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ORIGINAL RESEARCH

Ancestry indicative markers in HIV positive patients in the state of Mato Grosso, Brazil

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OBJECTIVE: Ancestry Indicative Markers are used to define the allelic frequency of genes from different ethnic groups in populations of certain localities of interest, for analysis of population ancestry and estimation of ethnic mixture. This work aimed to evaluate the frequency of occurrence of the ancestry Indicative Markers SB-19.3, APO, AT3 / ID and PV-92 and to determine the existence of polymorphisms for these markers in the state of Mato Grosso.
METHOD: The study aimed to estimate allelic and genotype frequencies, adherence to the Hardy-Weinberg equilibrium and genetic differentiation in the sample of 238 controls formed by HIV free individuals residing in twenty-six different municipalities in the state, collected at the Júlio Muller University Hospital and in a sample of 516 HIV-positive patients also residing in the state.
RESULTS: The Hardy-Weinberg equilibrium test revealed an imbalance between the observed and expected proportions of Sb19.3 and APO loci in the control population. Applying the genetic differentiation test, control populations and HIV-positive patients differed for the four loci analyzed.
CONCLUSION: The population of the state of Mato Grosso, Brazil proved to be very heterogeneous, confirming hypotheses about its history of colonization. Control populations and HIV-positive patients differed for the four loci analyzed.

KEYWORDS: Ancestry, Mato Grosso, population.

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INTRODUCTION

Studies of groups of individuals according to the population to which they belong or comparisons between individuals from the same population according to their ethnic groups are relevant to understand the characteristics of diseases, an important subsidy for Public Health.

This type of grouping of individuals according to phenotypic or social characteristics may be complicated, due to high levels of miscegenation presented by most contemporary populations, as is very much the case with the Brazilian population.

Genetic proportions of ancestry in several Brazilian population samples have been investigated with the use of markers. These markers, namely PSAs (Population-specific alleles) or AIMs (Ancestry Informative Markers) are molecular markers that present difference of allelic frequency between populations, being able to detect either the absence or the exclusive presence of a certain allele in a given population.[1] The use of genetic ancestry information can help map genes that influence susceptibility to complex diseases derived from ethnic factors.[2]

The most recent estimates made by the Brazilian Institute of Geography and Statistics-IBGE[3] indicate that the Brazilian population totals 208,042,639 inhabitants; 49% self-classify themselves as white, 37.3% as brown, 8.4% as black, 3.8% as indigenous and 1.5% as yellow.

However, the contribution of each ethnic group to the formation of our population was different, and

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according to Callegari-Jacques et al,[4] of immigrants who arrived in Brazil between 1500 and 1972, 58% were European, 40% African and 2% Asian.

After 500 years of miscegenation between Amerindians, Europeans and Africans, the Brazilian population has become one of the most heterogeneous in the world.[5] The state of Mato Grosso (3.5 million inhabitants) also presents this characteristic with the predominant miscegenation of three ethnic groups: Africans, Amerindians and Europeans.

The relationship between ancestry and genetic diseases is not restricted to any single population group, although differences in the prevalence of some diseases according to race and genotype are evident in several studies.[1-7] It is known that the vulnerability of individuals to certain diseases, as in the case of Acquired Immunodeficiency Syndrome (AIDS), is dependent on several ethnic, genetic, socioeconomic and environmental factors. These added factors drive the spread of HIV/AIDS.[6]

To understand the mechanism of infection and the host's response to the HIV-1 virus we must understand the genetic makeup of the host. It allows us to understand, at least in part, why some individuals are more resistant to certain infections than others. In order to understand the differential progression to AIDS, many studies have been carried out, but the understanding of the inclusion of different populations with different ancestries can contribute to the clarification of genetic factors that influence the infection.[7]

DNA markers such as AT3-I / D, APO, Sb19.3, PV92 point to divergences in the contributions of European, Amerindian, and African ancestry in each region. Such markers make it possible to recover the socio-historical and demographic events of the country.[8-10]

There is a scarcity of studies for the State of Mato Grosso (total area: 900.000 km^2, population 3.3 million; HDI: 0.72, equatorial/tropical climate) evaluating the incidence of AIDS according to the race/color variable. In order to overcome some of this deficiency, this study aimed to analyze the prevalence of ancestral informational marker polymorphisms in samples in HIV-free and HIV-1 virus carriers within the population of the state

MATERIALS AND METHODS

This is an observational, cross-sectional study. A total of 516 HIV-1 infected individuals were collected at the Júlio Muller University Hospital, in the state capital city of Cuiabá, Mato Grosso (population 600,000, HDI 0.78). As controls, samples from 238 seronegative individuals were collected from DNA banks of research projects already completed or in progress. All participants are residents of the state of Mato Grosso and have received basic information on this research, in addition to signing a free and informed consent form approved by a Research Ethics Committee, case # CEP UNIC: 2011-154. The data regarding their age, sex, city of birth, where they currently reside and self-assessment of their skin color is displayed in Table 1. The sampled participants are residents of 26 (out of 141) different municipalities in the state of Mato Grosso.

