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CLINICAL STUDY

Characterization of HIV-1 subtypes and drug resistance mutations in Slovakia: update 2017–2018

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ABSTRACT
OBJECTIVES: This study aimed to provide an overview on the HIV-1 subtypes circulating in Slovakia between 2017 and 2018 and to evaluate the risk of transmission of HIV-resistant strains.
BACKGROUND: The HIV epidemic in Slovakia is characterised by low incidence of new and pre-existing infections and a slightly elevated level of strain heterogeneity.
METHODS: Partial HIV pol gene sequences of 110 individuals newly diagnosed with HIV between 2017 and 2018 were analysed.
RESULTS: The genotypic analysis revealed sporadic occurrence of mutations linked to HIV resistance to antiretroviral therapy (ART). The HIV-1 B subtype has been found as predominant (84.55 %) and primarily linked to men who have sex with men (MSM). A total of eighteen individuals (15.45 %) were found to be infected with HIV-1 non-B subtypes.
CONCLUSION: The data suggest a minimal risk of a resistant HIV strain transmission and a marginal rise of HIV-1 subtypes´ diversity. The HIV-1 B subtype remains the most prevalent in the period 2017–2018 in Slovakia *(Tab. 2, Fig. 2, Ref. 37)*. Text in PDF *www.elis.sk*
KEY WORDS: HIV-1, subtypes, Slovakia, resistance, ART.

Abbreviations: AIDS – acquired immunodeficiency syndrome, ART – antiretroviral therapy, CRF – circulating recombinant form, HIV – human immunodeficiency virus, IVDU – intravenous drug user, INSTI – integrase strand inhibitor, INT – integrase, MSM – men having sex with men, NJ – neighbour-joining, NRTI – nucleoside reverse transcriptase inhibitor, NNRTI – nonnucleoside reverse transcriptase inhibitor, Pol – polymerase, PCR – polymerase chain reaction, PI – protease inhibitor, PR – protease, PrEP – preexposure prophylaxis, RT – reverse transcriptase, RT PCR – reverse transcriptase polymerase chain reaction, URF – unique recombinant form

Introduction

The diversity of HIV and its enormous evolutionary potential provide a significant barrier to controlling the spread of HIV/AIDS infection. Numerous HIV forms contribute to the global HIV/AIDS pandemic and may have implications for diagnostics, monitoring, therapy, and development of an effective vaccine.

In Slovakia, like in other European countries, owing to the history of homosexual/bisexual behaviour, the mode of HIV transmission through unprotected sexual intercourse among men having sex with men (MSM) has become predominant over the course of several decades (1). According to data collected by the Public Health Authority of the Slovak Republic in 2018 and 2022 (2, 3), MSM has been the most common mode of HIV transmission, accounting for 64 % and 70 % of cases, respectively. Unprotected heterosexual contact was reported in 23 % and 20 % of HIV infection cases during those same years. The proportion of HIV cases that have been transmitted through intravenous drug use and blood transfusion consistently remained at a low level over time.

In Slovakia, in addition to subtype B, new circulating HIV-1 subtypes have been identified in recent years. These include pure subtypes A, C, and F, as well as circulating recombinant forms (CRFs) 01_AE, 02_AG, 03_AB, and 12_BF (4-7). Additionally, the incidence of mutations associated with the loss of sensitivity to antiretroviral therapy (ART) within the group of ART-naïve individuals was reported to be low previously (8).

The objective of this study is to provide an overview on the occurrence of mutations resistant to ART as well as on the distribution of HIV-1 subtypes among individuals who have been newly diagnosed with HIV infection between 2017 and 2018 in Slovakia.

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Materials and methods

The genotyping and HIV-subtyping analyses were conducted on specimens obtained from a cohort of 110 HIV-1-infected individuals who were newly diagnosed between 2017 and 2018 in the National Reference Centre for HIV/AIDS Prevention in Bratislava. At the time of the investigation, all study participants were ART-naïve. Plasma samples were collected for routine viral load testing and subsequently for genotyping and drug resistance testing.

