Retrovirus infections in a sample of injecting drug users in Rio de Janeiro City, Brazil: prevalence of HIV-1 subtypes, and co-infection with HTLV-I/II

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Abstract

Background: Retrovirus infections among injecting drug users (IDUs), a core at-risk population for both HIV-1 and HTLV-I/II infections in Brazil, were assessed within an ongoing cooperative research. *Objective:* The study assessed the seroprevalences of HIV-1 and HTLV-I/II infections, as well as the prevalence of HIV-1 subtypes in a sample of IDUs from Rio de Janeiro, Brazil. An attempt to evaluate HIV incidence was carried out using a dual 'sensitive/less sensitive' testing strategy. *Study design:* Cross-sectional evaluation of 175 IDUs. Serostatus for HIV-1 and HTLV-I/II were established by enzyme-linked immunosorbent assays, and confirmed by western blot. The dual testing strategy aimed to estimate HIV-1 incidence rates. Differentiation between HTLV-I and -II was performed by western blot. DNA samples were polymerase chain reaction amplified by a nested protocol, and HIV-1 subtyping was determined by heteroduplex mobility assay. *Results:* Forty-six and 29 samples were found to be, respectively, positive for HIV-1 and HTLV-I/II, 15 of them co-infected by both viruses. Among HTLV-I/II-infected patients, 75.9% were infected by HTLV-I. Thirty-one HIV samples were identified as B subtype, with seven of them showing the typical 'Brazilian B' pattern in the gp120 V3 loop, and ten were identified as F subtype. The use of less sensitive assays for HIV infection wrongly identified a deeply immunocompromised patient as an incident case. *Conclusion:* Moderately high seroprevalences were found for both HIV-1 and HTLV-I/II infections, HIV-1/HTLV-I co-infections being of special concern. A non-statistically significant higher prevalence of F subtype was observed, when compared with the distribution of F/B subtypes among Brazilian patients from other exposure categories. No recent HIV-1 infections were detected, but

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a limitation of the 'sensitive/less-sensitive' testing strategy was made evident. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Injecting drug users; HIV; HTLV; HIV/HTLV co-infection; Sensitive/less sensitive dual testing strategy for HIV infection

1. Introduction

As of June 2000, 190 949 cases (AIDS Epidemiological Bulletin, 2000) of AIDS have been reported in Brazil, with approximately 19% of them reported among injecting drug users (IDUs). IDUs are the second most common form of exposure for HIV-1 infection and AIDS in Brazil, after cases acquired through heterosexual transmission, which accounts for 24% of the AIDS cases registered to date (AIDS Epidemiological Bulletin, 2000). The role of IDUs in the dynamics of the AIDS epidemic has changed dramatically since the middle of the 1980s. Between 1982 and 1986, IDUs represented only 3% of Brazilian AIDS cases. Since 1991, they have represented about 20% or more of the total number of cases. Cocaine is the main illicit drug injected in Brazil, and the reported AIDS cases among IDUs are concentrated along the main cocaine-trafficking routes (Bastos et al., 1999). The increasing number of AIDS cases in this exposure category has resulted in a further increase of the number of AIDS cases reported among IDUs' sexual partners and of the vertical transmission of HIV. In Brazil, around 23% of the women diagnosed with AIDS in the past 5 years were IDUs and, among heterosexuals, 33% were sexual partners of male IDUs (Telles et al., 1997).

Several studies about HIV diversity in Brazil have shown the presence of at least four HIV-1 subtypes so far: B, F, C, and D (Potts et al., 1993; Couto-Fernandez et al., 1994; Lowagie et al., 1994; Morgado et al., 1994, 1998a; WHO National Network for HIV Isolation and Characterization, 1994b; Janini et al., 1996), as well as B/F and B/C recombinant viruses (Sabino et al., 1994; Cornelissen et al., 1996; Gao et al., 1996). Data from the state of São Paulo, where roughly 75% of the AIDS cases among IDUs were registered (Bastos et al., 1999), suggested an association between HIV-1 subtype F and IDUs (Sabino et

al., 1996; Rossini et al., 2001). However, Brazil is a large country, and local differences on HIV/AIDS subepidemics and HIV-1 subtype distribution have been observed (Morgado et al., 1998a). The city of Rio de Janeiro has the second highest prevalence of AIDS cases in the country, with 28 219 cases reported until June 2000; approximately 6% of them among IDUs (AIDS Epidemiological Bulletin, 2000).

