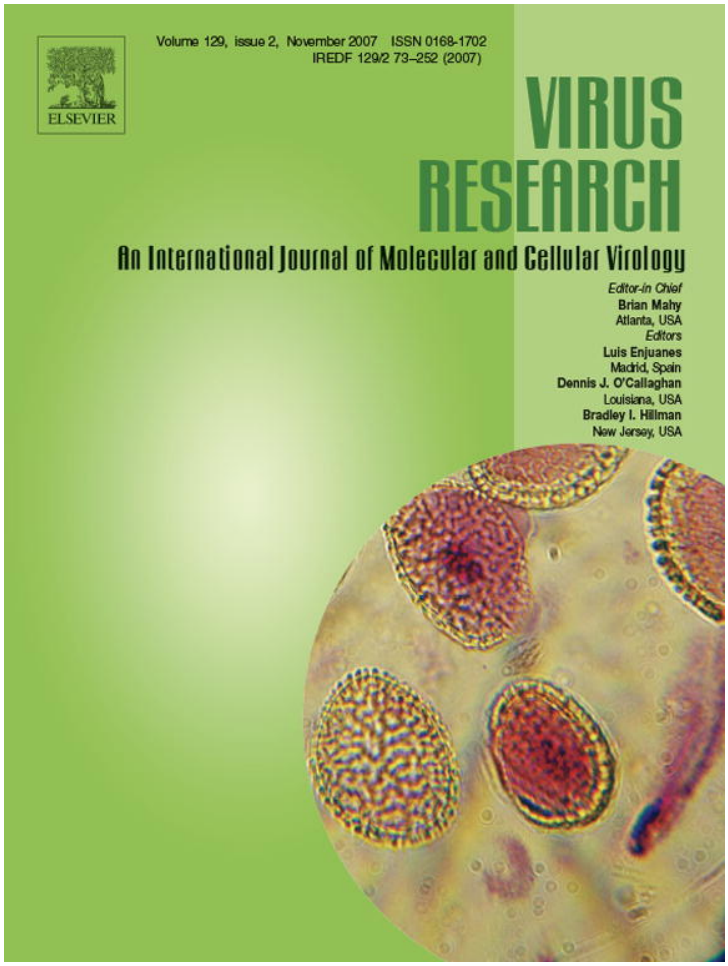

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Drug resistance among chronic HIV-1-infected patients naïve for use of anti-retroviral therapy in Sao Paulo city

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Abstract

Primary infection with drug-resistant HIV appears to be increasing in the regions where HAART is widely available, which may reduce efficacy of first-line antiretroviral therapy.

To determine prevalence of antiretroviral drug-resistant mutations in newly diagnosed subjects in a clinical setting where HAART has been widely used since 1997.

One hundred and thirty-six HIV-1-infected adult patients were diagnosed with HIV infection between January 2000 and December 2006 in the HIV out-clinic at the HC/FMUSP, Sao Paulo city. These antiretroviral naïve patients were mainly referred from the blood bank, situated in the same building or elsewhere in the city. The samples were genotyped to provide HIV protease and reverse transcriptase sequence data. Major antiretroviral drug resistance mutations were classified according to Shafer et al. [Shafer, R.W., Rhee, S.Y., Pillay, D., et al., 2007. HIV-1 protease and reverse transcriptase mutations for drug resistance surveillance. AIDS 21, 215–223].

Thirteen cases had no DNA amplification, and 123 patients were successfully analyzed, with a mean age of 37 years and 89 (72%) were males. Antiretroviral drug resistance mutations were detected in 8/123 patients (6.5%), all eight were heterosexuals and HIV asymptomatic, the mean of the CD4 cells count was 323 cells/mm^3, and the RNA plasma viral load was 4.7 $\log_{10}$/mL. We found NRTI ($n=2$, 1.6%), NNRTI-resistant ($n=2$, 1.6%) mutations, and one cases with PI mutation (0.8%). Three cases (2.4%) showed mutations for NRTI, NNRTI or PI, simultaneously. Eighty-two percent were HIV-1 B subtype, and HIV-1 F (6.5%), HIV-1 C (5.7%) and recombinant viruses (5.8%) were observed.

In an unselected cohort, primary drug resistance was seen in 6.5% of the naïve for drug ART use. These results indicate that HIV drug resistance testing should be a practical approach in monitoring first-line ART. In addition, HIV-1 C seems to be emerging in Sao Paulo city.

Keywords: HIV-1; Drug resistance; Brazil; Mutation

1. Introduction

In 1993, a federal law determined that antiretroviral drugs should be released free of charge by public health providers for all Brazilians with HIV/AIDS based on local guidelines. In the last 10 years, with this highly active anti-retroviral therapy (HAART) implementation program, Brazilian HIV-1-infected subjects have experienced a prolongation of survival, a drop in the number of opportunistic infections, and an improvement in the quality of life (Casseb et al., 1999; Teixeira et al., 2004). Sao Paulo city possess the largest absolute number of documented AIDS cases in the country, almost 40% of the Brazilian AIDS cases recorded in the last 25 years of the epidemic (Brazil, Boletim epidemiológico, 2004 and 2005).

