

HIV-1 subtypes and profiles of resistance to protease inhibitors in the Marajo Archipelago (Brazilian Amazon region)

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Abstract

The Marajo Archipelago, located in the rural area of the state of Para (Amazon region of Brazil), is the region with the lowest index of human development in the country, presenting poor health services, which makes adequate epidemiological surveillance of HIV-1 infection difficult. After a serological screening enrolling 1,877 samples, three samples were diagnosed with HIV-1 and the pro gene was sequenced to evaluate the presence of resistance mutations to protease inhibitors. Molecular analyses revealed, for the first time in the region of Marajo Island, the occurrence of HIV-1 subtypes B and D, as well as the presence of transmitted high and intermediate protease inhibitors resistance mutations. The results emphasize the importance of ongoing molecular epidemiological surveillance studies in the Brazilian Amazon region because antiretroviral (ARV) resistance mutations may limit treatment options, and the presence of certain subtypes seems to influence the progression to AIDS, particularly in areas where the entry and spread of the virus can be facilitated by socio-demographic problems that expose the population to sexually transmitted infections.

Key word: HIV-1, subtypes, mutation, resistance, Marajo Island

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Introduction

The genetic variability of human immunodeficiency virus 1 (HIV-1) leads to the classification into four groups (M, N, O, P); M includes the vast majority of infections and is divided into nine subtypes (A, B, C, D, F, G, H, J and K), circulating recombinant forms (CRF) and in unique recombinant forms (URF). The distribution of subtypes varies around the world, with subtypes A, C, D and E showing higher prevalence in Africa and Asia, and subtype B mostly present in Europe and the Americas⁽¹⁾.

In Brazil, four subtypes are detected B, C, D and F. Subtype B is found in urban areas, although it has been also found in indigenous populations^(2,3). Studies in the Amazon region of Brazil have revealed the predominance of subtypes B and F⁽⁴⁾, and the presence of subtypes C and D and of the recombinant form CRF02_AG has also been reported⁽⁵⁾.

Another consequence of the high genetic variability of HIV-1 is the emergence of strains with antiretroviral (ARV) resistance mutations, which has been a major concern because the transmission of these variants to other individuals may alter the progression of the infection and limit treatment options over time^(6,7). To monitor the circulating subtypes in the Amazon region of Brazil, as well as strains with acquired resistance mutations, is of great relevance as they may contribute to the development of epidemiological surveillance actions against HIV-1.

The Marajo Archipelago is located in the State of Pará, northern Brazil, and is one of the richest regions of the country in regard of water and biological diversity. The region consists of islands, which is the largest fluvial-maritime archipelago in the world, with 49,606 square kilometers⁽⁸⁾. Marajo Island is constituted of sixteen municipalities in which are inserted the following investigated cities: Chaves (13,085 Km², 22,566 inhabitants), Anajás (6,922 Km² and 27,540

inhabitants), São Sebastião da Boa Vista (1,632 Km², 25.161 inhabitants) and Portel (25,385 Km², 58.282 inhabitants)(9).

The population of the Marajo Archipelago is poor, very ill educated, starts sexual activities early in life and uses no measures to prevent pregnancies or sexually transmitted infections. These factors are more than relevant for the health authorities to develop strategic and action plans for the near future in order to change the chaotic health situation, which is continuously described in the island (9).

In the present study we report the first cases of HIV-1 subtypes B and D infections in the Marajo archipelago and the occurrence of mutations to protease inhibitors.

Materials and methods

In our previous serological screening performed in 1,877 blood samples from volunteers residing in four municipalities from Marajo Island: Chaves (N = 380), Anajas (N = 341), Sao Joao da Boa Vista (N = 359) and Portel (N = 797) we described three seropositive women(10). Participants were informed about and invited to take part of a health campaign in the public health center of each community. All subjects answered to an epidemiological questionnaire and had a blood sample (15 mL) collected. All the participants were informed about the project and those who accept to take part signed an informed consent. Individuals, under 18 years old, were authorized by the parents or guardians.

In the present study the three cases of HIV-1 infection described in our previous study(10) were submitted to partial sequencing of the protease region (pro) of the pol gene, as previously reported(5). PCR products (222 bp) were purified and subjected to automated Sanger sequencing(11), using an ABI PRISMTM 310/377 BigDye[®] Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA).