In addition to HIV transmission, the common use of injection equipment and drug injecting paraphernalia were shown to be associated with other infectious diseases (Garfein et al., 1996; Oliveira et al., 1999). Among them, human T-cell leukemia virus (HTLV) has been documented in injecting drug user populations in America and Europe (Desgranges et al., 1996). Previous studies have also demonstrated the presence of this virus in certain populations in Brazil (Casseb et al., 1997; Britto et al., 1998; Carvalho et al., 1998; Ishak et al., 1998). A survey in blood donors of five state capitals showed an overall HTLV-I seroprevalence of 0.4% and none of the samples were confirmed positive for anti-HTLV-II (Galvão-Castro et al., 1997).

A 'sensitive/less sensitive' assay testing algorithm has been proposed as a serological strategy to identify recently infected individuals (Janssen et al., 1998) and was validated for estimating HIV incidence in blood donors and homosexual men (San Francisco Men's Health Study) in the USA. Moreover, this serological strategy was recently employed to identify HIV-1 incident infections in a Brazilian prison (Diaz et al., 1999).

In this paper, we present data from a cross-sectional study of injecting drug users in the city of Rio de Janeiro, to provide information on risk behavior, rates of HIV-1 and HTLV-I/II infection, as well as the distribution of HIV-1 different subtypes. Moreover, we used the dual testing strategy to estimate HIV-1 incidence in the studied group.

2. Material and methods

2.1. Study group and questionnaire

A sample of 175 IDUs has been recruited in the streets and treatment centers for drug abuse, from 1994 to 1997, as part of an ongoing cooperative Multicenter Study on HIV/AIDS among IDUs. After signing an informed consent form, people were interviewed using a standard questionnaire based on the WHO Multicenter Study form (WHO International Collaborative Group, 1994a). The questionnaire addressed socio-demographic data, sexual and injecting risk behaviors, and information on the health status of interviewees. Clinical data were assessed by trained physicians, and the patients with HIV/AIDS were scored according to Centers for Disease Control and Prevention (CDC) clinical classification (Centers for Disease Control and Prevention, 1992).

2.2. Whole blood and CD4+ count evaluation

Five milliliters of ethylenediamine tetraacetic acid-anticoagulated blood and 10 ml blood without anticoagulant were obtained from each patient. CD4+ T-cell determinations were carried out by flow cytometry using whole blood, and scored as < 200 cells/mm^3, $200 < CD4 < 400$ cells/mm^3 and > 400 cells/mm^3. After centrifugation, the plasma was collected, aliquoted and stored at $-20°C$. The pellet (2–3 ml) was suspended (v/v) in 0.5% Saponin/0.4% NaCl, thoroughly vortexed, centrifuged ($400 \times g$, 5 min, room temperature), washed twice with phosphate-buffered saline by centrifugation in the same conditions, and stored for DNA extraction. Ten milliliters of blood without anticoagulant were also obtained and the sera were aliquoted, stored at $-20°C$ and used for serological tests.

2.3. HIV and HTLV serostatus

Serostatus for HIV-1 (Ortho HIV-1/HIV-2 Ab-Capture ELISA Test System; Ortho, NJ, USA; HIV-1 Uniform II; Organon, Boxtel, The Nether-lands) and HTLV-I/II (HTLV-I/II Cambridge Biotech, Worcester, USA) were determined by enzyme-linked immunosorbent assay (ELISA), and HIV-1 infection was confirmed by western blot assay (Cambridge Biotech Corp., Worcester, USA). Confirmation of HTLVI/II infection and differentiation between HTLV-I and -II were performed by western blot (HTLV Blot 2.4; Genelabs, Science Park Drive, Singapore).

2.4. DNA preparation and polymerase chain reaction amplification of HIV-1 env sequences

Genomic DNA was extracted using a phenol/chlorophorm protocol and DNA samples (≈ 1 µg) were polymerase chain reaction (PCR)-amplified by a nested protocol as previously described (Morgado et al., 1998a), using ED3/ED14 as the outer primer set and ED5/ED12, ES7/ES8 or ED31/ED33 as inner primer sets.

2.5. Heteroduplex mobility assay

HIV-1 subtyping was determined by heteroduplex mobility assay (HMA) as described elsewhere (Delwart et al., 1993). Briefly, 5 µl ED31/ED33 or ED5/ED12 PCR-amplified products were mixed with 5µl PCR-amplified plasmids (94°C for 3 min, followed by incubation in ice for 10 min) containing *env* fragments of the HIV-1 subtypes A–H reference samples (Delwart et al., 1995), provided by the NIH AIDS Research and Reference Reagent Program. Each unknown sample was tested respectively against three reference plasmids of HIV-1 subtypes B, and two of C, D and F.

