

Primary Antiretroviral Drug Resistance among HIV Type 1-Infected Individuals in Brazil

Eduardo Sprinz,¹ Eduardo M. Netto,² Maria Patelli J.S. Lima,³ Juvênao J.D. Furtado,⁴ Margaret da Eira,⁵ Roberto Zajdenverg,⁶ José V. Madruga,⁷ David S. Lewi,⁸ Alcyone A. Machado,⁹ Rogério J. Pedro,¹⁰ and Marcelo A. Soares¹¹

Abstract

Infection with drug-resistant human immunodeficiency virus type 1 (HIV-1) has been documented in all countries that have surveyed for it and may result in an unfavorable response to therapy. The prevalence and characteristics of individuals with transmitted resistance to antiretroviral drugs have been scarcely described in Brazil. We performed antiretroviral resistance testing prior to initiation of therapy in 400 subjects enrolled from 20 centers in 13 Brazilian cities between March and September 2007. Genotyping was conducted using PCR-amplified HIV *pol* products by automated sequencing, and genotype interpretation was done according to the IAS-USA consensus. Of 400 eligible participants, 387 (95.8%) were successfully tested. Seven percent of antiretroviral-naïve patients carried viruses with one or more major mutation associated with drug resistance. The prevalence of these mutations was 1.0% for protease inhibitors, 4.4% for nonnucleoside reverse transcriptase inhibitors, and 1.3% for nucleoside reverse transcriptase inhibitors. The frequency of multidrug resistance among the resistant strains was 13.6%. Among subjects infected with drug-resistant virus, the majority were infected with subtype B viruses (91%). Subjects from the city of São Paulo had higher transmitted resistance mutations compared to the rest of the country. Reporting a partner taking antiretroviral medications was associated with a higher chance of harboring HIV variants with major drug resistance mutations [odds ratio = 2.57 (95% confidence interval, 1.07–6.16); $p = 0.014$].

Resistance testing in drug-naïve individuals identified 7% of subjects with mutations associated with reduced susceptibility to antiretroviral drugs. Continued surveillance of drug-resistant HIV-1 in Brazil is warranted when guidelines for HIV prophylaxis and treatment are updated. Resistance testing among drug-naïve patients prior to treatment initiation should be considered, mainly directed at subjects whose partners are already on antiretroviral therapy.

Introduction

THE INTRODUCTION OF highly active antiretroviral therapy (HAART) in patients infected with human immunodeficiency virus type 1 (HIV) is associated with a marked reduction in morbidity and mortality^{1–3} and a significant recovery of the compromised immune function.^{4,5} Resistance to anti-

retroviral (ARV) drugs is a major cause of treatment failure in individuals with HIV infection and has been associated with higher mortality rates.^{6–9} The prevalence of ARV drug-resistant virus has been reported to vary between 1% and 18% in newly infected HIV persons in North America.^{10–14} While some authors did not find a significant trend in prevalence of transmitted resistance mutations over years,¹⁵ others have

¹Hospital de Clínicas–Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil.

²Hospital Universitário Prof. Edgard Santos, Salvador, Brazil.

³Hospital e Maternidade Celso Pierro da Pontifícia Universidade Católica, Campinas, Brazil.

⁴Hospital Heliópolis, São Paulo, Brazil.

⁵Instituto de Infectologia Emílio Ribas, São Paulo, Brazil.

⁶Projeto Praça Onze, Rio de Janeiro, Brazil.

⁷CRT/AIDS, São Paulo, Brazil.

⁸Universidade Federal de São Paulo, São Paulo, Brazil.

⁹Hospital das Clínicas da Faculdade de Medicina de Ribeirão Preto da Universidade de São Paulo, São Paulo, Brazil.

¹⁰Universidade de Campinas, Campinas, Brazil.

¹¹Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil.

reported an increasing proportion of new infections that involve drug-resistant virus in Europe.¹⁶ Moreover, the time to viral suppression might be longer and the time to virologic failure shorter among patients who are infected with drug-resistant viruses who start HAART.¹⁷

The transmission of virus with mutations that confer resistance to ARV drugs has been reported with all main routes of transmission.^{18–24} Increasing rates of resistance may not only limit future therapeutic options, but also affect HAART efficacy, even under postexposure prophylaxis.^{25,26} Thus, resistance testing before initiation of therapy may be of value in selected sites to help determine the most appropriate ARV combination regimen. More than a decade ago, Brazil was the first nation to offer universal and free access to ARV drugs to HIV-positive persons. Nevertheless, there are scant data about transmission of resistant virus to individuals who were supposed not to have been exposed to antiretroviral drugs.^{27,28} In the current study, we evaluated the prevalence of transmitted HIV mutations in a cohort of drug-naive, HIV-infected subjects from different regions of the country.

