HIV Genotyping and Phylogenetic Analysis in the System of Virological Monitoring of HIV Infection

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The aim of the study was to assess the possibility and effectiveness of using modern molecular-genetic technologies in determining the source of infection and cause-effect relationships in the epidemic focus of HIV infection.

Materials and Methods. Two samples of blood plasma from HIV-positive persons of the tested group and 18 samples from the infected patients of the comparison group have been genotyped. 13 genetically close nucleotide sequences of HIV genome from the GenBank sequence database were also used in our work.

ABI Prism 3100 and ABI 3500XL genetic analyzers (Applied Biosystems, USA), ViroSeq HIV-1 genotyping system (Abbott, USA), and AmpliSense HIV-Resist-Seq reagent kit (Central Research Institute of Epidemiology of Rospotrebnadzor, Russia) were used for genotyping. Subtyping is done online using COMET HIV-1/2 and HCV software and REGA HIV-1 Sybtyping Tool program (v. 3.0). Phylogenetic analysis and calculation of genetic distance were carried out using MEGA 5.2 program, Maximum Likelihood Estimation method, and the Kimura model (bootstrap level 1000).

Results. All examined viral isolates were found to belong to HIV-1 subtype A (A6). The conducted phylogenetic analysis showed that the samples of the tested group were not collected in a separate cluster, for each of them genetically close samples from the comparison group were found. The distance between the nucleotide sequences of the tested group samples was 0.050; but it varied from 0.007 to 0.058 (average 0.032) between the nucleotide sequences of the 2 samples from the tested group and the samples from the comparison group. The distance equal to 0.007 has been found to be between the nucleotide sequences of sample No.1048 of the tested group and sample No.1051 from the comparison group which indicated a high degree of their genetic proximity, confirming the presence of the epidemiological link between them.

Conclusion. The molecular genetic examination did not confirm the genetic relationship between the samples of the tested group. The results obtained were grounds for refusal to make a diagnosis of occupational HIV infection.

The application of modern technologies in the monitoring of HIV infection has demonstrated their practical significance and efficacy.

Key words: HIV-1; molecular genetic monitoring; HIV genotyping; phylogenetic analysis; nucleotide sequences; occupational infections.

Introduction

A long lasting period of HIV infection epidemic process with a high level of affected population over vast territories of the Russian Federation and an increased incidence of secondary diseases in HIV-infected people have predetermined the emergence of persons with HIV who need outpatient and inpatient treatment [1].

Due to the above-mentioned circumstances as well as the growing incidence of HIV infection among donors and medical providers, the problem of infecting patients with HIV in the course of rendering medical aid in healthcare settings and infecting medical personnel performing their professional duties has become of increasing concern [2].

Current specificity of HIV infection epidemic course and the increasing incidence of HIV infection with ambiguous interpretation of infection causes and conditions strongly suggested the necessity of conducting molecular genetic monitoring of HIV infection which became possible owing to the development of novel laboratory technologies [3, 4].

HIV genotyping and the subsequent phylogenetic analysis are the methods of high sensitivity and specificity which made it possible to obtain correct and reliable results with the establishment of genetic

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similarities or differences within one subtype between nucleotide sequences of the samples in the foci of HIV infection, to provide an evidence base on the presence/absence of the epidemiological link between them [5, 6].

Application of modern molecular genetic technologies in the system of virological monitoring of HIV infection extends scientific knowledge of biological and genetic characteristics of the causative agent, its interaction with the human organism, uncovers the mechanism of disease development, increases the capabilities of the laboratory and epidemiological diagnosis of the infection caused by HIV.

The aim of the study was to assess the possibility and efficacy of using modern molecular genetic technologies in determining the source of infection and cause-effect relationships in the epidemic focus of HIV infection.

Materials and Methods

Molecular genetic examination was carried out in the Laboratory for molecular genetic and serological methods of investigation of Privolzhsky District Center for AIDS Prevention and Control at Blokhina Research Institute of Epidemiology and Microbiology of Nizhny Novgorod. Two samples of blood plasma were included in the tested group: collected from nurse M. (No.1048) and the suspected source of infection, patient P. (No.1049). The comparison group comprised 18 blood plasma samples from HIV-infected patients from the same region of residence (No.1051–1058, 1060–1068, 1070). Nucleotide sequences (n=13) of the HIV genome from the GenBank sequence database were also used in the work. Building of the phylogenetic tree and calculation of the genetic distance was performed by the analysis of 33 samples.

