SPANDIDOS PUBLICATIONS

Frequency and diversity of human immunodeficiency virus type 1 mutations associated with antiretroviral resistance among patients from Southern Brazil failing highly active antiretroviral therapy (HAART)

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Abstract. The human immunodeficiency virus type 1 (HIV-1) epidemic in Brazil is spreading to small municipalities as well as the innermost parts of the country and scarce information has been reported on the frequency of HIV-1 resistance-associated mutations in these areas. To determine the frequency and diversity of the HIV-1 antiretroviral resistance-associated mutations among patients failing highly active antiretroviral therapy from Londrina in Southern Brazil, 127 HIV-1 genotyping tests that were assayed during January 2000 to July 2008 from 108 patients were evaluated. Sixty-nine patients (63.9%) were male and 39 (36.1%) were female and the age ranged from 10 to 68 years (mean, 40.8±9.2). All of them showed at least one HIV-1 antiretroviral resistance-associated mutation and in 72 (56.7%) genotyping tests, mutations for the three antiretroviral classes were detected simultaneously. Mutations associated with resistance to protease inhibitor (PI) were detected in 124 tests (97.6%), the main ones were L90M in 28 (22.0%), V82A in 27 (21.2%), M46I in 26 (20.5%), and I54V in 23 (18.1%). The main mutations associated with nucleoside reverse transcriptase inhibitor (NRTI) resistance were M184V in 82 (64.6%), and the thymidine analog mutations were D67N in 51 (40.1%) tests, K70R in 45 (35.4%), T215Y in 40 (31.5%), and M41L in 38 (30.0%). The most frequent major mutations associated with resistance to non-nucleoside RT inhibitors (NNRTI) were K103N in 47 (37.0%), G190A in 11 (8.7%), and G190S in 2 (2.6%) tests. Mutations associated with reduced susceptibility to NRTI and IP simultaneously were observed in 46 (36.2%) tests. The results obtained may contribute to the improvement of the treatment strategies and the management of the antiretroviral drug therapy of HIV-1-infected patients from this Brazilian region, reducing public costs for antiretroviral drugs which have not been efficient in therapy.

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Introduction

One of the most important characteristics of the human immunodeficiency virus type 1 (HIV-1) is the genetic variability resulting in different groups, subtypes and circulating recombinant forms (CRFs). The HIV-1 genomic heterogeneity is caused by different mechanisms such as the spontaneous mutations promoted by the reverse transcriptase (RT) that occurs during viral replication. Mutations in the *pol* gene are promoted by the selective pressure of antiretroviral drugs and the polymorphism in the *env* gene results in a different envelope protein gp120 as a mechanism of immune response evasion of the HIV-1. The different genetic forms may have variable biological properties and influence immune response and effectiveness of antiretroviral treatment (1,2).

To date, nearly 25 antiretroviral drugs have been licensed for the treatment of HIV-1, including nucleoside reverse transcriptase inhibitor (NRTI), non-nucleoside reverse transcriptase inhibitor (NNRTI), protease inhibitor (PI), fusion inhibitor, entry inhibitor (chemokine receptor CCR5 inhibitor), and integrase inhibitor. The highly active antiretroviral therapy (HAART) for management of HIV-1 infection that includes an association of the 2 NRTIs plus NNRTI and/or PI has been effective in suppressing HIV-1 replication. The Brazilian Ministry of Health has been sponsoring free access to HIV-1 treatment for AIDS patients since 1996 (3,4). However, >200 mutations are associated with antiretroviral resistance to drugs belonging to the six licensed antiretroviral classes (5). The development of mutations in the HIV-1 genome remains one of the most common reasons for failure of HAART in treated patients and accounts for the transmission of HIV-1

drug resistance variants to non-infected individuals. The identification of specific drug resistance mutations is increasingly used to avoid antiretroviral drugs that retain only minimal residual activity in favor of other residual activity that is likely to be either fully or almost fully active.