2.6. Restriction fragment length polymorphism determination

After the second round of PCR with ED31/ED33 primers, 10µl HIV-1-amplified DNA were digested with 6 U *Fok I* restriction enzyme for 2 h at 37°C, electrophoresed through 2% agarose gels, and the restriction fragments were evaluated under UV illumination, as previously described (Morgado et al., 1998a,b).

2.7. Dual testing strategy

A sensitive 3A11 ELISA immunoassay (EIA) (Abbott Laboratories, Chicago, IL, USA) and a less sensitive version of the same EIA were used according to Janssen et al. (1998). HIV incidence for each studied group will be measured based on the following formula proposed by the authors: $I_{ins} = (n_{ins}/N) \times (365/w) \times 100$, where I_{ins} is the number of persons with 3A11 reactive/3A11 insensitive reactive results, N is the number of persons with HIV-negative results plus the number with 3A11 reactive/3A11 insensitive test results, and w is the estimated mean in days between seroconvertion on the 3A11 and 3A11 insensitive test.

3. Results

From the 175 IDUs participating in this study, 44 (25.1%) were recruited in the streets and 131 (74.9%) in drug treatment centers. The sample was composed mostly of males (84.2%), and had a mean age of 33.3 years (S.D., 7.9). The vast majority was intravenous users of cocaine.

The serostatus for HIV-1 and HTLV-I/II was established for 171 samples. Four plasma samples were lost during laboratory manipulation, and the individuals were not available for further blood collection. Of the 171 samples analyzed, 46 (26.9%) were positive for HIV-1. From this group, 15 patients (32.6%) had CD4+ counts < 200/mm³, 11 patients (23.9%) had CD4+ counts between 200 and 400/mm³, and 19 (41.3%) had CD4+ counts > 400/mm³. The CD4+ count was not available for one sample.

As shown in Table 1, most interviewees were males in their early thirties, with a low educational level. Unsafe injecting practices were common, and strongly and consistently (through different variables) associated with both infections (HIV and HTLV). Most of the interviewees have been engaged in different sexual partnerships, most of them unprotected (data not shown), although not a single variable related to sexual risk behaviors was found to significantly differ between those with and without HIV and/or HTLV

infections, highlighting the core role of parenteral exposure in this population. Elsewhere (Oliveira et al., 1999; Telles et al., 1997), we explored in more detail the main risk factors for different infections (HIV and viral hepatitis) in this population through multivariate analyses.

3.1. 'Less-sensitive' enzyme immunoassay

Thirty-eight serum samples, two from HIV-1-seronegative and 36 from HIV-1-seropositive individuals were tested using the dual testing strategy to detect early infected individuals (less than 129 days in average) in order to evaluate the seroincidence of HIV infection in the IDUs from Rio de Janeiro. The two seronegative samples (used as internal controls) were confirmed negative and one of the seropositive samples was also negative in the less sensitive assay. However, contrary to what was expected, this sample was obtained from an individual seropositive since 1996, already immunocompromised, with CD4+ counts < 200/mm³. Therefore, no true incident case was found in the studied group.

3.2. HTLV-I/II co-infection

Positivity for anti-HTLV-I/II antibodies was detected in 29 (17.0%) of the 171 plasma samples tested. Western blot assay was able to discriminate 22 (75.9%) samples as HTLV-I, and seven (24.1%) as HTLV-II infections. HIV-1/HTLV-I/II co-infections were verified among 15 (32.6%) out of the 46 HIV-1-positive individuals. Indeed, ten (45.5%) out of the 22 HTLV-I-positive individuals and five (71.4%) out seven HTLV-II-positive ones were co-infected with HIV-1 (Table 2).

3.3. HIV-1 env subtyping by HMA and RFLP with Fok I restriction enzyme

From the 41 HIV-1-positive individuals analyzed by HMA, 31 (75.6%) could be identified as infected by B subtype and ten (24.4%) by subtype F. DNA samples from five patients did not yield PCR-amplified products to be typed. We assessed possible associations between HIV-1 subtypes and clinical and socio-demographical parameters; however, no association was found.