Nucleotide sequences of the HIV genome were identified by means of commercial genotyping test-systems ViroSeq HIV-1 (Abbott, USA) and AmpliSense HIV-Resist-Seq (Central Research Institute of Epidemiology of Rospotrebnadsor, Russia) by the method of sequencing amplified fragments of *pol* gene with the primers included in the BigDye Terminator v. 3.1 Cycle Sequencing Kit using ABI Prism 3100 and ABI 3500XL genetic analyzers (Applied Biosystems, USA). Nucleotide sequences of HIV-1 protease (*pro*) and reverse transcriptase (*rev*) gene region were used for the investigation.

Sequencing data were analyzed using ViroSeq HIV-1 genotyping system software v. 2.8 (Celera Diagnostic, USA) and DEONA software (Med IT Group, Russia).

HIV genotyping was done by real-time integration with the database of Stanford University, USA (https://hivdb.stanford.edu/).

COMET HIV-1/2 and HCV (https://comet.lih.lu/) software, and REGA HIV-1 Subtyping Tool v. 3.0 (http://RegaSubtyping/stanford-hiv/typingtool) were used to identify HIV-1 subtypes of the examined samples.

To detect the existing homologs of HIV strains in the databases, BLAST program (https://blast.ncbi.nlm.nih.gov/Blast) was employed.

Phylogenetic analysis and calculation of genetic distances were carried out using MEGA 5.2 program, Maximum Likelihood Estimation method, and the Kimura's two-parameter model (bootstrap level 1000).