Previous national and regional surveillance studies on the prevalence of mutation conferring antiretroviral drug resistance were carried out in large centers in Brazil (3,4,6-10). All of these studies have shown a low frequency of wild-type HIV-1 isolates and a high prevalence of virus strains containing resistance mutations among treated patients failing HAART. However, studies on HIV-1 genetic diversity in relatively small Brazilian municipalities are scarce (11). The HIV-1 Brazilian epidemic is spreading from large urban centers to small municipalities and the innermost parts of the country (12,13). During the period of 1980-2003, Londrina was in the 2nd and 39th positions for HIV/AIDS cases registered in Paraná State and in the country, respectively (14). Despite numerous studies characterizing the HIV-1 genetic diversity in the Brazilian population, there are no data on the HIV-1 genome variability among patients from the Londrina municipality, situated in the interior of the country. To address this question, the aim of this study was to determine the frequency and diversity of the HIV-1 antiretroviral mutations associated with antiretroviral resistance in HIV-1 infected patients failing HAART from Londrina in Southern Brazil.

Materials and methods

Design, patients and setting. The study was conducted at the State University of Londrina, Paraná, Brazil with a retrospective and descriptive design. The HIV-1 genotyping results, assayed during January 2000 to July 2008, from 108 patients, aged ≥10 years, both genders and at least one HAART failure were evaluated. All samples belonged to individuals who had viral load counts >1,000 copies/ml and were under antiretroviral drug treatment. Some patients had >1 HIV-1 genotyping test due to recurrent therapeutic failure in the period evaluated. The patients were from Londrina, a municipality located in the Northern region of Paraná State, in Southern Brazil, 390 km from Curitiba, the capital of Paraná, 500 km far from São Paulo (SP) and 1,000 km from Rio de Janeiro (RJ). A detailed sociodemographic and epidemiological characterization of the HIV-1 patients cohort from Londrina and the region were described previously (13). Medical appointments were offered at two different local outpatient clinics (Outpatient Clinic of State University of Londrina and Integrated Center of Infectious Diseases of 17th Health Regional of the Health Secretariat of Paraná State). Antiretroviral drugs are supplied for free to HIV-1 patients when clinically indicated, as part of the National Sexually Transmitted Disease and AIDS Program of the Brazilian Ministry of Health. HAART failure was qualified according to the governmental definition (15). The data on adhesion to antiretroviral treatment were collected from the medical records of patients and pharmacists responsible in these health services. Good adherence meant taking antiretroviral drugs correctly and taking the correct dose for the recommended length of time, as well as using and adhering to the guidelines of the health multi-professional team. The self-report and the laboratorial tests evaluating the therapy response such as CD4+T cell count and plasma HIV viral load were also considered. Regular adherence meant that patients adhered in a sporadic way and good adherence was not obtained, and poor adherence meant that frequently good

Table I. Sociodemographic and epidemiological characteristics of the human immunodeficiency virus type 1 (HIV-1)-infected individuals failing antiretroviral therapy attended in Southern Brazil.

Characteristic	No. of patients (n=108)	Percentage (%)
Gender		
Male	69	63.9
Female	39	36.1
Age (years)		
10-20	3	2.8
21-30	8	7.4
31-40	39	36.1
41-50	43	39.8
51-60	14	13.0
>60	1	0.9
Route of infection		
Sexual	78	72.2
Blood[a]	14	12.9
Sexual + Blood[a]	7	6.5
Vertical	2	1.9
Not reported	7	6.5
Sexual behavior		
Heterosexual	75	69.4
Homosexual	7	6.5
Bisexual	5	4.6
Transexual	1	0.9
Not reported	20	18.5
Education		
<8 years of school	58	53.7
≥8 years of school	34	31.5
Not reported	12	2.9
Adherence to antiretroviral treatment[b]		
Good	48	44.4
Regular	16	14.8
Poor	31	28.7
Not reported	13	12.0

[a]Includes blood contact through intravenous drug use, blood exposure through shared needles and syringes with HIV-1-infected individuals, history of blood or hemocomponent transfusion, or accident with blood. [b]Good adherence meant: i) taking antiretroviral drugs correctly and taking the correct dose for the recommended length of time; ii) using and adhering to the guidelines of the health multi-professional team; iii) laboratorial tests evaluating the therapy response such as CD4+T cell count and plasma HIV viral load were considered; regular adherence, sporadic but good adhesion was not obtained according to the self-report of the patients; poor adherence, frequently good adherence was not obtained (Brasil, 2008).

