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Original Article

Recent HIV infection rates among HIV positive patients seeking voluntary counseling and testing centers in the metropolitan region of Recife – PE, Brazil

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A B S T R A C T

The BED capture enzyme immunoassay test makes it possible to determine whether individuals were recently infected with HIV.

Objective: In this study, the overall HIV and recent infections prevalences were determined at five Voluntary Counseling and Testing (VCT) centers, in the Metropolitan Region of Recife, Northeastern of Brazil.

Material and methods: A cross-sectional study was conducted among users of five VCTs in the metropolitan region of Recife between July 2007 and April 2009. Out of the individuals who tested positive for HIV, 169 were analyzed to assess the prevalence of recent infection by means of the BED-CEIA (BED-Calypte®).

Results: Out of 46,696 individuals tested 916 (1.96%) turned out positive for HIV infection The highest prevalence was in Recife (3.9%). The prevalence was higher among males (3.93%), and men who have sex with men (MSM) (12.4%). The frequency of recent infections among the 169 subjects evaluated was 23.7%. Recent infections were more common among individuals under 25 years of age. There was slight predominance of men and higher frequency of heterosexuals in both groups, but still a significant portion of MSM (33%). Subtype B predominated, followed by a high proportion of subtype F.

Conclusions: Recent infection occurs mainly among young individuals and heterosexuals, despite a significant proportion of recent infection among MSM. These results suggest that preventive actions aimed at the MSM community remains a challenge and efforts focusing this group should continue to be a priority.

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Introduction

Differing methodologies have been used in epidemiological surveillance of HIV infection around the world, to assess and improve the monitoring of trends within the epidemic. Most of the epidemiological data available is based on cross-sectional prevalence studies, which vary considerably in terms of local conditions, study populations, and methodology used. The epidemiological and virological characteristics of the HIV epidemic in Brazil also vary according to the region and population studied. The Brazilian National Department for STD/AIDS and Viral Hepatitis of the Ministry of Health has estimated that around 630,000 individuals in this country are infected.[1]

The Brazilian epidemic is considered by the World Health Organization to be concentrated, i.e. there is low prevalence among the general population and much higher prevalence among groups at risk, particularly men who have sex with men (MSM), sex workers and injected drug users (IDUs).[2] In Brazil, the prevalence rate for HIV infection is less than 1% in the general population and has remained stable since 2004. However, among groups with increased risk of infection, HIV prevalence rates of around 5.9% were detected among IDUs, 10.5% among MSM, and 5.1% among female sex workers (FSW) in a study conducted in ten Brazilian state capitals between 2008 and 2009.[3] In another investigation, FSW, MSM and drug users (DU) had higher risk of acquiring HIV infection compared to a relatively low prevalence in the general population (~0.6%), whereas the prevalence among FSW was 6.2% (95% CI: 4.4-8.3), MSM was 13.6% (95% CI: 8.2-20.2), and among DU was 23.1% (95% CI: 16.7-30.2).[4] In Brazil, the AIDS epidemic has particular regional characteristics and the AIDS incidence rates have shown a trend towards stabilization, decreasing in the southeastern and central-western regions and increasing in the northern, northeastern and southern regions.[1,5]

On the other hand, ascertainment of HIV infection incidence may provide information with greater detail for monitoring the epidemic. Such measurements thus become an important tool for identifying groups that present greater vulnerability to infection, and they may be used to direct prevention and control programs.[6]

