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HIV genotypes and primary drug resistance among HIV seropositive blood donors in Brazil: role of infected blood donors as sentinel populations for molecular surveillance of HIV

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Abstract

Background—There are few surveillance studies analyzing genotypes or primary (transmitted) drug resistance in HIV-infected blood donors in Brazil. The aim of this study was to characterize patterns of HIV genotypes and primary resistance among HIV seropositive donors identified at 4 geographically dispersed blood centers in Brazil.

Methods—All HIV-infected donors who returned for counseling at the 4 REDS-II Hemocenters in Brazil from January 2007–March 2011 were invited to participate in a case-control study involving a questionnaire on risk factors. Viral sequencing was also offered to positive cases to

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assign genotypes and to detect and characterize primary resistance to RT and protease inhibitors according to WHO guidelines.

Results—Of the 341 HIV seropositive donors who consented to participate in the risk-factor and genetics study, *pol* sequences were obtained for 331 (97%). Clade B was predominant (76%) followed by F (15%) and C (5%). Primary resistance was present in 36 (12.2%; 95% confidence interval [CI] 8.2,15.5) of the 303 individuals not exposed to antiretroviral therapy (ART), varying from 8.2% (95%CI 2,7,13.6) in Recife to 19.4% in São Paulo (95%CI 9.5,29.2); there were no significant correlations with other demographics or risk factors.

Conclusion—Although subtype B remains the most prevalent genotype in all 4 areas, increasing rates of subtype C in Sao Paulo and F in Recife were documented relative to earlier reports. Transmitted drug resistance was relatively frequent, particularly in the city of Sao Paulo which showed an increase compared to previous HIV seropositive donor data from 10 years ago.

Keywords

HIV; Genotypes; Blood donors; Brazil

Introduction

Brazil had 608,230 reported cases of AIDS as of June 2011, the second highest number of cases in the Americas, exceeded only by the United States¹. Monitoring the genetic diversity of HIV-1 in Brazil (and elsewhere) is important to understand the molecular epidemiology and spread of the epidemic. The predominant HIV-1 clade in Brazil is B (~70%), but unlike in the U.S., Brazil also has appreciable numbers of HIV-1 infections caused by clades F, C and circulating recombinant forms (CRFs).^{2,3}

As early as 1991, the Brazilian Ministry of Health provided antiretroviral therapy (ART) through its extensive public health system. In 1996 a law was enacted guaranteeing free access of antiretroviral therapy to all Brazilians who required treatment, according to Brazilian guidelines. The widespread use of ART has led to a substantial decline in AIDS related mortality in Brazil.^{4,5} However, it is expected that the proportion of patients experiencing virologic failure and consequently harboring resistant strains will increase over time. Depending on the behavioral characteristic of these individuals, transmission of drug resistant strains may occur with increasing frequency.⁶ The transmission of resistant variants to uninfected individuals, termed “primary resistance”, raises serious clinical and public health consequences and may dramatically impair the capacity of treating HIV in the near future.^{7,8} Consequently monitoring rates of transmission of drug resistant strains is extremely important in a country like Brazil that provides free ART to approximately 200,000 HIV infected individuals., many of whom have low levels of education and modest resources making compliance with drug regimens and, hence, resistance a serious concern.

The objective of this study was to characterize HIV strains by genotype and primary resistance in recently identified HIV-infected blood donors for whom detailed demographics, risk factors and infection staging data were available. This study was designed to further inform Brazilian surveillance efforts and to complement similar

monitoring of molecular variants among HIV-infected blood donors in the US, other international REDS-II/III sites (China and South Africa), and other countries, thus allowing direct comparisons of these parameters in the donor base on a global level.

Methods

Study Design

This study was part of the Retrovirus Epidemiological Donor Study REDS-II international program.⁹ A case control study comparing HIV seropositive and seronegative blood donors was designed to understand risk exposures and motivations to donate blood among HIV seropositive relative to matched seronegative control donors.¹⁰ A blood sample was also collected from all enrolled HIV seropositive blood donors to characterize HIV genotype and drug resistance.

