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Rate and Incidence Estimates of Recent Human Immunodeficiency Virus Type 1 Infections among Pregnant Women in São Paulo, Brazil, from 1991 to 2002

Carmem A. de Freitas Oliveira,[1]* Mirthes Ueda,[1] Rosemeire Yamashiro,[1] Rosāngela Rodrigues,[1] Haynes W. Sheppard,[2] and Luís Fernando de Macedo Brígido[1,3]

Instituto Adolfo Lutz, Secretaria de Estado da Saúde,[1] and National Program on STD and AIDS, Ministry of Health,[3] São Paulo, Brazil, and Viral and Rickettsial Disease Laboratory, California Department of Health Services, Berkeley, California[2]

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The serological testing algorithm for recent human immunodeficiency virus (HIV) seroconversion (STARHS) was employed to estimate HIV incidence among pregnant women from São Paulo, Brazil. A cross-sectional study (1999 to 2002) showed an incidence of infection of 0.2 per 100 pregnant women per year (95% confidence interval, 0.041 to 0.608). Western blot profiles suggested an association between results of the STARHS analysis and gp41/gp31 bands.

Epidemiological data have shown that human immunodeficiency virus (HIV) and AIDS transmission patterns have been changing in Brazil in the last few years. The increase in the reported number of AIDS cases associated with heterosexual transmission was accompanied by an increase in the proportion of AIDS among females. In São Paulo, as well as in the rest of Brazil, the male/female ratio among AIDS cases reported for individuals older than 12 years has fallen from 6:1 in the middle 1980s to less than 2:1 in 2002 and 2003 (21, 22). Monitoring the spread of HIV among healthy, sexually active women is an important element in tracking epidemic trends among heterosexual people and in supporting strategies for the prevention of mother-to-child HIV transmission. According to the Brazilian Ministry of Health cross-sectional sentinel serosurveys, conducted since 1997, HIV seroprevalence among delivering mothers has ranged from 0.00 to 2.41% (95% confidence intervals [CI] range from 0.00 to 4.74%), depending on year of evaluation, geographic region, and age groups (23). An assessment of incident infections (recently acquired infections) might provide useful information to better understand the epidemic dynamics among this low-prevalence segment of the population, potentially in real time for prevention strategies. The serologic testing algorithm for recent HIV seroconversion (STARHS) (14), also referred to as the "detuned" enzyme immunoassay (EIA), employs a sensitive-less sensitive EIA testing strategy and has been used to estimate HIV incidence from HIV-reactive serum samples (3–5, 9–17, 24–26, 30–33). Other laboratory tools, also based on antibody assays, have been evaluated for the detection of recent HIV infections by assessing specific reactivity against virus antigens (8, 18–20, 28).

In the present study, we applied the STARHS in a cross-sectional analysis to estimate seroincidence among specimens from pregnant women (PW) from São Paulo, Brazil, and investigate the correlation of standard Western blot (WB) assay band patterns with the results of the STARHS.

We tested, unlinked and anonymously, 106 anti-HIV-positive serum samples from PW seeking antenatal care in public clinics in São Paulo from 1991 to 2002. Serum samples were obtained from the serum bank of the Serology Section of the Adolfo Lutz Institute (IAL), São Paulo, and comprise all 93 HIV-seroreactive specimens from PW received for confirmatory purposes from 1991 to 2002 and 13 of 14 (92.9%) HIV-seroreactive specimens from 4,247 PW who attended for testing purposes from 1999 to 2002. Recent HIV seroconversion (within the previous 170 days) was evaluated by employing the Organon Teknika/bioMérieux Vironostika HIV type 1 (HIV-1) less sensitive EIA (33). Estimates of HIV incidence were calculated for the 1999-to-2002 period by means of mathematical modeling proposed by Janssen et al. (14). An HIV-1 Western blot assay (Genelabs Diagnostics, Singapore) was performed according to the manufacturer's instructions. Each serum strip was interpreted and its banding patterns were scored in a blind fashion to avoid bias. Bivariate analysis was employed to assess the association (χ^2 test) between STARHS results and the independent variables: age groups (by 5-year strata) and WB banding profiles. A stepwise forward-based procedure was employed in a multiple logistic regression model analysis. Based on adjusted odds ratios (OR), a final logistic regression model was evaluated. This study is a part of the project IAL/CCD-BM 28/01, approved by the IAL Committee for Ethics in Research, in accordance with Brazilian policies for research involving human subjects.