Tests to detect recent HIV infection are being used in scientific studies to calculate the rate of new HIV infection. In 1998, the Centers for Disease Control (CDC) produced a standardized methodology for identifying recent HIV-1 infections, based on a serological test algorithm known as STARHS (Serologic Testing Algorithm for Recent HIV Seroconversion).[7] A new methodology called IgG BED capture enzyme immunoassay (CEIA) (Calypte Biomedical Corporation – USA) is based on detection of increasing proportions of specific IgG after seroconversion. The antigens used in this method are peptides derived from the immunodominant region of gp41, containing sequences of subtypes B, E and D, which are representative of many subtypes of HIV-1.[8,9] This assay has the capacity to determine what percentage of a population of HIV-infected individuals had been infected within the last six months prior to the date of sample collection, provided that this is done in conjunction with the patients' clinical and epidemiological data. It should not be used routinely for diagnosing HIV infection. Quantitative tests assess HIV-specific IgG in relation to total serum IgG and make it possible to calculate the increase in specific IgG that takes place at the start of the HIV infection. This increase is gradual and, over a period of usually up to 160 days, values that are below the lower limit of detection of the traditional tests. This period is thus considered to be the "window" period for seroconversion. The test presents sensitivity of 81.7% and specificity of 89.1% for identification of incident samples. The gold standard used is documented seroconversion, and the test performance is unaffected by HIV-1 diversity.[10]

It has been demonstrated that among different populations such as IDUs or pregnant women, or even on stored frozen serum samples, the BED-CEIA test provided consistent results for epidemiological surveillance studies.[8] Therefore, it would make it possible to ascertain trends in incidence level variation if samples from different years were available for testing.

VCTs have been set up to improve the access to testing, thereby stimulating early diagnosis and treatment of HIV infection, and are distributed throughout the national territory. In Pernambuco, there are 24 VCTs across the state, five of which are located at strategic points within the metropolitan region of Recife (RMR), where there is greater demand for HIV testing because of convenient location and easy access. Most individuals who approach the VCT are asymptomatic but had been exposed to some risk of contracting the infection. Thus, VCTs are excellent places for detecting new cases of HIV infection by means of a new technique enabling the monitoring of recent infections.[11]

The aims of the present study were to describe the prevalence of HIV infection at five VCTs in the metropolitan region of Recife, over the period from July 2007 to April 2009; to determine the frequency of recent infection among individuals who were recently diagnosed as seropositive for HIV-1; and to compare the sociodemographic and laboratory characteristics of individuals with recent and established infection.

Material and methods

A cross-sectional study was conducted among users of five VCTs in the metropolitan region of Recife, in the municipalities of Cabo de Santo Agostinho, Jaboatão dos Guararapes, Olinda, Paulista and Recife, who were recruited between July 2007 and April 2009. Individuals who had a diagnosis of HIV infection in accordance with the guidelines of the Brazilian Ministry of Health (available at http://www.aids.gov.br), when returning to the VCT to receive recent test results were approached to participate in this study during the post-test counseling session. Those who agreed to participate signed a free and informed consent statement and a blood sample collected for specific laboratory tests. These samples were sent to the virology sector of the Central Laboratory of Pernambuco (LACEN – PE), accompanied by the specific form for this project where blood was centrifuged and the plasma was stored at –70°C for subsequent processing.

Samples from 169 individuals who fulfilled the inclusion criteria of the study (recent diagnosis of HIV-1 infection and no previous antiretroviral treatment) were analyzed. The BED-CEIA test (BED-Calypte®) was used to detect recent infection. Controls (negative, strongly positive and weakly positive) and calibrators were tested together with the samples, in accordance with the manufacturer's instructions (Calypte Biomedical Corporation – Oregon, USA); briefly, serum plasma samples were added to wells of microplates coated with anti-human IgG, thus HIV specific and not specific IgGwill compete to bind with anti-human IgG on the solid phase. After incubations, a synthetic peptide containing the HIV-1 immunodominant epitope of gp41 subtype B, E and D was added and incubated, and after other steps, the reactions are revealed with tetramethylbenzidine (TMB) and read using a spectrophotometer (450 nm).

Results were expressed as optical density (OD), such that a sample with an OD $\leq$ 1.2 was taken to be positive but, after repetition in triplicate, only samples with an OD $\leq$ 0.80 were considered cases of recent infection. Incidence estimates were not calculated due to the small proportion of samples tested for detecting recent infection by BED–CEIA (169 out of 916 tested samples), and could lead to under or over estimation.