HIV RNA isolation from plasma was performed according to the manufacturers' recommendation using QIAamp Viral RNA Mini Kit (QIAGEN, Hilden, Germany) followed by nested RT PCR of HIV-1 *pol* region. The products of nested RT PCR have been sequenced with the use of in-house procedures according to optimised ANRS1 sequencing protocol (9). Sequences had 645 bp for reverse *transcriptase* (RT), 297 bp for *protease* (PR), and 805 bp for *integrase* (INT) in length.

All partial HIV-1 pol sequences were individually edited with BioEdit Software (10) and evaluated by the HIVdb algorithm of the Stanford HIV Drug Resistance Database (11) for identification of mutations linked to HIV resistance to ART. Subtyping was done primarily using REGA HIV-1 subtyping tool (12). The sequences were aligned using CLUSTAL W software (13) and subsequently, HIV-1 subtype determination was carried out by comparing patterns of sequences obtained from samples of study participants against consensus sequences representing a variety of HIV-1 subtypes available in the Los Alamos HIV-1 sequence database. (14) The phylogenetic trees were constructed by MEGA11 software (15) according to bootstrap analysis (17, 16). Statistical support for specific clades was obtained by bootstrapping (100 replicates) for the neighbour-joining (NJ) trees.

Results

Our cohort was comprised of 110 HIV-infected individuals, of whom 10 (9.1 %) were females and 100 (90.9 %) were males. The mean age of females and males at the time of initial HIV diagnosis was 32.4 years (median 29.5, with a range of 23 to 55) and 37.7 years (median 37.5 with a range of 20 to 73) years, respectively. The study sample consisted entirely of Slovak citizens, with the exception of a small subset of 6 individuals (5.45 %) originating from Serbia, Cyprus, Bulgaria, Vietnam, and Thailand. The distribution of groups at risk was as follows: men who have sex with men (MSM) (70; 63.64 %), heterosexual males (HTM) (6; 5.45 %), heterosexual females (HTF) (10; 9.09 %), intravenous drug users (IVDUs) (2; 1.82 %) and a group of individuals with unknown routes of HIV transmission (22; 20.00 %). All individuals who were aware of their mode of infection acquired the virus through unprotected sexual intercourse with the exception of two IVDUs.

Genotyping

Sequencing in the HIV *pol* region was successful for *protease* in 99 % (109/110 persons), for *reverse transcriptase in 100* % (110/110 persons), and for *integrase in* 75 % (83/110 persons).

Mutations associated with decreased susceptibility to at least one of four main groups of ART differentiated as PIs (protease inhibitors), NRTIs/NNRTIs (nucleoside/nonnucleoside reverse transcriptase inhibitors), and INSTI (integrase strand inhibitor) were found in 8 of 110 (7.3 %) study participants.

As demonstrated in Table 1, our results indicated that 102 out of 110 persons (92.7 %) were infected by HIV-1 strains sensitive to ART, and 8 persons (7.3 %) were infected with HIV-1 strains carrying resistance mutations. In particular, the mutations causing resistance to PI, NRTI/NNRTI and INSTI were found in 1.8 % (2 persons), 3.6 % (4 persons), and 1.8 % (2 persons), respectively. Except for one, all isolates that carried mutations causing resistance belonged to the subtype B variety. A single isolate was found to be clustered with strain F while possessing only mutations causing resistance to NRTIs.

Regarding the mode of transmission, men having sex with men were found to be the primary carriers of the majority of mutations associated with resistance to ART (total 5 out of 8 isolates possessed mutations associated with resistance).

Phylogenetic analysis

In our cohort, the phylogenetic analysis of 110 HIV-1 isolates revealed that the of HIV-1 B subtype (92; 83.64 %) had the highest incidence, followed by subtypes A (6.36 %), F (2.73 %), C (1.82 %), CRF12_BF (2.73 %), and CRF01_AE (2.73 %). The distribution of HIV-1subtypes among study participants by gender is illustrated in Figure 1.

