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ORIGINAL RESEARCH
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Prevalence of resistance mutations associated with integrase inhibitors in therapy-naive HIV-positive patients in Hungary

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ABSTRACT

Widespread introduction of HIV integrase inhibitors into clinical care may result in appearance of drug resistance mutations affecting treatment outcome. The aim of our study was to monitor the resistance patterns of integrase inhibitors beside protease and reverse transcriptase inhibitors in newly diagnosed therapy-naive HIV-positive patients in Hungary between 2017 and 2019.

Genotype-based resistance testing of HIV integrase, protease and reverse transcriptase was performed by amplification and Sanger population sequencing from plasma samples. Drug resistance mutations were identified by the algorithm of Stanford HIV Drug Resistance Database.

Potentially transmitted, non-polymorphic integrase major mutation was detected in 1 out of 249 samples, while accessory mutations were observed in further 31 patients (12.4%). The overall prevalence of transmitted drug resistance (TDR) mutations related to protease and reverse transcriptase inhibitors was 5.8% (10/173) between the end of 2017 and 2019. Nucleoside reverse transcriptase inhibitor associated resistance mutations were the most frequent indicators of TDR (6/173; 3.5%), followed by resistance mutations associated with protease (3/173; 1.7%) and non-nucleoside reverse transcriptase inhibitors (2/173, 1.2%).

The first detection of integrase major mutation and the changing patterns of other resistance mutations in Hungarian untreated HIV-positive population indicate the necessity of continuous molecular surveillance of Hungarian HIV epidemic.

KEYWORDS

integrase resistance mutations, Hungary, transmitted drug resistance

INTRODUCTION

Combination antiretroviral therapy (cART) has reduced HIV-related morbidity and mortality significantly [1]. In 2019, there were an estimated 38 million people living with HIV, and 25.4 million people were accessing antiretroviral therapy globally [2]. Although effective cART reduces the risk of HIV transmission [3], the emergence of drug resistant variants in the presence of antiretroviral drugs can lead to incomplete viral suppression and therapeutic failure. Transmission of resistant HIV strains may reduce the treatment options of newly HIV-infected patients, pointing out the necessity of baseline drug resistance testing and continuous drug development [4].

One of the newest drug class of antiretrovirals, namely integrase inhibitors (INIs), target the integrase enzyme of HIV by blocking the incorporation of reverse transcribed proviral DNA

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into the host genome. Due to their excellent tolerability, minimal toxicity, high efficacy and ease of use, integrase inhibitors became preferred agents for treatment-naïve or experienced patients and serve as novel treatment options in case of acquired and transmitted resistance in combination with other HIV drug classes [5]. The first HIV integrase inhibitor, raltegravir (RAL) was introduced into medical care in Europe in 2007, followed by the approval of elvitegravir (EVG) in 2013 [6]. The rapid emergence of drug resistance mutations against the first-generation integrase inhibitors and cross resistance to RAL and EVG indicated the development of second-generation INIs. These drugs, with a higher genetic barrier to resistance, include dolutegravir (DTG), bictegravir (BIC) and cabotegravir (CAB); they were approved in Europe in 2014, 2018 and 2020, respectively [5, 6].

Medical care for HIV-positive persons is centralized in Hungary, most of HIV-infected patients are followed and treated in Center for HIV, National Institute for Hematology and Infectious Diseases, South-Pest Central Hospital, Budapest. Integrase inhibitors are used since 2008 in Hungary, and they are included into first-line recommendations since 2009. Between 2008 and 2017, 617 of 2,232 registered patients received RAL or DTG in the Center for HIV [7]. Since 2017, INIs are dominantly selected for initial therapy in Hungary.

The aim of our study was to evaluate how the widespread use of INIs affected the prevalence of resistance mutations, associated with the clinically preferred integrase inhibitors, in newly diagnosed, treatment-naïve HIV-positive patients in Hungary. In addition, as a part of complete resistance pattern analysis, mutations to reverse transcriptase (RT) and protease (PR) were also monitored.

MATERIALS AND METHODS

Study population included 249 ART-naïve patients diagnosed as HIV-positive between 2017 and 2019. Plasma samples obtained from EDTA-anticoagulated peripheral blood were collected in Center for HIV, National Institute of Hematology and Infectious Diseases, South-Pest Central Hospital, Budapest, Hungary. Analysis of surveillance drug resistance mutations associated with integrase, protease and reverse transcriptase inhibitors were implemented from samples collected within 1 year (median: 0.7 month, interquartile range, IQR: 0.2–0.9 month) of HIV diagnosis. Clinical and demographical data, including CD4⁺ cell counts and viral load at the time of sampling, possible route of infection, gender and age were also documented. This study was approved by the Institutional Bioethics Committee of South-Pest Central Hospital (EB/14/2017, EB-21/2020).

