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LATE PRESENTATION AND LIMITED TRANSMITTED DRUG RESISTANCE IN NEWLY DIAGNOSED HIV-1 PATIENTS FROM THE NORTH-EASTERN REGION OF ROMANIA

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Abstract. Objective. To evaluate the prevalence of resistance mutations in antiretroviral (ARV) treatment-naïve patients from the north-eastern region of Romania. **Methods.** Adult patients from the HIV/AIDS Regional Centre of Iasi were investigated between January 2007 and June 2008. HIV genotyping was performed by using the ViroSeq Genotyping System. The prevalence of transmitted resistance was calculated by using the WHO recommended list of mutations. **Results.** Fifty patients were included in the study. Most of them were late presenters, 56% had AIDS. Subtype F1 predominated, although other subtypes (B, C and CRF02_AG) were detected. A statistical significant association between older patients, risk-related behaviour and non-F1 subtype infection was found. In the 41 HIV sequences analyzed, only two resistance related mutations were detected: T215S (2 cases) and K103N (1 case). **Conclusions.** The transmitted HIV resistance has low prevalence and is limited to single NRTI and NNRTI resistance mutations. These results might be influenced by the large proportion of late presenters in the studied group, with a much higher prevalence in recently infected cases.

Keywords: HIV resistance, late presenters

Introduction

The development of resistance to antiretroviral (ARV) drugs is one of the major limitations to a long-term successful treatment of the HIV-1 infection. HIV resistance mutations are frequently detected in patients with treatment failure [1-3], generating an increased risk of transmission of drug-resistant (TDR) viral strains [4]. Once transmitted, a drug-resistant virus can persist for months to years without reversion to wild-type [5] and thus could influence the response to the first-line antiretroviral therapy [6-8]. For these reasons, the current guidelines recommend to perform genotypic resistance testing in all ARV naïve patients [9,10,11].

The evaluation of transmitted drug resistance provides a feedback on the efficacy of HIV-1 prevention strategies and essential data for the design of optimal first-line ARV regimens. The prevalence of transmitted resistant HIV varies widely among different reports [12]. Several factors might explain the different estimated rates: the design of the study, the time between the infection and the diagnosis, the methodology used for resistance testing, the list of mutations used to define drug resistance, the HIV diversity, the time and the region evaluated [12].

To estimate the magnitude of this phenomenon in the North-eastern region of Romania, we investigated newly diagnosed HIV-1 infected patients.

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Our study is aimed to estimate i) the prevalence of the mutations associated with resistance to reverse-transcriptase (RT) and protease (PR) inhibitors ii) the factors that might influence the rate of the transmitted drug resistance and iii) the diversity of HIV-1 infective strains.

Methods

Patient and data collection

Adult patients from the HIV/AIDS Regional Centre of Iasi with a recent diagnosis of HIV infection, i.e. less than 6 months from the time of the diagnosis, were invited to participate in the study. For all the participants, enrolled between January 2007 and June 2008, clinical, immunological, behavioural, virological data and blood samples were collected.

Informed consent was obtained from participants. All of the information gathered during the study was analyzed in a completely anonymous way.

HIV sequencing

HIV genotype analysis was performed on plasma samples by using a commercially available kit (ViroSeq HIV-1 genotyping system; Celera Diagnostics) according to the manufacturer's recommendation. Briefly, the HIV RNA was extracted, reverse-transcribed and then amplified in a polymerase chain reaction (PCR). RT-PCR product was sequenced bidirectionally on an ABI Prism 3100 Avant genetic analyser (Applied Biosystems) by using six different primers. The raw analysis of the sequences was made using Sequencing Analysis Software v 3.7 and then they were assembled with ViroSeq 2.5/2.7 HIV-1 genotyping system software. The correctness of each electropherogram interpretation was validated by the operator and then sequences were saved in FASTA format. The resistance report generated by ViroSeq provides a list of nucleotide mutations detected in the PR (codons 1—99) and the RT gene (codons 1—335) known to determine resistance to antiretroviral drugs. The Fasta files were then used to interpret the results by the HIVSeq Program, available at the Stanford database (http://hivdb.stanford.edu). For subtyping purposes we used the publicly available algorithm from REGA HIV-1&2 Automated subtyping tool version 2.0 (http://jose.med.kuleuven.be/genotypetool/html/subtypinghiv.html).

