Estimating HIV-1 Incidence Using the Serologic Testing Algorithm for Recent HIV Infections at HIV Counseling and Testing Centers in the City of São Paulo, Brazil

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The network of HIV counseling and testing centers in São Paulo, Brazil is a major source of data used to build epidemiological profiles of the client population. We examined HIV-1 incidence from November 2000 to April 2001, comparing epidemiological and socio-behavioral data of recently-infected individuals with those with long-standing infection. A less sensitive ELISA was employed to identify recent infection. The overall incidence of HIV-1 infection was 0.53/100/year (95% CI: 0.31-0.85/100/year): 0.77/100/year for males (95% CI: 0.42-1.27/100/year) and 0.22/100/year (95% CI: 0.05-0.59/100/year) for females. Overall HIV-1 prevalence was 3.2% (95% CI: 2.8-3.7%), being 4.0% among males (95% CI: 3.3-4.7%) and 2.1% among females (95% CI: 1.6-2.8%). Recent infections accounted for 15% of the total (95% CI: 10.2-20.8%). Recent infection correlated with being younger and male (p = 0.019). Therefore, recent infection was more common among younger males and older females.

Key-Words: HIV-1/immunology, HIV infections/epidemiology, HIV seroprevalence, seroepidemiologic studies.

In December 2001, 51,841 AIDS cases were reported in the city of São Paulo, corresponding to 22% of all AIDS cases reported in Brazil as a whole (http://www.aids.gov.br). The HIV-1 epidemic in Brazil is characterized by the number of reported AIDS cases and the number of HIV-infected pregnant women (and their infected children), as well as HIV-1 sentinel surveillance of certain population groups. In several studies, recent HIV-1 infection has been described using a less sensitive ELISA [1-4]. This detection strategy, known as the serologic testing algorithm for recent HIV seroconversion (STARHS) [3], has been used to estimate HIV-1 incidence and to investigate epidemiological aspects in newly-infected individuals.

The city of São Paulo, where nearly 25% of all Brazilian AIDS cases occur, is recognized as the epicenter of the AIDS epidemic in Brazil (http://www.aids.gov.br). The São Paulo Municipal STD/HIV Network comprises 22 outpatient clinics located throughout the city. One important element of this network is the service provided by HIV counseling and testing (HIV CT) centers, which conduct STD/HIV testing and provide prevention counseling. The health care system is also an important source of data, enabling studies of HIV-1 prevalence and epidemiological profiles of client populations.

In the STARHS methodology, serum samples testing positive for HIV-1 in the initial ELISA are retested. In the second test, a less sensitive ELISA is performed. The less sensitive ELISA protocol uses higher serum dilutions and shorter incubation periods. If a sample tests positive in the conventional ELISA and negative in the less sensitive ELISA, the viral infection is presumed to have occurred within the preceding 170 days, and the patient is classified as having been recently infected with HIV-1. This system allows the incidence of recent infection to be estimated.

We used the STARHS methodology to calculate the number of recent HIV-1 infections in samples collected at HIV CT centers between November 2000 and April 2001. We also studied the epidemiological and socio-behavioral aspects of HIV-positive individuals that had been recently infected, as well as those of individuals presenting long-standing infection.

Material and Methods

Study Design

This was a cross-sectional study using information gathered from questionnaires administered between November 2000 and April 2001 during routine HIV CT center visits before and after testing. Five HIV CT centers were involved in this study and are identified by their locations: Henfil, Lapa, Pirituba, Santo Amaro, and São Miguel.

STARHS

Patient blood samples were collected in vacutainer tubes (Becton-Dickinson, Rutherford, NJ, USA). On the same day, samples were transferred to one of the three public reference laboratories for storage. All samples were tested twice, using conventional HIV-1 ELISA. Double-negative samples were considered HIV-1 negative. Samples for which the ELISA results were inconclusive, as well as those testing positive for HIV-1 only once, were further analyzed by Western blot according to the guidelines established by the Brazilian Health Ministry [3].

Samples classified as HIV-1 positive were sent to the Retrovirology Laboratory of the Federal University of São Paulo to be analyzed using STARHS. The Vironostika HIV-1 Micro-ELISA System kit was used (LS HIV-1 EIA; Organon Teknika, Durham, NC, USA), according to the manufacturer's instructions.

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Serum samples were diluted to 1:20,000, and incubation times were modified. Vironostika negative controls, Centers for Disease Control and Prevention calibrators, high-positive controls, and low-positive controls were also diluted and incubated according to the manufacturer's instructions. Sample selection and control analysis were performed in triplicate. Sample optical density (SOD) was calculated using the equation:

$$SOD = (OD_{sample} - OD_{median\ negative\ control}) / (OD_{median\ calibrator} - OD_{median\ negative\ control})$$

Samples in which the SOD screening value was below 2.0 were tested in triplicate as a confirmatory measure. The SOD was calculated based on median OD values measured in triplicate. Samples in which the confirmed SOD value was below 1.0 were determined to have originated from patients having acquired the HIV-1 infection within the preceding 170 days [3].