Materials and Methods

Study subjects

HIV-seropositive patients were enrolled from 20 sites in 13 Brazilian cities (Ribeirão Preto, Santo André, Santos, Nova Iguaçu, Rio de Janeiro, Belo Horizonte, Curitiba, Florianópolis, Salvador, Brasília, 2 in Campinas, 2 in Porto Alegre, and 6 in São Paulo City) between March and September 2007. This selection comprised eight states from four of the five major macroregions in Brazil, but for the sake of comparison we have grouped cities according to their relevance for the Brazilian AIDS epidemic. Therefore, subjects were grouped geographically as from São Paulo City, São Paulo State (Ribeirão Preto, Santo André, and Campinas), Santos (which has reported the highest primary resistance in the country),²⁹ Southern Brazil (Curitiba, Florianópolis, and Porto Alegre), and others (all remaining cities). Eligibility criteria included age ≥ 18 years and no previous exposure to ARV drugs before the time of sampling according to the medical chart review (if a chart was available) and personal interview. After informed consent was obtained, demographic, risk behavior, and clinical information was obtained using standardized interviews. Results from CD4⁺ T lymphocyte count and plasma HIV RNA levels, measured within the previous 3 months of enrollment, were also obtained from medical charts. Blood specimens for drug resistance testing were obtained from each consenting participant. The study was approved by the institutional review boards affiliated with the centers in which participants were recruited.

Resistance testing

Protease (PR) and reverse transcriptase (RT) genotyping was conducted on the basis of HIV PCR-amplified products with the ViroSeq system (Celera, Rockville, MD) according to the manufacturers' specifications. Briefly, viral RNA was isolated from plasma of HIV-positive enrolled individuals and subject to RT-PCR to amplify a 1.7-kb viral genomic fragment corresponding to the whole coding region of HIV PR and the first 335 codons of RT, which comprises all positions associated with drug resistance.³⁰ PCR products were

further sequenced using the BigDye termination chemistry (Applied Biosystems, Foster City, CA) with seven primers provided with the ViroSeq kit. DNA sequencing was performed in an ABI Prism Genetic Analyzer DNA (Applied Biosystems) and sequence reads were automatically assembled in the Viroseq software, which also interpreted genotypic resistance and HIV-1 viral subtype in an automated fashion. Genotypic resistance was defined as the presence of one or more resistance-related major mutation as specified by the consensus of the International AIDS Society (IAS; Spring 2008 version).³¹

Statistical analyses

Patients were grouped into those presenting primary drug resistance and those who did not. Demographic characteristics and clinical, laboratory, and molecular (HIV subtype) data were compared between those groups and statistically significant differences were assessed by Fisher's exact test of contingency tables.

The odds ratio (OR) and corresponding 95% confidence interval (CI) for transmitted drug-resistant HIV were calculated for those characteristics that were significantly different between the abovementioned groups. All statistical analyses were performed using Statistica software version 5.1/97.

Results

Study population

Four hundred and one patients were enrolled (20 per center, with the exception of one center that enrolled 21 patients). One patient was excluded from the analyses due to a protocol violation, having had previous exposure to ARV therapy. One patient under 18 years of age, considered as a protocol deviation, was analyzed after informed consent was obtained from a legally authorized representative. Table 1 summarizes the demographic and clinical characteristics of the study population. The majority of patients (69%) were white, and two-thirds were male. Forty-three percent of the patients were men who reported sex with other men (MSM), 2.5% were intravenous drug users (IDU), and 54% were heterosexual and/or had other risk factors for HIV acquisition. At baseline, the median CD4⁺ T cell lymphocyte count was 375 cells/ μ l [interquartile (IQR) range, 2–2455], and 5.4% of patients had a CD4 count lower than 50 cells/ μ l. Viral load information was available for 76.5% of the patients, and the median HIV RNA level was 24,896 copies/ml (IQR range, 184–941,910).

