

# Distribution of HIV-1 subtypes in patients with HAART therapeutic failure in the States of Pará and Amazonas, Brazil: 2002 to 2006

Distribuição de subtipos de HIV-1 em pacientes com falha terapêutica à HAART nos Estados do Pará e Amazonas, Brasil, entre 2002 e 2006

Distribución de subtipos de VIH-1 en pacientes con fallo terapéutico al HAART en los Estados de Pará y Amazonas, Brasil, entre 2002 y 2006

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## ABSTRACT

The aim of this study was to determine the distribution of HIV-1 subtypes in patients undergoing highly active antiretroviral therapy (HAART) therapeutic failure in Amazonas and Pará, two States in northern Brazil, from 2002 to 2006. This study was performed using plasma collected from individuals with human immunodeficiency virus-1 (HIV-1) and/or acquired immunodeficiency syndrome (AIDS) who were selected from the National Genotyping Network (*Rede Nacional de Genotipagem* – RENAGENO). From 2002 to 2006, a total of 127 plasma samples from the States of Amazonas and Pará, in northern Brazil, were obtained from AIDS and/or HIV-positive patients and subjected to genotyping and resistance testing using the ViroSeq™ Genotyping System kit. Using the genetic information obtained from the HIV-1 protease and/or reverse transcriptase regions, HIV-1 subtype B was identified in 85% of the cases, followed by subtype F1 (4.6%) and the recombinant forms BF1 (4.6%) and CF1 (0.8%). The results of this study were similar to the results of other studies conducted in other regions of Brazil, with the exception of the detection of recombinant CF1, which was described for the first time in the Amazon Region.

**Keywords:** HIV-1; Subtypes; CF1 Recombinant Form; HAART Therapeutic Failure.

## INTRODUCTION

Since the late 1970s, patients have lived with human immunodeficiency virus (HIV), a retrovirus that causes acquired immunodeficiency syndrome (AIDS). HIV, a member of the *Lentivirus* genus, is classified into two types, HIV-1 and HIV-2, which differ in the molecular weights of their proteins, their accessory genes and their epidemiological dispersal patterns. Although the spread of HIV-2 is restricted, HIV-1 has spread rapidly worldwide and has become a pandemic that is responsible for thousands of deaths every year<sup>1,2</sup>.

In Brazil, from 1980 to June 2011, 608,230 cases of HIV infection were reported. According to the Ministry of Health (MS), from 1980 to 2010, 241,469 people died in Brazil due to AIDS, and in 2010 alone, 11,865 Brazilians died from AIDS. Early diagnosis and treatment were key to reducing deaths of patients infected with HIV<sup>3,4,5,6</sup>.

The course of the disease varies widely among infected individuals. The transition from acute infection to the development of AIDS is defined by a CD4<sup>+</sup> T cell count between 200 and 350 cells/mm<sup>3</sup> for asymptomatic individuals. This definition was adopted by the Brazilian Advisory Committee of the Ministry of Health, which also recommended an earlier initiation of antiretroviral (ARV) treatment compared with the previous regimen<sup>7</sup>, a change that aimed to prevent lymphocyte counts from reaching 200 cells/mm<sup>3</sup>, and to prevent the onset of opportunistic infections<sup>8,9</sup>.

In the course of a natural infection by HIV-1, an initially high rate of viral replication occurs, leading to the production of approximately 10<sup>10</sup> viral particles per day and a mutation rate of approximately 3.5 x 10<sup>-5</sup> nucleotides per

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replication cycle. This high mutation rate contributes to the appearance of viral quasiespecies, i.e., closely related variants of the virus that are genetically distinct from each other and that can infect the same cell<sup>9,10,11</sup>.

The HIV-1 subtypes are classified based on phylogenetic analysis and are distributed into the main (M), outlier (O) and non-M/non-O (N) groups. The most prevalent is group M, which is divided into subtypes, subtypes (A1, A2, B, C, D, F1, F2, G, H, J, K) and circulating recombinant forms (CRFs) that are hybrids between different subtypes<sup>12,13,14,15</sup>.