Subject Recruitment, Enrollment and Questionnaire

We invited all HIV seropositive blood donors who had been counseled for HIV from 2007–2011 in the 4 Brazilian sites of the REDS II study (Fundação Pro-Sangue, Hemominas, Hemope, HemoRio).¹¹ HIV positive donors who were counseled from January 2007 to June 2009 were recruited by a letter, followed by a phone call; subsequent to June 2009 donors were prospectively recruited. If consent was obtained, a questionnaire about HIV risk factors and motivations for donation was administered through a computer audio system (ACASI).¹⁰ For consenting HIV-seropositive subjects, we performed genotype and drug resistance testing on plasma samples obtained at the time of counseling which were rapidly processed and frozen in order to optimally preserve HIV RNA; in contrast the index donation samples was used to define recent infection status (see below), since that sample would best represent the stage of seroconversion and hence duration of infection at the time of the original seropositive donation.

Classification of Donors as Recently Infected by Incidence Assay Testing

For all HIV seropositive donors who had donated during the REDS II period (2007–2010) in the 3 primary REDS-II centers (Hemope, Hemominas and FPS), the index donation sample was saved and submitted to BSRI for testing using a Less Sensitive (LS) or “detuned” enzyme immunoassay (LS-EIA: Vironostika HIV-1 MicroElisa, bioMérieux, Durham, NC, USA) or LS chemiluminescent immunoassay (LS-ChIA: Vitros HIV-1/2 Assay, Ortho Diagnostics, Rochester, NY, USA) as previously described¹². For the Hemorio center, LS-EIA could only be performed if the index donation sample was available.

HIV-1 Clade Typing and Drug Resistance Testing in Sao Paulo

Subtype and resistance analysis was performed at FPS/HSP as previously described.¹³ We have sequenced a fragment encompassing the protease gene and approximately 700 base pairs of the reverse transcriptase (RT) gene¹³. The calibrated population resistance tool (CPR) Version 5.0 beta (available through the Stanford University HIV Drug Resistance Database <http://cpr.stanford.edu/cpr.cgi>) was used to identify transmitted drug resistance mutations in untreated persons.¹⁴ Mutations listed as causing or contributing to resistance are non-polymorphic in untreated persons and apply to all HIV-1 subtypes in accordance

with World Health Organization guidelines.¹⁴ Sequences were submitted to GenBank under the number JQ237931-JQ238236

HIV Sequencing and Viral Load Testing at Abbott Diagnostics

All samples that were refractory to PCR amplification and sequencing in Sao Paulo, representative successfully amplified samples that were classified as clade B, and all samples classified as non-B clade were sent to Abbott Diagnostics (Abbott Park, IL, USA) for viral load testing using the Abbott Real-Time HIV-1 assay (Abbott Molecular, Des Plaines, IL, USA). In addition, samples that were negative by all PCR methods employed in Sao Paulo were retested at Abbott Diagnostics using the ViroSeq HIV-1 Genotyping System v2.0 (Celera, Alameda, CA, USA).

Statistical Analysis

We investigated the relationships between primary drug resistance and HIV subtype with donation characteristics (site, donation type, and first time/repeat donor), demographic characteristics (gender, age, education, and sexual orientation), HIV infection characteristics (recent or long-standing infection and subtype for primary drug resistance), and HIV risk factors. The HIV risk factors were chosen based on their statistically significant relationship ($p < 0.05$) with HIV infection in the HIV Risk Factor Study¹⁰. In addition to the unadjusted relationships shown in Tables 2 and 4, we applied stepwise logistic regression modeling, conditioned on hemocenter, with entry into the model set at $p < 0.20$ and $p < 0.05$ to stay in the model. None of the candidate predictor variables remained in the stepwise models for either the model of correlates of primary resistance or HIV subtype distributions. All statistical analyses were performed using SAS version 9.2 (SAS Institute Inc., Cary, NC).

