Primary resistance of human immunodeficiency virus type 1 in a reference center in Recife, Pernambuco, Brazil

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To assess the prevalence of primary resistance of human immunodeficiency virus type 1 (HIV-1) to antiretrovirals, 84 patients chronically infected with HIV without prior antiretroviral treatment from Northeast Brazil were studied. Genotyping was performed using the ViroSeq™ Genotyping System. Thimidine analog mutations occurred in 3 (3.6%) patients. Accessory mutations related to NRTI occurred in 6 (7.1%) and related to PI in 67 (79.8%). Subtypes B (72.6%), F (22.6%), B/F 3 (3.6%), and C (1.2%) were detected. A low prevalence of major mutations related to NRTI in patients chronically infected by HIV was observed.

Key words: human imunodeficiency virus type 1 - primary resistance to drugs - genotyping - subtypes - Brazil

Highly active antiretroviral therapy (HAART) has modified the course of the acquired immunodeficiency syndrome (AIDS) and prolonged the life of its victims (Palella et al. 1998, Mocroft et al. 2003). However, failure of HAART has been reported, attributed to several factors, resistance to drugs being the most important (Hanna & D'Aquilla 1999). When the resistance occurs without the prior use of antiretrovirals, it constitutes the primary resistance and is generally transmitted at the time of acquisition of the viral infection (Boden et al. 1999). The problem becomes even more serious when one notes that the resistance to two or more classes of drugs can also be transmitted in primary infections (DeGruttola et al. 2000, Tamalet et al. 2000, Little et al. 2002).

The genotyping resistance assay allows the mapping of regions with a high prevalence of primary resistance, where the genotyping test performed in patients without prior treatment would be indicated and would help to determine the initial drugs of choice for empirical treatment (Hirsch et al. 2003).

The universal provision of medication by the Brazilian Ministry of Health, which began in 1996 dramatically changed the mortality related to AIDS in Brazil (Marins et al. 2003). However the massive use of these medications brings the risk of resistance (Clavel & Hance 2004), which must be continuously monitored.

In this paper we describe the prevalence of primary resistance of HIV-1 in individuals without previous treatment attending a reference health center for HIV/AIDS in Recife.

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MATERIALS AND METHODS

Cases - A total of 101 individuals infected by HIV-1 in consecutive attendance at the Federal University of Pernambuco Hospital in Recife, between February 2002 and January 2003, were studied. None of them had used antiretrovirals previously. The participants received the relevant information and signed the consent form. The study was approved by the Ethics Commission of the University's Health Sciences Center (Protocol no. 158/2001-CEP/CCS).

HIV-1 resistance genotyping determination - The ViroSeq™HIV-1 Genotyping System (Celera Diagnostic, Abbott Laboratories, US) was used to identify the resistance-associated mutations in the HIV-1 polymerase (pol) gene. The methodology included the isolation and purification of plasma viral RNA followed by cDNA synthesis and genomic amplification by polymerase chain reaction (PCR) assay of the HIV-1 pol fragment (RT-PCR and PCR module), spanning the entire protease (PR) gene and approximately two thirds of the RT gene. The amplified PCR products were sequenced using the BigDye Terminator sequencing chemistry (Sequencing Module – Big Dye v.2.0) and analyzed on an ABI Prism ABI 3100 Genetic Analyzer (Applied Biosystems, US) coupled to the DNA sequencing analysis software. Mutations resistance profiles were classified according to the International AIDS Society US consensus (D'Aquilla et al. 2002). For the determination of the genetic subtypes of the HIV-1 all sequences were analyzed using the Stanford Sequence Resistance Database (http://hivdb.stanford.edu).

RESULTS

A total of 101 patients had their blood samples collected. Seventeen (16.8%) of the samples were excluded because they did not generate fragments in the PCR for the region of reverse transcriptase or protease. The remaining 84 patients have their data described in this paper.

Most of patients were men (64.3%), aged 18-40 years (67.9%), with up to 8 years of formal education (64.4%). The mode of transmission of HIV-1 infection was mostly sexual. Heterosexual practices (58.2%) were detected in the majority of cases, followed by homosexual ones (41.8%). Transmission through IV drugs or blood transfusion occurred in just 3 (3.5%) patients. The mean CD4 lymphocyte count was 178.7 cells/mm^3; the mean viral load was 269,305 copies RNA/ml and the majority of patients were symptomatic (90.5%).

Three samples (3.6%) showed primary mutations related to NRTI (all of them Thymidine Analog Mutations-TAMs) as follows: 2 (2.4%) M41L and 1 (1.2%) K219E (Table). No primary mutations related to NNRTI or PI were detected.

