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Keywords:	HIV-1, Latvia, drug resistance mutations, primary resistance, transmitted drug resistance

Low Prevalence of Transmitted Drug Resistance among Newly Diagnosed HIV-1 Patients in Latvia

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Shortened title: HIV transmitted drug resistance in Latvia.

ABSTRACT

Transmitted drug resistance (TDR) is a concern because it may reduce the efficacy of antiretroviral treatment. Plasma samples of 119 HIV-1 infected patients who were newly diagnosed at the Infectology Center of Latvia in 2005 and 2006 were analyzed by an in-house genotypic resistance assay to determine the prevalence of transmitted drug resistance in Latvia. TDR was identified using the WHO 2009 list of mutations for surveillance of TDR as implemented in the Stanford Calibrated Population Resistance tool. Neighbor-joining phylogenetic analyses were used to determine genetic subtype and investigate the relatedness of the sequences. Resistance testing was successful in 117 of 119 patients. The study population represented approximately 20% of all patients that were diagnosed in Latvia in 2005 and 2006 and was well distributed between gender, transmission routes and areas of residence. Four patients showed evidence of TDR, which represents a prevalence of TDR of 3.4% (95% CI: 0.9% - 8.5%). All four patients displayed single, but different resistance mutations (M46I, F53L, M41L, and G190A). All patients, except one, were predicted to respond well to standard first-line therapy in Latvia. The prevalence of TDR in Latvia was low, which partly may be due the low proportion of HIV-1 patients who receive antiretroviral therapy. The results indicate that routine resistance testing in Latvia currently should be focused on patients who display treatment failure, rather than treatment naïve patients.

KEY WORDS: HIV-1, Latvia, drug resistance mutations, primary resistance, transmitted drug resistance

INTRODUCTION

With the increased availability and use of antiretroviral treatment (ART) for HIV-1 infected patients transmitted drug resistance (TDR) has become an important clinical and epidemiological problem. The reported prevalence of TDR has ranged from 0-24% [Avi et al., 2009; Chaix et al., 2009; Little et al., 2002; Payne et al., 2008; Poggensee et al., 2007; Ross et al., 2007; Shet et al., 2006; The SPREAD programme 2008; UK Collaborative Group on HIV Drug Resistance et al., 2007; Weinstock et al., 2004; Yerly et al., 2007]. TDR represents a challenge for the control of HIV-1 infection since it can reduce the efficacy of first-line ART and thereby contribute to treatment failure [Leigh Brown et al., 2003]. The World Health Organization (WHO) recommends that countries that have installed ART programs should establish sentinel surveillance systems of TDR and make evidence-based recommendations for prevention of HIV drug resistance [Bennett et al., 2008a; Bennett et al., 2008b]. Furthermore, international treatment guidelines recommend that resistance testing should be performed in newly diagnosed patients [Hirsch et al., 2008].

In Latvia, the first HIV infected patient was diagnosed in 1987. In the first ten years the epidemic evolved slowly and mainly involved infections among men who have sex with men. In 1997 an explosive outbreak of HIV-1 infections began among intravenous drug users. The outbreak culminated in 2001 when a total of 665 new infections were diagnosed among intravenous drug users. By December 2009, 4614 HIV-1 cases had been reported in Latvia. Of these 824 had developed AIDS and 486 patients had died from AIDS or other causes [Infectology Center of Latvia, 2010a]. Combination antiretroviral treatment (cART) has been available in the Latvia since 1996. Until December 2009, 990 patients had started therapy, but approximately half of these patients have either died or terminated therapy and 439 patients were reported to be under therapy at the end of 2009. The first-line regimen in Latvia usually consists of the non-nucleoside reverse transcriptase inhibitor (NNRTI) efavirenz (EFV) together with two nucleoside reverse

transcriptase inhibitors (NRTIs); lamivudine (3TC) and zidovudine (AZT) or 3TC and abacavir (ABC) [Infectology Center of Latvia, 2010b]. There is limited information about the prevalence of TDR in Latvia, as well as in other parts of the former Soviet Union [Avi et al., 2009; Pandrea et al., 2001; Rumyantseva et al., 2009; Vazquez de Parga et al., 2005]. The aim of the study was to investigate prevalence of TDR in newly diagnosed treatment-naïve HIV-1 patients in Latvia. This study shows that the prevalence of TDR in 2005-2006 was low, 3.4%, which in part can be explained by the low proportion of HIV-1 patients under cART.