Table 1. Overview of mutations causing resistance to ART in newly diagnosed HIV-infected ART-naïve individuals in Slovakia in 2017–2018.

Patient	Subtype	Amino acid positions associated with drug resistance to inhibitors				Mode of transmission
		NRTIs	NNRTIs	PIs	INSTIs	
1	B	–	–	M46L	–	MSM
2	B	–	–	M46L	–	not known
3	B	–	V108I	–	–	MSM
4	B	K70S	Y188F	–	–	MSM
5	F	M41L, E44D, T215S	–	–	–	IVDU
6	B	S68G, T215C	–	–	–	MSM
7	B	–	–	–	S153F	MSM
8	B	–	–	–	T66TI	not known

NRTIs – nucleoside reverse transcriptase inhibitors, NNRTIs – nonnucleoside reverse transcriptase inhibitors, PIs – protease inhibitors, INSTIs – integrase strand inhibitors, MSM – men who have sex with men, IVDU – intravenous drug user

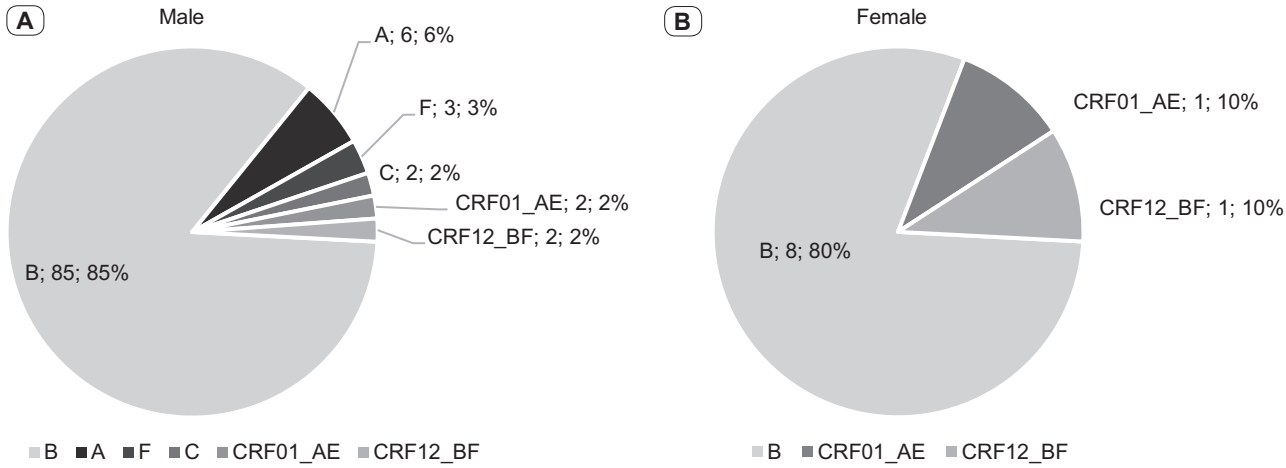

Fig. 1. Distribution of HIV-1subtypes among male (a) and female (b) study participants by gender

HIV-1 B subtypes

Except for two men from Serbia and Cyprus, all individuals infected with the HIV-1 B subtype were Slovaks. Regarding gender, 84.0 % of men (80 out of 100) and 80.0 % of women (8 out of 10) were infected with the HIV-1 B subtype. The HIV-1 B subtype predominated across all study groups. The distribution of HIV-1 subtypes among study participants in the relationship to risk of exposure is shown in Table 2. Regarding the mode of transmission, the majority of cases was among MSM (85.7 %; 60 out of 70), followed by female study participants (8 out of 10; 80 %), heterosexual males (5 out of 6; 83 %), while 81.7 % of individuals from the group with unknown mode of transmission (18 out of 22) were found to be infected with HIV-1 B subtype.