Accessory mutations related to reduced sensitivity to commonly used NRTI or NNRTI (TAM-associated mutations) occurred in the positions V118I in 5/84 (5.9%) and E44D in 1/84 (1.2%) patient.

Minor mutations related to PI occurred in 67 (79.8%) sequences of the PR gene in the following positions: L63P in 40/84 (47.6%), M36I in 29/84 (34.5%), L10I/V in 14 (16.7%), V77I in 13/84 (15.5%), K20R in 9/84 (10.7%), A71T/V in 8/84 (9.5%) and I54P in 1/84 (1.2%) patient (Table). Nine samples (10.7%) presented mutations related to NRTI and IP simultaneously.

Subtype B was detected in 61 patients (72.6%), F in 19 (22.6%), and B/F in 3 (3.6%). Subtype C occurred in one patient (1.2%), who had arrived recently from the Southeast region of Brazil.

DISCUSSION

This study revealed a low prevalence of primary mutations of resistance related to NRTI in individuals chronically infected without previous treatment.

A major criticism of the study is that it analyzes primary resistance in chronically infected individuals. One can argue that, in these patients, the acquisition of the infection must have happened several years before and the mutations of resistance would no longer be detected in the absence of the drug selection pressure (Clavel & Hance 2004). That is actually true in patients with secondary resistance (Diaz 2004); however, several studies have demonstrated the persistence of mutations of primary resistance for periods as long as five or seven years (Brenner et al. 2002, 2004, Barbour et al. 2004, Pao et al. 2004), supporting the rationale for testing the chronically infected (Novak et al. 2005).

The three cases (3%) of major mutations found in the present study were TAMs, which lead to resistance to the majority of NRTI, especially zidovudine and stavudine. NRTI was the first group of antiretrovirals introduced into clinical use; one could therefore infer that these viruses represent the circulating virus at the time of infection acquisition, probably several years ago. While considered to be of minor importance, the following other mutations should be noted: the E44D mutation, which commonly accompanies the TAMs and, when associated with V118I or TAMs, causes low-level resistance to lamivudine and other NRTI (Montes & Secondy 2002, Houtte et al 2003); the A71I mutation, which occurs in 5 to 10% of untreated

TABLE

Age, sex, risk behavior, subtypes, and mutations associated with nucleoside reverse-transcriptase inhibitors (NRTI), non-nucleoside reverse-transcriptase inhibitors (NNRTI), and protease inhibitors (PI)

					ARV classes		
Code	Age	Sex	Risk behavior	Subtype	NRTI	NNRTI	PI
AAB	25	M	MSM	B			L63P, A71V, V77I
AAS	50	M	HTS	B			M36I
ALMR	24	M	HTS	B			
ALS	24	M	MSM	B			V77I
AMAS	56	F	HTS, HEM	F			
AMLF	35	F	HTS	F			M36I
APS	22	F	HTS	B			L10I, L63P
ARC	22	M	MSM	B			V77I
CBB	32	M	HTS	B			L63P
CBS	59	M	HTS	B			L63P, M36I
DCB	80	F	HTS	B			L10I, A71T, L63P, V77I
DSBM	39	M	MSM	B			L63P
EAE	37	M	MSM	BF			M36I
EAM	45	F	HTS	B			V77I
EAS	31	M	MSM, IVDU	B			
EAS	35	M	MSM	F			K20R, M36I
ECC	38	M	HTS	B			L63P
ECS	41	M	HTS	B			L63P
EF	49	M	HTS	B			
EJO	29	M	MSM	B			M36I, L63P
EJS	54	F	HTS	B			L63P
EMS	35	M	MSM, IVDU	B			M36I, L63P