Most HIV-1 samples that have been tested fall into a defined set of subtypes. However, a small number of HIV-1 samples contain genomes with regions from multiple subtypes; these circulating, recombinant forms are found in geographical areas where multiple subtypes of the virus coexist. These hybrid samples are the products of recombination events that occur in HIV-1. If two different subtypes infect a single cell, a mosaic virus can be generated that includes regions from each of the two subtypes.

Equatorial Africa presents a great diversity of group N and O variants. All group M subtypes and several CRFs co-circulate in Cameroon, Equatorial Guinea, Gabon and the Democratic Republic of the Congo<sup>16</sup>. In Brazil, subtype B is the most prevalent, followed by F, C and CRFs<sup>17,18,19</sup>.

This study aimed to assess the proportional distribution of HIV-1 subtypes in individuals living in the States of Pará and Amazonas, Northern Brazil, in the period from 2002 to 2006.

## MATERIALS AND METHODS

### STUDY POPULATION

The material in this retrospective study, conducted from 2002 to 2006, was processed according to the following procedures. All patients presented with highly active antiretroviral therapy (HAART) therapeutic failure.

The study population consisted of 127 patients. A total of 95 patients were from Pará State, and 32 were from Amazonas State, all of whom were monitored by RENAGENO (the National Genotyping Network) physicians. The project that originated this study, registered under CEP/IEC N° 0030/07 and CAAE 0027.0.072.000-07, was submitted and approved on December 19 2007 by the Ethics Committee of the Instituto Evandro Chagas (IEC).

### INCLUSION CRITERIA

We used the RENAGENO network forms, which contained personal, clinical, laboratory and epidemiological information. Subsequently, this group was evaluated using the RENAGENO criteria listed below. Patients who agreed to participate in the study signed informed consent forms from the National STD/AIDS Control Program (CN DST/AIDS) of the Brazilian Ministry of Health and from the RENAGENO network. Plasma samples (1 mL) were collected from patients who met the inclusion criteria for the study being stored in low temperature freezers at the IEC until its timely use.

Patients who were selected for genotyping examination had submitted evidence of adequate adherence to ARV medication to avoid unnecessary use of the test.

### MOLECULAR ANALYSIS

The molecular analyses began with the isolation and purification of viral RNA from patient plasma, followed by cDNA synthesis and polymerase chain reaction (PCR) amplification of the HIV-1 *pol* fragment, which spans the entire protease gene and approximately two thirds of the reverse transcriptase (RT) gene. The resulting 1.8-kb fragments were sequenced using the BigDye Terminator Sequencing Kit v2.0 on the ABI Prism® 3100 Genetic Analyzer (Applied Biosystems, USA) coupled to DNA sequence analysis software. To determine the FASTA sequence, the sequence generated by the software was compared with the reference base sequence (HXB-2) of the B subtype of HIV-1. After comparing both sequences, it was possible to identify different genetic subtypes and/or CRFs.

Genotyping was performed on all of the samples using the ViroSeq™ HIV-1 Genotyping System (Celera Diagnostic, Abbott, USA)<sup>20</sup>.

All of the nucleotide sequences were analyzed using the Stanford Sequence Resistance database<sup>21</sup> and the National STD/AIDS Program algorithm (version from February 2005) from the Brazilian Health Surveillance Department (Secretaria de Vigilância em Saúde - SVS/MS).

This identification was performed using FASTA and genotype sequence analyses with the PN/STD/AIDS Brazilian algorithm (version from February 2004) of the SVS/MS<sup>22</sup>.

## RESULTS

### STUDY POPULATION CHARACTERISTICS

This study included 32 (25.2%) patients from Amazonas, 19 (59.4%) of whom were male, and 95 (74.8%) patients from Pará, 75 (78.9%) of whom were male (Table 1). All of the patients received highly active antiretroviral therapy (HAART) and met the inclusion criteria for this study.