Results

We enrolled 341 HIV seropositive donors (72 from São Paulo, 110 from Recife, 51 from Belo Horizonte and 108 from Rio de Janeiro). Detailed results of risk factor interviews for these infected donors relative to a group of matched non-HIV-infected controls are reported elsewhere¹⁰.

PCR amplification of HIV *pol* sequences enabling genotype and resistance analyses was successful in the Brazil Central Laboratory for 302 (88.6%) of the 341 cases; 75% were classified as subtype B. Using the ViroSeq HIV-1 genotyping system v2.0, informative sequence data was obtained for 29 of 39 samples that were initially refractory to amplification in Brazil, resulting in a total of 331 (97%) of the 341 genotype results. Of the 29 samples, only 62% were subtype B, suggesting that the original primers used in Brazil were not as sensitive as Abbott's assay for amplification of *pol* sequences and genotyping non-B subtypes.

Figure 1 compares the viral load distributions of the 331 cases that were successfully amplified and genotyped with the 10 refractory samples. Of the 10 samples that were not amplifiable by both the Brazil Central Lab and Abbott, 7 were below the level of RNA detection in the Abbott Real-Time HIV-1 assay and the remaining 3 samples had viral loads of 1.7, 2.8 and 4.3 log₁₀ copies/mL.

Table 1 summarizes the HIV-1 subtype results by collection site. Subtype B was predominant (76%), followed by subtype F (15%) and subtype C (5%). Subtype F had a clear predominance in relation to C in Recife and Rio de Janeiro, while in Sao Paulo and Belo Horizonte the proportion of C was similar to F.

Table 2 presents the subtype results, grouped as B or non-B, according to donor demographics, stage of infection and major behavioral risk factors. The distribution was similar for most variables, although males overall and men who acknowledged homosexual risk behaviors had a higher proportion of subtype B relative to non-B infections, with p values of 0.06. Recent infection status could be determined for 246 index donation samples from donors who had not received ART, with 43 cases (17.5%) classified as having acquired infection within approximately 6 months prior to the seropositive donation; the frequency of non-B HIV-1 subtypes was similar for donors with recent and long standing infections (28% and 24%, respectively). The distributions of HIV viral loads were similar among donors with subtype B and non-B infections (Figure 1).

Of the 331 samples successfully amplified and sequenced, 303 had never been exposed to ART, of these 241 had the index donation plasma sample available for recent infection status. Primary drug resistance could be detected in HIV strains from 36 (11.8%) of the 303 characterized individuals. The majority of the samples (31) were resistant to one drug class (17 to NNRTI, 8 to protease inhibitors and 6 to NRTI), 4 samples to 2 drug classes (NNRTI and NRTI) and 1 to all 3 drug classes. Supplemental Table 1 describes the relevant mutations detected in this population and the impact of these mutations on drug response. Resistance mutations to Efavirenz (EFV) that is used in most first-line treatment regimens in Brazil were detected in 20 (6.6%) cases.

Table 3 presents the drug resistance status of HIV-1 sequences according to the demographic and risk factor characteristics of the donors. Drug resistance could not be associated with any risk factors. There was no significant difference in the rates of resistance relative to duration of infection at the time of donation based on incidence assay testing (15.4% for recently acquired vs. 8.9% for long-standing infections).

Discussion

We analyzed 341 HIV seropositive blood donors from 4 geographically disbursed blood centers in Brazil. This represents successful recruitment and blood sampling for molecular studies of approximately 50% of all HIV seropositive donors identified by these blood centers during the eligibility period. Use of residual plasma samples from the index donations to determine genotypes and particularly primary resistance would be the best study design to avoid bias related to donor return; however Brazilian IRBs do not consistently approve studies using such samples due to lack of specific consent for genetic studies. Consequently our study only generated sequence data on samples from individuals who returned for counseling and consented to participate in the study. No differences were observed in accrual rates by known demographic parameters (not shown), suggesting no ascertainment bias, although this does represent a potential limitation of our study.