The prevalence of HIV infection was computed by analyzing available test results of the database of each VCT (laboratory test result system of the State of Pernambuco's STD/AIDS Coordination Office) and the sociodemographic data from the users' histories issued by the SIREX database. Viral load results and CD4 T lymphocyte counts were retrieved from the SISCEL database (laboratory test control system of the National Department for STD/AIDS and Viral Hepatitis). The data on the individuals tested for recent infection were transcribed to a specific research form for the project and were double-entered into the Epi-Info software version 6.0 and into Microsoft Office Excel spreadsheets (2007 version). The study was approved by the ethics committee of the Health Sciences Center, Federal University of Pernambuco, under registration number CEP/CCS/UFPE 120/07-SISNEP FR-134429.

Results

According to the data obtained for the period from July 2007 to April 2009, 46,696 individuals were tested and 916 individuals turned out positive, with a general prevalence of 1.96%. The prevalence of HIV infection ranged from 1.2 to 3.9% at the five VCTs, and it was highest at the VCT in Recife (3.9%) and Jaboatão dos Guararapes (2.2%). Among those tested for recent infection, the overall positivity rate was 23.7%, being highest in the municipalities of Recife (34.7%), Cabo de Santo Agostinho (31.2%) and Jaboatão dos Guararapes (23.0%) (Table 1).

Information about sex, age group and exposure category of the individuals tested at the VCT in the municipalities of Cabo de Santo Agostinho, Olinda and Jaboatão dos Guararapes is presented in Table 2. The great majority of the individuals tested were female and under the age of 25 years. On the other hand, the positivity rate was greater among males (4%) than among females (1.02%). The prevalence of infection was higher among MSM (14%) than heterosexual subjects (1.49%), and among individuals over 40 years of age (2.8%).

Out of the 169 individuals who underwent the BED-CEIA test to check for recent infection, 40 (23.7%) were classified as presenting recent infection or recent seroconversion (RS). The variables of gender and exposure category were not significantly different in two groups of HIV infection. However, individuals with recent infection were younger than those with chronic infection (p < 0.05). The infection rate was higher in the heterosexual group, but the rate of recent infection in the MSM group was 33%. The median CD4 T lymphocyte count in individuals with recent infection was 560 cells/mm^3 versus 409 cells/mm^3 among those with chronic infection. The median viral load was 795 copies of RNA/mL among patients with recent infection versus 8,540 copies of RNA/mL in those with chronic infection, with no significant difference between the two groups. The molecular characterization of the samples to verify viral subtypes did not show any difference between the two groups. There was a predominance of subtype B followed by subtype F (Table 3).

Table 1 - Prevalence of HIV-1 at five VCTs in the metropolitan region of Recife and samples tested to detect recent infection in the period of July 2007 to April 2009

VCT	Number of samples			Prevalence (%)	Number of samples	
	Tested for HIV	Positive	Negative	95%CI	Tested using BED	Recent infection
Olinda	9,297	119	9,178	1.27 1.06-1.53	20	2 (10%)
Cabo	11,440	148	11,292	1.29 1.09-1.52	64	20 (31.2%)
Jaboatão	11,519	256	11,263	2.2 1.96-2.51	39	9 (23%)
Paulista	6,544	81	6,463	1.23 0.98-1.54	32	4 (12.5%)
Recife	7,896	312	7,584	3.9 3.53-4.40	14	5 (34.7%)
Total	46,696	916	45,780	1.96 1.84-2.09	169	40 (23.7%)

Table 2 - Prevalence of HIV-1 at three VCTs* located in the metropolitan region of Recife, according to gender, age group and exposure category in the period of July 2007 to April 2009

Variables	Number of samples tested	HIV-positive samples			p-value
		Number	%	95% CI	
Total	32,256	523	1.62	1.48-1.76	-
Gender					
Male	6,718	264	3.93	3.48-4.42	< 0.001
Female	29,538	259	0.88	0.77-0.99	
Exposure category					
HTS	30,156	445	1.48	1.34-1.62	< 0.001
MSM	427	53	12.4	9.43-15.9	
Age group					
Under 25 years	16,161	132	0.82	0.68-0.97	< 0.001
25 to 40 years	11,877	276	2.32	2.06-2.61	
Over 40 years	4,218	115	2.72	2.26-3.26	

*VCTs, Cabo de Santo Agostinho, Olinda and Jaboatão dos Guararapes; CI, confidence interval; HTS, heterosexual; MSM, men who have sex with men.