HIV-1 non-B subtypes

Eighteen out of 110 study participants (16.36 %) were found to be infected by non-B HIV-1 subtypes, of whom two individuals were female.

The results of the non-B HIV-1 subtype analysis revealed that the pure subtypes (12/18; 66.67 %) were more prevalent than circulating recombinant forms (6/18; 33.33 %). The results show that subtype A exhibited the highest frequency (7/18, 38.9 %), followed by subtype F (3/18; 16.67 %) and subtype C (2/18; 11.11 %). Two distinct CRFs were detected, namely CRF_01AE and CRF_12BF, each accounting for three cases (16.7 %).

Regarding the country of origin, the majority of persons carrying non-B HIV-1 subtypes, (14/18; 77.78 %) were Slovaks, 2 were of Vietnamese origin (11.11 %), 1 was of Thai origin, (5.56 % and 1 was of Bulgarian origin (5.56 %1).

As shown in Figure 2, non-B isolates revealed in our study were distributed within 3 monophyletic clades (A(A1), C, and F), and two subclades (AE and BF).

All isolates clustered with subtypes (A(A1), C, and F) were obtained from Slovak males with the exception of one belonging to a consensus strain A(A1) from a male with Thai origin.

Regarding CRF, two strains identified from a heterosexual couple with Vietnamese origin and one strain isolated from a Slovak male with unknown source of HIV infection were clustered with the consensus strain CRF01_AE. Moreover, two strains similar to CRF12_BF were isolated from 2 Slovak MSM subjects and one CRF12_BF isolate from a female from Bulgaria.

Discussion

Genotyping and HIV resistance to ART

The HIV drug resistance testing represents a valuable tool for identifying individuals with HIV infection and managing their treatment (17), as it allows for targeted, efficient, and cost-effective measures to be implemented in the ART of HIV-infected individuals (18). As described in previous studies, the occurrence of HIV

Table 2. HIV-1 subtypes distribution in newly diagnosed HIV-infected ART-naïve individuals in Slovakia in 2017–2018 in the relationship to risk of exposure.

Risk of exposure	HIV-1 subtype					
	B	A	F	C	CRF12_BF	CRF01_AE
MSM	60	4	2	2	2	–
HTS Male	5	–	–	–	–	1
HTS Female	8	–	–	–	1	1
IVDUs	1	–	1	–	–	–
Unknown	18	3	–	–	–	1
Total abs (%)	92 (83.64)	7 (6.36)	3 (2.73)	2 (1.82)	3 (2.73)	3 (2.73)

MSM – men who have sex with men, HTS – heterosexual, IVDUs – intravenous drug users

drug-resistant mutations among ART-naïve individuals remains low within the region of Central Europe (19, 20). Similarly, only 7.3 % (8) of the individuals in our study group had mutations associated with HIV resistance to ART.

The emergence of drug resistance transmission exhibits variations in prevalence among risk groups (20, 21). In Slovakia, from the beginning of the epidemic, MSM represent the group with the highest risk of HIV infection. Therefore, as expected in our study, the highest proportion of isolates with resistant mutations were also found in this high-risk group. MSM were found to be the primary carriers of the vast majority of mutations associated with resistance to all classes of ART, except for NNRTI resistance mutations observed in one IVDU. Similarly, in the study among MSM in the USA, a high level of HIV drug resistance was detected in 31 % of individuals; among them, some (12 %) had multiclass resistance and 11 % had INSTI resistance (22). In contrast, in Portugal, the HIV-1 epidemic is exhibiting redistribution among risk group populations, with heterosexuals showing increasing levels of HIV-1 transmission and transmission drug resistance (21).

