HIV genotyping among female sex workers in the State of Santa Catarina

Genotipagem do HIV em mulheres profissionais do sexo no Estado de Santa Catarina

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ABSTRACT

The objective of this study was to investigate the frequency of HIV infection among female sex workers in the port area of Imbituba (State of Santa Catarina), and to identify the viral subtype and its susceptibility to antiretroviral medications. Ninety women were interviewed between December 2003 and February 2004. Six (6.7%) were HIV-positive. Genotyping for HIV, performed on four samples, detected subtype C in three of them, which is predominant in Africa and Asia, and subtype B in one of them, which is prevalent in Brazil, USA and Europe. The results suggest that the Port of Imbituba may be one of the gateways for HIV-1 subtype C to enter Brazil, and for its dissemination to the rest of the country and the Mercosul area, along the highway BR-101. This points towards the need for preventive work to reduce the introduction and dissemination of HIV subtype C in Brazil.

Key-words: *HIV. Sex workers.* ***Subtype C. Brazil.***

RESUMO

O objetivo deste estudo foi verificar a freqüência da infecção pelo HIV em profissionais do sexo, atuantes em Imbituba (SC), identificar o subtipo viral e a suscetibilidade do vírus aos medicamentos antiretrovirais. De dezembro de 2003 a fevereiro de 2004, foram entrevistadas 90 mulheres, profissionais do sexo, e a freqüência de HIV nessa população foi de 6,7%. O teste de genotipagem para o HIV, realizado em quatro amostras, detectou em três delas o subtipo C, que é predominante na África e Ásia, e em uma o subtipo B, prevalente no Brasil, EUA e Europa. Os resultados sugerem que o Porto de Imbituba pode ser uma das portas de entrada para do HIV-1 subtipo C no Brasil, e a partir dessa localidade ocorrer sua disseminação para o restante do País e países do Mercosul pela rodovia BR-101. Isto aponta para a necessidade de trabalhos de prevenção, com a finalidade de reduzir a introdução, transmissão e disseminação do HIV subtipo C.

Palavras-chaves: *HIV. Profissionais do sexo.* ***Subtipo C. Brasil.***

The human immunodeficiency virus (HIV) is an emerging pathogen with great genetic polymorphism. Two types of virus (HIV-1 and HIV-2) have been identified as etiological agents for AIDS. HIV-2 is concentrated on the African continent, with a small number of cases in Europe and the United States. HIV-1 has spread worldwide. In order to identify the different subtypes of HIV-1, it has been divided into three large groups: M (Major), O (Outlier), and N (non-M and non-O)[9,14,15].

At least 10 subtypes derived from the M group have already been detected, and these have been classified with the letters from A to J. These variations generally occur in the *env* and *gag* regions of the virus[17]. Studies conducted around the world have determined the distribution of the viral subtypes according to their predominant geographic localization: subtype A predominates in central Africa; B, in the United States, Europe, South America and Thailand; C, in India, southern Africa and Brazil[8,13]; D, in central Africa; E, in Thailand and the Central African Republic; F, in Brazil, Romania and the Democratic Republic of the Congo (DRC; former Zaire); G, in DRC, Gabon and Taiwan; G, in DRC, Gabon and Taiwan; H, in DRC and Gabon[8].

Up to June 2006, 433,067 cases had been notified[12] in Brazil, and the number of studies on the viral genotypes of HIV is still small. A study conducted among 107 patients who underwent HIV genotyping tests in Porto Alegre (State of Rio Grande do Sul), from 1994 through 1997, found prevalences of 75% for subtype

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B and 22% for subtype C[9]. A study performed in Rio de Janeiro from 2002 to 2003, among 547 HIV-infected patients undergoing antiretroviral treatment, found subtype C in only 0.4% of the sample[4]. Knowledge of the distribution of these HIV genotypes is essential for better understanding of HIV epidemiology, pathogenesis and response to antiviral treatments, and with regard to the appearance of resistance to medications, development of vaccines, and updating of diagnostic methods.

MATERIAL AND METHODS

Between December 2003 and February 2004, 135 women were contacted. They were all sex workers at the port City of Imbituba (State of Santa Catarina; SC), and were registered with the non-governmental organization Indústria da Solidariedade (ISO). The Research Ethics Committee of the University of Southern Santa Catarina (Unisul) had approved the study. However, only 90 of these women accepted participation. After they agreed to sign the informed consent statement, blood samples were collected for anti-HIV antibody detection. The HIV-1 serological test was performed at the Clinical Laboratory of Unisul using the Abbott® HIV kit, M.E.I.A. in the automated *Axsym System* and the Roche® immunoassay HIV kit, with ELISA methodology in automated Cobas Core. The positive or undetermined results were confirmed by indirect immunofluorescence or Western blot analysis, as a confirmatory test for HIV[11]. At the test stage, a second sample was collected in the case of positive or undetermined results for HIV, for each assay. The confirmatory tests were performed at the Public Health Reference Laboratory (LACEN), in Florianópolis (SC).

