HIV subtype, epidemiological and mutational correlations in patients from Paraná, Brazil

ABSTRACT

Objective: Analyze patients with HIV infection from Curitiba, Paraná, their epidemiological characteristics and HIV RAM. **Methods:** Patients regularly followed in an ID Clinic had their medical data evaluated and cases of virological failure were analyzed with genotypic report. **Results:** Patients with complete medical charts were selected (n = 191). Demographic and clinical characteristics were compared. One hundred thirty two patients presented with subtype B infection (69.1%), 41 subtype C (21.5%), 10 subtype F (5.2%), 7 BF (3.7%) and 1 CF (0.5%). Patients with subtype B infection had been diagnosed earlier than patients with subtype non-B. Also, subtype B infection was more frequent in men who have sex with men, while non-B subtypes occurred more frequently in heterosexuals and women. Patients with previous history of three classes of ARVs (n = 161) intake were selected to evaluate resistance. For RT inhibitors, 41L and 210W were more frequently observed in subtype B than in non-B strains. No differences between subtypes and mutations were observed to NNTRIs. Mutations at 10, 32 and 63 position of protease were more observed in subtype B viruses than non-B, while positions 20 and 36 of showed more amino acid substitutions in subtype non-B viruses. Patients with history of NFV intake were evaluated to resistance pathway. The 90M pathway was more frequent in subtypes B and non-B. Mutations previously reported as common in non-B viruses, such as 65R and 106M, were uncommon in our study. Mutations 63P and 36I, previously reported as common in HIV-1 subtypes B and C from Brazil, respectively, were common. **Conclusion:** There is a significant frequency of HIV-1 non-B infections in Paraná state, with isolates classified as subtypes C, F, BF and BC. Patients with subtype C infection were more frequently female, heterosexual and had a longer average time of HIV diagnosis.

Keywords: HIV, aids, subtypes, subtype B, subtype non-B, genetic diversity, antiretroviral, Brazil.

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INTRODUCTION

It is unresolved whether the extent that HIV-1 genetic diversity has impacts in the outcome of its treatment or its biological behavior. At least 9 subtypes, 5 sub-subtypes and 34 circulating recombinant forms (CRFs) have been reported worldwide.[1] Most knowledge about HIV is related to HIV-1 subtype B, but 90% of HIV-1 infection in the globe is caused by HIV-1 subtype non-B.[2]

Brazil has the greatest number of HIV-infected patients in South America.[3] The HIV subtype that predominates is B, but increased detection of others subtypes, mainly F in northern and C in southern part of the country, has been described.[4,5,6] Also, Brazil has provided free access to antiretroviral (ARV) medications for all HIV infected patients since 1996, providing a good setting to study the impact of HIV diversity on resistance mutation patterns.[7] Paraná is a state localized in south Brazil and very scarce data have been published about its HIV-1 subtype prevalence. In this report, we analyze patients with HIV-1 infection from Paraná and discuss their epidemiological characteristics and RAM.

METHODS

Patients regularly followed in an Infectious Diseases Clinic in Curitiba, Paraná, participated in this study. Two hundred eleven patients were initially enrolled. Inclusion criteria were age above 18 years, HIV-1

infection, available laboratory and ARV treatment history and genotype report with subtype. The genotype methods were VircoType HIV-1® and Phenosense GT® and a region pool was analyzed. Clade assignment was restricted to region analyzed and was described as reported by resistance test. Patients with previous history of intake and virological failure to 3 ARV class were also evaluated by resistance profile according to different subtype. Main mutations of each class were compared in different subtypes. Statistical analysis was used to verify for significant differences between demographic and clinical profiles and frequencies of RAMs in different subtypes. Multivariate analysis was used to evaluate the effect of characteristics with a p value of less than 0.25 in the univariate analysis. Logistic regression was used, considering Wald test to analyze the level of significance. RAMs were selected according to recent literature.[8] This study was approved by the Internal Review Board *Comitê de Ética do Hospital de Clínicas da Universidade Federal do Paraná.*

RESULTS

From 1987 to 2008, 211 patients had specimens submitted for resistance tests because of virological failure, and 191 m*et al*l inclusion criteria (Table 1).

Patients were diagnosed with HIV infection between 1987 and 2008, presenting from 20 to 72 years old. One hundred forty patients were male and 51 females.