Sequence alignment was performed in two steps: the first was to obtain the consensus sequence of the samples, and the second step was to evaluate the alignment of the consensus sequence with reference sequences of all HIV-1 M group subtypes obtained in the database of the Los Alamos National Laboratory (NIH, USA) using the ClustalW tool in BioEdit software version 7.1.9.

Phylogenetic inference was performed using the Bayesian statistical method, which selects the phylogram with the highest probability according to the nucleotide substitution model GTR+I+G (which best explains the data set) selected through the jModeltest 0.1.1 software. A phylogenetic tree was generated using MrBayes software version 3.2. The reliability of the branches was calculated by bootstrap analysis based on 1000 resampling to determine the consistency of the branches. The associations of the sequences were supported by a significant bootstrap ($\geq 80\%$; $p \leq 0.05$) or a highly significant bootstrap ($\geq 95\%$; $p \leq 0.01$). TRACER v1.5 software was used to evaluate the convergence of all the parameters sampled by the analysis. The first 25% of the sampled trees was discarded as burn-in using TreeAnnotator software, and the generated tree was

visualized using FigTree software.

Drug resistance mutations (DRMs) and all antiretroviral susceptibilities were analyzed using the calibrated population resistance tool CPR version 6.0 and the Stanford HIV drug resistance database HIVdb version 8.4.

The study was submitted to the Research Ethics Committee of the Center for Hematotherapy and Hematology Foundation of Para (HEMOPA) and approved under the protocol number 0003.0.324.000-10. The individuals were informed of the project's goals, and those who agreed to take part in the study, signed an Informed Consent Form.

Results

The phylogenetic tree obtained from the comparison of nucleotide sequences of HIV-1 samples with reference strains (Figure 1) showed that two samples were grouped to subtype B (BRPA_129; GenBank: MG460604 and BRPA_497; GenBank: MG460605) and one to subtype D (BRPA_116; GenBank: MG460603).

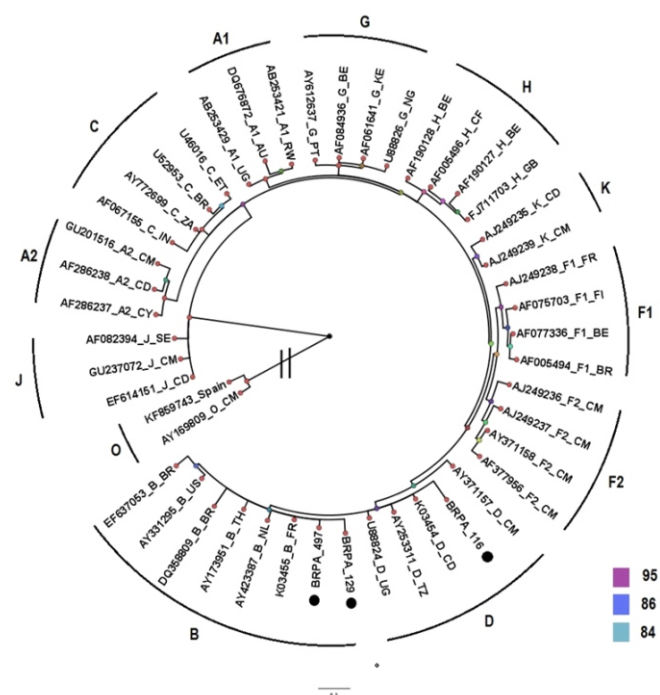


Fig. 1. Phylogenetic tree inferred by the Bayesian method, based on the pro gene showing the relationship between the investigated samples and the reference sequences from the GenBank (investigated samples, colored squares: bootstrap values).

The two subtype B sequences (BRPA_129 and BRPA_497) showed mutations that were not correlated with ARV resistance (BRPA_129: K43R, R57K, I62V, L63P, I72E, V75I and V77I; BRPA_497: L63H, I64V and V77I). In contrast, the subtype D (BRPA_116) sequencing showed mutations related to intermediate and high resistance to protease inhibitors (PIs) (V23I, M46I, I47V and V82A), in addition to the accessory mutation K43T (Table 1).