Primary infection with drug-resistant HIV appears to be increasing in the United States (Wheeler et al., 2007) and other countries where anti-retroviral therapy (ART) is widely available

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(Palma et al., 2007; Booth et al., 2007). Brazil has approximately 200,000 HIV/AIDS cases under HAART in the last 10 years (Galvão, 2002). Despite this long use, little data have been reported on the prevalence of mutations in naïve antiretroviral therapy patients in Brazil. In a study of 341 specimens collected at the major blood bank in Sao Paulo city between July 1998 and March 2002, 21 (6.3%) had drug-resistant mutations (Barreto et al., 2006). In this study, we looked at the prevalence of genetic HIV subtypes and the antiretroviral drug-resistant mutations in newly infected subjects in Sao Paulo city, Brazil.

2. Materials and methods

2.1. Study population

HAART naïve patients were selected from 350 HIV-1-infected individuals who have been followed at the Secondary Immunodeficiencies, Dermatology Department at the HC-FMUSP, from March 2002 to December 2006. Naïve cases were considered who had never been exposed to any ART lifelong. The participants had support from nurses, nutritionists and psychologists, as do all patients in follow up. The HC/FMUSP ethical board approved the protocol and written informed consent was obtained from all participants. The demographical data and self-reported HIV infection transmission route were obtained using a questionnaire at the baseline visit.

2.2. HIV resistance genotyping test

After viral RNA extraction, cDNA was generated by RT-PCR and used in nested PCR protocols with primers for protease (PR), and reverse transcriptase (RT) of HIV-1. The PCR strategy used external primers K1/K2, and as internal primers, DP10/F2 or DP16/RT4 (Kozal et al., 1996; Frenkel et al., 1995). The amplicons were purified by a PCR Purification kit (Qiaquick, Qiagen Inc., Valencia, CA, USA). Genomic sequencing was conducted to define HIV-1 subtypes and drug resistance mutations, as previously described (Johnson et al., 2006). Major antiretroviral drug resistance mutations were classified according to Stanford online HIV Drug Resistance Database and more recently publication (Shafer et al., 2007).

2.3. Statistical analysis

Statistical analysis was performed using the Statistical Package program (GraphPad Prism 3.0). Differences between the means of groups were analyzed using an independent, two-tailed *t*-test. The probability measures for all calculations were also provided and values of $P < 0.05$ were considered significant.

3. Results

Table 1 depicts the demographical and clinical data of the patients. The mean age was 37 years and 26% were women. The most frequent transmission route was unprotected heterosexual exposure for both genders. Few cases of severe immunosuppression were seen, and the viral and T CD4+ cells counts were 4.5 $\log_{10}$ copies/mL and 458 cells/mm^3, respectively. Of the 136 samples tested, 13 (9.5%) patients had no DNA amplification. The major cause of no amplifications was low RNA plasma viral load, below the method sensitivity (>5000 copies/mL), which was observed in all samples.

Subtype B was found in 101/123 (82%), and HIV-1 F was found in 6.5%, followed by HIV-1 C (5.7%) and other recombinant forms of the circulating viruses in Sao Paulo (Table 2). Antiretroviral drug resistance mutations were detected in 8 (6.5%) of 123 patients, NRTI ($n = 2$, 1.6%), two cases with NNRTI-resistant mutations, one case showed PI resistance (0.8%), and three cases (2.4%) yielded more than one mutation for each class (Table 3). Table 4 depicts the characteristics of the eight naïve HAART patients who presented any HIV drug resistance mutation. In this group, the mean of T CD4 cells count was 323 cells/mm^3 and the plasma viral load was 4.7 $\log_{10}$. All were heterosexuals (data not shown), one was woman, and three cases had a multi-drug resistance profile.

Table 1
Demographical, virological and immunological characteristics of the Naïve HAART patients

Characteristics	Resistance Analysis
n	123
Mean age, S.D. (years)	37 ± 12
Male sex (%)	89 (72%)
White race (%)	90
CD4 T cells count, mean (cells/mm^3)	338 ± 243
Mean HIV-1 RNA ($\log_{10}$ copies/mL)	4.5 ± 0.85
AIDS-related symptoms (%)	None

Note: Only T CD4 cell count and HIV viral load available for 89 patients.

Table 2
Subtypes and recombinants viruses in the pol gene of the 123 patients enrolled in this analysis

Subtype protease/RT	Number	%
B/B	101	82.0
F/F	8	6.5
C/C	7	5.7
B/F	5	4.1
B/C	2	1.7
Total	123	100

RT: Reverse transcriptase.

Table 3
Antiretroviral drug resistance mutations distribution

Prevalence of resistance mutations by drug class, *n* (%)	
Any	8 (6.5)
NRTI	2 (1.6)
NNRTI	2 (1.6)
PI	1 (0.8)
Multidrug	3 (2.4)

Note: Three patients with more than one mutation for each ART class.