HIV-1 RNA was extracted from plasma samples using the NucliSENS miniMAG nucleic acid purification system and NucliSens Magnetic Extraction Reagent (bioMérieux, France) according to the manufacturer's instructions. HIV-1 integrase region was reverse transcribed and amplified using KVL068/KVL069 outer primers [8] with SuperScript III

One-Step RT-PCR System with Platinum Taq DNA Polymerase kit (ThermoFisher Scientific Inc., USA) or Verso 1-Step RT-PCR Hot-Start kit (ThermoFisher Scientific Inc., USA) and further amplified using KVL070/KVL084 [8] or INT1/INT2 [9] inner primers and 2x MyFi Mix (Meridian Life Science Inc., USA) in a nested-PCR reaction. Amplification of protease and reverse transcriptase regions was described previously [10, 11].

Purified IN and PR/RT amplicons obtained from nested PCRs were subjected to Sanger sequencing using BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, USA) and appropriate sequencing primers [8–10, 12]. Sequencing and base-calling was performed on ABI3500 Genetic analyzer system (Applied Biosystems, USA). HIV subtypes, resistance profile of IN, PR and RT and the clinical relevance of mutations were determined from obtained sequences by Stanford HIV Drug Resistance Database algorithm [13]. In the case of prevalence of transmitted drug resistance the 95% confidence intervals (CI) were calculated using Wilson score interval [14].

HIV-1 RNA quantification was performed using the NucliSENS nucleic acid purification (NucliSENS miniMAG nucleic acid purification system and NucliSens Magnetic Extraction Reagent) and amplification (NucliSENS EasyQ[®] HIV-1 kit) system (bioMérieux, France) according to the manufacturer's instructions.

RESULTS

In total, 249 newly diagnosed, HIV-positive, ART-naïve patients were enrolled in our study investigating the prevalence of surveillance drug resistance to integrase inhibitors between 2017 and 2019. The majority of patients were male (95.2%) and the main risk factor for HIV infection was MSM (93.2%), followed by heterosexual contact (6.8%). The median age of study population was 32 years (IQR: 26–40). At the time of resistance testing the median CD4⁺ cell count and median viral load was 418 cells/ μ l (IQR: 260–592) and 70,000 cps/ml (IQR: 22,000–270,000), respectively.

HIV-1 drug resistance analysis of 249 samples revealed a low prevalence of surveillance drug resistance mutations associated with integrase inhibitors in therapy-naïve patients (Fig. 1/A). The only detected major integrase mutation ($n = 1$; 0.4%; 95% CI: 0.1–2.2), T66A, decreases susceptibility to elvitegravir and raltegravir. However, polymorphic accessory integrase mutations conferring low-level or no resistance to integrase inhibitors were identified in 31 additional cases (12.4%). No clinical resistance to dolutegravir or bictegravir was observed. Surveillance drug resistance mutations associated with PR or RT inhibitors were not detected among patients harbouring HIV-1 strains with major or accessory integrase resistance mutations. The most common integrase accessory polymorphism, L74I ($n = 17$; 6.8%) was predominantly found among non-B subtypes ($n = 16$; 6.4%), while L74M ($n = 7$; 2.8%) was exclusively associated with subtype B virus strains, similar to T97A ($n = 1$; 0.4%) and V151I