The inclusion criteria were defined based on the analysis of the records of care and follow-up of these patients and consultation with an infectious physician from the State Health Department, taking into account the ELISA test for anti-HIV antibodies, confirmed by Western blotting and antiretroviral treatment.

Laboratory analysis: Peripheral blood samples from HIV positive patients were collected by venipuncture in tubes containing 0.1% EDTA (final volume in 1 Mg/ dL concentration) and the genomic DNA was extracted from 200 mL using a kit for DNA purification GFX (GE Healthcare Life Sciences of Brazil®), with adaptations.

Peripheral blood samples from seronegative controls (2-4 ml) were collected as described above; the DNA was extracted according to Higuchi & Ochman,[10] with modifications, or through the use of two commercial extraction kits (GE Healthcare Life Sciences of Brazil® and Invitrogen – Thermo Fisher Scientific).

DNA quantifications were performed by Agarose Gel Electrophoresis (140V applied for 30 minutes). After this period, the gel was observed in the translucent (UVP®) and the analysis of the bands indicated the respective DNA characteristics.

We then performed the analysis of the ancestry indicative markers of the ALU insertion types: SB19.3, PV92 and APO and (Indel) – (AT3-I/D). The ALU locus Sb19.3, previously described by Parra et al,[11] belongs to the subfamily Yb8 and is located in chromosome 19p12.[12] The presence of this insert generates a fragment of approximately 457bp and characterizes the Sb 19.3*1 allele. The ALU APO locus described by Batzer et al[13] is close to the apolipoprotein AI-CIII-AIV gene complex on the long arm of chromosome 11. The presence of this insert generates a fragment of approximately 409bp and characterizes the APO*1 allele. The ALU PV-92 locus is located in chromosome 16 and the presence of this insert generates a fragment of approximately 400bp and characterizes the PV92*1 allele; its absence generates a 110bp fragment that characterizes the PV92*2 allele. The AIM AT3-I/D, described by Liu et al.[14] is an insertion/deletion type, also called "indel", which is characterized by the presence of the insertion of exon 1, with 76bp, located in chromosome 1 (1q25.1). The AT3*1 allele, representative of the insert corresponds to the heavier band (572bp) and the AT3*2 allele represents the lighter fragment (Table 2).

Table 1 - Anthropometric data on included patients

	N	Female	Male	Age (years)
HIV-1 infected	516	247	269	40.7
Controls	238	172	66	37.5

Table 2 - Indicating Loci, type of polymorphism and population where allele *1 is more frequent.

Locus	Polymorphism	Allele*1	Allele*2	Population with more frequent Allele*1
Sb 19.3	Insertion ALU	457pb	150pb	European
APO	Insertion ALU	409pb	110pb	European
PV-92	Insertion ALU	400pb	110pb	Amerindian
AT3/ID	76bp indel	572pb	496pb	African

Table 3 - PCR conditions and primer sequences used of the four analyzed AIMs.

Locus	Denaturation (T°C/ time)	Annealing (T°C/ time)	Extension (T°C/ time)	Type	Primer sequence
Sb19.3	94°C/1 min.	58°C/2 min.	72°C/2 min.	Insertion Alu	F 5' TCTAGCCCCAGATTTATGGTAACTG 3' R 5' AAGCACAATTGGTTATTTTCTGAC 3'
APO	94°C/1 min.	55°C/2 min.	72°C/2 min.	InsetionAlu	F 5' AAGTGCTGTAGGCCATTTAGATTAG 3' R 5' AGTCTTCGATGACAGCGTATACAGA 3'
PV92	94°C/1 min.	58°C/2 min.	72°C/2 min.	Insertion Alu	F 5' AACTGGGAAAATTTGAAGAGAAAGT 3' R 5' TGAGTTCTCAACTCCTGTGTGTTAG 3'
AT3-I/D	94°C/45seg.	55°C/1 min.	72°C/1 min.	76bp indel	F 5' CCACAGGTGTAACATTGTGT 3' R 5' GAGATAGTGTGATCTGAGGC 3'

DNA was amplified by PCR (Polymerase Chain Reaction) using an Eppendorf® thermocycler in a total reaction volume of 25µl. After an initial denaturation step of 5 minutes at 94ºC, the samples were amplified for 30 cycles at the denaturation / annealing / extension temperatures specified for each loci, followed by a final extension step of 5 minutes at 72ºC. Table 2 displays specific parameters, type and primer sequences used.