Table 4
Epidemiological, virological and immunological characteristics of the eight naïve HAART patients who presented HIV drug resistance

Case	Date of HIV infection	Date of sampling	Age (year)	Sex	CD4	VL	PI	NRTI	NNRTI
1	1/8/99	7/5/03	26	M	220	5.0	None	A62AG, V75L	None
2	1/1/04	22/3/04	39	M	90	4.99	M46L	D67G, T69D	None
3	5/2/04	19/4/04	34	M	NA	NA	None	None	K103N
4	14/6/04	9/8/04	24	M	235	4.09	None	None	K103N
5	1/10/92	23/11/04	45	F	69	5.77	L90M	None	None
6	10/5/05	7/7/05	30	M	409	4.33	L90M	D67N, M184V	None
7	1/10/03	21/11/05	37	M	704	3.52	None	V75M	None
8	24/2/06	20/4/06	25	M	534	5.16	None	M184V	K103N, P225H

Age in years; M: male; F: female; VL; HIV RNA plasma viral load (copies/mL); all were subtype B/B; CD4: T CD4+ cells count at the moment of the genotyping testing; PI: protease inhibitor; NRTI: analogue transcriptase reverse inhibitor; NNRTI: non-analogue transcriptase reverse inhibitor; NA: not avaliable.

4. Discussion

Since November 1996, the Brazilian government has provided free of charge HAART to Brazilian patients infected with HIV. This policy has resulted in decreased rates in mortality, morbidity and costs. However, the availability of HAART may have some influence on HIV drug resistance, especially in those patients with low adherence to the treatment regimen (Brígido et al., 2001).

Our major finding was that 6.5% of the 123 successfully analyzed patients yielded drug resistance to anti-retrovirals used in the clinical practice among newly diagnosed HIV-1-infected subjects in the last 4 years. The prevalence was the same as noted by a study done in Sao Paulo, among those with long-standing infections collected previous to 2001 (Barreto et al., 2006). Taken together, it seems that prevalence of HIV drug resistance seems to be stable among chronic infected subjects in Sao Paulo city.

This rate may be related to the wide use of HAART, lack of adherence to the treatment protocol, or both. Despite the high prevalence of primary drug resistance to ART in Sao Paulo, only three (3.6%) patients with NRTI resistance were diagnosed in Northeast (De Medeiros et al., 2006) or South Brazil (Rodrigues et al., 2006). In contrast, this rate in Sao Paulo city was lower than in noted in Santos city, 40 miles apart, when among chronic patients, an accumulative rate of 29% of primary drug resistance was ascribed recently (Sucupira et al., 2007). These regional differences may be related to the time which the HAART is available, migratory flux and tourism exchange with other Brazilians or those from abroad.

Moreover, this number may reflect some changes in new guidelines, which included new codons considered mutations. More recently, it was shown that 10% of the newly diagnosed patients possessed drug resistance mutations in a large nationwide study in the USA. In London, the prevalence of resistance mutations was 7/85 (8.2%) and 10/154 (6.5%) in persons with recent and established infection, respectively. In an unselected UK cohort, the prevalence of resistance mutations was 7.1% and was highest in those born in the UK (Booth et al., 2007). Thus, the high rate and stability in Sao Paulo may reflect the longer time of availability of ART, which has been provided since 1997 in Brazil, and this city is the epidemic center of the HIV infection.

As expected, HIV-1 B was the most prevalent subtype, found in 82% of the subjects. These data are in accordance with a previous study of the cohort (Sabino et al., 1996), and more recently published, where 81% of the samples were classified as subtype B, 25 (7.3%) as subtype F, 13 (3.8%) as subtype C, and 26 (7.6%) as recombinant strains (Barreto et al., 2006). However, HIV-1 C was punctual subtype described in the 80 and 90 in Sao Paulo and from a person out-side of the city (Csillag, 1994), our data indicated similar prevalence for both viruses. Thus, it is possible to infer that subtype C may overwhelm subtype F epidemic in the near future. In fact, it is worth noting because HIV-1 C is becoming the major subtype in South Brazil, where it accounts for almost 30%–45% of the epidemic (Soares et al., 2005; Monteiro et al., 2007), and where the epidemic is growing more rapidly. In contrast, recent data from Rio de Janeiro city, 400 miles from Sao Paulo city, found no HIV-1 C (Sa-Ferreira et al., 2007). However, further epidemiological studies should be done to assess this possibility.

It is important to stress that our data are from a convenient sample rather than a representative sample and that the patients are not primary infections but are treatment naïve, chronic infections. Moreover, our findings should be viewed with caution, since no incidence case was seen. The high T CD4+ cell counts may indicate that our patients have probably been infected less than five years, but this assumption is highly speculative, and studies with high-risk populations should be performed to address this question.

Finally, this drug resistance rate noted here is a concern, since the current guidelines in Brazil does not indicate HIV resistance testing in naïve HIV infected subjects. The antiretroviral therapy-naïve subjects who are at early stages of infection are usually diagnosed during voluntary screening tests for blood donation. Although Brazil does not recommend HIV drug resistance testing of those antiretroviral therapy-naïve subjects, our findings indicated this approach would maximize ART efficacy and could result in a higher rate of mortality in these patients (Lohse et al., 2007; Hogg et al., 2006).

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