Distribution of the human immunodeficiency virus type 1 (HIV-1) mutations detected by 127 genotyping tests from individuals failing highly active antiretroviral therapy from Southern Brazil according to the antiretroviral class.

Antiretroviral class	Mutations detected		Mutations associated with antiretroviral resistance	
	n	%	n	%
NRTI	121	95.3	107	88.4
NNRTI	75	59.1	66	88.0
PI	124	97.6	78	62.9

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

adherence was not obtained (16). This research project was approved by the Institutional Research Ethics Committee of Londrina State University.

Genotyping tests. A total of 127 consecutive HIV-1 genotyping tests were included in the study. During the period evaluated, blood was collected in EDTA tubes and plasma was separated and stored at -80˚C. Furthermore, plasma samples were sent to reference laboratories of the Genotyping National Network (Renageno) of the National Sexually Transmitted Disease and AIDS Program of Brazilian Ministry of Health to perform the genotyping tests. A fragment of the HIV-1 *pol* gene, spanning both RT and protease regions, was amplified by reverse transcriptase polymerase chain reaction (RT-PCR) and nucleotides from the dominant HIV-1 strain were sequenced. In 84 (66.7%) of the 127 genotyping tests evaluated, the HIV-1 subtype was also determined by an amplification of a fragment of the HIV-1 *env* gene, spanning the envelope protein gp120. The results were interpreted according to the international guidelines using the Trugene™ HIV-1 Program.

Statistical analysis. A database with the results was set up using the Microsoft Office Excel Program. The qualitative variables were presented in frequency expressed by number (n) and percentage (%). The quantitative variables were reported as range, mean, standard deviation and median. For the comparison of the proportions, the chi-square test was used, with two grades of freedom, and it was considered significant at $p<0.05$.

Results

Characteristics of HIV-1-infected patients failing HAART. From January 2000 to July 2008, a total of 108 HIV-1-infected adult patients failing HAART attending two different local outpatient clinics were evaluated by the genotyping tests in order to detect the HIV-1 antiretroviral resistance-associated mutations. Table I shows some sociodemographic, epidemiological and treatment characteristics of these patients. Of them, 69 (63.9%) were male and 39 (36.1%) female, with a male:female ratio of 1:8. The age ranged from 10 to 68 years old (mean, 40.8±9.2 years; median, 41 years old). Eighty-five (78.7%) patients were exposed to three or more antiretroviral regimens and 96 (88.9%) had started antiretroviral therapy after 1996 with HAART. The time of the HIV-1 diagnosis ranged from 2 to 21 years (mean, 10.3±4.3 years; median, 10 years). All patients enrolled were in the AIDS stage of the HIV-1 infection, according to the Brazilian AIDS definition (17) and had been receiving HAART (2 NRTIs plus 1 PI/r, or 2 NRTIs plus 1 NNRTI as the first option) for a period ranging from 2 to 16 years (mean, 8.1±3.3 years; median, 9 years). Two patients were using the fusion inhibitor enfurvitide in association with NRTI and IP. The HIV-1 genotyping test was solicited after the first therapeutic failure in 20 (23.8%) patients, after the second therapeutic failure in 16 (19.0%) and after multiple failures in 48 (57.1%) patients. Of the patients evaluated, 48 (44.4%) presented good adhesion to antiretroviral treatment.

The $CD4^+$ T-cell counts evaluated before the genotyping test were available in 103 patients and the values ranged from 4 to 851 cells/mm^3, with a mean of 314.3±161.5/mm^3 and median of 329.0/mm^3. The HIV-1 plasma viral load also evaluated before the genotyping test were available in 105 patients and the values ranged from 1,150 to >750,000 copies/ml; mean, 22,024±25,738 copies/ml; median, 9,760 copies/ml.