Patients with subtype non-B were more frequently female (45.8% *vs* 18.2%, p < 0.001) and reported more commonly heterosexual exposure as their single HIV risk for infection than patients with subtype B infection (78% *vs* 47.7%, p < 0.001). The average duration of HIV-1 diagnosis was longer in patients with subtype B than subtype non-B (p = 0.005) (Table 2).

Age, gender, risk factor for HIV infection (MSM versus heterosexual) and average time of diagnosis were analyzed in a multivariate model and is showed in Table 3.

We observed that in non-adjusted (univariate) and in adjusted (multivariate) analyses, the associations involving female sex, heterosexual practice and longer time of HIV diagnosis reached statistical significance and were independently related to HIV subtype non-B infection.

There were no other significant differences in clinical or demographic characteristics among subtypes.

From 191 patients, 115 had a previous history of treatment with all of the original antiretroviral classes and virological failure.

NRTI resistance mutations

There was no statistical difference between mutations comparing subtype B and non-B (Table 5). Mutation V106A/M was found in only 1 patient with subtype B and 1 subtype C.

Table 1. Shows patients demographic and clinical profiles

		Subtype				
		B **(n = 132)**	**C** **(n = 41)**	**F** **(n = 10)**	**BF** **(n = 7)**	**CF** **(n = 1)**
Age	yr±SD	42.0 ± 9.7	40.6 ± 8.7	43.2 ± 8.4	36.9 ± 8.1	24
Sex (%)	Male	108 (81.8)	24 (58.5)	4 (40.0)	4 (57.1)	0
	Female	24 (18.2)	17 (41.5)	6(60.0)	3 (42.9)	1
HIV-1 risk factor (%)	MSM	68 (51.5)	9 (22)	2 (20.0)	1 (14.3)	0
	Heterosexual	63 (47.7)	31 (75.6)	8 (80.0)	6 (85.7)	1 (100)
	IVDU	0	1 (2.4)	0	0	0
	Congenital	1 (0.8)	0	0	0	0
Race (%)	Caucasian	118 (89.4)	38 (92.7)	10 (100)	7 (100)	1 (100)
	Black	14 (10.6)	3 (7.3)	0	0	0
CDC Clinical Stage (%)	A	60 (45.4)	23 (56.2)	6 (60.0)	1 (14.3)	1 (100)
	B	31 (23.5)	9 (21.9)	2 (20.0)	2 (28.6)	0
	C	41 (31.1)	9 (21.9)	2 (20.0)	4 (57.1)	0
Average time of diagnosis	yr ± SD	9.0 ± 5.0	7.0 ± 3.8	8,0 ± 4,0	7.1 ± 3.4	4.0
CD4 (cels/mm³)	Average ± SD	222 ± 134	237 ± 130	263 ± 133	236 ± 163	498
Viral load (copies/mL)	Average ± SD	99,700 ± 210,344	114,853 ± 224,916	132,536 ± 238,279	59,165 ± 97,861	782

Table 2. Demographic and clinical characteristics of HIV-1-positive patients infected with subtype B and non-B

		Subtype		p (B vs non-B)
		B (n = 132)	non-B (n = 59)	
Age	yr ± SD	42.0 ± 9.7	41.0 ± 8.5	0.480[a]
Sex (%)	Male	108 (81.8)	32 (54.2)	
	Female	**24 (18.2)**	**27 (45.8)**	**< 0.001[c]**
HIV-1 risk factor (%)	MSM	68 (51.5)	12 (20.3)	
	Heterosexual	**63 (47.7)**	**46 (78.0)**	**< 0.001[c*]**
	IVDU	0	1 (1.7)	
	Congenital	1 (0.8)	0	
Race (%)	Caucasian	118 (89.4)	56 (94.9)	
	Black	14 (10.6)	3 (5.1)	0.278[c]
CDC Clinical Stage (%)	A	60 (45.4)	31 (52.5)	
	B	31 (23.5)	13 (22.0)	
	C	41 (31.1)	15 (25.4)	0.635[b]
Average time of diagnosis	yr ± SD	**9.0 ± 5.0**	**7.1 ± 3.8**	**0.005[a]**
CD4 (cels/mm³)	average ± SD	222 ± 134	225 ± 120	0.458[a]
Viral load (copies/mL)	average ± SD	99,700 ± 210,344	114,853 ± 224,916	0.653[a]
Average time of ARV treatment	yr ± SD	6.59 ± 2.83 (n = 115)	6.16 ± 3.10 (n = 45)	0.395[a]

[a] Student's t-test, $p < 0.05$; [b] Qui-square test, $p < 0.05$; [c] Fischer's exact test , $p < 0.05$; * MSM *vs* Heterosexual.