For marker Sb19.3, the amplified products were separated by electrophoresis on 10% non-denaturing polyacrylamide gels[15] and 1% agarose. APO, and PV92 markers were analyzed on 2% agarose gel and stained with GelRed™ (Uniscience®). For the AT3-I/D marker, 10% polyacrylamide gel was used with silver nitrate.

Statistical Analysis: Allele frequency estimates, deviations from Hardy-Weinberg equilibrium expectations and the exact test of population differentiation based on allele or genotype frequencies were performed using GENEPOP software (http:// genepop.Curtin.edu.au).

RESULTS

This study used samples from 238 individuals from the control group, of whom 172 were female and 66 were male; 516 were HIV positive patients, of whom 247 were female and 269 were male.

In the control group, 161 individuals were interviewed and self-assessed for skin color, categorized as yellow, white, indigenous, black or brown.

A total of 215/238 (90.3%) control subjects presented positive results in the amplification of the markers studied. Of the HIV-positive patients, only 277/516 (53.7%) individuals presented positive results in the amplification of the markers studied. The allele frequencies obtained are listed in Table 4.

The Hardy-Weinberg equilibrium test revealed an imbalance between the observed and expected proportions of Sb19.3 and APO *loci* in the control population (Table 5).

Applying the genetic differentiation test, control populations and HIV-positive patients differentiated for the four loci analyzed (Table 6).

DISCUSSION

The history of each population studied is essential in genetic studies of populations that attempt to group individuals according to ethnicity . The genetic constitution of a population with respect to the genes that it possesses is described by the gene frequency, the identification of which alleles are present in each of the loci and their proportions.[17] The challenge of genetic studies of human history is to use genetic differentiation among populations to infer the history of human migrations; because most alleles are common, genetic differences between human populations come mainly from gradations in allelic frequencies.[18]

According to data from IBGE in 2000,[3] there is a greater participation of Africans in the settlement of the northeast region, Amerindians in the North and Europeans in the South.

Considering markers Sb19.3 and APO and their higher frequencies in European populations, it is possible to suggest that miscegenation of the population of the state of Mato Grosso began with populations of European origin; this is justified because of the high frequency of

Table 4 - Allele frequencies

Locus	Allele	Allele frequencies in the control population (215 individuals)	Allelic frequencies in HIV patients (277 individuals)
Sb19.3	1	0.7183	0.9167
	2	0.2817	0.0833
AT3ID	1	0.4000	0.1571
	2	0.600	0.8429
PV92	1	0.1957	0.3920
	2	0.8043	0.6080
APO	1	0.7727	0.7465
	2	0.2273	0.2535

Table 5 - Test for the Hardy-Weinberg Equilibrium (H-W)

Locus	H-W in the controls	H-W in HIV patients
Sb19.3	0.0012 (**)	0.0504 (*)
AT3ID	1.0000 (NS)	0.7193 (NS)
PV92	0.1619 (NS)	0.2380 (NS)
APO	0.0000 (**)	0.0720 (NS)

NOTE: NS: not significant; *: significant; **: highly significant

Table 6 - Population Differentiation Test.

Locus	P value	Standard deviation
Sb19.3	0	0
AT3ID	0	0
PV92	0.00658	0.000756545
APO	0.40881	0.0105555

the *1 alleles of Sb19.3 and APO in this population. This is shown in Table 4.

The observed deviations are due to the great gene flow and high level of miscegenation of the population, which is a marked characteristic of the population of the state of Mato Grosso.

This result resembles that found in the study by Lins et al[2] when studying Brazilian population samples using ancestral the information content of 28 ancestry-informative Single-Nucleootide Polymorphisms (SNPs): they reported that more than half of the individuals sampled had a predominant European ancestry, 52% in the total sample and 32.5% in the sample from the center-west region.

Pena et al[19] carried out an estimation of ancestry in the four Brazilian regions using 40 Indel markers and as a result also observed the predominance of European ancestry with proportions ranging from 60.6% in the Northeast region to 77.7% in the South region.

Using several Microsatellite Markers and AIMs including the AT3-I/D locus, Pedrosa[9] investigated four remaining Brazilian communities of *quilombos* and found an allele frequency for the locus ranging from 0.438 to 0.671 and 0.329 to 0.563 for The AT3 *1 and AT3 *2 alleles, respectively. This difference in comparison to our results can be explained by the fact that the remnant *quilombo* populations have ancestry strongly linked to the Africans, considering the historical context of their formation, the refuge and establishment of the slaves in places of difficult access that gave rise to the isolation.[20,21] Consequently the allelic frequency of insertion in this locus tends to be greater. In the present study, we are dealing not with small and isolated communities, but integrally with the large and mixed population of a very large Brazilian state.