Regarding drugs used for pre-exposure prophylaxis (PrEP) in our study, a mutation causing resistance to Tenofovir was found in 1 (12.5 %) strain (in one MSM patient) out of 8 isolates presenting resistance mutations. A higher resistance of 16 % to drugs used for PrEP was detected among MSM in a study conducted in the USA (22). Based on our findings, the identified mutations in our ART- naïve study participants are related with low levels of HIV resistant to the currently used ART. However, given their presence in the group with the highest risk of HIV infection, there is a possibility for the trend of transmission of mutated strains to grow. Therefore, it is imperative to monitor this risk, establish targeted therapy based on genotyping, and pay close attention to the adherence of treated patients.

Phylogenetic analysis

Certain strains regularly display distinct correlations with specific geographic regions and mechanisms of transmission; however, direct associations between particular subtypes and specific routes of transmission are still a matter of controversy (23). Previous studies have shown that subtypes are associated with disease progression (24–26) and mother-to-child HIV transmission (27). However, most studies show that after antiretroviral therapy, HIV subtypes do not affect the outcomes (28); although this conclusion may be influenced by sociodemographic factors.

As per a review study conducted in 2019 (29), apart from other introduced subtypes, subtype B is predicted to be one of the most prevalent viral genotypes, predominating particularly in Europe, North America, South America, the Middle East, and North Africa. Our study has confirmed the previously described situation (5–7) in Slovakia, where the highest prevalence of HIV-1 B subtype was identified among HIV-positive individuals. This finding indicates that there has been no significant change in the predominant prevalence of this subtype in the country. The prevalence of HIV-1 B subtypes remains highest among MSM, primarily transmitted through unprotected sexual intercourse. Furthermore, our study findings indicate that the HIV-1 B subtype is prevalent among both female and heterosexual male participants. Similarly, phylogenetic analyses conducted in the Czech Republic (30), Slovenia (31), and Austria (32), showed that the HIV-1 B subtype remains the predominant form, although other HIV subtypes such as A, C, and F, as well as recombinant circulating forms CRF01_AE and CRF02_AG, CRF02_AB are seen to be less frequent. Moreover, unique recombinant forms (URFs) were infrequently detected in Slovenia by obtaining near full-length genome sequences and performing detailed recombination analysis (33).

In our study, 16.4 % of persons were infected with HIV-1 non-B subtypes. There is an assumption that subtype A was initially introduced in Ukraine, a neighbouring country to Slovakia, where the transmission of both subtype A and subtype B is widespread through unprotected heterosexual contact and intravenous drug use. Following the simultaneous appearance of subtypes A and B in Ukraine, subtype A spread extensively, giving rise to one of the most rapidly expanding global epidemics. The study accomplished among IVDUs in Ukraine supported the notion that subtype A was