The 32 samples from the Amazonas patients were received between 2005 and 2006, and most of the samples (87.5%) belonged to asymptomatic individuals. The percentage of asymptomatic individuals was lower (58.9%) in the serum samples collected in Pará between 2002 and 2006. In both states, most patients had no clinical symptoms. In both states, the samples were collected primarily from men, and the majority of these patients (64%) were diagnosed with HIV-1 in the 1990s, 18% of whom were diagnosed in the year 1997 alone. Therefore, these patients had been living with HIV for at least ten years.

The patients' ages ranged from 19 to 72 years old (with a mean of 39 years), and most patients (107, 84.3%) were between 20 and 49 years old.

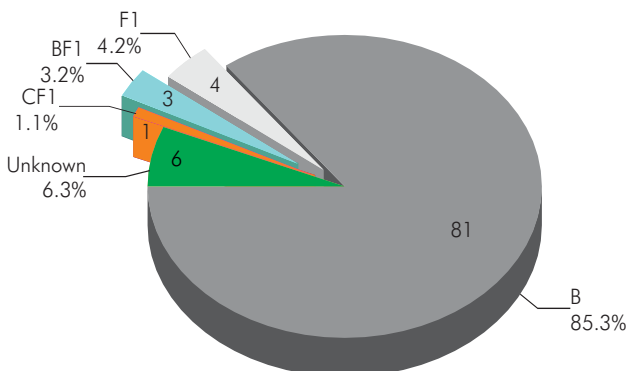
At the time of genotyping, 84 (66.1%) individuals from both States were asymptomatic, and the diagnosis of HIV-1 infection was performed between 1991 and 1999 for 63.8% of the patients (Table 1).

**Table 1** – The distribution of the general epidemiological data from 127 patients presenting with antiretroviral (ARV) therapeutic failure in the States of Amazonas and Pará, from 2002 to 2006

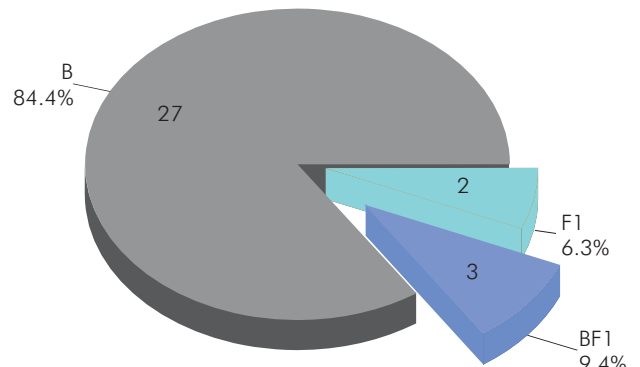
Variable	N	%
Origin		
Amazonas	32	25.2
Pará	95	74.8
Gender		
Female	33	26
Male	94	74
Age group (years)		
Younger than 20	2	1.6
20-49	107	84.2
≥ 50	17	13.4
Unknown	1	0.8
Symptoms		
Yes	43	33.8
No	84	66.1
Year of diagnosis		
Before 1990	2	1.6
1991-2000	81	63.8
2000-2005	40	31.5
Unknown	4	3.1
Viral Load (copies RNA/mL)		
> 100,000 and < 500,000 copies RNA/mL	48	37.8
From 10,000 to 100,000 copies RNA/mL (mean = 42,910)	74	58.3
< 10,000 copies RNA/mL	4	3.1
Unknown	1	0.8
Cd4 (cells/mm <sup>3</sup> )		
< 200 cells/mm <sup>3</sup> (mean = 95.8)	73	57.5
From 200 to 349 cells/mm <sup>3</sup>	34	26.5
≥ 350 cells/mm <sup>3</sup>	17	13.4
Unknown	3	2.4
<b>Total</b>	<b>127</b>	

A total of 58.3% of the patients possessed a viral load between 10 thousand and 100 thousand RNA copies/mL (with a mean of 42,910 RNA copies/mL). The CD4<sup>+</sup> T lymphocyte count in 57.5% of the patients at the time of inclusion in the study was below 200 cells/mm<sup>3</sup> (with a mean of 95.8 cells/mm<sup>3</sup>) (Table 1).