The women whose biological samples showed reactivity in the tests were invited to give a new blood sample, which was analyzed using the BD® Vacutainer system with EDTA. The tubes that contained the biological samples were centrifuged for 15 minutes at 2,500rpm, and the plasma obtained was frozen at –20°C until the time of performing the genotyping and detection of *pol* gene mutations, which are associated with the sensitivity to antiretroviral tests. These tests were performed at the Virology Service of the Adolfo Lutz Institute, São Paulo State Department of Health, and followed the procedures described below:

Molecular analysis. HIV-1 RNA was extracted from 500ul of plasma using Trizol LS reagent (Invitrogen, USA). Reverse transcription was performed with 200U of SuperScript II, (Invitrogen, USA), 300ng of random hexamer primers (Invitrogen, USA), 0.5mM of deoxynucleotide triphosphate, 7.5U of RNase Inhibitor (Invitrogen, USA) and 10mM of dithiothreitol, in a final volume of 20µl at 42°C for 90 min in 1x reaction buffer. Polymerase chain reaction (PCR) of the target sequences was performed using a nested protocol to amplify protease (PR) and reverse transcriptase (RT) genes. The relative average positions of sequences to HXB2 were: PR (2,240-2,548) and RT (2,664 – 3,214) (Los Alamos National Laboratory - Sequence Locator Tool). For de-amplification of the *pol* gene, the first PCR allowed an amplicon product of 1.2Kb to be obtained. The nested PCR allowed the coding genes for PR and RT to be obtained. The samples with negative amplification were recovered using alternative primers or a commercial kit (Viroseq 2.1; Abbott). Nested PCR products were purified using the Concert™ Rapid PCR purification system (Invitrogen, USA) and quantified on agarose 2% gel with low DNA Mass Ladder™ (Invitrogen, USA). Sequencing of both the PR and RT was performed using fluorescent dideoxynucleotides (ddNTPs) from the ABI Prism Big Dye™ Terminator (Applied Biosystems Inc., Foster City, CA, USA), resolved on 3,100 automatic sequencer (Applied Biosystems Inc., Foster City, CA, USA).

The genetic sequencing of PR and RT allowed genetic information to be obtained for genotype analysis. The analysis and editing of the genetic sequences were performed using the Sequence Navigator software, in which *forward* and *reverse* sequences formed a consensus file in FASTA format. The molecular data were subjected to genotype resistance analysis using the *Stanford HIV Drug Resistance Database* website (hivdb.stanford.edu), which produces a report on susceptibility to antiretroviral drugs. After alignment using the Clustal X program against a reference set from Los Alamos Laboratory (http://www.ncbi.nih.gov/projects/genotyping/formpage.cgi) and manual correction using the BioEdit software, neighbor joining (NJ) trees 9 were constructed under HKY 85 distance matrices, using PAUP*4.10b10 for PR and/or RT. HIV-1 group O was used as the outgroup. Bootstrap values (1,000 replicates) above 70% were considered significant.

RESULTS

The mean age of the 90 female participants in the study was 27 ± 8 years, ranging from 18 to 60. The data showed that 77 (85.6%) were white, 76 (84.4%) had children (with reports of previous maternal breastfeeding), the educational level was low (incomplete elementary school) and the socioeconomic classification was low, predominantly class D, according to the Brazilian criteria[5]. Furthermore, only four of the women were born in Imbituba, and the women stated that they were frequently moving from one city to another, and from state to state (Table 1).

With regard to sexual practices and risky behavior, a statistical association was found between the number of clients attended per day and HIV infection ($p = 0.008$). There were also associations between HIV infection and sexual relations without frequent use of condoms ($p = 0.015$), and between HIV infection and the use of inhaled illicit drugs ($p = 0.053$) (Table 2).

Six (6.7%) women, out of all the women who took part in this study, presented reactivity in the anti-HIV test. Among these six, five agreed to undergo a new blood sample collection for the HIV-1 genotyping test. In four samples it was possible to obtain amplified genetic material. HIV-1 subtype C was found in three of the samples and HIV-1 subtype B in the remaining sample. The mutations confering resistance to antiretroviral drugs indicated that three samples were susceptible to protease inhibitors, nucleosides and non-nucleoside

Table 1- Demographic and socioeconomic characteristics of the female sex workers surveyed in Imbituba (State of Santa Catarina; SC), from December 2003 to February 2004.