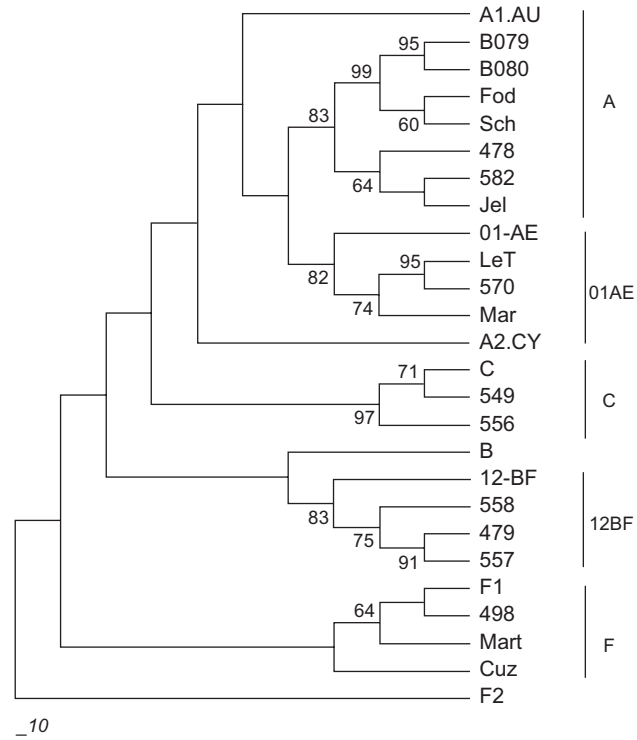

Fig. 2. Neighbor-joining tree of HIV-1 non-B subtypes. Phylogenetic relationships of 18 non-B subtype strains based on 615 nucleotide *reverse transcriptase* of the HIV-1 *pol* gene with consensus strains from the *Los Alamos HIV Sequence Database*. Bootstrap values > 60% are shown. The scale bar indicates 10% nucleotide sequence divergence. The tree was rooted by using HIV-1 B strain as the outgroup. The evolutionary history was inferred using the neighbor-joining method. The bootstrap consensus tree is inferred from 100 replicates. The evolutionary distances were computed using the Kimura 2-parameter method. The rate variation among sites was modeled with gamma distribution. This analysis involved 26 nucleotide sequences. There was a total of 527 positions in the final dataset. Evolutionary analyses were conducted in MEGA X (15).

the most frequent (66 %), followed by subtypes B (30 %), C (2 %), D (1 %), and a CRF03_AB recombinant form (1 %) (34).

In our study, HIV subtype A had the highest transmission rate among non-B HIV subtypes, with seven recorded cases, four of which were males from the MSM group. However, the countries of origin of these cases are unknown, with the exception of one strain carried by an individual who had an extended work assignment in Russia and is presumed to have contracted HIV in that region. One possible explanation for the occurrence of HIV-1 non-B subtypes discovered by this study is that they may have originated in regions with a consistent geographic distribution. For instance, CRF01_AE strain is prevalent in China and Southeast Asia, as demonstrated by the fact that two out of three individuals carrying the CRF01_AE strain in our study are of Vietnamese origin. Moreover, Asia is known to harbour various subtypes of viruses and has been recognized as a region where recombinant viruses are prevalent. Reports indicate an elevated number of new CRFs and URFs in this region, characterizing it as a "hotbed" for such viruses (29).

Contrary to the common opinion associating the transmission of HIV-1 non-B subtypes with heterosexual transmission, our study revealed that the majority of non-B HIV-1 subtypes discovered, such as subtypes A, C, F, and CRF12_BF, were predominantly found among MSM.

Subtype C HIV-1 is prevalent in Southern Africa, India, and Ethiopia, accounting for 46 % of all global infections. It is remarkable that one subtype achieved global dominance despite its prevalence being limited only to several geographical locations (35). Subtype F HIV-1 represents about one third of non-B infections in some countries in South America. Subtype F1 was found among heterosexuals, gays, and IVDUs in Brazil, suggesting that both sexual and iatrogenic routes may have played a role in viral transmission (36). Presumably, BF CRFs viruses (namely 12_BF and 38_BF) were generated during the 1980s, shortly after the estimated introduction of subtype F1 in South America. Subsequently to the initial phase of rapid exponential expansion, the rate of dissemination of CRFs 12_BF and 38_BF epidemics appears to have declined (37), following a demographic trend similar to that previously observed for HIV-1 outbreaks in Brazil, the United States, and Western Europe.

Even though the origin of the majority of identified non-B isolates in our study is unknown, their sporadic identification suggests they were imported from outside Slovakia. Nonetheless, the introduction of diverse HIV subtypes in Slovakia is facilitated by travel and immigration.

Conclusion

In years 2017–2018, in Slovakia, HIV resistance mutations were only occasionally found in newly diagnosed naïve HIV-positive individuals and were associated with MSM population, the most vulnerable category engaging in high-risk behaviour. Therefore, it is important to address concerns regarding the potential increase in the transmission of mutated strains, particularly within this highest-risk population. Our findings further highlight the need for improved HIV care in MSM and the identification of alternative PrEP regimens in Slovakia.