The RT and protease gene could be amplified and sequenced in 97% of the samples after supplementing the original PCR assay performed in the Central Lab in Brazil with results from the ViroSeq HIV-1 Genotyping System v2.0. Subtype B was found to be the most prevalent in all four sites, varying from 73% in Recife to 80% in Sao Paulo.

Subtype F was present in 24% (95% CI 15.9,31.9) of HIV-infected donors in Recife, confirming recent results from a study from Cavalcanti¹⁵. Subtype C infections are mainly detected in the South of Brazil, where they represent 50% of recent infections and are associated with heterosexual transmission.^{16–19} In the early 90s, this subtype was not detected in the city of Sao Paulo^{20,21}. Subsequently, we detected clade C in 3.8% of HIV seropositive donations from 1998 to 2002 in the same Sao Paulo center that participated in the current study¹³. Prevalence of subtype C of less than 5% of successfully genotyped infections was also described by others.^{22,23} In the current study, the prevalence of subtype C has increased to 9% (95% CI 2.1,15.3), suggesting that the proportion of infections due to this clade may be slowly increasing in the city of Sao Paulo.¹⁸ Recently, a similar trend was observed by Brígido and colleagues who detected subtype C sequences in 11% of recently diagnosed patient samples from the city of Sao Paulo²⁴.

There are few studies that have evaluated rates of transmitted drug resistance in large sample sets of newly diagnosed subjects in Brazil.^{3,13,25–27} Only three of these studies characterized more than 100 samples from different regions of Brazil. The rates of primary drug resistance in these five large studies varied from 2.2% to 8.1%.^{3,13,25–27} Smaller studies, however, have called attention to the possibility of higher rates of primary resistance in Brazil. Sucupira and colleagues analyzed 75 drug naïve HIV positive individuals in the city of Santos, a port city in southern Brazil, and found 21 (28%) harboring resistant strains²⁸, while Bermudez-Aza et al. evaluated 99 ART naïve homosexual men and found 21% with drug resistance, raising concern that in certain areas or population groups transmission of drug resistant strains may be rapidly increasing.²⁹ Our data show an overall prevalence of primary ART resistance of 12.2% (95% CI 8.2, 15.5), a rate considered “intermediate” by WHO. In Sao Paulo, however, the rate was 19.4% (95% CI 9.5, 29.2), significantly higher ($p=0.02$) than our previous study that detected a prevalence of 6.3% among blood donors from 1998–2002¹³.

Although we have tried to understand the correlates of drug resistance by performing a detailed questionnaire on risk factors including sexual behavior, no clear associations could be found. Recently Bermudez-Aza analyzed homosexual men from 10 cities in Brazil using respondent-driven sampling and found 21% acquired ART resistant viruses²⁹, similar to the prevalence found among homosexual males in our study (17.7%). It is possible that this group has a higher rate of resistance, but our samples size was not large enough to detect a significant difference in relation to other sexual orientation groups.

In conclusion, our data show an increasing diversity of HIV clades and a moderate rate of primary resistance in four sites in Brazil, and in particular an increase in clade diversity and transmitted ART resistance in the city of Sao Paulo. These results further illustrate the important contribution of studies of infected blood donors as sentinel populations for molecular characterization of HIV and other transfusion-transmitted infectious agents.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Correlation of HIV viral load in representative HIV infected blood donations with ability to successfully PCR amplify and sequence HIV RNA (upper panel) and distribution of HIV viral loads in donations determined to harbor genotype B vs non-B infections (lower panel).

Table 1

HIV-1 genotype results for all successfully characterized samples by Hemocenter sites.