Studies with two other Brazilian *quilombo* populations were carried out by Gontijo.[22] The allelic frequencies found ranged from 0.500 to 0.742 for the AT3*1 allele and 0.258 to 0.500 for AT3*2.

Parra et al[23] studied 9 autosomal AIMs loci (APO, AT3-I / D, GC 1S, GC 1F, FY-Null, LPL, OCA 2, RB 2300 and Sb19.3) in 10 African-American populations of the United States and Jamaica. Their results indicated that the European contribution corresponds to 6.8% in Jamaica and to 22.5% in New Orleans. This finding weakens the concept of grouping considering only the phenotype expressed by an individual.

In another study with the objective of evaluating the genomic ancestry of the Brazilian population, Parra et al[23] sampled (A): 173 individuals from a rural southeastern community, visually classified as whites, blacks, and intermediates; the also sampled (B): 200 individuals from the North, Northeast, Southeast and South regions of the country, using the "African Ancestry Index" (IAA). The results revealed that in Brazil skin color is a poor indicator of ancestry.

Luizon[24] evaluated the polymorphism of eight AIMs (FY-Null, RB, LPL, AT3-I / D, Sb19.3, APO, PV92 and CYP1A1*2C) in three remaining *quilombo* communities compared to samples from two urban populations. This work evidenced a greater miscegenation in the urban populations when compared to remnants of *quilombos* and indigenous tribes, but the subpopulations studied presented significant differences of genetic frequencies among themselves. Due to the population isolation, this portion of individuals tends to differentiate from larger populations that occupy a larger area and resemble favoring a higher frequency of certain alleles within themselves.

Other interethnic estimates also held in Brazil by Santos[25] using 48 markers of indel type, indicate that, as expected, individuals in southern Brazil have an almost exclusively European descent. The indels AIMs panel MID group were optimized and the findings validated the valuable tools for individual and overall estimate of ethnic proportions in admixed populations.

In the state of Mato Grosso specifically, there are no studies that record genetic information of this nature and

there is still a gap in the genetic makeup of the population. New data on the contribution of ancestral populations in HIV patients and in the control population may support a better understanding of the prevalence of differential ancestry within the diseased and may help to advance the use of genomic medicine.

CONCLUSION

This study showed that the use of markers can provide useful data on genetic structure in samples of mixed populations. The population of the state of Mato Grosso proved to be very heterogeneous, confirming hypotheses about its history of colonization. For a better characterization of this population, it would be interesting if more markers were to be used. We also found that control populations and HIV-positive patients differentiated for the four loci analyzed.

CONFLICT OF INTEREST

Authors Declare no conflict of interest relating to this project.

AUTHOR PARTICIPATION

Lenicy Lucas de Miranda Cerqueira: Conceived and designed the experiments; performed the experiments; Analyzed the data; Contributed reagents, materials, analysis tools; wrote the manuscript.

Claudinéia Araújo: Conceived and designed the experiments; performed the experiments; Analyzed the data; Contributed reagents, materials, analysis tools; wrote the manuscript.

Beatriz Santos Ribeiro: Performed the experiments; wrote the manuscript.

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MARCADORES INDICATIVOS DE ANCESTRALIDADE EM PACIENTES HIV POSITIVOS NO ESTADO DE MATO GROSSO, BRASIL.

OBJETIVO: Os Marcadores Indicativos de Ancestralidade (AIMs) são usados para definir a frequência alélica de genes de diferentes grupos étnicos em populações de determinadas localidades de interesse, para análise de ascendência populacional e estimativa de mistura étnica. Este trabalho teve como objetivo avaliar a freqüência de AIMs (SB-19.3, APO, AT3 / ID e PV-92) e verificar a existência de polimorfismos para esses marcadores no estado de Mato Grosso.

MÉTODO: O estudo teve como objetivo estimar as freqüências alélicas e genotípicas, a aderência ao equilíbrio de Hardy-Weinberg e a diferenciação genética na amostra de controles formada por indivíduos residentes em vinte e seis municípios do estado, coletados no Hospital Universitário Júlio Muller e em uma amostra de pacientes HIV positivos também residentes no estado.

RESULTADOS: O teste de equilíbrio de Hardy-Weinberg revelou um desequilíbrio entre as proporções observadas e esperadas dos loci Sb19.3 e APO na população de controle. Aplicando o teste de diferenciação genética, a população controle e os pacientes HIV positivos diferenciaram-se para os quatro loci analisados.

CONCLUSÃO: A população do estado de Mato Grosso mostrou-se heterogênea, confirmando hipóteses sobre sua história de colonização. A população controle e os pacientes HIV positivos diferenciaram-se para os quatro loci analisados.

PALAVRAS-CHAVE: Ascendência, Mato Grosso, população.

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