HIV-1 B subtype continues to be the most prevalent subtype in Slovakia, especially among MSM, with a slight rise in the variety of HIV-1 subtypes. Regardless of differing views on the impact of subtypes on clinical outcomes, it is crucial to monitor changes in the diversity and distribution of HIV strains from epidemiological and clinical standpoints. The risk of emerging and spreading of new CRFs and URFs could have implications for diagnostics, surveillance, therapy, and the future development of an effective vaccine.

References

1. Staneková D, Kramárová P, Wimmerová S, Hábeková M, Takáčová M, Mojzesová M. HIV and risk behaviour among men who have sex with men in Slovakia (2008–2009). Cent Eur J Public Health 2014; 22 (4): 239–244.

2. https://www.uvzsr.sk/documents/41637/608222/Sprava_o_plneni_NPP_HIV_AIDS_v_SR_2017_2020_za_rok_2018.pdf .

3. https://www.uvzsr.sk/web/uvz/hiv-aids/-/asset_publisher/favz/content/vyskyt-hiv-infekcie-v-slovenskej-republike-k-30-6-2022.

4. Mayer V, Apetrei C, Habekova M et al. HIV-1 diversity in heterosexual population in Slovakia, Ukraine and the Czech Republic. AIDS 1998; 12 (9): 1106–1108.

5. Habekova M, Takacova M, Lysy J et al. Genetic subtypes of HIV type 1 circulating in Slovakia. AIDS Res Hum Retroviruses 2010; 26 (10): 1103–1107.

6. Chabadová Z, Habeková M, Truska P, Drobková T, Mojzesová M, Staneková D. Distribution of HIV-1 subtypes circulating in Slovakia (2009–2012). Acta Virol 2014; 58 (4): 317–324.

7. Čereš A, Valkovičová Staneková D, Hábeková M, Drobková T, Mojzesová M. Occurrence of HIV subtypes and HLA-B*57:01 allele in HIV-positive patients newly diagnosed in 2015–2016. Medical overview: professional journal of the Slovak Health University in Bratislava 2017; 66 (7–8): 276.

8. Valkovičová Staneková D, Čereš A, Hábeková M, Truska P, Drobková T, Mojzesová M. Resistance to ART in HIV-positive patients newly diagnosed in SR. 2015-2016-incidence and significance of follow-up Internal medicine: a peer-reviewed, postgraduate-focused professional medical journal 2017; 17, no. S4: 18.

9. http://www.hivfrenchresistance.org.

10. Hall TA. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl Acids Symp Series 1999; 41: 95–98.

11. Liu TF, Shafer RW. Web resources for HIV type 1 genotypic-resistance test interpretation. Clin Infect Dis 2006; 42 (11): 1608–1618.

12. https://www.genomedetective.com/app/typingtool/hiv.

13. Larkin MA, Blackshields G, Brown NP, et al. Clustal W and Clustal X version 2.0. Bioinformatics 2007; 23 (21): 2947–2948. DOI: 10.1093/bioinformatics/btm404.

14. https://www.hiv.lanl.gov.

15. Tamura K, Stecher G, Kumar S. MEGA11: Molecular Evolutionary Genetics Analysis version 11. Mol Biol Evol 2021; 38 (7): 3022–3027.

16. Salemi M, Vandamme AM (Eds). The Phylogenetic Handbook. A practical approach to DNA and Protein Phylogeny. Cambridge University Press 2003: 126–127.

17. Vandamme AM, Camacho RJ, Silberstein FC et al. European recommendations for the clinical use of HIV drug resistance testing: 2011 Update. AIDS Rev 2011; 13 (2): 77–108.

18. Sax P, Islam R, Walensky R et al. Should resistance testing be performed for treatment-naive HIV-infected patients? A cost-effectiveness analysis. Clin Infect Dis 2005; 41 (9): 1316–1323.

19. Parczewski M, Bander D, Urbańska A, Boroń-Kaczmarska A. HIV-1 integrase resistance among antiretroviral treatment naive and experienced patients from Northwestern Poland. BMC Infect Dis 2012; 12: 368.