Site	Subtypes, n(%)						
	Total	A	B	C	D	F	C/F B/F
Recife	110	0	80 (73)	2 (2)	0	26 (24)	0 1 (1)
Belo Horizonte	51	0	39 (78)	4 (8)	0	5 (10)	0 2 (4)
Sao Paulo	72	1 (1)	55 (80)	6 (9)	0	7 (10)	0 0
Rio de Janeiro	108	0	78 (76)	4 (4)	1 (1)	12 (12)	2 (2) 6 (6)
Total	331	1 (0.3)	252 (76)	16 (5)	1 (0.3)	50 (15)	2 (1) 9 (3)

Table 2

Subtype results by donation characteristics, demographics, HIV infection characteristics, and HIV risk factors.

Characteristic	Subtype B N(%)	Non-subtype B N(%)	P value
Total	252 (76.1)	79 (23.9)	
Site			
Recife	80 (73.4)	29 (26.6)	0.79
Belo Horizonte	39 (78.0)	11 (22.0)	
Sao Paulo	55 (79.7)	14 (20.3)	
Rio de Janeiro	78 (75.7)	25 (24.3)	
Donation Type			
Community	158 (79.0)	42 (21.0)	0.06
Replacement	71 (68.3)	33 (31.7)	
Other	23 (85.2)	4 (14.8)	
Gender			
male	214 (78.1)	60 (21.9)	0.07
female	38 (66.7)	19 (33.3)	
Age			
<30	123 (78.9)	33 (21.2)	0.27
30	129 (73.7)	46 (26.3)	
Sexual Orientation			
Straight/Heterosexual/Refused/DK	152 (73.1)	56 (26.9)	0.06
Bisexual	42 (75.0)	14 (25.0)	
Gay/Homosexual	58 (86.6)	9 (13.4)	
Infection status			
Recent	31 (72.1)	12 (27.9)	0.54
Long term	163 (76.5)	50 (23.5)	
IVDU of Sex Partner of IVDU			
Yes	21 (72.4)	8 (27.6)	0.63
No	231 (76.5)	71 (23.5)	
Sex with HIV+ Partner			
Yes	35 (74.5)	12 (25.5)	0.77
No	217 (76.4)	67 (23.6)	
MSM/Sex Partner of MSM			
Yes	110 (79.1)	29 (20.9)	0.27
No	142 (74.0)	50 (26.0)	

Table 3

Number and percent of subjects with evidence of primary ARV resistance, by donor demographics, HIV risk factors, donation characteristics, and genotypes.

	Total	N(%) of resistance	Likelihood ratio Chi-Square p-value
Total	303	36 (11.9)	
Site			
Recife	98	8 (8.2)	0.21
Belo Horizonte	47	6 (12.8)	
Sao Paulo	62	12 (19.4)	
Rio de Janeiro	96	10 (10.4)	
Donation Type			
Community	185	24 (13.0)	0.64
Replacement	96	9 (9.4)	
Other	22	3 (13.6)	
Gender			
Male	251	30 (12.0)	0.93
Female	52	6 (11.5)	
Age			
<30	144	20 (13.9)	0.30
30	159	16 (10.1)	
Sexual Orientation			
Straight/Heterosexual/Refused/DK	188	19 (10.1)	0.30
Bisexual	53	6 (11.3)	
Gay/Homosexual	62	11 (17.7)	
IVDU of Sex Partner of IVDU			
Yes	25	4 (16.0)	0.52
No	278	32 (11.5)	
Sex with HIV+ Partner			
Yes	40	8 (20.0)	0.11
No	263	28 (10.7)	
MSM/Sex Partner of MSM			
Yes	129	18 (14.0)	0.34
No	174	18 (10.3)	
Infection Status			
Recent	39	6 (15.4)	0.24
Long term	202	18 (8.9)	
Genotype			
B	230	31 (13.5)	0.11
Non B	73	5 (6.9)	