Based on the genotyping data, in Pará, 81 (85.3%) HIV-1 viral samples were classified as subtype B, four samples (4.2%) as subtype F1, three samples (3.2%) as BF1 recombinants and one sample (1.1%) as a CF1 recombinant. Additionally, six samples (6.3%) were of an unknown genotype (Figure 1). In Amazonas, 27 samples (84.4%) were classified as subtype B, two samples (6.3%) as subtype F1 and three samples (9.4%) as BF1 recombinants (Figure 2).



**Figure 1** – HIV-1 subtype distribution in Pará State, Brazil, 2002-2006



**Figure 2** – HIV-1 subtype distribution in Amazonas State, Brazil, 2005-2006

## DISCUSSION

This study, similarly to other RENAGENO studies, investigated adult patients (with a median age of 39 years old) who were chronically infected with HIV-1 and were undergoing ARV treatment. This study included patients from the States of Amazonas and Pará, both located in northern Brazil.

All 127 patients were in virological failure; the median viral load was 67 thousand RNA copies/mL, and the median CD4<sup>+</sup> T cell count was 171.5 cells/mm<sup>3</sup>. The viral load and the CD4<sup>+</sup> T cell count are the primary markers used to monitor the course of AIDS in patients who are treated with ARVs<sup>4</sup>. In particular, a count of fewer than 200 CD4<sup>+</sup> T cells/mm<sup>3</sup> in the peripheral blood indicates the onset of an important immunodeficiency condition that is directly associated with an increase in opportunistic infections and, consequently, with the possibility of presenting a less complete therapeutic response<sup>23</sup>.

According to Morgado et al<sup>24</sup>, 131 patients in Rio de Janeiro were divided into subtypes as follows: 80.9% subtype B, 15.3% subtype F and 1% subtype D. An additional study performed a year later in this city (involving 43 samples from "blood donors") obtained similar results: 76.7% of the samples were identified as subtype B, 14% as subtype F and 9.3% as B/F or B/D recombinants<sup>18</sup>.

Brazil possesses a variety of HIV-1 subtypes, which are distributed unevenly throughout the country's geographical regions. However, in general, subtype B is the most prevalent subtype in the country<sup>25,26,27,28</sup>.

In the Brazilian Amazon, the prevalence of subtype B was greater than the prevalence of subtype F in the Cities of Belém and Macapá, Pará State. However, genetic analysis in Belém revealed the presence of *env* segments from subtypes B, F, D and C and the presence of *pro* segments from subtypes B, F, D and CRF02\_AG<sup>29</sup>.

In this study, the molecular epidemiology of HIV-1 samples collected from 127 infected patients in the States of Pará and Amazonas was characterized. In addition, the proportional distribution of different subtypes in these states was estimated. Our results for the Amazon Region are similar to the results of previous studies conducted in other regions of Brazil<sup>30</sup>.

The proportion of HIV-1 subtype B infections found in this study was higher than that of subtype F1, confirming a previous report by Machado<sup>29</sup> from the Cities of Belém and Macapá. This study differs, however, from the study by Vicente et al<sup>31</sup> with respect to the prevalence of subtype F in Amazonas, which we observed to be nearly equal to that of subtype B. Indeed, in the present study, HIV-1 subtype B comprised 85.3% Pará and 84.4% Amazonas of HIV-1 circulating in these states, which contrasts with the results previously obtained in Amazonas State<sup>31</sup>, but another study conducted in the Amazonas revealed the high prevalence of subtype B over F and C, respectively<sup>32</sup>.

One example of HIV-1 subtype C was isolated from blood donors in Amazonas recently<sup>33</sup>.

These results support the higher prevalence of subtype B and indicate the introduction of other non-B subtypes. However, our results are consistent with those obtained by Machado<sup>29</sup> for Pará. In contrast to subtype B, the prevalence of subtype F1 was low [Pará (4.2%), Amazonas (6.3%)], and its occurrence even included the recombinant forms B/F1 [Pará (3.2%), Amazonas (9.4%)] and CF1

(isolated only once). Notably, the detection of the latter recombinant was the first reported in Pará and the Amazon Region, and recombinant CF1 has also been described in a newly infected individual in Curitiba, Paraná State, southern Brazil<sup>34</sup>.