Mutations associated with antiretroviral drug resistance. The highest number of mutations was detected for the PI class of antiretroviral, present in 124 (97.6%) among the 127 tests evaluated (Table II). All patients showed at least one HIV-1 mutation associated with antiretroviral resistance and mutations for the three antiretroviral classes were detected simultaneously, in 72 (56.7%) genotyping tests. Mutations associated with resistance to the NRTI and PI were observed in 46 (36.2%) genotyping tests simultaneously. Resistance to PI was isolated in 6 (4.7%) and to NRTI and NNRTI simultaneously in 3 (2.4%) genotyping tests.

The main NRTI resistance mutation was M184V, observed in 82 cases (64.6%), and the thymidine analog mutations (TAMs) such as D67N in 51 cases (40.1%), K70R in 45 (35.4%), T215Y in 40 (31.5%), M41L in 38 (30.0%), V118I in 28 (22.0%), L210W in 27 (21.2%), K219Q in 25 (20.0%), T215F in 18 (14.2%), K219E in 15 (12.0%) and T69N in 14 (11.0%) cases. The additional accessory mutations were also detected such as L214F in 85 (67.0%) cases, R211K in 52 (41.0%), H208Y in 12 (9.4%), E44D in 9 (7.1%), D67G in 3 (2.4%) and V75A in 1 (0.8%) genotyping test. Multi-nucleoside resistance mutations were also detected such as F116Y in 8 (6.3%), Q151M in 6 (5.0%) and V75I in 4 (3.1%) cases.

Table III. Nucleoside reverse transcriptase inhibitor-resistance mutations.[a]

Mutation	No. of genotyping tests (n=127)	Percentage (%)
M184V	82	64.6
Thymidine analog mutations (TAMs)		
D67N	51	40.1
K70R	45	35.4
T215Y	40	31.5
M41L	38	30.0
L210W	27	21.2
K219Q	25	20.0
T215F	18	14.2
K219E	15	12.0
T69N	14	11.0
Accessory mutations		
L214F	85	67.0
R211K	52	41.0
V118I	28	22.0
H208Y	12	9.4
E44D	9	7.1
D67G	3	2.4
V75A	1	0.8
R211K +L214F	41	32.3
M184V + R211K+ L214F	23	18.1
M184V+R211K+ L214F+H208Y	4	3.1
Multi-nucleoside resistance mutations		
F116Y	8	6.3
Q151M	6	5.0
V75I	4	3.1
Non-thymidine analog-resistance mutations		
L74V	7	5.5
V75M	7	5.5
Y115F	2	1.6
V75T	2	1.6

[a]Frequency and diversity of the nucleoside reverse transcriptase inhibitor (NRTI) resistance mutations in human immunodeficiency virus type 1 (HIV-1)-infected individuals failing highly active antiretroviral therapy enrolled in 127 genotyping tests from Southern Brazil.

Detected mutations occurring in the absence of thymidine analogs were L74V in 7 (5.5%), V75M in 7 (5.5%), Y115F in 2 (1.6%) and V75T in 2 (1.6%) genotyping tests. All the NRTI resistance-associated mutations detected in the current study are shown in Table III.

The most frequent primary NNRTI resistance mutations were K103N observed in 47 (37.0%) genotyping tests and G190A in 11 (8.7%). Major secondary NNRTI resistance mutations were L100I detected in 6 (4.7%) and P225H in 4 (3.1%) genotyping tests. Minor NNRTI resistance mutations detected were A98G, detected in 14 (11.0%) and V108I in 9 (7.1%). The frequency of all the NNRI resistance mutations detected in this study is shown in Table IV.

Table IV. Non-nucleoside reverse transcriptase inhibitor-resistance mutations.[a]

Mutation	No. of genotyping tests (n=127)	Percentage (%)
Primary		
K103N	47	37.0
G190A	11	8.7
K103S	3	2.4
Y181Y/C	2	1.6
Y188L	2	1.6
G190S	2	1.6
V106M	1	0.8
K103N/S	1	0.8
Major secondary		
L100I	6	4.7
P225H	4	3.1
M230L	2	1.6
K103R	2	1.6
K101P	1	0.8
Minor		
A98G	14	11.0
V108I	9	7.1
V179D	3	2.4
K101E	2	1.6
V179E	1	0.8

[a]Frequency and diversity of the non-nucleoside reverse transcriptase inhibitor (NNRTI) resistance mutations in human immunodeficiency virus type 1 (HIV-1)-infected individuals failing highly active antiretroviral therapy enrolled in 127 genotyping tests from Southern Brazil.