Drug resistance analysis

Three hundred and eighty-seven (96%) subjects underwent drug resistance testing. For the remaining subjects, resistance analysis was not possible due to lack of PCR amplification for genotyping. Twenty-two of 387 (5.7%; 95% CI, 4.4–9.6%) ARV-naive subjects carried viruses with one or more major resistance-related mutation. The prevalence of resistant genotypes varied according to the drug class: 1% (4/387) for protease inhibitors (PI), 4.4% (17/387) for nonnucleoside reverse transcriptase inhibitors (NNRTIs), and 1.3% (5/387) for nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs). Table 2 shows the primary protease and RT mutations found in all isolates with resistance mutations in the study population. Of the 22 subjects with resistance-related mutations,

TABLE 1. STUDY POPULATION CHARACTERISTICS

Characteristic	Total	Persons with primary drug resistance	p-value ^a
Total	400	22	
Age in years			
Median	36	35	
Range	15–66	23–52	
Gender			
Male	265 (66.3%)	16 (72.7%)	
Female	135 (33.8%)	6 (27.3%)	
Race/ethnicity			
White	276 (69.0%)	17 (77.3%)	
Black	68 (17.0%)	2 (9.1%)	
Mixed race	54 (13.5%)	3 (13.6%)	
Asian	1 (0.3%)	0 (0%)	
Native Brazilian	1 (0.3%)	0 (0%)	
Geographic region			
São Paulo (capital)	140 (35.0%)	13 (59.1%)	0.038
São Paulo State	60 (15.0%)	2 (9.1%)	
Santos	20 (5.0%)	0 (0%)	
Southern Brazil	80 (20.0%)	5 (22.7%)	
Others	100 (25.0%)	2 (9.1%)	
HIV risk behavior			
Male-to-male sex	173 (43.2%)	9 (40.9%)	
Injection drug use ^b	10 (2.5%)	0 (0%)	
Heterosexual exposure/other ^b	217 (54.3%)	13 (59.1%)	
Time of infection in years			
Median	1	2	
Interquartile range	0–18.0	0–8	
CD4 ⁺ count (cells/ μ l)			
Median	375	449	
Interquartile range	2–2,445	50–869	
HIV RNA level (copies/ml)			
Median	24,896	19,825	
Interquartile range	184–941,910	7,110–27,400	
HIV subtype ^c			
B	264 (66.0%)	20 (91%)	0.03
C	51 (12.8%)	1 (4.5%)	
Non-B non-C	68 (17.0%)	1 (4.5%)	
Not available	17 (4.3%)	—	
Partner taking HIV medications			
Yes	92 (23.0%)	10 (45.5%)	0.014
No	226 (56.5%)	7 (31.8%)	
Unaware	82 (20.5%)	5 (22.7%)	

^aOnly significant *p*-values (≤ 0.05) are shown.

^bDoes not include individuals who also reported male-to-male sex.

^cHIV subtype prevalence refers to 387 patients, for which HIV molecular data (subtype and drug resistance) could be obtained.

19 (87%) had mutations associated with only one drug class, 2 (9%) with two drug classes, and 1 (4%) with all three drug classes. Other mutations recently listed as transmitted drug resistance related,³² but which are not considered as resistance mutations according to the IAS-USA list, were also observed. That was the case of mutations T215S (found in viruses from three subjects), T215D (two viruses), and T69D (three viruses). Five of these mutations were found in viruses with other drug-related mutations, while three (two T215S and one T69D) were found alone (Table 2).

The rate of transmission of drug resistance in each region varied from 0% (0/18) in the city of Santos to 9.9% (13/118) in the city of São Paulo. Indeed, we found a trend of association between higher transmitted resistance and individuals from São Paulo City ($p = 0.038$; Table 1). Although 66% of the total

subjects were infected with subtype B viruses, the proportion in the 22 subjects with drug resistance was 91%, suggesting higher transmitted resistance among subtype B-infected patients ($p = 0.03$). Finally, in the 304 subjects for whom such information was available, reporting a partner taking ARV medications was associated with a higher chance of harboring resistant HIV variants (OR = 2.57; 95% CI, 1.07–6.16; $p = 0.014$).