Genotypic resistance analysis

To estimate the prevalence of resistance, we used the World Health Organization 2009 List of mutations for surveillance of transmitted drug resistant HIV strains [13]. This updated list has 34 nucleoside reverse-transcriptase inhibitors (NRTI) -associated resistance mutations at 15 RT positions, 19 non-nucleoside reverse-transcriptase inhibitors (NNRTI) -associated resistance mutations at 10 RT positions, and 40 protease inhibitors (PI) -associated resistance mutations at 18 protease positions: M41L, K65R, D67N/G/E, T69D, T69ins, K70R/E, L74V/I, V75A/M/T/ S, F77L, Y115F, F116Y, Q151M, M184V/I, L210W, T215C/D/E/F/I/S/Y/V, K219E/N/Q/R for NRTI, L100I, K101E, K103N/S, V106A/M, V179F, Y181C/I/V, Y188C/H/L, G190A/E/S, P225H, M230L for NNRTI and L23I, L24I, D30N, V32I, M46I/L, I47A/V, G48V/M, I50L/V, F53L/Y, I54A/L/M/S/T/V, G73A/C/S/T, L76V, V82A/C/F/L/M/T/S, N83D, I84A/C/V, I85V, N88D/S, L90M for PI (13).

Statistical analysis

Categorical data were analyzed by using chi – squared and Fisher exact test. A *p* value less than 0.05 was used to determine statistical significance.

Results

Study population

A number of 93 newly diagnosed HIV infected patients were registered in the HIV/AIDS Regional Centre of Iasi over the 18 months period of the study. More than half (50 patients) accepted to participate in our study. Forty-one RNA samples were successfully amplified and sequenced. The main characteristics of the patients are summarized in table I

Sex distribution was different according to the age: male patients were equal in number with female patients in the age group of less than 25 years, but predominated significantly if age was more than 25 years ($p = 0.01$), and even more significantly if age was more than 20 years ($p = 0.001$). More than half (28 patients) had AIDS related diseases and/or severe immunosupression (CD4 count less than 200 cells/μL) at the time of diagnosis. The AIDS diagnosis was more frequent in patients aged of less than 20 years.

Risk related behaviour was declared in twenty patients (40%): multiple anonymous heterosexual

Patient characteristics	All patients (n = 50)	Patients with non-F subtype (n = 7)	p value	Patients with age >20 years (n = 34)	p value
Male (%)	35 (70%)	6	0.22	29	0.001
Median age (years)	26 (17-58)	29 (21-58)		30 (21-58)	
15-24 years (%)	22 (44%)	1	0.20	6	
Risk exposure	20	6	0.009	19	0.002
Viral load, median, (log copies/ml)	5.19	4.96		5.17	
CD4 count, median, cell /mm3	238 (3 – 1136)	288 (27-619)		288(27-619)	
CD4 count >350/mm3	14 (28%)	3	0.02	12	0.1
CDC Clinical stage					
A	12	3	0.16	11	0.07
B	19	3		14	
C	19	1	0.09	9	0.03
Subtype					
F	34	0	NA	19	0.03
non-F	7	7	NA	7	0.03

Table 1 Demographic, clinical and virological characteristics of the newly diagnosed patients from the HIV/AIDS Regional Centre of Iasi

partners (13 cases), HIV- positive heterosexual partner (5 cases), homo/bisexual contact (2 cases). Risk behaviour was significantly associated with age of more than 20 years, but not with male sex. There was no correlation between known risk factors and AIDS diagnosis.

Subtype analysis

As expected, the subtype F1 was the most prevalent subtype, being detected in 34 of the 41 patients with complete sequences (83%). We could identify also other HIV-1 subtypes: B (4 patients), C (2 patients) and one circulating recombinant form: CRF02_AG (1 patient).

An interesting observation was that the patients infected with other subtypes than F1 had significantly higher CD4 cell count (p = 0.02) and more risk factors (p = 0.009). The diagnosis of infection with subtypes other than F1 was significantly associated with patients older than 20 years (p = 0.03), but not with the male sex.

Resistance analysis

The resistance mutations in the RT and the PR genes provided by the ViroSeq and the HIVSeq Program were compared with the WHO 2009 list of mutations for surveillance of transmitted drug resistant HIV strains.

Reverse-transcriptase mutations

The mutation T215S was present in two patients. This mutation, which is a transition between wild type and the mutations Y and F, does not reduce NRTI susceptibility. However its presence suggests that T215Y or F may also be present and that NRTI resistance can appear sooner than expected if the drug is prescribed.

The mutation K103N, which causes high-level resistance to nevirapine and efavirenz, but has no effect by itself on etravirine susceptibility, was present in one patient.