MATERIALS AND METHODS

Patients

The study included 119 Latvian HIV-1 patients who were newly diagnosed in 2005 – 2006 and from whom plasma samples frozen at -70°C obtained within 180 days after diagnosis and prior to any ART were available at the Infectology Center of Latvia. The study was part of the European SPREAD (Strategy to Control SPREAD of HIV drug resistance) and EHR (EuropeHIVResistance) projects (www.europehivresistance.org). SPREAD/EHR is a multinational, multicentre surveillance program aimed to determine the prevalence of TDR in Europe. The study protocols and data collection forms from SPREAD/EHR were used. The Regional Medical Ethics Board in Stockholm, Sweden (Dnr 2008/4:1) and The Central Medical Ethics Committee in Latvia (24 march, 2005, A-4, decision nr.4) had approved the study. Written informed consent was obtained from all study subjects.

Measurements of plasma HIV-1 RNA levels and CD4 cell counts

HIV-1 RNA levels in plasma were measured using the COBAS Amplicor HIV-1 monitor version 1.5 assay (Roche Molecular Systems, Basel, Switzerland) and CD4 cell counts were measured using flow cytometry.

Genotypic HIV-1 resistance testing

Genotypic HIV-1 resistance testing was performed using a published in-house method that targets amino acids 1-99 in the protease and 1-253 in reverse transcriptase (RT) [Lindstrom and Albert, 2003; Lindström and Albert, 2003; Murillo et al., 2010]. The test has been used several years for national surveillance TDR in Sweden and has successfully undergone regular quality control within ENVA genotyping proficiency programme [Pandit et al., 2008].

The sequences were assembled and edited using the SequencherTM software (Gene Codes Corporation, Ann Arbor, MI, US). TDR was identified using the WHO 2009 list of mutations for surveillance of TDR as implemented in the Calibrated Population Resistance tool (v5.0 beta) [Bennett et al., 2009; Gifford et al., 2009] available at the Stanford HIV Drug Resistance Database (hivdb.stanford.edu). The susceptibility of the viruses to antiretroviral drugs was predicted using the Stanford HIVdb, ANRS and Rega algorithms as implemented in the HIValg tool also available at the Stanford HIV Drug Resistance Database.

To determine the HIV-1 genetic subtype of the viruses, the *pol* sequences were manually aligned with the subtype reference data set from the Los Alamos HIV Sequence Database (www.hiv.lanl.gov) using the BioEdit software [Hall, 1999] and neighbor-joining (NJ) trees were constructed with maximum composite likelihood substitution model using Mega v4.1 [Tamura et al., 2007]. BLAST searches in the Los Alamos HIV Sequence Database were used to retrieve the 25 most closely related database sequences to each of four samples with evidence of TDR to investigate their evolutionary relationship in NJ trees. The Latvian sequences have been deposited in GenBank database under accession numbers GU945072-GU945077; GU945079-GU945083; GU945085-GU945092; GU945094-GU945098; GU945100-GU945102; GU945104-GU945118; GU945120-GU945127 and GU945129-GU945195.

Statistical analyses

The 95% confidence interval of the prevalence of TDR was calculated using the binomial distribution and the exact method.

RESULTS

General characteristics of the study subjects

Genotypic resistance testing was successful for 117 of the 119 study subjects. The two patients without results from resistance testing were excluded from further analyses. The characteristics of the 117 remaining subjects are shown in Table 1. Briefly, the reported infection route was heterosexual for 21% of the patients; intravenous drug use for 38%, sex between men for 8.5% and unknown for 32.5% of the patients. Approximately 59% of the patients were males and 39% were females. The median age of the study population was 29 years (range 17-69). The median time between diagnosis and sampling for resistance testing was 6 days (range 0 – 166 days). A majority of patients (n=98; 84%) were infected with subtype A1 and the remaining 19 patients (16%) were infected with subtype B. Overall we sampled around one fifth of all patients that were diagnosed in Latvia during 2005-2006 and the study subjects were well distributed between genders, transmission routes and areas of residence, but the study population included slightly lower proportions of patients from the homosexual transmission group and residents from Riga (Table 1). The age distribution of the study subjects in each transmission group did not differ from the age distribution in respective transmission groups among patients diagnosed in 2005 and 2006 (data not shown).