Table V shows PI resistance mutations detected in 124 (98.0%) of the genotyping tests evaluated. The most frequent were L90M in 28 (22.0%), V82A in 27 (21.2%), M46I in 26 (20.5%), I54V in 23 (18.1%), and L33F in 22 (17.3%). Accessory PI resistance mutations frequently observed were L63P in 72 (57.0%) genotyping tests, M36I in 71 (56.0%), I93L in 51 (40.1%), L10I in 40 (31.5%), A71V in 37 (29.1%), I62V in 37 (29.1%), and I13V in 31 (24.4%). Additional PI-selected accessory mutations detected were I54S in 5 (4.0%) genotyping tests, l33I in 2 (1.6%), and V82C in 2 (1.6%). In addition to the mutations described in Table III, the polymorphism L33V was also detected in the protease region of the *pol* gene not associated with PI therapy in 1 (0.8%) genotyping test.

Regarding the HIV-1 subtype, the *env* gene was amplified and nucleotide sequencing was determined in 84 genotyping tests. Of them, the subtype B was the most frequent, detected in 50 (59.5%) genotyping tests, followed by HIV-1 BF1 CRF detected in 16 (19.0%), subtype C in 7 (8.3%), and F in 5 (5.9%). Other subtypes and CRFs were also detected including BF in 2 (2.4%), BC in 2 (2.4%), A1 in 1 (1.2%) and F1 in 1 (1.2%) tests.

Protease inhibitor resistance mutation.[a]

Major			Accessory			Additional		
Mutation	n	%	Mutation	n	%	Mutation	n	%
L90M	28	22.0	L63P	72	57.0	I54S	5	4.0
V82A	27	21.2	M36I	71	56.0	L33I	2	1.6
M46I	26	20.5	I93L	51	40.1	V82C	2	1.6
I54V	23	18.1	L10I	40	31.5	L24F	1	0.8
L33F	22	17.3	A71V	37	29.1	F53Y	1	0.8
I50L	12	9.4	I62V	37	29.1	G73C	1	0.8
G48V	11	9.0	I13V	31	24.4	G73A	1	0.8
F53L	10	8.0	V77I	26	20.5			
I84V	9	7.1	L10V	24	19.0			
G73S	9	7.1	K20R	21	16.5			
N88S	7	5.5	T74S	18	14.2			
N88D	7	5.5	K20I	11	9.0			
D30N	6	5.0	L10F	10	8.0			
L24I	6	5.0	H69K	10	8.0			
V32I	5	4.0	A71T	8	6.3			
M46L	4	3.1	Q58E	8	6.3			
L76V	4	3.1	K43T	8	6.3			
I54M	2	1.6	T74A	5	4.0			
V82F	2	1.6	E35G	4	3.1			
V82S	2	1.6	K20M	2	1.6			
I84C	2	1.6	K20V	2	1.6			
G73T	2	1.6	N83D	2	1.6			
I54T	2	1.6	T74P	2	1.6			
I47V	2	1.6	A71I	1	0.8			
V82T	1	0.8	V82A/V	1	0.8			
I54A	1	0.8	L10F/V	1	0.8			
G48M	1	0.8						
I54L	1	0.8						
V82A/T	1	0.8						
I50V	1	0.8						
V82L	1	0.8						

[a]Frequency and diversity of the protease inhibitor (PI) resistance mutations in human immunodeficiency virus type 1 (HIV-1)-infected individuals failing highly active antiretroviral therapy enrolled in 127 genotyping tests from Southern Brazil.