Table 3 - Population characteristics according to condition of HIV infection

Characteristics	Recent infection		Chronic infection		Total		p-value
	n	%	n	%	n	%	
Total	40	23.7	129	76.3	169	100.0	
Age group							
Under 25 years	12	30.0	15	11.6	27	16.0	0.011
25 to 40 years	23	57.5	80	62.0	103	60.9	
Over 40 years	5	12.5	34	26.4	39	23.1	
Gender							
Female	16	40.0	60	46.5	76	45.0	0.470
Male	24	60.0	69	53.5	93	55.0	
Exposure category							
Male							
MSM	9	37.5	18	26.1	27	29.0	0.569
Heterosexual	15	62.5	51	73.9	66	71.0	
Female							
Heterosexual	16	100.0	60	100.0	76	100.0	-
Originating TCC							
Cabo	20	50.0	44	34.1	64	37.9	0.114
Jaboatão	9	22.5	30	23.2	39	23.1	
Olinda	2	5.0	18	14.0	20	11.8	
Paulista	4	10.0	28	21.7	32	18.9	
Recife	5	12.5	9	7.0	14	8.3	
CD4 (cells/ml)							
Median (P_{25}; P_{75})	560 (119; 884)		409 (215; 672)		418 (208; 696)		0.376
Viral load (copies/ml)							
Median (P_{25}; P_{75})	795 (179; 39.174)		8.540 (208; 39.121)		7.491 (198; 39147)		0.421
HIV subtype							
B	15	60.0	44	53.0	59	54.6	0.796
F	8	32.0	35	42.2	43	39.8	
C	1	4.0	2	2.4	3	2.8	
BF	1	4.0	2	2.4	3	2.8	

** Association between recent infection and exposure category, independent of sex.
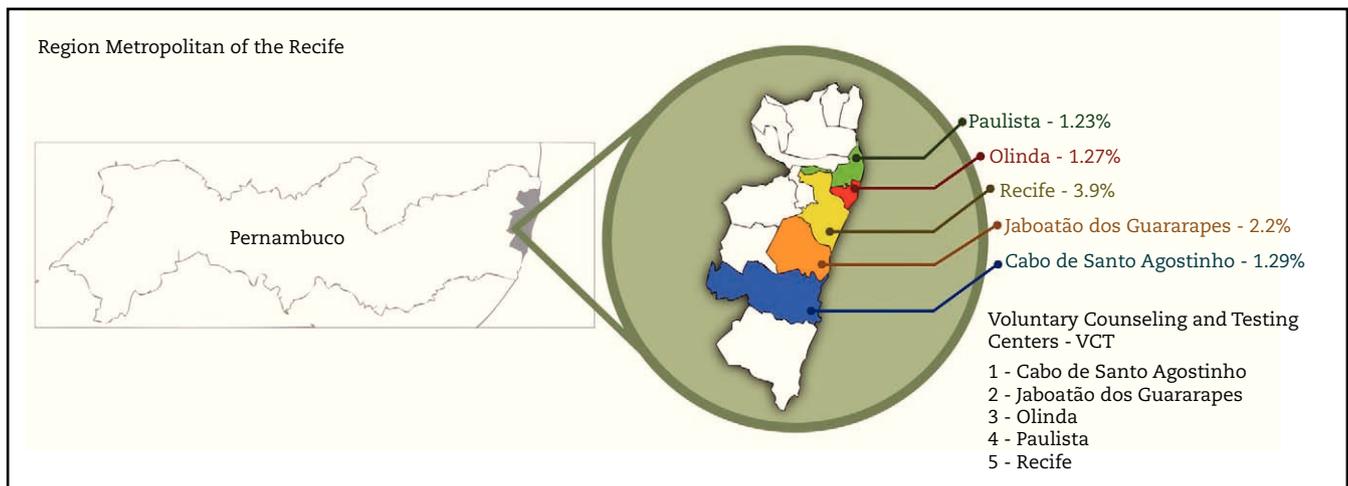
Fig. 1 - Metropolitan region of Recife with the VCTs studied.

