV drug resistance among newly HIV type-1 diagnosed patients in St. Petersburg. HIV Infection and Immunosuppressive Disorders. 2021;13(1) P.70-79 (In Russian) https://doi.org/10.22328/2077-9828-2021-13-1-70-79

7. Kumar S., Stecher G., Tamura K. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Mol. Biol. Evol. 2016; 33(7) P. 1870–4. DOI: 10.1093/molbev/msw054.

8. Lebedeva N.N., Zverev S.Y., Kulagin V.V., Kurina N.V., Pronin A.Yu., Mikova O.E., Milovanova I.I., Polovitsa N.V., Sandyreva T.P., Sizova N.V., Sklyar L.F., Tertyshnaya Yu.N., Belkina N.S., Shemshura A.B., Bobkova M.R. HIV drug resistance early warning indicators and their assessment in some regions of Russia. HIV Infection and Immunosuppressive Disorders. 2018;10(4) P.67-75. (In Russian) https://doi.org/10.22328/2077-9828-2018-10-4-67-75

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