## CONCLUSION

Based on these results, we conclude that HIV-1 subtype B was the most prevalent subtype in the samples of patients with treatment failure in the States of Amazonas and Pará, followed by subtype F1 and recombinant subtype BF1 in Pará. However, in Amazonas, recombinant subtype BF1 was more prevalent than subtype F1. In Pará, the first occurrence of recombinant CF1 in that State and the Amazon Region of Brazil was described.

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## Distribuição de subtipos de HIV-1 em pacientes com falha terapêutica à HAART nos Estados do Pará e Amazonas, Brasil, entre 2002 e 2006

### RESUMO

O objetivo deste estudo foi determinar a distribuição dos subtipos de HIV-1 em pacientes com falha terapêutica à highly active antiretroviral therapy (HAART) nos Estados do Amazonas e Pará, Região Norte do Brasil, entre 2002 e 2006. Este estudo foi realizado utilizando-se soro coletado de indivíduos infectados com o vírus da imunodeficiência humana tipo 1 (HIV-1), e/ou portadores da síndrome da imunodeficiência adquirida (aids), selecionados a partir do banco de dados da Rede Nacional de Genotipagem (RENAGENO). Entre 2002 e 2006, foram obtidas 127 amostras sorológicas de pacientes portadores de aids e/ou HIV-positivos dos Estados do Amazonas e Pará, as quais foram submetidas a genotipagem e teste de resistência com o kit ViroSeq<sup>TM</sup> Genotyping System. Considerando as informações genéticas obtidas das regiões da protease e/ou transcriptase reversa do HIV-1, o subtipo B foi identificado em 85% dos casos, seguido do subtipo F1 (4,6%) e as formas recombinantes BF1 (4,6%) e CF1 (0,8%). Os resultados dessa pesquisa foram semelhantes aos encontrados em estudos realizados em outras regiões do Brasil, exceto pela detecção da forma recombinante CF1, que foi descrita pela primeira vez na Região Amazônica.

**Palavras-chave:** HIV-1; Subtipos; Forma Recombinante CF1; Fracasso Terapêutico da HAART.

## Distribución de subtipos de VIH-1 en pacientes con fallo terapéutico al HAART en los Estados de Pará y Amazonas, Brasil, entre 2002 y 2006

### RESUMEN

El objetivo de este estudio fue de determinar la distribución de los subtipos de VIH-1 en pacientes con fallo terapéutico al highly active antiretroviral therapy (HAART) en los Estados de Amazonas y Pará, Región Norte de Brasil, entre 2002 y 2006. Este estudio se realizó utilizando suero colectado de individuos infectados con el virus de inmunodeficiencia humana tipo 1 (VIH-1), y/o portadores del síndrome de inmunodeficiencia adquirida (sida), seleccionados a partir del banco de datos de la Red Nacional de Genotipado (RENAGENO). Entre 2002 y 2006, se obtuvieron 127 muestras serológicas de pacientes portadores de sida y/o VIH-positivos de los Estados de Amazonas y Pará, las que fueron sometidas a genotipado y a prueba de resistencia con el kit ViroSeq<sup>TM</sup> Genotyping System. Considerando las informaciones genéticas obtenidas de las regiones de la proteasa y/o transcriptasa reversa del VIH-1, el subtipo B fue identificado en un 85% de los casos, seguido del subtipo F1 (4,6%) y las formas recombinantes BF1 (4,6%) y CF1 (0,8%). Los resultados de esa investigación fueron similares a los encontrados en estudios realizados en otras regiones de Brasil, excepto por la detección de la forma recombinante CF1, que fue descrita por primera vez en la Región Amazónica.

**Palabras clave:** VIH-1; Subtipos; Forma Recombinante CF1; Fracaso Terapéutico de la HAART.





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