Table 3. Multivariate analysis of multiple factors and infection by subtype B and non-B

	p	OR	95% CI	
Age	0.601	1.01	0.97	1.05
Gender	0.023	2.55	1.13	5.75
Risk factor for HIV infection				
(MSM or heterosexual)	0.023	2.65	1.14	6.15
Avarage time of diagnosis	0.019	0.91	0.83	0.98

Table 4. Mutations for NRTIs and their frequency in patients with HIV-1 B and non-B

Mutation	B% (n = 115)	Non-B% (n = 45)	p*
41L	**59 (51.3)**	**13 (28.9)**	**0.013**
62V	9 (7.8)	2 (4.4)	0.729
65N/R	2 (1.7)	0 (0)	1
67N	47 (40.9)	16 (35.6)	0.592
69ins	1 (0.9)	0 (0)	1
70R	25 (21.7)	14 (31.1)	0.225
74V/I	14 (12.2)	6 (13.3)	0.796
75T/M/I	11 (9.6)	5 (11.1)	0.773
77L	3 (2.6)	0 (0)	0.560
116Y	1 (0.9)	2 (4.4)	0.191
151M	2 (1.7)	2 (4.4)	0.314
184V	87 (75.7)	29 (64.4)	0.171
210W	39 (33.9)	7 (15.6)	0.021
215Y/F	**72 (62.6)**	**22 (48.9)**	**0.153**
219Q/E	25 (21.7)	14 (31.1)	0.225

* Fisher's exact test, $p < 0.05$.

Figure 1 shows the frequency of NRTIs mutations in subtype B and non-B. The mutation 184V was more frequently seen in subtype B than in non-B viruses. Positions 41 and 210 of reverse transcriptase were more frequently mutated (41L and 210W) in subtype B than subtype non-B (51.3% *vs* 28.9%, p = 0.13; 33.9% *vs* 15.6%, p = 0.021). 65R was rarely seen, even in subtype C, being found in only two patients, both with subtype B infection. Tables 4 and 5 show the frequency of NRTIs and NNRTIs mutations for subtype B and non-B.

Figure 1: HIV-1 subtype frequency.
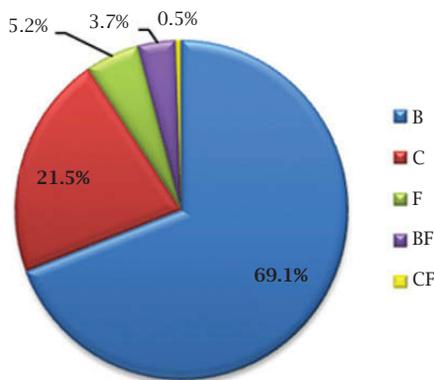
PI mutations

Major and minor mutations were analyzed and the positions 20 (64.4% *vs* 35.7%, p = 0.001) and 36 (86.7% *vs* 50.4%, p < 0.001) were more commonly mutated in patients with subtype non-B than B. Substitutions at positions 10 (64.3% *vs* 44.4%, p = 0.031), 32 (13.9% *vs* 2.2%, p = 0.042) and 63 (73.9% *vs* 35.6%, p < 0.001) were more common in subtype B than non-B viruses. Table 6 shows RAM analyzed and their frequencies.

Patients with previous history of nelfinavir intake and virological failure (n = 81 patients, 61 subtype B, 20 subtype C and 11 subtype F) were studied regarding NFV resistance pathway (Figure 2). All patients that used NFV had used other PIs in a subsequent ARV regimen. The 90M mutation pathway was more frequent for all subtypes, occurring in 78.7% of subtype B and 38.7% of non-B viruses. The pathway of mutation 30N was rarely seen, with 9.8% of subtype B viruses presenting compared to 3.2% of non-B virus (Table 7).