The geographical locations of the five VCTs studied can be seen in Fig. 1. The distribution of the prevalence of HIV infection and frequency of recent infection showed that both were highest in Recife, followed by the municipalities of Jaboatão dos Guararapes and Cabo de Santo Agostinho.

Discussion

In the present study the overall prevalence of HIV infection among the individuals tested at five VCTs in the metropolitan region of Recife between 2007 and 2009 was 1.96%. The highest prevalences were in Recife (3.9%) and Jaboatão dos Guararapes (2.2%). The prevalence was higher among men; in the exposure category of MSM (12.4%); and in the age group over 40 years. The overall frequency of recent infection among the 169 individuals evaluated was 23.7%. Comparison between individuals with recent versus chronic infection showed that recent infection was more frequent among individuals under 25 years of age and in the municipalities of Recife (35.7%), Cabo de Santo Agostinho (31.2%), Jaboatão dos Guararapes (23%), which are in the south of the metropolitan region. There was slight predominance among men and greater frequency among heterosexuals in the two groups, but there was still a significant proportion of MSM (33%). There was similarity between the viral subtypes, with predominance of subtype B, although with a high proportion of subtype F (around 40%), both in cases of recent infection and in cases of chronic infection.

This study presents data from VCTs located in five populous municipalities in the greater metropolitan region of Recife, which has a total of 3,042,426 inhabitants. Recife is the capital of the state of Pernambuco, located in the northeastern region of Brazil, one of the poorest and most populous regions of Brazil. The rate of recent infection of 23.7% indicates that this epidemic is still growing in the metropolitan region. The data show that Recife is the municipality with the highest prevalence of diagnosed infection and highest frequency of recent infection, among the five municipalities evaluated.

This finding is in agreement with data from the Brazilian Ministry of Health (2010), which indicate that Recife ranks fourth among Brazilian cities in terms of AIDS incidence, with 58.4 cases/100,000 inhabitants, whereas in 1999, the incidence was 20.4 cases/100,000 inhabitants.[1] On the other hand, municipalities located in the south of the metropolitan region have also evolved with high frequencies of recent infection, as is the case of Cabo de Santo Agostinho (31.2%) and Jaboatão dos Guararapes (23%). These rates differ from those reported in municipalities located on the northern part of the coast, such as Olinda and Paulista, with rates of recent infection of 10 and 12.5%, respectively. It should be pointed out that the state of Pernambuco has been experiencing significant economic growth, with an increase in Gross Domestic Product (GDP) of 9.3% in 2010. This growth has occurred mainly as a result of greater activity in the Port of Suape and the industrial center and shipbuilding industry located in the municipality of Cabo de Santo Agostinho, with migration of young male workers from all over the state and even from southeastern Brazil.[12] Although this is positive from an economic point of view, it may have reflected in the increased HIV infection rate observed in this study.

With regard to gender, while the majority of the tests were performed on women, infection was found predominantly among men. The large numbers of women tested was primarily due to the characteristics of VCT in Brazil, which are places where pregnant women are referred for HIV testing. The inclusion of pregnant women may have reduced the prevalence of infection among women, which was only one quarter of the rate seen among men, given the probable inclusion of many women who were not at a risk situation. On the other hand, the men who sought the VCTs possibly did so because they were at greater risk of exposure. However, the evaluation relating to recent infection showed that the frequency among men was only 1.5 times higher than that among women. This is in agreement with the frequencies of AIDS cases notified to the Ministry of Health in 2010 and other recent years, which showed a ratio of 1.5 men to each woman with AIDS in Brazil over the last decade.[1]

With regard to exposure category, the sexual orientation predominated, with a significant proportion of heterosexuals, both in cases of recent infection and in cases of chronic infection. However, the characteristic of a concentrated epidemic (i.e. with groups at higher risk) still seems to be occurring, given that in evaluating both prevalence and recent infection, MSM constituted a significant proportion of the infected individuals. This group presented an infection rate that was nine times greater than that of heterosexuals and comprised one third of the men with recent infection.