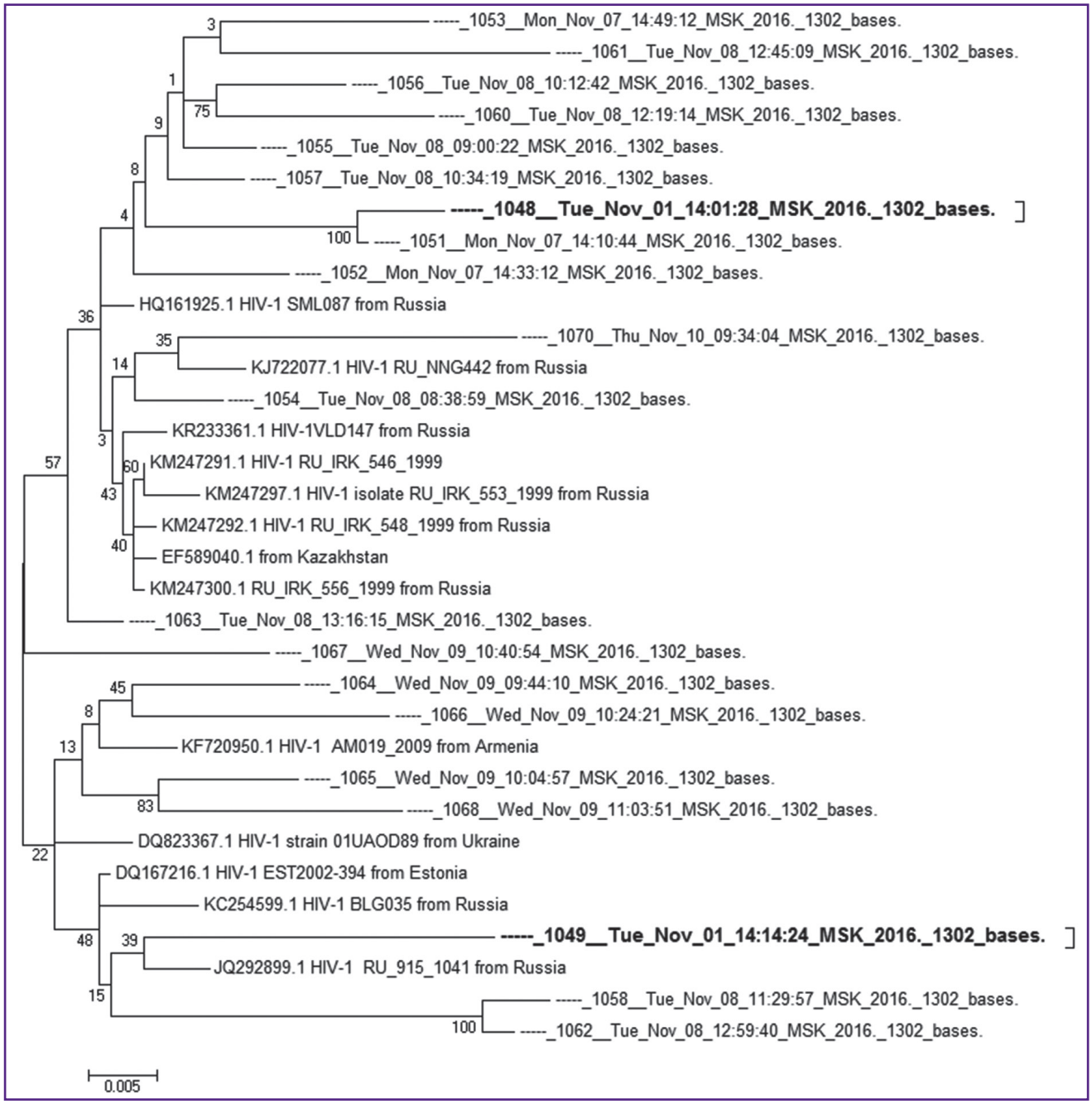
Results and Discussion

HIV genotyping and subsequent phylogenetic analysis are an important supplement, and sometimes the only objective proof of the presence or absence of epidemiological link between the examined samples. They enable one to clear up the complicated situations of identifying the causes and sources of HIV infection. In our study, the effectiveness of these methods is considered on a concrete case.

Thus, according to the information presented by the healthcare workers of the Center for AIDS Prevention and Control (AIDS Center), one of the subjects of Privolzhsky Federal District, on July 30, 2016, an emergency situation occurred during disassembling the system for intravenous administrations used for patient P. with a previously known positive HIV status: nurse M. (who was alone in the procedure room at the time of the accident) pricked her distal phalanx of the index finger of the right upper extremity. All required emergency measures and post-exposure chemoprophylaxis were fulfilled to the full extent but on-site express-testing for HIV infection at the time of the accident was not performed. The results of previous tests for HIV infection markers for this nurse (April 2016) were negative. The result of Western blotting of August 17, 2016 was equivocal and of September 6, 2016 positive, she was diagnosed to have "occupationally acquired HIV infection?".

In order to confirm or deny this fact, genotyping and subsequent phylogenetic analysis of genome nucleotide sequences of the viruses isolated from the blood plasma of the tested group (nurse M. and patient P.) and comparison group as well as the samples from the GenBank database were performed in the laboratory of the Privolzhsky District AIDS Center.

The genetic typing has established that viral isolates used including the samples of the tested group (No.1048 and No.1049) referred to the HIV-1 subtype A (A6). This situation required the performance of phylogenetic analysis which showed that samples No.1048 and No.1049 did not form a separate cluster, for each of them genetically close samples from the comparison group were found (see the Figure). The distance between the nucleotide sequences of the tested group samples was 0.050; whereas it varied from 0.007 to 0.058 (average 0.032) between the nucleotide sequences of the 2 samples from the tested group and the samples from the comparison group. Thus, genetic proximity between the samples of the tested group was not



Phylogenetic tree for samples of the tested group (No.1048, No.1049) and the comparison group constructed using nucleotide sequences of HIV-1 pol gene

higher than between the samples of this group and the comparison group. This fact is evidence of low likelihood of epidemiological link between nurse M. and patient P.

But at the same time, according to the data presented also by the healthcare workers of the AIDS Center, the nurse M. might have epidemically significant contacts with HIV-infected patient N. (No.1051 from the comparison group) previously followed-up at the Center. The results of phylogenetic analysis showed that the calculated distance between the nucleotide sequences of samples No.1048 and No.1051 was equal to 0.007 which signified a high degree of genetic proximity of these isolates. They reliably formed a common cluster on the phylogenetic tree (see the Figure) confirming the epidemiological link between them.

Thus, in this particular case, the molecular genetic examination using modern technologies of genotyping and subsequent phylogenetic analysis allowed us to establish that the sample collected from nurse M. (No.1048) was genetically closer to the sample from the

comparison group, i.e. from patient N. (No.1051) than to the suspected source of infection, patient P. (No.1049). The conclusion drawn does not confirm reliably the fact of occupational acquisition of HIV infection.

Conclusion

Genotyping with subsequent phylogenetic analysis used for molecular genetic examination, conducted in case of suspected occupational exposure to HIV infection, did not validate genetic relationship between the samples of the tested group but detected genetically more close samples from the comparison group. The results obtained were grounds for refusal to make a diagnosis of occupationally acquired HIV infection.

Application of the current molecular genetic methods showed their practical value and efficacy.

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