By applying the STARHS strategy, we found 18 of 106 specimens (17.0%) with putative recent infection reactivity. This overall proportion of HIV infections is similar to rates reported by other authors also evaluating low-risk populations (14, 19), although we observed that 30.8% of specimens (4 of 13) showed a reactivity pattern consistent with recent infection for the most recent years studied (1999 to 2002) (Table 1). The estimated annual HIV seroincidence, calculated for the 1999-

* Corresponding author. Mailing address: Seção de Sorologia, Instituto Adolfo Lutz-Secretaria de Estado da Saúde de São Paulo, Av. Dr. Arnaldo, 351, 10° andar, 01246-902 São Paulo, Brazil. Phone: (55) 11 3068 2885. Fax: (55) 11 3085 3505. E-mail: freitas@ial.sp.gov.br.

TABLE 1. Recent HIV-1 infections according to age group and year and HIV incidence data from 1999 to 2002[a]

Variable	Category	No. (%)				Incidence/100 PW/yr (95% CI)
		Tested	HIV positive	Available for STARHS	Recent seroconvertions	
Age (yr)[b]	Unknown	NA	20 (ID)	20 (100.0)	05 (25.0)	ID
	15–19	NA	10 (ID)	10 (100.0)	01 (10.0)	ID
	20–24	NA	28 (ID)	28 (100.0)	05 (17.9)	ID
	25–30	NA	24 (ID)	24 (100.0)	04 (16.7)	ID
	30–35	NA	16 (ID)	16 (100.0)	03 (18.8)	ID
	> 35	NA	08 (ID)	08 (100.0)	0 (0.0)	ID
Period of specimen collection	1991–1993[c]	NA	31 (ID)	31 (100.0)	8 (25.8)	ID
	1994–1996	NA	45 (ID)	45 (100.0)	4 (8.9)	ID
	1997–1998	NA	13 (ID)	13 (100.0)	4 (20.0)	ID
	1999–2002[d,e]	4,251	18 (ID)	17 (94.4)	2 (20.0)	ID
Annual HIV seroincidence	1999 or 2000	2,888	7 (0.24)	6 (85.7)	2 (33.3)	0.17 (0.01–0.64)
	2001 or 2002[e]	1,359	7 (0.52)	7 (100.0)	2 (28.6)	0.32 (0.02–1.37)
	1999–2002[e]	4,247	14 (0.33)	13 (92.9)	4 (30.8)	0.22 (0.04–0.61)

[a] Data are from sera from HIV-positive pregnant woman analyzed at the Central Laboratory of the Adolfo Lutz Institute (1991 to 2002). CI, confidence interval; NA, not available; ID, insufficient data for calculations.
[b] Bivariate analysis. $P = 0.708$ (χ^2 test).
[c] February to December 1991.
[d] Including 4,247 samples received for testing and 4 samples received for confirmation purposes only.
[e] January to April 2002.

to-2002 period, was 0.22 per 100 PW per year (95% CI, 0.04 to 0.61 per 100 PW per year) (Table 1). Unfortunately, information on the number of HIV screening tests performed on samples received from 1991 to 1998 was limited, which hindered the evaluation of the incidence trends.