Low prevalence of transmitted drug resistance in Latvia

Four of the 117 study subjects displayed resistance mutations indicative of TDR according to the WHO 2009 list of mutations for surveillance of transmitted drug resistance (Table 2). Thus, the prevalence of TDR was low in this study population (3.4%; 95% CI: 0.9% - 8.5%). Two of the four patients with TDR had reported intravenous drug use as the route of infection, while the route of infection was unknown for the other two patients. All four patients with TDR were infected

with HIV-1 of subtype A1, which is the dominant subtype among HIV-1 infected intravenous drug users and heterosexually infected patients in Latvia [Balode et al., 2004].

Each of the four patients displayed a single, but different, resistance mutation, i.e. F53L, M41L, G190A and M46I, respectively (Table 2). Two of the viruses, those with M46I and F53L, were predicted to be fully susceptible to standard NNRTI-based first-line cART in Latvia, despite having mutations indicative of TDR. Many of the viruses (n=36, 31%) had the A62V mutation in RT. All these viruses were of subtype A1. The mutation is characteristic for the subtype A1 variant that has spread throughout the former Soviet Union, including Latvia and is considered as common natural sequence polymorphism [Sukhanova et al., 2005].

Phylogenetic tree analyses with Latvian and database *pol* sequences indicated that the four patients with TDR were epidemiologically unlinked (data not shown), which was expected since their viruses displayed different resistance mutations. Furthermore, the phylogenetic tree analyses did not reveal any unexpected sequence similarities, which argues against the occurrence of PCR contamination or sample mix-up.

DISCUSSION

This study estimated that the prevalence of TDR was 3.4% among Latvian HIV-1 patients diagnosed in 2005 and 2006. The estimate of TDR in Latvia agrees well with published data on Latvian patients sampled in 2006-2007 and a recent conference report on 2006-2008, which estimated a prevalence of TDR of 7% and 5.3% respectively [Dusacka et al., 2009; Kolupajeva et al., 2008]. However, it is difficult to directly compare or combine the data from these studies on TDR in Latvia since there were differences in the inclusion criteria, the representativeness and the resistance algorithms used. The prevalence of TDR in Latvia was low compared to most other European countries, but there is limited data from Eastern Europe and the former Soviet Union and most published studies are small, which makes the estimates of TDR uncertain. In available reports the prevalence of TDR ranges from 0% in Estonia to 14.7% in Poland [Babic et al., 2006; Rummyantseva et al., 2009; Santoro et al., 2008; Stanczak et al., 2007; Vazquez de Parga et al., 2005].

The finding that the prevalence of TDR in Latvia was low in 2005 and 2006 is encouraging because it indicates that patients with failing therapy are not a major source of new infections. However, the low prevalence of TDR was not unexpected since the proportion of HIV-1 patients who receive therapy is relatively low in Latvia [de Joncheere et al., 2009]. Thus, approximately 570 Latvian patients had started therapy until 2006, which represent 16% of the approximately 3600 patients that had been diagnosed at this time. The data indicated that the prevalence of TDR in Latvia was below 5% (95% CI 0.9%-8.5%) in 2005 and 2006. The point estimate in report from 2006-2008 was higher, 5.3% [Dusacka et al., 2009], but the difference is not statistically significant. Nevertheless, the recommendation by the WHO would be to review antiretroviral therapy monitoring data, investigate potential problems and repeat the survey in following year [Myatt and Bennett, 2008]. Reassuringly, the results from our study indicate that the standard

183 first-line treatment regimen in Latvia (EFV + 3TC + AZT or ABC) would be effective in all,
184 except one, of the study subjects.

185 The fact that TDR was only observed in patients infected with subtype A1 virus was
186 somewhat surprising, because the subtype A1 epidemic in Latvia is relatively young [Balode et
187 al., 2004; Ferdats et al., 1999], which means that some patients are not yet in need of therapy.
188 Furthermore, most of the subtype A1 infections have occurred among intravenous drug users who
189 sometimes are excluded from therapy because they are considered to be at increased risk of
190 displaying poor adherence to treatment [de Joncheere et al., 2009]. Thus, one would have expected
191 that TDR would have been more common in patients infected with subtype B virus, because this
192 subtype was introduced earlier in Latvia (Balode et al, unpublished) and predominate among men
193 who have sex with men who generally have better access to ART than intravenous drug users.
194 However, it should be pointed out that this study was too small to allow reliable estimates of TDR
195 in different subgroups of patients. The phylogenetic analyses indicated that the four patients with
196 TDR were not epidemiologically linked. Thus, there were no networks of HIV drug-resistant
197 strains of the type that have been reported from other countries, such as Switzerland and Sweden
198 [Lindstrom et al., 2006; Yerly et al., 2009].