Discussion

The HIV-1 epidemic is spreading from large urban centers to small and the innermost parts of Brazil. Regarding the number of documented cases of AIDS in the country, Londrina, an innermost municipality located in the Northern region of Paraná State, Southern Brazil, was in the 39th position, with 1,164 cases from 1980 to 2003 (14) but scarce data exist on the genetic variability of HIV-1 that is circulating in that area. The sociodemographic and epidemiological characteristics of the patients enrolled in this study were consistent with the major features of the HIV/AIDS epidemic in Brazil and Londrina (13). The higher gender frequency was male, although a small male to female ratio was observed as a result of an increasing proportion of female patients. Other predominant characteristics of the study cohort were age ranging from 31 to 50 years, heterosexual transmission and lower level of education. With these characteristics, the study cohort may be considered a representative sample of the background population.

The current study presents the first survey describing the antiretroviral drug resistance in Londrina, an inland Brazilian municipality and the results showed high frequency of antiretroviral resistance mutations in HIV-1 patients failing HAART. This study has a selection bias since the results obtained in the current study are based on patients failing therapy, where the treating doctor already has found the need to perform a resistance test. Evidently this bias leads to an overestimation of the frequency of resistant among the population on treatment. However, the results obtained are consistent with the findings previously reported in HIV-1-infected patients failing HAART from other regions of Central America (18) and Brazil (4,8-10,19). As expected, the rates were higher than those detected among HIV-1-infected

patients naïve for use the antiretroviral therapy where Brazil has shown primary drug resistance rates that were on average lower than in most developed countries analysed (20,21). There are no previous reports in Brazil showing the occurrence of multidrug-resistant HIV-1 strains circulating in drug naïve patients in contrast to some countries (20). However, among HIV-1 treated patients and failing HAART, high rates of drug-resistance mutations are detected, probably related to the wide use of HAART, lack adherence to the treatment protocol, or both.

Mutations in the *pol* gene in the protease region were the most frequent (98.0%), consistent with previous reports (5), followed by the mutations in the RT region. However, the low frequency was verified of PI resistance mutations (62.9%) when compared with the NRTI resistance mutations (88.4%) and NNRTI (88.0%). This result is explained by the low genetic barrier to NNRTI resistance, where only one or two mutations are required for high-level resistance. High levels of clinical cross-resistance exist among the NNRTI because many of the NNRTI resistance mutations reduce the susceptibility to multiple NNRTI and the low genetic barrier to resistance allows a single NNRTI to select for multiple NNRTI resistance mutations (5).

Although more mutations were selected by PI than any other antiretroviral class, the multiple protease mutations are often required for HIV-1 to develop clinically significant resistance to a ritonavir-boosted PI. Many protease mutations are accessories, compensating for the replication impairment of other resistance mutations or reducing PI susceptibility only in combination with other PI resistance mutations (5).

The M184V mutation was the most frequently detected in the HIV-1 patients failing HAART evaluated in this study. M184V emerges rapidly in patients treated with lamivudine and can occur within weeks during monotherapy. This mutation is associated with high-level (>100-fold) resistance to NRTI such as lamivudine (3TC), 1.5- and 3.0-fold reduction in susceptibility to didanosine (ddI) and abacavir (ABC), respectively. However, this mutation increases the susceptibility to zidovudine (ZDV), stavudine (d4T) and tenofovir (TDF). Moreover, the phenotypic and clinical significance of M184V is influenced by the presence or absence of other NRTI-resistant mutations. The presence of K65R or L74V in combination with M184V is sufficient for high-level resistance to both ABC and ddI (22).

Either TAMs type I (T215Y, M41L and L210W) or type II (D67N, K70R, T215F, and K219Q/E) patterns were detected in high frequency in this study. This result was also consistent with previous reports (23). TAMs are selected by the thymidine analogs ZDV and d4T and decrease susceptibility to these NRTI and to a lesser extent to ABC, ddI, and TDF. The K70R mutation is usually the first mutation to emerge during ZDV therapy and mediates low-level resistance. T215Y/F causes intermediate resistance to ZDV alone but antagonizes the effect of K70R. Therefore, the two mutations are rarely seen together, and K70R will disappear (24). TAMs are common in low-income countries in which fixed-dose combinations containing thymidine analogs are the mainstays of the therapy. TAMs are also common in viruses from persons who began therapy in the pre-HAART era with incomplete suppressive thymidine analog-containing regimens that result in an incomplete suppression of the viral replication. Type I TAMs cause higher levels of phenotypic and clinical resistance to the thymidine analogs and cross-resistance to AABC, ddI, and TDF than type II TAMs (5).