Table 1
Protease inhibitor resistance mutations in the HIV-1 pro gene found in three samples

Sample	Subtype	Mutations	Resistance Level	Protease Inhibitor
BRPA_116	D	V82A, V32I, M46I, I47V	High	Atazanavir/r, Fosamprenavir/r, Indinavir/r, Lopinavir/r, Nelfinavir/r
			Intermediate	Saquinavir/r, Darunavir/r, Tipranavir/r
		K20I, R41K, I62V, L63S, I64V, E65D, T74A	ND	ND
BRPA_129	B	K43R, R57K, I62V, L63P, I72E, V75I, V77I	ND	ND
BRPA_497	B	L63H, I64V, V77I	ND	ND

ND: not determined

Discussion

The prevalence of HIV-1 subtype B is similar in almost all Brazilian regions(4,12-15). The phylogenetic analysis of the pro gene showed the predominance of this subtype also in the Amazon region, followed by the F subtype(5). Finding subtype B for the first time in the Marajo Archipelago corroborates the previous results, which show this subtype as the most prevalent in Brazil. These results also highlight the need for constant epidemiological surveillance of the virus circulation, which is currently extending to a rural area of the state of Para where there is the lowest human development index.

The subtype D, one of two subtypes described in Marajó archipelagos, occurs mainly in East Africa, especially in Uganda(16) and in the Republic of Congo(17), but it is uncommon in Brazil, where occurs only in isolated cases(18), that highlight the need to evaluate the primary resistance in the Marajó island. In the northern region of the country, subtype D, from pro gene analysis, was described for the first time, with a frequency of 1.7% (1/60) in the city of Belem(5). However, analysis of other virus genes has shown that the frequency of this subtype ranged from 1 to 16%(14,15). HIV-1 subtype (pro region) was detected in one of the samples of the Marajo Archipelago indicating the spread of the subtype to a poor rural area of the Amazon region, what is worrisome as most residents of the region have limited access to public health services, which makes it more difficult to diagnose the infection. In addition, subtype D has recently been associated with more rapid progression to AIDS(19). But supposing the sequence would not be long enough to provide phylogenetic signal, further study with a longer sequence would be necessary to confirm our findings.

In the analysis of PI resistance mutations (Table 1), subtype D (BRPA_116) presented several mutations of

transmitted resistance, with high resistance to almost all of the PIs except for saquinavir, as previously described(6,7). Identification of HIV-1 infection in blood donor candidates showed that 16% of individuals had transmitted PI resistance mutations(6). All resistance mutations found in the investigated gene (pro) are related to antiretrovirals used in Brazil, which are available for use in Reference Treatment Units located in urban areas. This is an important alert, as the infected subject (BRPA_116) was ARV naïve; indeed, until the moment of examination, the three individuals were unaware of their infection. Thus the data shows the importance of monitoring resistance to ARV for treatment that is effective and inhibitory to sequential human transmission.

Recently, Eisinger and Fauci pointed out that the end of HIV/AIDS pandemic necessarily implies in the implementation of social, cultural, demographic and political studies at local, regional and national levels(20). The present manuscript brings important and additional information to the previous detection of HIV-1 infection in the Marajo Archipelago(10), showing the occurrence of protease inhibitor resistance mutations among HIV-1 infected subjects. This is a crucial information in order to monitor ARV drug resistance which could compromise the effective success of a free, regular, national program that provides diagnostic and treatment for the infected population. The Marajo Archipelago is an important touristic point in the Amazon region (North of Brazil), which receives visitors from several parts of Brazil and from abroad. The report of inhibitor resistance mutations may result in the implementation of public health policy measures in the local and regional area.

Conclusions

The analysis of the pro gene of HIV-1 showed the presence of subtypes B and D in the Marajo Archipelago, a rural area with the lowest human development index in Brazil and highlight the importance of continuously performing molecular studies of epidemiological surveillance, particularly in areas where the entry and spread of the virus can be facilitated by socio-demographic problems that expose the population to sexually transmitted infections.

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Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

ACRV, MOGI and RI designed the study. RI was the general coordinator of the Marajo project. FBF, MBS, STMG and SAA provided technical assistance and executed the experiments. MBS and MAFQ analyzed all data. MBS performed phylogenetic analysis. MBC, MAFQ, ACRV and IMVC wrote the manuscript with input from all authors. All authors read and approved the final manuscript.

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