20. Frentz D, Vijver D, Abecasis A et.al. Patterns of transmitted HIV drug resistance in Europe vary by risk group. PLoS One 2014; 9 (4): e94495.

21. Pingarilho M, Pimentel V, Diogo I et al. Increasing Prevalence of HIV-1 Transmitted Drug Resistance in Portugal: Implications for First Line Treatment Recommendations. Viruses 2020; 12 (11): 1238.

22. Fogel J, Sivay MV, Cummings V et al. HIV drug resistance in a cohort of HIV-infected MSM in the United States. AIDS 2020; 34 (1): 91–101.

23. http://www.ncbi.nlm.nih.gov/pubmed/21249770.

24. Agwale SM, Zeh C, Robbins KE et al. Molecular surveillance of HIV-1 field strains in Nigeria in preparation for vaccine trials. Vaccine 2002; 20 (16): 2131–2139.

25. Siemieniuk RA, Beckthold B, Gill MJ. Increasing HIV subtype diversity and its clinical implications in a sentinel North American population. Can J Infect Dis Med Microbiol 2013; 24 (2): 69–73.

26. Kanki PJ, Hamel DJ, Sankalé J et al. Human immunodeficiency virus type 1 subtypes differ in disease progression. J Infect Dis 1999; 179 (1): 68–73.

27. Yang C, Li M, Newman RD et al. Genetic diversity of HIV-1 in western Kenya: subtype-specific differences in mother-to-child transmission. AIDS 2003; 17 (11): 1667–1674.

28. Bhargava M, Martinez Cajas J, Wainberg MA, Klein MB, Pant Pai N. Do HIV-1 non-B subtypes differentially impact resistance mutations and clinical disease progression in treated populations? Evidence from a systematic review. J Int AIDS Soc 2014; 17 (1): 18944.

29. Bbosa N, Kaleebu P, Ssemwanga D. HIV subtype diversity worldwide. Curr Opin HIV AIDS 2019; 14 (3): 153–160.

30. Linka M, Brůcková M, Malý M et al. A study of HIV-1 genetic diversity in the Czech Republic: 1986–2007. Cent Eur J Public Health 2008, 16 (4): 175–177.

31. Mezei M, Balog K, Babic DZ et al. Genetic variability of gag and env regions of HIV type 1 strains circulating in Slovenia. AIDS Res Hum Retroviruses 2006; 22 (1): 109–113.

32. Kusejko K, Tschumi N, Chaudron SE et al. Similar but Different: Integrated Phylogenetic Analysis of Austrian and Swiss HIV-1 Sequences Reveal Differences in Transmission Patterns of the Local HIV-1 Epidemics. J Acquir Immune Defic Syndr 2022; 90 (4): e4–e12.

33. Lunar M, Mlakar J, Zorec MT, Poljak M. HIV-1 Unique Recombinant Forms Identified in Slovenia and Their Characterization by Near Full-Length Genome Sequencing. Viruses 2020; 12 (1): 63.

34. Nabatov AA, Kravchenko ON, Lyulchuk MG, Shcherbinskaya AM, Lukashov VV. Simultaneous Introduction of HIV Type 1 Subtype A and B Viruses into Injecting Drug Users in Southern Ukraine at the Beginning of the Epidemic in the Former Soviet Union. AIDS Res Hum Retroviruses 2002; 18 (12): 891–895.

35. Gartner MJ, Roche M, Churchill MJ, Gorry PR, Flynn JK. Understanding the mechanisms driving the spread of subtype C HIV-1. EBioMedicine 2020; 53: 102682.

36. Lai A. Ciccozzi M, Franzetti M et al. Local and global spatio-temporal dynamics of HIV-1 subtype F1. J Med Virol 2014; 86 (2): 186–192.

37. Bello G, Aulicino PC, Ruchansky D. et al. Phylodynamics of HIV-1 circulating recombinant forms 12_BF and 38_BF in Argentina and Uruguay. Retrovirology 2010; 7: 22.

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