The combination of the M184V with additional mutations L214F, R211K, and H208Y were also detected in the genotyping tests evaluated in this study. Some studies have shown that the combination of the additional mutations L214F and R211K that are selected by the NRTI can abrogate phenotypic susceptibility to ZDV restored by M184V (25,26) and the HIV-1 presents and increased of 7.4- or 21-fold ZDV-resistant when the R211K/L214F or H208Y/R211K/L214F mutations, respectively, were added to a highly ZDV-resistant virus (27). However, some data are contradictory and show that the mutations R211K and/or L214F are not invariably responsible for high-level phenotypic resistance to ZDV or to d4T in patients naïve to ZDV, and further mutations or polymorphisms are necessary to determine high-level phenotypic resistance to thymidine analogs when the combination of R211K, L214F, and M184V mutation is present (28).

The cross-resistance is a complex problem with NRTI drugs. The mutation Q151M that was detected in 6 (5.0%) genotyping tests of this study have been associated with multinucleoside resistance, conferring high-level resistance to the NRTI (5).

The findings of this study showed high frequency of the primary NNRTI mutations including K103N, G190A, K103S, Y181Y/C, and Y188L that cause high-level resistance to nevirapine and variable resistance to efavirenz, ranging from 2-fold for Y181C, 6-fold for G190A, 20-fold for K103N and >50-fold for Y188L (29-31).

Both the highest number (97.6%) of mutations associated with PI observed in 127 genotyping tests evaluated in this study and the lowest (62.9%) number of mutations associated with HIV-1 antiretroviral resistance are consistent with previous published data (5). Thirty-one different major mutations were detected in the protease gene, with frequencies ranging from 22.0% for the L90M and 0.8% for the V82L. The most frequent, L90M and others including G48V (9.0%) and I84V (7.1%) are contraindications to the use of saquinavir associated with ritonavir (SQV/r) (32-34). The mutations I84V (7.1%), V32I (4.0%), I47V (1.6%), and I54L (0.8%), are contraindications to the use of fosamprenavir associated with ritonavir (FSP/r) (35-37). The number of licensed PI antiretroviral drug class has expanded and the number of mutations associated with PI resistance is higher when compared with other antiretroviral drug classes. However, multiple protease mutations are often required for HIV-1 to develop clinically significant resistance to PI.

The frequency of PI accessory mutations confirmed the polymorphism in the protease gene in some specific positions such as 20 (k20R, K20I, K20M, K20V, observed in 16.5, 9.0, and 1.6%, respectively), and the position 63 (L63P observed in 57.0% of the tests). These polymorphisms upregulate protease processivity to compensate for the decreased fitness associated with the major PI resistance mutations (38-42). Many protease mutations are accessory, compensating for the replication impairment of other PI resistance mutations or reducing PI susceptibility only in combination with other PI resistance mutations (5).

patients were using enfurvitide but there is no data the mutation associated with this antiretroviral because the region was not sequenced. Since the fusion inhibitor therapy is currently used in some patients from the study population, further studies should include the HIV genomic region associated with this antiretroviral therapy.

Although the B subtype was the most frequent in this sample, other subtypes including F, C, and the CRFs BF, BC, and BF1 were also detected. It was observed that a low frequency of the pure form of the subtype F and the presence of forms with high variability such as F1 and the CRFs BF and BF1, in agreement with previous studies carried out in other Brazilian regions (19,43,44). The prevalence of different subtypes may differ significantly across geographical regions, what account, in part, for the differences in estimates found in different studies. In the south region of Brazil, the prevalence of subtype B equals that of subtype C. Subtype F was reported as the second most prevalent subtype in most parts of Brazil (45).