Table 1
Main socio-demographic characteristics, parenteral and sexual risk behaviors among injection drug users, with and without HIV and/or HTLV infection, Rio de Janeiro, 1994–1996[a]

Variables	Non-infected (i.e. HIV−/HTLV−) (n = 111)	Infected by HIV and/or HTLV (n = 60)	P value	HIV-infected (n = 46)	HTLV-infected (n = 29)	HIV-/HTLV-infected (n = 15)
Gender, male (%)[b]	90/107 (84.1%)	50/59 (84.7%)	0.914	37/45(82.2%)	23/28(82.1%)	10/14(71.4%)
Age (years) (mean)[c]	32.82	33.37	0.680	32.22	36.9	36.73
Educational level (<8 years)	37/104 (35.6%)	28/58 (48.3%)	0.114	22/44(50.0%)	14/28(50.0%)	8/14 (47,1%)
Injected at least once before 18 years old	42/101 (41.6%)	24/55 (43.6%)	0.804	19/43(44.2%)	12/25(48.0%)	7/13(53.8%)
Needle sharing (%) (ever)	62/102 (60.8%)	50/56 (89.3%)	0.000	38/43(88.4%)	24/26(92.3%)	12/13(92.3%)
Needle sharing (last month)	8/109 (7.3%)	9/51 (17.6%)	0.049	7/37 (18.9%)	4/26 (15.5%)	2/12 (16.7%)
Shared with more than two different persons in the last month	3/103 (2.9%)	9/57 (15.8%)	0.009	6/43(14.0%)	5/28(17.9%)	2/14(14.3%)
Had more than two occasional partners (past 6 months)	4/103 (38.8%)	22/56 (39.3%)	0.956	17/93(39.5%)	13/26 (50.0%)	8/13(61.5%)
Any male homosexual intercourse (%)[d]	69/87 (79.3%)	34/48 (70.8%)	0.268	26/35(74.3%)	15/22(68.2%)	7/9 (77.8%)
Shared injection equipment outside of the town	18/101 (17.8%)	21/56 (37.5%)	0.006	19/44(43.2%)	6/26(23.1%)	4/14 (28.6%)

[a] Comparisons and P values refer to interviewees neither infected by HIV nor by HTLV versus those infected by at least one of these viruses
[b] Chi-square or Fisher's exact test was used for categorical variables.
[c] Student t for means was used for discrete variables.
[d] Refers to any homosexual intercourse in the past 5 years (n = 166 men).

One important aspect to be assessed by studies in the field of HIV-1 molecular epidemiology in Brazil is the distinction between the classical North American/European subtype B samples, from the typical Brazilian HIV-1 subtype B samples presenting the antigenically distinct GWGR amino acid sequence in the crown of the V3 loop. The digestion of the 600 bp ED31/ED33 PCR-amplified DNA products, previously typed by HMA as B subtype, with *Fok I* restriction enzyme, generates two patterns of two (400 bp–200 bp) or three (400 bp–120 bp–80 bp) restriction fragments. Using this approach, seven (22.6%) out of the 31 subtype B samples from Rio de Janeiro City were digested with *Fok I* and identified as corresponding to the typical GWGR subtype B samples found in Brazil, whereas the other B samples were not digested with this enzyme.

4. Discussion

The seroprevalence of 26.9% for HIV-1 antibodies in IDUs, verified in this study, was similar to the other studies conducted in Rio de Janeiro, which show, in general, moderate to high HIV seroprevalence rates among IDUs in Rio (14–33%), and high levels of risk behavior (Telles et al., 1997; Bastos et al., 1999). No significant difference between the HIV seroprevalences in females and males was observed, unlike those detected in a recent survey in Salvador (BA),

Table 2
Summary of serologic data for HIV-1, HTLV-I and HTLV-II in a sample of IDUs in Rio de Janeiro, Brazil 1994–1996

	HIV-1 (+)	HIV-1 (−)	Total
HTLV-I (+)	10 (45.5%)	12 (54.5%)	22 (13.0%)
HTLV-II (+)	05 (71.4%)	02 (28.6%)	07 (4.0%)
HTLV-I/II (+)[a]	–	01 (100%)	01 (0.6%)
HTLV-I/II (−)	29 (21%)	109(79%)	138 (80.7%)
ND[b]	02 (66.7%)	01 (33.3%)	03 (1.7%)
Total	46 (27%)	125 (73%)	171 (100.0%)

[a] Without discrimination between HTLV-I and -II.
[b] Not determined.

where the seroprevalence of HIV infection in the female IDUs was 74.4%, higher than observed in the male IDUs (44.1%) studied in the same group (Dourado et al., 1999).

A higher prevalence, although non-statistically significant, of the F subtype was verified in the IDU group when compared with other exposure categories prevalent in Rio de Janeiro City, Brazil, as described in a previous report (Morgado et al., 1998a). Moreover, the subtype distribution showed to be associated only with gender, as three out four HIV-1-seropositive females evaluated in this study were infected with the F subtype. However, a very low number of female injecting drug users could be enrolled for this study, probably reflecting a true restricted number of female IDUs in the Rio de Janeiro's drug scene (Telles et al., 1997; Bastos et al., 2000), a picture very different from the one observed in other Brazilian settings, for instance, with a much higher number of female IDUs in Santos, state of São Paulo (Carvalho et al., 1996).