199 Some limitations of the study should be mentioned. First, the study was relatively small,
200 which makes the estimate of TDR somewhat uncertain [Marks et al., 2010], but the estimate
201 agrees quite well with two other reports from Latvia [Dusacka et al., 2009; Kolupajeva et al.,
202 2008]. Second, the patients were diagnosed in 2005 and 2006 and therefore the results do not
203 necessarily reflect the current prevalence of TDR in Latvia. Third, population-based sequencing
204 does not detect minority resistance variants [Jakobsen et al., 2010], but this limitation is present
205 also in almost all other surveys of TDR. Some strengths of the study should also be mentioned.
206 Thus, the study population represented approximately 20% all patients that were diagnosed in
207 Latvia in 2005 and 2006 and was well distributed between gender, transmission route and area of

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residence. Furthermore, the results are directly comparable to data from many other European countries because it was a part of the EU supported SPREAD/EHR programme [The SPREAD programme 2008; Vercauteren et al., 2009].

Several drug resistance algorithms and mutations lists have been developed: Stanford HIVdb, Rega and ANRS Algorithms and International AIDS Society (IAS) [Bennett et al., 2009; Johnson et al., 2009; Liu and Shafer, 2006; Van Laethem et al., 2002]. According to a study which compared Stanford HIVdb algorithm and Stanford Drug resistance mutation (SDRM) and IAS-USA lists, the choice of genotyping definition has a minor influence on the estimated rate of TDR, but have larger impact at individual level [Green et al., 2008]. In the present study we used the WHO 2009 list of mutations for surveillance of TDR as implemented in the Calibrated Population Resistance tool (v5.0 beta) [Gifford et al., 2009], which specifically has been developed for surveillance of TDR [Bennett et al., 2008b].

In conclusion, this study shows that the prevalence of TDR in Latvia is low and indicates that routine resistance testing should be focused on patients that display treatment failure, rather than treatment naïve patients.

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For Peer Review

Table 1. Characteristics of 117 study subjects diagnosed and sampled during 2005 and 2006

Characteristics	Study subjects	No. of diagnosed patients in 2005-2006	Coverage (%)
Patients	117	598	20
Sex [n (%)]			
Male	69 (59)	380 (64)	18
Female	45 (38.5)	218 (36)	21
Unknown	3 (2.5)		
Age (years)			
Median (range)	29 (17-69)	29 (0-69)	
Route of transmission [n (%)]			
Heterosexual	25 (21)	183 (31)	14
Intravenous drug use	44 (38)	222 (37)	20
Homosexual	10 (8.5)	30 (5)	35
Mother-to-child	0 (0)	6 (1)	0
Unknown	38 (32.5)	157 (26)	24
Area of residence [n (%)]			
Riga	74 (63)	476 (80)	16
Other	38 (33)	122 (20)	31
Unknown	5 (4)		
HIV-1 subtype [n (%)]			
Subtype A1	98 (84)		
Subtype B	19 (16)		
Clinical Stage [n (%)]			
Stage A	92 (79)		
Stage B	5 (4)		
Stage C	15 (13)		
Unknown	5 (4)		
CD4 counts (cells/ μ l)*			
Median (range)	388 (27-2002)		
Viral load (log ₁₀ copies/ml)#			
Median (range)	4.5 (3.0-6.9)		

*The data were not available for 5 patients

#Not available for 4 patients

Table 2. Characteristics of the four study subjects with indications of transmitted drug resistance (TDR)

Sequence ID	Gender	Age	Transmission route	Days from diagnosis to sampling	Subtype	Mutations indicative of TDR		
						PI	NRTI	NNRTI
S2023	Female	23	Intravenous drug use	1	A1	F53L		
S2025	Male	23	Unknown	1	A1		M41L	
S2114	Male	37	Intravenous drug use	14	A1			G190A
S2264	Female	22	Unknown	11	A1	M46I		

PI, protease inhibitors; NRTI, nucleoside reverse transcriptase inhibitors; NNRTI, non-nucleoside reverse transcriptase inhibitors.