Two patients presented different subtypes of HIV when analysed by more than one genotyping test in the period evaluated. According to previous studies (46,47) many cases of people coinfected with two or more strains have been documented. All cases of coinfection were once assumed to be the result of people being exposed to the different strains more or less simultaneously, before their immune systems had a chance to react. However, it is now believed that superinfection also occurs. In these cases, the second infection occurs several months after the first. It would appear that the host immune response against the first virus is sometimes not enough to prevent infection with a second strain, especially with a virus belonging to a different subtype. Resistance assays measure only the HIV-1 dominant species at the time the test is performed and resistance variants that comprise <20% of the total viral population in blood are not detected. The difference in the genotypic pattern of the subtypes of HIV-1 could result in a variable plasma viral load that changes the dominant subtype detected in the two genotyping tests assayed in the same patient evaluated in this study.

The HIV-1 genetic variation at the positions associated with NNRTI resistance such as V106M and A98S was substantially greater in the samples from subtype C-infected patients than in subtype B-infected patients (48). Tissue culture experiments have shown that subtype-C isolates the developed V106M mutation, conferring high-level cross-resistance to all NNRTI. Thus, V106M seems to be a signature mutation in subtype C-infected patients treated with efavirenz (49). Although a limited number of patients evaluated in the present study were infected by the subtype C, the results obtained corroborate with previous studies. The mutation V103M was detected in one sample from a subtype C-infected patient whose therapeutic failure was observed during the treatment with efavirenz, a NNRTI that could select a virus with this mutation and conferring to this patient a cross-resistance to all NNRTI.

The antiretroviral therapy failure is caused by many factors including lack of adherence, reduced potency of the antiretroviral regimen, pharmacologic failure due to reduced drug delivery to the site of infection (due to malabsorption, protein binding, or drug interactions), and resistance. In general, most failures in the first 24 weeks of treatment using the recommended HAART regimens in treatment-naïve patients are due to lack of adherence or inadequate potency, and most late failures that follows good virologic response are due to resistance (50). One weakness of this study is the inclusion of patients that did not present good adherence to antiretroviral therapy. Although the adherence was considered good in most patients (50.5%), 47 (49.5%) patients did not use the antiretroviral correctly, which could also contribute to the emergence of strains of HIV-1 with resistance to antiretroviral drugs. This situation could reflect in the high frequency of the mutations observed for the three classes of antiretrovirals that are available and recommended by the Brazilian Ministry of Health. After a period of interruption in the antiretroviral treatment, the dominant wild-type virus can return and mutations are not detected in these patients. This phenomenon, particularly evident with the M184V mutation that confers resistance to lamivudine, results in a false-negative result, since the patient returns the adherence to the antiretroviral therapy, the resistant virus returns to be dominant and the therapeutic response is not obtained (16). In our present study, the presence of HIV-1 strains with high frequency of M184V mutation can also occur, by the poor adhesion in the antiretroviral treatment.

Another significant concern, which arises from the results obtained in this study, is the impact of the high frequency of antiretroviral resistance in treated patients failing HAART on HIV-1 transmission. These mutated isolates may be efficiently transmitted to other individuals and retain the antiretroviral resistance conferring mutations (51). In a cohort of 8 newly HIV-1-infected individuals the prevalence of HIV-1 variants with known resistance-conferring genotypes to any antiretroviral agent was 16.3% (52). In Brazil, in a cohort of 136 HIV-1 infected adult patients naïve for use of antiretroviral therapy, primary drug resistance was seen in 6.5% of them (21). It was recently observed that the prevalence of primary drug resistance-associated mutations among Brazilian HIV-1 vertically infected children was 9.8% (53). The high frequency and rapid selection of drug resistance in treated patients from countries such as Brazil where the patients have free access to antiretroviral therapy demonstrates a need to monitor the emergence of drug resistance and transmission of mutations to drug-naïve patients.

Collectively, the results underscore the increasing importance of the inclusion of the HIV-1 resistance testing as a component of HIV-1-infected patient care. The knowledge of the genetic characteristics of the HIV-1 that circulates in Londrina and the region mainly those related with the resistance profile and susceptibility to the antiretroviral regimens currently used in Brazil could contribute to the improvement of the treatment strategies and the management of the antiretroviral drug therapy of the HIV-1-infected patients from this population, making possible a reduction of the public costs with antiretroviral drugs which do not show efficacy in therapy.

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