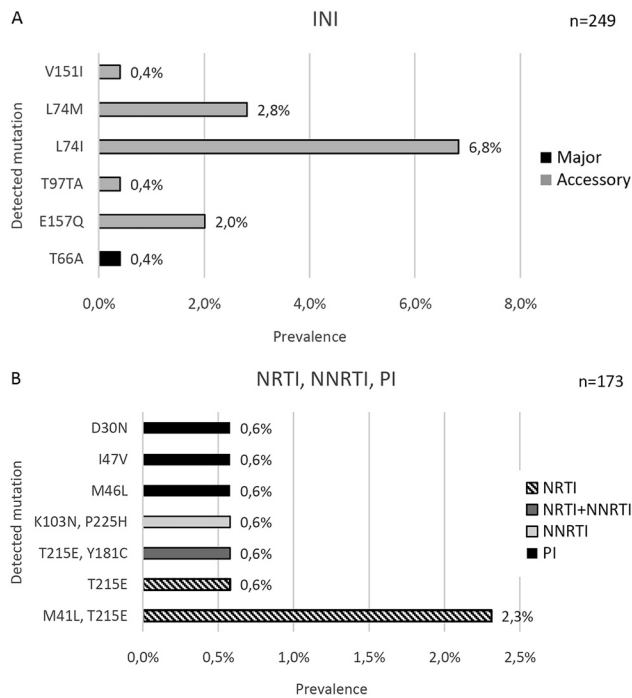


Fig. 1. Frequency of detected HIV-1 drug resistance mutations associated with INIs (A) or NRTIs, NNRTIs and PIs (B) among treatment-naïve patients diagnosed as HIV-positive between 2017 and 2019. In the case of INI resistance mutations, the results of the analysis of 249 samples are illustrated, while the depicted frequencies of RTI and PI resistance mutations originate from the 173 examined samples diagnosed as HIV-positive between the end of 2017 and 2019 (76 samples collected at the beginning of 2017, were characterized for mutations related to PR and RT inhibitors, but not for INIs, in our previous study [11])

INI: integrase inhibitor; NRTI: nucleoside reverse transcriptase inhibitor; NNRTI: non-nucleoside reverse transcriptase inhibitor; PI: protease inhibitor.

($n = 1$; 0.4%). E157Q polymorphic accessory mutation ($n = 5$; 2.0%) was identified in subtype B virus strains in 4 samples and in a subtype G virus strain in 1 sample. The overall prevalence of accessory mutations was significantly higher in viruses with non-B subtypes and CRFs (17 out of 52; 32.7%), than in subtype B virus strains (14 out of 194; 7.2%).

In this study, in addition to the mutations associated with integrase resistance, transmitted drug resistance mutations related to protease and reverse transcriptase inhibitors were also determined in 173 out of 249 examined samples, whereas 76 samples collected at the beginning of 2017, were characterized for mutations related to PR and RT inhibitors, but not for INIs, in our previous study [11]. From the end of 2017–2019, the overall prevalence of transmitted HIV drug resistance mutations (TDRMs) related to nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs) and protease inhibitors (PIs) was 5.8% (10 out of 173; 95% CI: 3.2–10.3). Among the 173 treatment naïve patients, the most frequently detected drug resistance mutations were associated with NRTI resistance ($n = 6$; 3.5%; 95% CI: 1.6–7.4), including thymidine analogue mutation, M41L, and a T215 revertant variant

(T215E) alone, or in combination. SDRMs associated with PIs were identified in 3 cases (1.7%; 95% CI: 0.6–5.0), while NNRTI resistance was observed in 2 out of 173 (1.2%; 95% CI: 0.3–4.1) studied samples (Fig. 1/B). Genotypic evidence for two-class resistance of HIV inhibitors (NRTI and NNRTI) was detected in a single patient ($n = 1$; 0.6%). All patients carrying mutations conferring resistance to PIs or RTIs were infected with subtype B HIV-1 strains. Regarding to the cumulative analysis of 249 samples, in total, the overall prevalence of TDRMs related to RTIs and PIs was 7.6% ($n = 19$) between 2017 and 2019. 5.6% ($n = 14$) of patients carried HIV-1 strains with NRTI resistance, while PI and NNRTI resistance was observed in 3 (1.2%) and one (0.4%) cases, respectively. Dual class resistance (NRTI and NNRTI) was identified for an additional sample.

According to the obtained IN and PR/RT sequences, the predominant HIV-1 subtype in the examined 173 samples was subtype B ($n = 133$; 76.9%) among patients diagnosed as HIV-positive between the end of 2017 and 2019. Subtype F was detected in 10.4% of patients ($n=18$), subtype A in 5.2% ($n = 9$), subtype C in 0.6% ($n = 1$), followed by recombinants CRF01_AE in 3.5% ($n = 6$), CRF02_AG in 1.2% ($n = 2$) and CRF06_cpx in 0.6% ($n = 1$). In case of 3 samples HIV-1 subtypes could be not clearly determined. The cumulative prevalence of detected HIV-1 subtypes and circulating recombinant forms (CRFs) according to our earlier [11] and present study are depicted in Fig. 2.