No significant association between STARHS results and age groups could be found ($P = 0.708$). Except for the PW aged 35 to 39 years, for which only established infections were identified, similar proportions of recent HIV infections were observed. With regard to antibody reactivity profiles, a negative or indeterminate pattern for one of the gp41, p31, p51, p66, and gp120 regions was a predictor of recent HIV-1 seroconversion (OR = 108.75, $P < 0.001$; OR = 7.36, $P < 0.001$; OR = 4.03, $P = 0.015$; OR = 4.46, $P = 0.023$; and OR = 5.38, $P = 0.105$, respectively). After multiple logistic regression model analysis, only gp41 remained an independent factor associated with recent HIV-1 seroconversion (adjusted OR = 91.19, $P <$ 0,001). On the other hand, comparisons among different combinations of bands present in the tested strip showed that a strong reactivity for all *env*- or *pol*-encoded proteins and strong reactivities for combined gene-encoded proteins, especially gp41 and p31 antigens, might be correlated with an established HIV infection outcome (Table 2). These results are in accordance with data obtained by other authors who investigated the differential maturation of human humoral immune responses against HIV antigens and evaluated the responses as markers to predict disease progression (6, 7, 27, 29). Our results suggest that a specific antibody response examined by means of standard WB procedures may serve as a predictor of recent HIV infection and may be useful in conjunction with STARHS results. Both methodologies, STARHS and WB, can reveal patterns in antibody kinetics maturation that may be potentially helpful not only in timing infection but also in other fields of AIDS research. Validation of these tools and eventually clues for knowledge on HIV pathogenesis may be provided from follow-up studies of antibody response maturation. The WB approach would have the advantage of making it possible to evaluate data retrospectively, even when biological specimens are no longer available, as long as hard data have been recorded. Longitudinal studies based on serial samples should be carried out to corroborate these findings.

Serum samples analyzed in the present study were collected from female individuals whose test was performed due to suspected or confirmed pregnancy between 1991 and 2002. It corresponds to a period when HIV infection may have been expanding among females in Brazil, as suggested by the continuous decrease in the male/female ratio for AIDS cases. The estimation of the outcome in this study is based on a relatively small number of samples, from which a few misclassified samples might have a major impact on incidence estimates. The limitations of single samples from routine diagnostic proce-

TABLE 2. Assessment of the strength of different combinations of WB bands with matched strongly positive patterns in predicting an established infection among PW in São Paulo from 1991 to 2002

Combination of WB bands	Crude OR (crude 95% CI)	*P* value
Only *env* encoded antigens		
gp160 + gp120 + gp41	24.86 (6.25–140.52)	<0.001[a]
Only *pol* encoded antigens		
p66 + p51 + p31	4.97 (2.33–10.41)	<0.001[b]
Only *gag* encoded antigens		
p55 + p24 + p17	1.03 (0.49–2.08)	0.930[b]
Mixed profile		
gp41 + p31	18.79 (6.50–56.23)	<0.001[b]
gp41 + p66	15.00 (5.14–45.20)	<0.001[a]
gp41 + p51	11.22 (4.31–29.09)	<0.001[b]
gp41 + p31 + p51	9.76 (4.53–20.92)	<0.001[b]
gp41 + p31 + p51 + p66	8.12 (4.18–15.65)	<0.001[b]
gp160 + gp120 + gp41 + p24	8.60 (3.06–25.15)	<0.001[a]
gp160 + gp120 + gp41 + p31 + p24	7.58 (3.45–16.75)	<0.001[b]

[a] Fisher exact *P* value.
[b] Yates corrected *P* value.

dures must be considered. However, the relative stability among the periods may suggest that these estimates can be used as a baseline for HIV incidence in PW in São Paulo State for the period analyzed. False detuned assay results have been reported for samples from patients with advanced disease and low CD4 values or for those with controlled viremia due to highly active antiretroviral therapy (26). Although all samples analyzed in this anonymous study have been collected for diagnostic purposes, we cannot rule out these possibilities. Moreover, STARHS performance may differ for viruses in the non-B clade. Although we have not evaluated this issue in the present study, clade B viruses have been responsible for most HIV-1 infections in this area (1, 2).

Our data show that the STARHS is a feasible, inexpensive, and time-saving tool to identify recent HIV infections in banked sera from a well-defined low-risk population. Public health laboratories, and clinical laboratories as well, can play an essential role in the implementation of surveillance strategies based upon antibody assays, especially in retrospective studies. In spite of limitations with regard to an anonymous cross-sectional study design, our results may provide information on infections on a per-incident basis. In conclusion, such evaluations might be useful to understand in more detail the trends in epidemics in different segments of the population, such as among sexually active women, contributing to the prevention strategies that focus on heterosexual and mother-to-child HIV transmission.

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