Other mutations detected by the HIVSeq Program, but not present in the WHO list, were V106I (1 patient), E138A (4 patients) and V179D (2 patients). These mutations are reported as polymorphisms associated with decreased etravirine response in the DUET studies. The V179D mutation cause low-level reduction in susceptibility to each NNRTI, but its effect is considered insignificant in the absence of other mutations.

Protease mutations

No PI resistance mutations described in the WHO list for surveillance of transmitted drug resistance were present in the analysed sequences. Several clade-specific polymorphisms are considered as secondary resistance mutations by the Viroseq and the HIVSeq Program, which use a subtype B consensus sequence [13]. These mutations occurred at codons 10, 16, 20, 36, 62, 63, and 74 in more than 10% of the analyzed sequences: L10I/V (19 cases), G16E (8 cases), K20R (22 cases), M36I (35 cases), I62V (12 cases) L63T (34 patients), T74S (10 cases).

Discussions

The HIV epidemic in Romania has as characteristic the existence of a large cohort of young people who were infected as children in the late 1980s by parenteral transmission [14,15,16]. Most of these patients have been under treatment for more than ten years and many of them have experienced years of either monotherapy (AZT) or bitherapy (AZT with 3TC or zalcitabine (ddC). As many of these adolescents are becoming sexually active, the possible transmission of HIV resistant strains is a real concern. Extensive mobility of the Romanian adults in recent years might also increase the prevalence of drug resistance associated mutations. According to the National AIDS Committee Report, in 2008 9372 HIV /AIDS patients were in active medical surveillance; 14 % of them were living in the North-eastern region.

A large prospective European study showed an overall prevalence of 8.4% transmitted HIV drug resistance in Europe between 2002 and 2005, in the majority of cases single drug resistance related mutations being detected [17,18].

The prevalence of transmitted HIV resistance in the north-eastern region of Romania We evaluated between January 2007 and June 2008 was 7.3%, with 4.8% for NRTI and 2.4% for NNRTI. No PI resistance mutations were detected. Although comparable to the results reported in other surveillance studies in Europe, our results could be influenced by the limited number of patients enrolled and the large proportion of late presenters.

Four criteria were used for the selection of surveillance drug resistance mutations (SDRMs) in the WHO list: SDRMs should be present on three or more of five expert lists of drug resistance mutations: ANRS, HIVdb, IAS-USA, Los Alamos, and Rega algorithm lists, should be non-polymorphic or not occur at highly polymorphic positions. The mutation list has to be applicable to the eight most common HIV-1 subtypes and should be parsimonious, excluding mutations resulting exceedingly rarely from drug pressure [13]. This approach is particularly useful in Romania, where the HIV epidemic is produced mainly by the F1 subtype [14, 19], with polymorphisms in positions associated with drug resistance to protease inhibitors 10V, 20R, 36I, 62V, 69K, 77I or non-nucleoside reverse-transcriptase inhibitors 106I, 138A, 179D [19, 20].

One important observation is that most of the patients newly diagnosed between January 2007 and June 2008 in the North-eastern Region of Romania were late presenters. According to the statements of the European Working Group on late presentation, around 30% of patients with HIV infection across Western Europe present with a CD4 count of <200 cells/μL and/or clinical AIDS; however no published data on late presentation are available for many European countries, particularly those in Eastern Europe. In our study 56% of the patients fulfilled those criteria.

There is no consistently used definition for late presentation and, thus, estimates of the incidence of late presentation vary widely. The European Working Group recommends as standard definition for late presentation a CD4 count of < 350 cells/μL and /or an AIDS-defining condition. The fact that 74% of the newly diagnosed patients in our study were in this category suggests the need of better HIV testing strategies.

Late presenters have an increased risk of morbidity and mortality, compared with patients who present early. This higher risk is associated with both AIDS and non-AIDS related causes, like myocardial infarction, non-AIDS- defining malignancy, pancreatitis, liver-related disease. Although several studies reported that late presentation occurs more likely in older individuals and female patients, our results didn't showed a correlation between AIDS diagnosis and age or sex. A possible explanation could be the characteristic of the HIV epidemic in Romania, with a large number of young adults who were infected as children in the late eighties.

Conclusions

Most of the patients diagnosed between 2007 and 2008 in the HIV/AIDS Regional Centre of Iasi are late presenters. Risk behaviour was significantly associated with an age of more than 20 years, but there was no correlation between recognized risk factors and AIDS diagnosis. Infection with other subtypes than F1 was detected in 17% of cases, being significantly associated with sexual transmission risk factors. We found that the transmitted HIV resistance has low prevalence and is limited to single NRTI and NNRTI resistance mutations, but our results might be influenced by the large proportion of late presenters in the studied group.

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