Discussion

Currently, resistance testing is recommended at the time of diagnosis of HIV infection in the United States as part of the initial comprehensive patient assessment, as well as in all cases of virologic failure settings as a guide to the optimal

TABLE 2. HIV ISOLATES WITH ANTIRETROVIRAL DRUG RESISTANCE FOUND IN THE STUDY

Sample ID	HIV-1 subtype	PR mutations	RT mutations	SDRM ^a
18-01	B	M46L		
13-02	B		A62V	
18-02	B		K103N	
02-03	C		V106I/M	
13-03	B		V108I	
16-03	B	D30N, L90M	M41L, D67N, Y181V	T69D, T215D
18-03	B		V108I	
13-04 ^b	B			T215S
19-05	B		A62V	
03-06	F1		M41L, G190A	T215S
13-06	B		K103N	
14-07	B		K103N	
16-07	B		G190A	
11-09	B		D67N, K103N, G190A, K219Q	T69D
03-10	B		K103N	
05-11	B	V82L		
02-12	B		K103N	
06-12	B		K103N	
07-12	B		K103N	
13-12	B		K103N	
09-17 ^b	B			T215S
15-18	B		V108I	
16-18	B	I84V		T215D
06-20	B		Y188L	
06-21 ^b	B			T69D

^aSDRM (surveillance drug resistance mutations³³).

^bVirus with only one SDRM and no primary DRMs.

choice of treatment strategies.³³ However, especially in developing countries, cost issues weigh heavily on the implementation of the test for drug-naïve patients. Therefore, knowing the local prevalence and the pattern of resistance-related mutations has obvious implications on a discussion about performing resistance testing prior to initiation of therapy. The results of this study, carried out in 20 health care centers in Brazil, showed that 5.7% of HIV-1-infected subjects who had never received antiretroviral therapy carried viruses with one or more resistance-related mutation. This is in agreement with another nationwide study conducted in Brazil in 2002, in which a rate of 6.6% was found.²⁸ Our study, however, involved a larger number of genotyped viruses and a larger geographic coverage of the Brazilian territory. When considering the most recent list of transmitted drug resistance markers,³² the proportion virtually did not change, because all but one virus carried additional, established drug resistance mutations. This indicates that current estimates of drug resistance are still valid for epidemiologic assessments, at least in our scenario. A rate of 5.7% can still be considered a modest estimate when compared to those of developed nations, where rates of 9–16% have been reported.^{34–40} However, recent reports from Europe have pinpointed a stabilization of or a decrease in the rates of drug resistance,^{41,42} indicating rates similar to or even lower than those found herein, suggesting that Brazil is reaching a primary drug resistance profile of developed settings.

Although we tried to cover most of the important regions of the country and this was precisely the reason we included as many individuals as our budget permitted, our study has some weaknesses. Our population had a median count of 375 CD4⁺ lymphocytes, reflecting a more chronic course of dis-

ease than the scenario of some years ago, when these individuals likely acquired HIV. We also were expecting a higher presence of resistance mutations in the city of Santos, in São Paulo state, as previously reported.²⁹ However, we were not able to detect such a profile. This fact might have reflected “microdifferences” that could occur in the same region, indicating that HIV-positive individuals in different stages of infection could be assisted in the same city, but at diverse sites. Therefore, the site included in our study from Santos unfortunately might have not been as representative as previously expected.

The resistance assays currently in routine use detect resistance only in the predominant viral population at the time of testing. Therefore, when reversion to a wild-type variant occurs, the transmitted mutations are no longer routinely detectable.⁴³ Thus, the rate of transmitted resistance observed in this study may underestimate the true rates that could be determined by resistance testing performed at the onset of infection. Even if it is no longer detectable, transmitted drug-resistant variants have been reported to persist in reservoirs for many years after infection and may reappear under the selective pressure exerted by antiretroviral therapy.^{44,45}

Although our casuistic was significantly represented by men who have sex with men (MSM; 43.2%), no particular risk behavior population had higher levels of transmitted HIV drug resistance, as the increased proportion of MSM among persons carrying resistant viruses was not significant (Table 1). On the other hand, considering the reported increases in sexually transmitted diseases among MSM,^{33,46,47} our results may also indicate the need for improvement in the health care system regarding prevention of further transmission of HIV in this particular risk group.

We found a higher occurrence of transmitted drug resistance in persons from the city of São Paulo, the capital of São Paulo state, when compared to the rest of the country. This is in agreement with the history of the HIV/AIDS epidemic in Brazil, since São Paulo was among the first cities in Brazil reporting AIDS cases, and remains the city with the highest number of reported AIDS cases in the country.

In the present study, subjects reporting partners who took ARV medications had a 2.5-fold risk of harboring HIV variants with major drug resistance mutations, suggesting that these viruses may have been transmitted directly from their partners undergoing treatment. This finding implies that a proportion of HIV-infected individuals receiving ARV medication is still engaging in risk-related behavior, despite awareness of their infection status.⁴⁸ These patients are receiving medical attention, and healthcare providers should strongly consider them as main targets for prevention programs.