The present study did not show any clear relationship between HIV-1 subtypes and IDUs when compared with other exposure categories in Rio de Janeiro, mainly sexually transmitted infections (heterosexual, homosexual and bisexual), as made evident by former publications of our research group (Morgado et al., 1998a), where similar frequencies of subtypes B and F have been observed. This finding differs from published data based on the patients attended in an AIDS clinic from city of São Paulo (Sabino et al., 1996), which was further confirmed based on an expanded survey (Rossini et al., 2001).

In this study, we also tried to estimate the incidence of HIV-1 infection in IDUs from Rio de Janeiro using the EIA double testing strategy. No specific effort was made to recruit new injectors (Friedman et al., 1998), making this a sample of quite old injectors as described elsewhere (Oliveira et al., 1999). Only one sample, corresponding to a subtype B infected individual, was found to be negative by the 'less sensitive' assay. However, this individual has been long-term infected at the time of blood collection and was already immunocompromised, confirming that caution has to be taken in the interpretation of this assay when the

broad immunological profile is not available. Both subtype B and F individuals gave similar seropositive results in the 'sensitive' and 'less sensitive' assays used for this study. A possible complication in the use of the 'less sensitive/sensitive' dual testing strategy could be rendered by the higher anti-HIV-1 antibody reactivity observed by multivariate statistical analysis for those IDUs in comparison with sexually infected individuals (Bongertz et al. 1999).

High prevalence of HTLV-I/II infection and HIV-HTLV co-infection was observed in these samples. Although the frequency of HTLV-II was lower than the HTLV-I in our IDU study group, it was highly associated with HIV-1 co-infection. No previous data on HTLV-I/II infection among IDUs were available for Rio de Janeiro, so comparisons could only be made with data from Santos, São Paulo (Carvalho et al., 1998), and Salvador, Bahia (Dourado et al., 1999). Samples from IDUs, including those presented in the present study, and from former studies carried out in IDUs in Salvador (Dourado et al., 1999) and Santos (Carvalho et al., 1996), show a clear gradient in the seroprevalences for HTLV infection ($P = 0.000$), with the highest seroprevalence levels found for Salvador, intermediate levels for Santos, and the lowest for Rio de Janeiro.

The aforementioned national study showed exactly the same pattern for blood donors (Galvão-Castro et al., 1997), suggesting IDUs, due to their risky parenteral and sexual behaviors, are under particular risk for HTLV infection, but that, far from being a segregated population, they seem to exacerbate background infection patterns prevailing in the so-called general population, probably due to transmission of HTLV through unprotected sex between injecting drug users and their non-injecting sexual partners.

HIV/HTLV-I/II co-infection can pose problems in the field of clinical research and therapeutics, as was demonstrated by former studies (Schechter et al., 1997). The higher CD4 + lymphocyte counts observed in HIV/HTLV-I/II co-infection do not provide an immunological benefit, and may rather reflect HTLV-I-associated non-specific lymphocyte proliferation. However, we could not verify high levels of CD4 + counts in the co-in-

fected individuals. Indeed, in the present study, 7/10 individuals co-infected with HTLV-I and 2/5 individuals co-infected with HTLV-II had CD4 counts below 200/mm³.

Activities directed towards the prevention of infectious diseases, especially in developing countries, where both resources and expertise are restricted, have basically addressed HIV/AIDS prevention. Viral hepatitis is also a matter of recent concern (Oliveira et al., 1999). HTLV-I/II infections have not merited attention thus far, although recently available data (Carvalho et al., 1996; Andrade et al., 1998) and the present data point to a disquieting picture among Brazilian IDUs.

In contrast with the high prevalence of HTLV-II infections in North American and European IDUs, most infections among the Brazilian IDUs were caused by HTLV-I, which is of concern since HTLV-I is the etiologic agent of both tropical spastic paraparesis and T-cell leukaemia/lymphoma. Comprehensive surveys with larger samples are urgently needed, to allow analyses of risk factors for HTLV-I/II infection in different settings.

Health care workers must be trained to systematically screen for HTLV-I/II for patients reporting risk behaviors and/or from endemic areas, a task that presents many difficulties in routine practice (Carvalho et al., 1998). Recent infections must be diagnosed promptly, in order to prevent further spread among injecting and sexual partners of IDUs and their offspring.

Preventive initiatives targeting the different blood-borne infections should be fully implemented in this population, including counseling, testing, provision of condoms and sterile injection equipment, enrollment of the infected individuals in follow-up studies, and the offer of the best practice available for the patients already ill.

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