Figure 2: Mutations for NRTIs and their frequency in patients with HIV B and non-B.
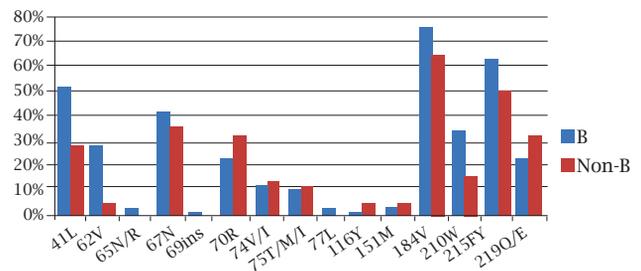

Table 5. RAMs for NNRTIs in patients infected with HIV-1 subtype B and non-B

Mutation	B% (n=115)	Non-B% (n=45)	P* (B *vs* non-B)
101E/P	10 (8.7)	7 (15.6)	0.254
98G	11 (9.6)	3 (6.7)	0.759
100I	5 (4.3)	1 (2.2)	1
103N/S	44 (38.3)	21 (46.7)	0.373
106A/M	1 (0.9)	1 (2.2)	0.485
108I	15 (13)	6 (13.3)	0.999
179D/E/F	5 (4.3)	0 (0)	0.323
181C/I/V	21 (18.3)	7 (15.6)	0.819
188L/H/C	6 (5.2)	2 (4.4)	1
190A/S/E	29 (25.2)	9 (20)	0.541
225H	11 (9.6)	3 (6.7)	0.759
227L/C	1 (0.9)	1 (2.2)	0.485
230L	0 (0)	1 (2.2)	0.281
236L	1 (0.9)	0 (0)	1
238N/T	9 (7.8)	2 (4.4)	0.729

* Fisher's exact test, p < 0.05.

Table 6. RAMs for PIs in patients infected with HIV-1 subtype B and non-B

Mutation	B% (n = 115)	Non-B% (n = 45)	p*
58E	16 (13.9)	5 (11.1)	0.797
16E	3 (2.6)	1 (2.2)	1
60E	15 (13.0)	4 (8.9)	0.592
10I/F/V/C/R	**74 (64.3)**	**20 (44.4)**	**0.031**
13V	33 (28.7)	8 (17.8)	0.226
20M/R/T/I/V	**41 (35.7)**	**29 (64.4)**	**0.001**
23I	8 (7.0)	4 (8.9)	0.740
24I	6 (5.2)	2 (4.4)	1
30N	6 (5.2)	1 (2.2)	0.674
32I	**16 (13.9)**	**1 (2.2)**	**0.042**
33F	26 (22.6)	7 (15.6)	0.389
35G	0 (0)	0 (0)	---
36I/L/V	**58 (50.4)**	**39 (86.7)**	**< 0.001**
43T	8 (7)	0 (0)	0.107
46I/L	54 (47)	14 (31.1)	0.077
47V/A	10 (8.7)	2 (4.4)	0.512
48V/M	0 (0)	0 (0)	---
50L/V	10 (8.7)	4 (8.9)	0.996
53L	13 (11.3)	2 (4.4)	0.237
54A/L/M/V/T/S	49 (42.6)	18 (40.0)	0.859
62V	24 (20.9)	7 (15.6)	0.511
63P	**85 (73.9)**	**16 (35.6)**	**< 0.001**
64L/M/V	12 (10.4)	2 (4.4)	0.353
71V/I/T/L	58 (50.4)	17 (37.8)	0.163
73S/A/C/T	21 (18.3)	3 (6.7)	0.084
74P	3 (2.6)	2 (4.4)	0.619
76V	5 (4.3)	1 (2.2)	1
77I	31 (27.0)	6 (13.3)	0.094
82A/F/L/S/T/I	49 (42.6)	17 (37.8)	0.598
83D	1 (0.9)	0 (0)	1
84V	19 (16.5)	3 (6.7)	0.129
85V	12 (10.4)	2 (4.4)	0.353
88D/S	9 (7.8)	2 (4.4)	0.729
90M	48 (41.7)	12 (26.7)	0.102

* Fisher's exact test, p < 0.05.