Statistical Analysis

In our analysis, the outcome variable was the timing of HIV-1 infection (recent or not). Explanatory and control variables included ethnicity, education, marital status, type of exposure, and use of condoms. The annual HIV-1 incidence, expressed as the percentage of new cases/year that were identified as cases of recent infection, was calculated for the population as a whole and by gender using the estimator included in the Vironostika-LS protocol:

$$\%Per\ year = \frac{number\ of\ recently\ infected\ patients}{Number\ of\ HIV-1\ negative\ individuals + number\ of\ recently\ infected\ patients} x \frac{365.25^{*} x100}{170}$$

*Study period = 182.5 days.

The prevalence of HIV-1 infection was calculated as the ratio between the number of HIV-1-positive individuals and the number of individuals tested in the first sample. The 95% confidence intervals (95% CIs) were calculated for incidence and prevalence of recent HIV-1 infections. These 95% CIs were used for estimation and to compare prevalence and incidence by gender. Correlations between the outcome and explanatory variables were identified using the chi-square test or Fisher's exact test, together with linear trend tests. The Epi Info, version 6.0 [5], and Stata, version 7.0 (Stata, College Station, TX, USA) programs were used to construct and analyze the database.

The Ethics in Research Committee of the Training and Reference Center for STD/AIDS (São Paulo State Health Secretariat) approved the study, and all participants gave written informed consent.

Results

Six thousand individuals (3,486 males and 2,514 females) were tested, of which 194 (140 males and 54 females) were found to be HIV-1 positive and were included in our study sample. Among those 194, 105 had been tested at Henfil, 10 at Lapa, 9 at Pirituba, 23 at Santo Amaro, and 31 at São Miguel. The overall prevalence of HIV-1 infection was 3.2% (95% CI: 2.8–3.7%). Prevalence varied by gender, being 4.0% among males (95% CI: 3.3–4.7%) and 2.1% among females (95% CI: 1.6–2.8%). Among the individuals identified as HIV-1 positive, 15% were classified as having been recently infected (95% CI: 10.2–20.8%). The overall incidence of recent HIV-1 infection, regardless of gender, was 0.53/100/year (95% CI: 0.31–0.85/100/year), being 0.77/100/year for males (95% CI: 0.42–1.27/100/year) and 0.22/100/year for females (95% CI: 0.05-0.59/100/year). Cases of recent infection tended to occur more frequently in individuals under 29 years of age (p = 0.043: 19/29; 65.5% *versus* 67/165; 40.6%). This tendency was stronger for men under 29 years of age (p = 0.019 for men:18/24; 62.9% *versus* women: 1/5; 5.6% - data not shown). The patterns of age distribution among recently infected individuals differed by gender (Figure 1). The incidence among males increased significantly by 20 years of age, peaking at 25 and dropping markedly thereafter. Among women, there was a trend towards increased incidence after 25 years of age, peaking at 35 and decreasing thereafter. t Recent HIV-1 infection (Table 1) did not correlate significantly with ethnicity (p = 0.702), education (p = 0.555), marital status (p = 0.178), employment status (p = 0.810), income (p = 0.549), use of condoms (p = 0.480), type of exposure (p = 0.314), or number of partners (p = 0.851).

Discussion

We examined the incidence of HIV-1 among individuals seeking counseling or treatment at the HIV CT centers of the STD/AIDS network in the city of São Paulo. Rates of recent infection were estimated using the STARHS methodology, which has been successfully employed for this purpose in various studies [2,6-9].

The HIV-1 prevalence that we observed (3.2%) was lower than the 4.4% reported among HIV CT center users in a previous study conducted in the city of São Paulo[10] and the 7.1% found among such clients in the city of Santos, also located in the state of São Paulo[11]. However, it was higher than the 2.1% observed in the city of San Francisco, California (USA) [2].

Similar numbers of men and women were tested at the HIV CT centers. However, HIV-1 infections were more prevalent among men, likely reflecting historical aspects of the local epidemic in terms of demographics and exposure. Alves et al. found the same to be true for HIV CT center users in the city of Santos [11]. We found that the incidence of recent HIV-1 infection was similar for men and women, which is also in agreement with the findings of Alves et al. [11] In view of the trend towards a decrease in the male-to-female ratio of AIDS cases in Brazil, which dropped from 23:1 in the mid-1980s to 1.5:1 in 2005 (www.prefeitura.sp.gov.br/dstaids), these findings were expected.

The incidence of recent HIV-1 infection observed in our study (0.53/100/year) was lower than that described in

Table 1. Distribution of individuals by socio-demographic and HIV-1 serological status. Data compiled from November 2000 to April of 2001 from four counseling and testing centers of the São Paulo Municipal Health Network System.

