PREVALENCE OF DRUG RESISTANT HIV-1 FORMS IN PATIENTS WITHOUT ANY HISTORY OF ART IN THE REPUBLIC OF GUINEA

Shchemelev A.N., Ostankova Yu.V., Zueva E.B., Semenov A.V.

Saint-Petersburg Pasteur Institute, Saint-Petersburg
ERIVI, FBRI SRC VB "Vector", Rospotrebnadzor, Ekaterinburg

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Resume: *Serological markers of HIV were detected in 239 people, which represents 11.02% of the entire sample. HIV RNA was detected in 58 people. Was revealed subtypes: HIV CRF02_AG (41.9%); A1 (29.1%); A3 (12.9%); URF A1_G (12.9%); G (3.2%). In 25% of patients, at least one significant mutation was encountered leading directly to HIV drug resistance. The mutations encountered cause resistance to NRTI and NNRT. Major resistance to PI was not seen.*

Relevance. The human immunodeficiency virus (HIV) epidemic continues to spread rapidly around the world. According to Joint United Nations Programme on HIV/AIDS (UNAIDS) estimates, the number of people currently infected with HIV globally is about 31.6 - 44.5 million, while the number of new infections amounted to 1.2 - 2.2 million cases in 2019 [1]. The African continent is one of the most HIV-affected regions in the world. It is currently home to 25.6 million people living with HIV (PLHIV), which is 67.37% of all registered HIV infections globally. The overwhelming majority (80.86%) are in the countries of Eastern and Southern Africa [2].

The Republic of Guinea is a located in West Africa. HIV prevalence in Guinea was approximately 1.6% in 2014. In 2019, it was practically unchanged, but treatment coverage also remains one of the lowest in the world, with less than a quarter (23%) of people living with HIV on antiretroviral therapy (ART) [1, 3].

The Republic of Guinea is one of the countries affected by the Ebola epidemic. As such, there were significant difficulties in providing treatment to patients with HIV infection in the 2014-2015 period. This, in turn, may have affected the prevalence of drug resistance (DR). Therefore, more careful monitoring of HIV resistance is required in this region. However, the most recent data on drug resistance in Guinea reported in the literature dates back to 2009, when the prevalence of primary drug resistance was 8.9% [4]. In 2016, it was not possible to analyze a sufficient number of samples to identify the prevalence of DR in the Republic of Guinea [4]. In addition, in the above study, patients with an existing diagnosis were examined, but due to the low awareness of HIV infection in the Guinean population, patients who do not know about their infection can make a significant contribution to the genetic diversity of the virus.

Aim: analysis of primary drug resistance in adult HIV patients in the Republic of Guinea.

Objective: 1. To study the genetic diversity of HIV in the territory of the Republic of Guinea; 2. Draw conclusions about the prevalence of primary drug resistance in the Republic of Guinea.

Materials and methods. Patient samples were tested for the presence of HIV antigens and antibodies to the virus by ELISA. Quantitative analysis of HIV RNA was carried

УДК 61:001(06) ББК 5 Ф 94

out with a commercial kit, AmpliSens® HIV-Monitor-FRT (Central Research Institute of Epidemiology, Russia), with a sensitivity threshold of 500 copies/ml. Samples with a detectable viral load (VL) were analyzed using RT-PCR and Sanger sequencing. For reverse transcription and amplification of HIV RNA, the RT-PCR-kit-Pro/Rev and PCR-kit-Pro/Rev commercial kits (Central Research Institute of Epidemiology, Russia) were used. Sequencing reactions were performed using the AmpliSens® HIVResist-Seq kit (Central Research Institute of Epidemiology, Russia) according to the instructions, as described earlier [5]. For HIV genotyping, we used a 1302 nucleotide sequence spanning the pol gene (nt. 2253–3554). Coordinates given for the data represent the GenBank entry for HIV HXB2 (K03455.1). Analysis of sequence reaction products was performed using an ABI Prism 3500 genetic analyzer (Applied Biosystems, USA).

Sequence data were analyzed using the NCBI Blast program to compare nucleotide sequences with those in the GenBank international database. Alignment of nucleotide sequences was executed with the MEGA (7.0) program using the ClustalW algorithm [6]. For the construction of phylogenetic trees and subsequent phylogenetic analysis, the Neighbor-joining algorithm was used, which allows optimization of trees by the criterion of "balanced minimum evolution". When assessing the reliability of phylogenetic relationships, we used multiple generations of samples using the bootstrap method for 1000 independent constructions of each phylogenetic tree.

Isolates were analyzed for recombination features using the REGA HIV-1 Subtyping Tool (3.0). Samples were also analyzed for phylogenetic relationships using MEGA and reference sequences from GenBank. This made it possible to more accurately assess the distribution of HIV-1 subtypes in the studied population. Analysis of sequences for the presence of drug resistance mutations was performed using the Stanford University HIV Drug Resistance Database.

Results and their discussions. In total, materials were studied from 2168 patients. Their ages were within the range 18-58 years, with a median age of 38 years (β = 0.95, (37.58; 39.72)). Most of the examined patients were males (68.17% (β = 0.95; (66.21%; 70.13%))). Viral RNA was detected in 58 patients, aged 18 to 54 years. The median age was 36 years (β = 0.95, (32.53; 39.47)). The predominant age group was those from 31 to 45 years old (62.07%). The most represented in the sample were men. They made up 63.79% of the study group (β = 0.95, (52%; 76%)), while women represented 36.21% (β = 0.95, (24%; 49%)).