Table 7 – Patients with history of NFV intake and virological failure and the resistance pathway

Mutation	Subtype B	Subtype C	Subtype F	Subtype non-B
D30N (%)	6 (9.8)	1 (5)	0 (0)	1 (3.2)
L90M (%)	48 (78.7)	7 (35)	5 (45.5)	12 (38.7)

Most of our knowledge about HIV is related to HIV-1 subtype B, but the available data about other subtypes are increasing as viruses from developing countries have been studied.[9,10] In Brazil, data from southeast and north regions are available.[11] From the three states of south region, Paraná has less available information about HIV-1 clade prevalence.[12]

Subtype B was the predominant HIV-1 subtype in our sample, but other subtypes showed a significant frequency. The three main subtypes in Brazil are B, C and F, with an increasing occurrence of recombinants BC and BF.[4]

It was previously reported that subtype non-B viruses were introduced later in Brazil than subtype B.[13,14] In our work, for subtype B, patient's oldest diagnosis was in 1983 and for subtype C was 1991. The moment that the patients became aware of their HIV status cannot predict the length of infection, but we could suppose that non-B viruses were also introduced later than subtype B in Paraná.

USA and Europe reported an increasing number of cases of HIV-1 non-B cases, mainly in specific ethnical group and women and heterosexuals.[15] Brazil is a multiracial country and the prevalence of Caucasians patients in south region is higher, as showed in all subtypes of viruses in our sample. But the higher prevalence of heterosexuals and woman in subtype non-B and MSM in subtype B followed the previous pattern reported by others.[16] It was suggested that subtype C virus presents functional variations that favors its heterosexual transmission.[17]

Mutations 41L and 210W were more frequently found in virologic failure in subtype B. These are thymidine associated mutations and they occur after viral replication in the presence of thymidine analogues (stavudine and zidovudine).[8] When virologic failure occurs, there is a dichotomization of resistance pathway to 41L, 210W and 215Y or 67N, 70R and 219Q18. In Brazil, initial ARV regimens included stavudine and zidovudine.[19] The length of use of ARVs in our patients did not differ between subtypes. Others suggested that the pathway for resistance can be influenced by subtype,[20] with viruses from Brazil showing higher use of pathway 41L in subtype B than in subtype C.[21,22] In Botswana, it was described a distinct resistance pathway for subtype C after thymidine analogues use, presenting 67N, 70R and 215Y.[20] The resistance pathway used by the virus can predict future options of nucleoside analogues.

65R is a mutation that "*in vitro*" was described to occur more frequently in subtype C virus.[23] This substitution occurs after failing a regime that included TDF, ddI or ABC.[8] We observed only two viruses presenting 65R and both were subtype B. Authors from Brazil and other countries with higher prevalence of subtype C infection haven't seen higher incidences of K65R in clinical samples.[22,24,25] At least in Brazil, with a frequent use of thymidine analogues in first ARV regimen, the presence of thymidine associated mutations (TAMs) could avoid the appearance of K65R and that could be the reason of its lower prevalence.

We did not observe differences in frequency of NNRTIs RAMs in our patients. Subtype C viruses with NNRTI resistance might develop 106A mutation instead of 106M, characteristic of subtype B viruses.[26] The changes 106A and 106M were rarely seen, and both occurred in one patient with subtype B and one patient with subtype C.

It was suggested that a Brazilian subtype C virus had specific amino acid signatures, not present in the world consensus C.[14] Authors showed higher frequency of changing of 36 position in protease gene, and a higher prevalence of 63L mutation in Brazilian subtype B viruses. We observed higher frequency of 36 and 20 codon mutations in subtype C, as previously reported.[27]

90M was the preferential pathway of protease resistance in patients with a failing regimen with nelfinavir. It is possible that the higher frequency of 36 position mutation observed in subtype non-B favors the 90M pathway.[28] Also, because patients used another PI after NFV and the virus was not under selective pressure at time of genotyping, is possible that some patients had NFV related mutations that were archived but not showed in genotyping.

In conclusion, as other states from Southern region of Brazil, we observed in a group of patients from Paraná a significant prevalence of non-B subtype HIV-1 viruses and they infect more frequently women and heterosexuals. Patients with different subtype viruses showed no other demographics of clinical significant differences. Some preferences for resistance pathway and prevalence of mutations were detected comparing subtype B and non-B viruses. The clinical impact of HIV-1 subtype diversity and difference frequency of mutations need more investigation.

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