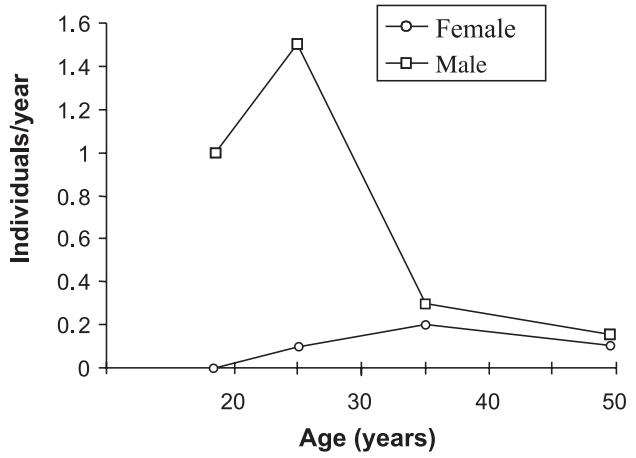
Characteristic	Recent infection				p
	Yes		No		
	N	%	N	%	
Gender					
Male	24	17.1	116	82.9	0.168*
Female	5	9.3	49	90.7	
Age (years)					
<20	3	33.3	6	66.7	0.043†
20–29	16	20.8	61	79.2	
30–39	5	7.6	61	92.4	
>40	5	11.9	37	88.1	
Ethnicity					
White	16	15.5	87	84.5	0.702†
Black	4	21.1	15	79.0	
Mixed	8	18.2	36	81.8	
Asian	0	-	7	100.0	
Education					
None	12	15.6	65	84.4	0.555†
Elementary School	7	15.2	39	84.8	
High School	9	17.7	42	82.4	
University Degree	0	-	12	100.0	
Marital Status					
Single	21	18.9	90	81.1	0.178*
Married	7	11.1	56	88.9	
Employment Status					
Employed	22	14.6	129	85.4	0.810*
Unemployed	7	16.3	36	83.7	
Income‡					
0	3	11.5	31	20.7	0.549†
1–2	3	11.5	27	18.0	
3–4	13	50.0	62	41.3	
5–6	7	26.9	30	20.0	
Exposure Category					
Heterosexual	11	12.5	77	87.5	0.314*
Homosexual	10	22.2	35	77.8	
Bisexual	7	18.0	32	82.1	
Use of Condoms					
Always	6	11.5	46	88.5	0.480†
Sporadic	17	19.5	70	80.5	
Never	5	14.7	29	85.3	
Number of Partners					
Single	8	17.0	39	83.0	0.851*
Multiple	19	15.8	101	84.2	

*Pearson's chi-square test. †Fisher's exact test. ‡Expressed as the number of times the local minimum wage.

previous studies involving diverse population groups (men who have sex with men, injectable drug users, and others) and analysis of stored serum samples. Studies conducted in Brazil have reported incidence rates varying from 1.2 to 2.8/100/year [10,11].

In our study, 60% of the individuals tested were men who have sex with men. Nevertheless, we found no significant difference between men who have sex with men and heterosexual men in terms of recent infection (p = 0.314; Table 1). This might indicate either similarity between the two exposure groups or low statistical power to detect differences (related to the small sample size). A sound estimate of HIV-1 incidence and the epidemiological aspects of newly-infected populations will only be possible when larger numbers of

Figure 1. Distribution of HIV-1 patients by gender and age. Data compiled from November 2000 to April 2001 from four counseling and testing centers within the São Paulo Municipal Health Network System.



recently-infected individuals are identified using STARHS. We noted that a significant correlation between recent infection and age was observed only for males that were bisexual; among 39 bisexual males 100% (7/7) presented recent infection *versus* 28% of the individuals with long standing infection (9/32, p = 0.003). This group seems to be at greater risk of acquiring HIV-1 infection in this region of Brazil; similar findings have been reported for young gay men in other areas of the world [12]. However, among women living in this region, the risk of recent HIV-1 infection seems to peak at a later age (Figure 1). Further prospective studies should be conducted in order to confirm these differences and seek to explain them. In addition, molecular epidemiological studies characterizing cases of recent HIV-1 infection will certainly lead to a better understanding of HIV-1 epidemics and their trends in terms of the profile of the virus and primary resistance to antiretroviral drugs. We recognize the fact that the retrospective nature of this study, as well as the small number of individuals analyzed, might have limited our evaluation of epidemiological aspects. However, as previously mentioned, preliminary analyses generated here could provide insight for the design of future studies. We also understand that analysis of demographic and behavioral data from HIV-seronegative individuals seeking testing in the Counseling and Testing Centers would provide more insight into the nature of HIV epidemics in Brazil; unfortunately, these data were not available to us.

Identifying cases of recent HIV-1 infection has clinical implications, allowing the implementation of early intervention measures, which could result in better control of virus replication and propagation during primary infection. This strategy might also help define the inclusion criteria for therapeutic and pathogenesis studies. In addition, since it also identifies populations at greater risk for HIV-1 infection, profiling the recently infected could be considered a prevention tactic. Furthermore, the inclusion of HIV-1 seronegative individuals in studies focusing on recent infections may help us understand other components of this epidemic and thereby lead to the development of better intervention measures.

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