DISCUSSION

To the best of our knowledge, this is the first study monitoring the prevalence of drug resistance mutations associated with HIV integrase inhibitors in ART-naïve patients in Hungary. Although integrase inhibitors are routinely applied drugs for ART-naïve and chronically infected HIV-positive patients in Hungary, this study revealed a low prevalence (0.4%) of major resistance mutations associated with integrase inhibitors in a therapy-naïve population. The identified T66A mutation conferring low-level resistance to raltegravir and high-level resistance to elvitegravir is non-polymorphic, therefore it is preferably thought to represent a transmitted

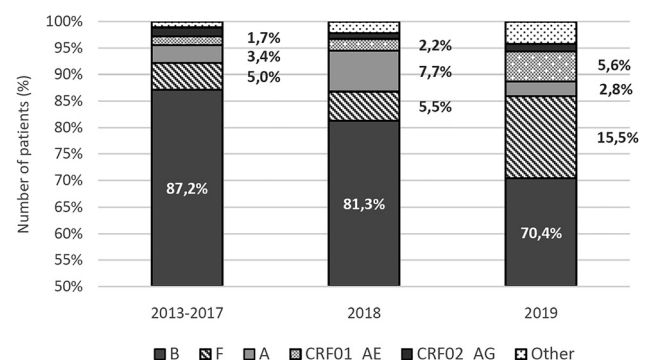
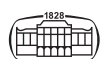


Fig. 2. Prevalence of detected HIV-1 subtypes and CRFs between 2013 and 2019 (cumulative data from earlier [11] and present study) in Hungary



drug resistance mutation, rather than a naturally arisen substitution. This first case of potentially transmitted integrase resistance mutation was detected 9 years after the first integrase inhibitor was introduced into the medical care in Hungary. The very low number of detected major integrase resistance mutations in therapy-naïve patients possibly reflects the remarkably low case number of treatment failures among INI recipients. Between 2006 and 2007, the European SPREAD programme revealed no resistance mutations to any of INIs, although potentially relevant polymorphisms could be observed before the introduction of integrase inhibitors [15]. A possible outcome of increasing role of INIs in ART worldwide is, however, the emergence of primary integrase resistance mutations among untreated patients as observed in our study and in different European countries [16–21].

12.4% of studied patients carried HIV-1 strains with polymorphic accessory integrase mutations (L74I/M, E157Q, T97A, V151I) contributing to reduced susceptibility of INIs mainly in combination with other major integrase mutations. These highly or minimally polymorphic mutations occur in less than 10% of viruses from ART-naïve patients depending on HIV subtype [22]. The majority of detected accessory integrase mutations in Hungary were also observed in different proportion in Austria [18], Poland [23], Spain [16], Scotland [17], Switzerland [20], UK [24] and Italy [21]. Prevalence of major and accessory integrase resistance mutations occurring in different studies is not easily comparable because of different time periods, sampling strategies, mutation identifying methodologies and datasets.

The overall prevalence of transmitted drug resistance related to PIs and RTIs (5.8%) decreased significantly compared to our earlier study (10.7%, [11]), despite the moderate increase in PI resistance among treatment-naïve patients. The most remarkable changes were observed in the number and proportion of resistance mutations associated with NRTI resistance (3.5% versus 9.5% in our present and previous study, respectively). In 2019, only 1 of 71 (1.4%) investigated samples carried HIV-1 strains with NRTI resistance. The phylogenetic analysis in our previous study revealed potential transmission clusters and a monophyletic group containing NRTI-associated resistance mutations. The dynamics of Hungarian HIV epidemic appear to undergo a significant change as the forward spread of NRTI resistant HIV-1 strains decreased dramatically, partly due to approaching the first 90 target (diagnose 90% of people living with HIV) of Joint United Nations Programme on HIV/AIDS [25, 26]. To identify even more HIV-positive people as early as possible together with the “treat all” strategy results the decrease of HIV transmission at population level.

According to our current study, subtype B remains the predominant HIV-1 subtype in Hungary, similarly to Central- and Western European countries [27]. It is remarkable, however, that the proportion of non-B subtypes almost doubled compared to our previous study. While the prevalence of non-B subtypes was 12.5% in Hungary between 2013 and 2017 [11], from the end of 2017–2019 this rate increased to 23.1% with a predominance of subtype F (10.4%). Despite the increased proportion of non-B subtypes, drug resistance

mutations were still associated with subtype B virus strains. These facts together with the alterations in the pattern of drug resistance mutations possibly reflect the changes in national transmission networks and genetic diversification of Hungarian epidemic, similarly to other European countries [27–31].

In conclusion, this is the first study monitoring the prevalence of drug resistance mutations associated with integrase inhibitors among treatment-naïve, HIV-positive patients in Hungary. We identified potentially transmitted integrase resistance mutation in only 1 out of 249 samples. Although the prevalence of transmitted INI resistance mutations is low, the patterns of such mutations may change due to the extensive usage of INIs. For this reason, transmission of INI resistance should be continuously monitored. The increasing proportion of non-B subtypes and the remarkable alteration of NRTI resistance observed among untreated, HIV-positive population in Hungary are important indicators of necessity of continuous molecular surveillance.

Conflict of interest: The authors declare no competing interests.

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