The great majority of subjects harboring drug-resistant HIV were infected with subtype B (91%) rather than with non-B viruses (9%). This finding is in agreement with recent estimates reported from southern Brazil, where a significant proportion of subtype C ensures such comparison.⁴⁹ Since subtype B virus is the predominant subtype in Brazil,⁵⁰ differences in the prevalence of resistance according to virus subtype may thus likely reflect the longer period during which subtype B viruses have been exposed to antiretroviral drugs. Alternatively, the fitness of subtype B viruses might be less affected by the presence of transmitted drug resistance, therefore allowing resistance mutations to persist for longer times in that subtype. Large prospective cohorts infected with distinct subtypes followed from acute infection will be necessary to evaluate such alternatives.

We found a higher prevalence of mutations associated with NNRTI resistance, which may reflect the extensive use of NNRTI-based regimens compared with PI-based regimens. Additionally, there may be a higher level of NNRTI resistance in the general population, given that just one point mutation can be sufficient to confer resistance to this class of drugs.⁵¹ Finally, NNRTI mutations have been shown to impact modestly on viral fitness,⁵² and studies have found a high persistence of these mutations in drug-naïve subjects.⁵³

In summary, viruses with resistance to at least one drug were found in 5.7% of antiretroviral-naïve patients in this Brazilian cohort. Taking into account the important implications for treatment and prophylaxis of HIV infection, resistance testing for mutations associated with reduced susceptibility to ARV drugs (mainly when including an NNRTI in HAART) might be considered prior to the initiation of therapy, and should be considered in individuals who have partners who are already on ARV therapy (who are at greater risk of having drug resistance mutations).

Collaborating Investigators In Brazil

Alcyone A. Machado (Hospital das Clínicas da Faculdade de Medicina de Ribeirão Preto da Universidade de São Paulo, Ribeirão Preto, São Paulo); Antônio F.B. Miranda (Hospital Nereu Ramos, Florianópolis, Santa Catarina); Breno Riegel dos Santos (Hospital Mãe de Deus, Porto Alegre, Rio Grande do Sul); David S. Lewi (Universidade Federal de São Paulo, São Paulo); Dirceu B. Greco (Faculdade de Medicina-UFMG, Belo Horizonte, Minas Gerais); Eduardo Martins Netto

(Hospital Universitário Prof. Edgard Santos-HUPES, Salvador, Bahia); Eduardo Sprinz (Hospital de Clínicas – UFRGS, Porto Alegre, Rio Grande do Sul); Eliana Bicudo (Hospital Dia, Brasília, Distrito Federal); João S. de Mendonça (Hospital do Servidor Público Estadual, São Paulo, São Paulo); José Luiz Andrade Neto (Instituto A. Z. de Pesquisa e Ensino, Curitiba, Paraná); José V. Madruga (CRT/AIDS, São Paulo, São Paulo); Juvêncio J.D. Furtado (Hospital Heliópolis, São Paulo, São Paulo); Marcelo A. Soares (Universidade Federal do Rio de Janeiro, Rio de Janeiro, Rio de Janeiro); Margareth da Eira (Instituto de Infectologia Emílio Ribas, São Paulo, São Paulo); Maria Patelli J. Souza Lima (Hospital e Maternidade Celso Pierro da Pontifícia Universidade Católica, Campinas, São Paulo); Marília S. de Oliveira (Hospital Geral, Nova Iguaçu, Rio de Janeiro); Max Igor B.F. Lopes (Casa da AIDS, São Paulo, São Paulo); Olavo Henrique M. Leite (Faculdade de Medicina do ABC, Santo André, São Paulo); Ricardo Leite Hayden (Hospital Beneficência Portuguesa, Santos, São Paulo); Roberto Zajdenverg (Projeto Praça Onze, Rio de Janeiro, Rio de Janeiro); and Rogério de Jesus Pedro (UNICAMP, Campinas, São Paulo).

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Address correspondence to:

Eduardo Sprinz

Hospital de Clínicas de Porto Alegre

Serviço de Medicina Interna—Unidade de Infectologia

Rua Ramiro Barcellos 2350, 7º andar, sala 700

Porto Alegre-RS, Brazil

E-mail: esprinz@hcpa.ufrgs.br