ERRO	20	F	HTS	B		
ESA	35	M	MSM	B		L63P, A71T
ESB	21	F	HTS	F		K20R, M36I
ESC	31	F	HTS	F		K20R, M36I
FAS	32	M	HTS	F		M36I
FAS	33	M	HTS	F		L10V, M36I
FFS	43	M	MSM	BF		M36I
GL	29	M	MSM	B		M36I
HSB	50	M	HTS	B		
ICMS	33	F	HTS	B		
IFG	36	M	MSM	B		
JBS	43	M	MSM	B		L63P, A71V
JBV	46	F	HTS	B		I54P, L63P
JCES	45	M	MSM	B		
JCL	30	M	MSM	B		L63P
JCS	41	F	HTS	B		L63P
JCSN	19	M	HTS	F		K20R, M36I
JDP	36	M	MSM	F		K20R, M36I
JDSL	28	F	HTS	F	E44D	M36I
JFS	31	M	MSM	F	V118I	M36I, L10V
JGS	45	M	MSM	B		L63P
JJS	31	M	HTS	B		L63P, A71T
JLA	27	M	MSM	B		V77I, L63P
JLN	41	M	MSM	B		
JMC	66	M	HTS	B		
JRS	37	M	MSM	B		L63P
JSM	42	F	HTS	B	V118I	M36I, L63P
JSPN	49	M	HTS	F		M36I, L63P
JSS	39	M	HTS	B		V77I
LGS	33	F	HTS	B	V118I	
LHSP	29	M	MSM	B		L63P, V77I
LJNM	27	M	HTS	B		
LMM	43	M	MSM	B		L63P
MCA	19	F	HTS	B		L63P, V77I
MCFS	41	F	HTS	C		M36I
MCL	48	M	MSM	B		L63P
MCS	35	F	HTS	B		M36I, V77I
MGPA	33	F	HTS	B		
MIL	36	M	HTS	B		L10V, V77I
MJC	36	M	MSM	B	V118I	L63P, A71T, V77I
MJOP	20	M	MSM	B		L63P
MJS	23	F	HTS	B		L63P
MJSF	42	M	MSM	B		
MSGS	18	F	HTS	F		K20R, M36I
MVGRN	29	F	HTS	B		K20R, L63P, A71T
MEMS	61	F	HTS	F		L10V, K20R, M36I
PBC	34	M	MSM	B		L63P
PPS	29	F	HTS	B		L63P
RAR	39	M	MSM	B		L63P
RFO	38	M	MSM	B		L10V, L63P, A71T
ROSC	24	F	HTS	F		M36I, L63P
RSF	33	M	MSM	F		M36I
SAF	36	F	HTS	B	M41L	L10I, L63P
SCB	41	F	HTS	B		
SGL	30	M	HTS	B		
SMS	42	M	HTS	F		L10V, M36I, L63P
SSI	34	M	MSM	B		L10V, L63P
SVCS	35	M	MSM	BF	V118I	L10V, A77I, L63P
SVNC	39	F	HTS	F	M41L	L10I, M36I
VESA	27	F	HTS	B		L10I
VJCL	31	M	MSM	B		M36I, L63P
VTS	46	F	HTS	F	K219E	L10V, M36I, K20R

MSM: men who have sex with men; HTS: heterosexual; HEM: hemophiliac; IVDU: IV drug user.

persons with the subtype B, but increases dramatically by as much as 60 to 80% in heavily treated patients (Hertogs et al. 2000, Wu et al. 2003); the V77I mutation, which is specifically associated with the use of nelfinavir (Wu et al. 2003); and the mutation K20R, which occurs at higher rates in subtype F than in subtype B isolates (Gonzales et al. 2001). It should not be overlooked that the concomitant occurrence of mutations related to NRTI and PI was found in 10.7% of the samples. Moreover a high frequency of minor mutations related to PI has been detected, mutations that seem to play a major role in the viral fitness recovery but have only a small impact on resistance (Diaz 2004).

Between 1996 and 1998 Brazilian investigators conducted limited regional surveys on HIV primary drug resistance that revealed rates of 0 to 9.3% of resistance (Brindeiro et al. 1999, Dumans et al. 2002). In 2003, a study that comprised 535 chronically infected individuals from all Brazilian regions showed a frequency of 6.6% of resistance to antiretrovirals (Brindeiro et al. 2003). Furthermore, Eyer-Silva and Morgado (2005), studying 27 individuals chronically infected in a small municipality of the state of Rio de Janeiro, detected no mutations related to resistance. All these data, in conjunction with the findings of this study, suggest that Brazil has lower rates of resistance to antiretrovirals than Europe and the US (Little et al. 2002, Eiros et al. 2004, Novak et al. 2005) and that in the Northeast region, particularly in Pernambuco, these rates are also very low. One might suspect, albeit without conclusive evidence, that this lower frequency of primary resistance in Pernambuco and in the Northeast as a whole is due to a later access to medication. However studies point to a worrying trend. In 2006, a study that comprised 341 blood donors from São Paulo from 1998 to 2002 revealed rates of 5% of resistance to antiretrovirals in long-standing infections and higher rates of 12.7% in the recently-infected individuals, and this difference was significant (Barreto et al. 2006). Two other studies also point to rising rates of resistance in the Southern region of Brazil. The first of these investigated 56 military personnel, and revealed 14% of NRTI associated mutations in the chronically infected patients (Pires et al. 2004). The second one, conducted in Santos in 2001 (the first Brazilian city where antiretrovirals were supplied through the public health service), showed 36% of resistance to antiretrovirals in recently-infected individuals (Caseiro 2001). One may infer that this trend probably applies also to new infections in the Northeast region.

This study confirms subtype B as the most prevalent, with subtype F as the second most important, as described elsewhere (Brindeiro et al. 2003). It also shows the circulation of the B/F recombinants and the rarity of subtype C in this region.

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