Whereas the prevalence study showed higher prevalence of infection in age groups over 40 years, the recent infections showed a trend towards new cases among younger individuals (under 25 years of age). In fact, while developed countries are moving towards infection at more advanced ages,[2] the situation in Brazil is that among younger age groups in poorer regions, the numbers of AIDS cases are advancing significantly and affecting individuals who become sexually active at an early age, without safe sexual practices, and with loss of many productive years.[13]

In Brazil, where the AIDS epidemic has regional characteristics, several studies on recent infection have also been conducted. The proportion of recent infection has been analyzed in some VCT centers in the city of São Paulo, and a rate of recent infection of 15% was found among 194 individuals who were tested using the Vironostika-Biomerieux assay.[14] Recent infections were more frequent among younger individuals (under 25 years of age). In Curitiba, among 32 HIV-positive samples analyzed by the BED-CEIA assay, recent infection was identified in 25% of the cases, and subtype C was found to be the most common among these recently infected (63%).[15] In Ceará, in samples collected in 2008/2009, a rate of recent infection of 6.8% was found among 74 patients with no previous antiretroviral treatment, who presented at two STD/AIDS referral hospitals in that state.[16] A study in Rio de Janeiro, on 434 frozen samples that were collected at three VCTs in the metropolitan region between November 2004 and October 2005, found a rate of recent infection of 14% by BED-CEIA.[17]

The laboratory tests showed that the patients generally presented a low stage of immunosuppression, with median CD4 T lymphocyte counts greater than 400 cells/mm^3 and viral loads less than 10,000 copies/mL, both in cases of recent and chronic infections. These findings underscore the importance of seeking testing centers at earlier stages, which leads to reduced transmission and better therapeutic results. However, this contrasts with the time at which individuals present themselves for medical care in the northeastern region, i.e. generally at a late stage and with advanced immunosuppression.[18,19] Failures of post-test counseling to convey the benefits of early medical follow-up, or difficulties in accessing medical care are important factors in the previously described disparity between the time of diagnosis at the VCT and the presentation for medical care in Brazil.[11]

There was no great variation in viral subtypes found between recent and chronic infections, with predominance of subtype B followed by subtype F. Differing from the southern region of the country, the frequency of subtype C remained low in both groups (less than 5%). However, a high frequency of subtype F was observed: around 32% in cases of recent infection and

42.2% in cases of chronic infection. This rate was very high compared to other regions of Brazil, and even to other states in the northeastern region, thus showing that the circulation of this subtype in Recife was indeed different.

The BED–CEIA has differences in the window periods when applied to different subtypes.[20] We emphasize the large proportion of subtypes B and F reported in this paper. The BED–CEIA has been well validated for subtype B.[21] There are no data on its performance for the subtype F; however, a higher prevalence of subtype B samples classified as recent infection has been observed in a study which showed a considerable proportion of samples belonging to subtype F.[22]

This study presents some limitations, such as the lack of separate analysis excluding the pregnant women when calculating the prevalence of HIV infection. Another limitation is a possible selection bias in relation to groups preferentially involved in recent HIV infection, as it assessed only the population seeking the voluntary testing centers, a group that generally has a higher perception of their risk of becoming ill. It may therefore have underestimated the rates of recent infection and prevalence in populations with traditionally low risk for infection such as heterosexual men. Another limitation was the small number of samples tested to determine the cases of recent and chronic infection, thus compromising the calculation of incidence. However, this was an analysis on a very populous region, with a large number of individuals tested, in an area with a growing epidemic and sparse published data on the topic. We consider that this study may enrich the discussion on the best strategies for preventing HIV infection in localities at greater risk and among younger individuals presenting greater vulnerability.

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Conflict of interest

All authors declare to have no conflict of interest.

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