Serological markers of HIV were detected in 239 people, which is 11.02% (β = 0.95; (9.74%; 12.42%)) of the total sample. Note that 69.45% (β = 0.95; (63.19%; 75.23%)) of patients with identified HIV markers were young people aged 20 to 39 years. HIV RNA was detected in 58 people, which represents 24.27% (β = 0.95 (18.97%; 30.21%) of patients in the seropositive group (2.68% of the total group).

The HIV pol gene of all patients with a detectable VL was sequenced and submitted to GenBank (MT874291-MT874321, MT874310-MT874321, MT919401-MT919427). Analysis made it possible to identify the following HIV-1 subtype ratios. The circulating recombinant form (CRF), 02_AG (56.90% (β = 0.95; (43.23%; 69.84%))), prevailed in the study group compared to: HIV A3 (13.79% (β = 0.95; (6.15%; 25.38%))); A1 (12.07%; (β = 0.95; (4.99%; 23.30%))); G (8.62% (β = 0.95; (2.86%; 18.98%))); CRF_06cpx (5.17%;

УДК 61:001(06) ББК 5 Ф 94

(β = 0.95; (1.08%; 14.38%))); URF between A1 and G (1.72%; (β = 0.95; (0.04%; 9.24%))); and A6 (1.72%; (β = 0.95; (0.04%; 9.24%))).

According to the analysis, 25% of patients had at least one significant mutation leading directly to HIV drug resistance for their virus subtype. The mutations encountered cause resistance to nucleoside and non-nucleoside reverse transcriptase inhibitors. In one case, several mutations were encountered simultaneously, causing resistance to both classes of drug. Among them is the K65R mutation, which causes resistance to most NRTIs, together with the S68G mutation, which partially restores the replication defect associated with K65R [7]. No major mutations associated with resistance to protease inhibitors were found.

The genetic diversity identified is consistent with the literature on HIV subtypes circulating in West Africa and the high genetic diversity of HIV in Africa in general. In the overwhelming majority of cases, the circulating recombinant form, 02_AG, is found. In this situation, special attention should be paid to isolates, the genotyping of which showed that more refined analysis (i.e. of recombination) is necessary. To gain a more complete picture, we additionally performed phylogenetic analysis together with isolates obtained in the 2016 study. On both phylogenetic trees, sample 18 was isolated, subtyped in REGA 3.0 as a recombinant of genotypes A1 and G, but not belonging to CRF02_AG. It is interesting to note that it forms a cluster with the isolate submitted under the number LT976766, which was assigned to CRF02_AG in 2016; this can be explained by the lack of information on the protease gene sequence in this sample. It is important to note that samples 44 and 46, identified in REGA 3.0 as unknown recombinant forms, are most closely clustered with CRF02_AG in both phylogenetic trees. It should be noted that samples 44, 46, and 13, according to the results of REGA HIV-1 analysis, are also recombinants between the known CRF_02AG and A1 subtypes.

The 24 mutations identified in naive patients here would most likely have approximately the same frequency of occurrence in a larger sample set. This is to be expected as there is no pressure on the virus from antiretroviral drugs in the study group, and mutations spontaneously arise and disappear in generations of the virus.

It is interesting to note that, in more than half of the cases (72%), there were substitutions at the twentieth position (pol gene). It can be seen that, of these mutations, K20I stands out the most. It is a polymorphic variant for subtypes CRF_02AG and G, but reduces sensitivity to nelfinavir in subtypes B and C. However, there is evidence that this mutation may enhance viral replication in non-B subtypes [8]. This mutation had a significantly higher incidence than all the others (67.24% (β = 0.95, (53.66%; 78.99%)), despite the absence of selection factors in the form of ART; this may be a consequence of an advantage received by carriers (virus) of this mutation.

A non-polymorphic variant of K20V has also been identified, which is rare, and its effect on HIV susceptibility to ARVs is poorly understood. Also, two mutations at the tenth position were identified. One of which, detected in one case (L10LF), is a minor mutation of resistance to PI. The other, L10I, was found in 29.03% of cases and increases the replication of viruses with other resistance mutations to SP [9]. Two rare mutations were identified: M46M_M and N88NH. Mutations at these positions have been associated with

УДК 61:001(06) ББК 5 Ф 94

resistance to protease inhibitors. These variants, however, have not been described among them.

Among the mutations associated with HIV resistance to non-nucleoside reverse transcriptase inhibitors, a relatively rare, non-polymorphic V179T mutation has been shown, which is sometimes selected for in patients receiving NNRTIs. This is due to a minimal decrease in the sensitivity to etravirine and rilpivirine [8. 10]. Several substitutions were also encountered at position 238: K238T, which reduces susceptibility to nevirapine and efavirenz by about 5-fold; K238R, which is a common polymorphism that does not reduce susceptibility to NNRTIs [10]; and K238E, a rare mutation at this position, the effect of which is not well described in the literature.

The identified mutations associated with drug resistance, both arising spontaneously and as a result of transmission by patients with treatment-resistant infections, can actively spread among new patients due to: the low awareness of the population about HIV infection; and inaccessibility of medical care and contraception. A consequence of low awareness of blood-borne infections is also an increased risk of trauma based transmission of the virus, the possibility of which has been shown in various studies.

Conclusion: we note that even a joint analysis, with data obtained in the course of this study and in 2016, was insufficient to fully clarify the genetic diversity of HIV in the Republic of Guinea. More detailed studies, on larger sample sets, are needed to elucidate the genetic profile and etiological structure of the virus in a territory with such a complex epidemiological situation. One aspect is clear however. The detection of HIV-1 mutations associated with drug resistance, in individuals who have never received antiretroviral therapy, is a cause for concern. It suggests that: new infections are occurring with strains that already have resistance; and the expansion of resistance is not always directly associated with selective drug pressure.

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УДК 61:001(06) ББК 5 Ф 94