Characteristics	Non-reactive to HIV		Reactive to HIV		
	nº	%	nº	%	p
Age					
≤ 25.5	42	50.0	3	50.0	0.96
> 25.5	42	50.0	3	50.0	
Race					
white	74	88.1	3	50.0	0.001
black	2	2.4	2	33.3	
mulatto	8	9.5	1	16.7	
Children					
yes	71	84.5	5	83.3	0.94
no	13	15.5	1	16.7	
Socioeconomic class – Brazilian criteria*					
B2	3	3.6	0	-	
C	24	28.6	1	16.7	0.39
D	43	51.1	2	33.3	
E	14	16.7	3	50.0	
Education background					
illiterate	4	4.8	0	-	
elementary school (incomplete)	52	61.9	5	83.3	
elementary school	10	11.9	1	16.7	0.72
secondary school (incomplete)	15	17.9	0	-	
secondary school	3	3.5	0	-	
Place of birth					
Imbituba	4	4.8	0	-	
other cities in SC	32	38.1	3	50.0	0.76
other Brazilian states	48	57.1	3	50.0	
Length of residence					
in transit	31	36.9	2	33.6	
< 2 years	12	14.4	1	16.6	0.99
≥ 2 < 5 years	16	19.0	1	16.6	
≥ 5 d ≤ 10 years	9	10.7	1	16.6	
> 10 years	16	19.0	1	16.6	

*The Brazilian economic classification criteria categorize the economic classes (A1, A2, B1, B2, C, D, and E) based on the family's income by combining the purchasing power with the educational background.

analogs. One sample presented low or intermediary resistance to protease inhibitors, high resistance to non-nucleoside analogs and lamivudine, low resistance to abacavir, and susceptibility to zidovudine and stavudine Table 3.

DISCUSSION

Out of the 90 women interviewed in this study, six were infected with HIV, which corresponds to a seroprevalence rate of 6.7%. This seroprevalence rate is similar to those found in other studies among sex workers in Brazil, such as the one carried out in Paranaguá (State of Paraná; PR) which reported an HIV infection rate of 4.5% among 132 women[1]. A similar study performed by the STD/AIDS reference center in Vitória (State of Espírito Santo; ES),

Table 2 - Sexual practices and risky behavior among female sex workers surveyed between December 2003 and February 2004 in Imbituba (SC).

Characteristics	Non-reactive to HIV		Reactive to HIV		
	nº	%	nº	%	p
Length of time as a sex worker					
≤ 2.5 years	26	31.0	2	33.3	0.61
> 2.5 years	58	69.0	4	66.7	
Number of clients per day					
≤ 2	73	86.9	4	66.7	0.008
> 2	11	13.1	2	33.3	
Sex during menses					
yes	35	41.7	1	16.7	0.22
no	49	58.3	5	83.3	
Condom use					
yes	14	16.7	1	16.7	0.02
no	70	83.3	5	83.3	
Previous reported STDs					
yes	46	54.8	4	66.7	0.45
no	38	45.2	2	33.3	
Use of drugs					
yes	41	48.8	6	100.0	0.01
no	43	51.2	0	-	
Blood transfusion					
yes	11	13.1	0	-	0.45
no	73	86.9	6	100.0	

Table 3 - Profile of susceptibility to HIV-1 found in female sex workers surveyed between December 2003 and February 2004 in Imbituba (SC).

	Sample 1 subtype C	Sample 2 subtype C	Sample 3 subtype B	Sample 4* subtype C
Protease inhibitors				
amprenavir	susceptible	susceptible	susceptible	low-level resistance
atazanavir	susceptible	susceptible	susceptible	low-level resistance
indinavir	susceptible	susceptible	susceptible	low-level resistance
lopinavir and ritonavir	susceptible	susceptible	susceptible	low-level resistance
nelfinavir	susceptible	susceptible	susceptible	low-level resistance
ritonavir	susceptible	susceptible	susceptible	low-level resistance
saquinavir	susceptible	susceptible	susceptible	low-level resistance
Nucleoside				
lamivudine	susceptible	susceptible	susceptible	high-level resistance
abacavir	susceptible	susceptible	susceptible	low-level resistance
zidovudine	susceptible	susceptible	susceptible	susceptible
stavudine	susceptible	susceptible	susceptible	susceptible
zalcitabine	susceptible	susceptible	susceptible	low-level resistance
didanosine	susceptible	susceptible	susceptible	potential low-level resistance
enfurvitide	susceptible	susceptible	susceptible	high-level resistance
tenofovir	susceptible	susceptible	susceptible	susceptible
Non-nucleosides				
delavirdine	susceptible	susceptible	susceptible	high-level resistance
efavirenz	susceptible	susceptible	susceptible	high-level resistance
nevirapine	susceptible	susceptible	susceptible	high-level resistance

*Using antiretroviral drug therapy.

revealed an infection rate of 8.6% among 140 women[16]. Another study conducted among 45 street sex workers in Santos (State of São Paulo; SP), presented an infection rate of 11.1%[2]. Therefore, the results found in this study are similar to those found by other researchers in different regions of Brazil.

In 1996, the HIV infection rate among sex workers was approximately 18% in Brazil, according to the Ministry of Health. The last survey by the Ministry of Health, in cooperation with Brasília University in 2002, revealed that the HIV infection rate was 6% among sex workers in different regions of Brazil (n = 3,000), which is similar to that found in this study. The prevalence rate among sex workers in Brazil was lower than that found in Canada (15%), China (10%) and Thailand (19%), and is higher than that found in India (5%) and Argentina (4%)[10].

In the present study, HIV-1 subtype C was found in three (75%) of the four samples in which HIV was recovered. This subtype is usually found in Africa, along with subtype D. HIV-1 subtype B is usually found in the Americas and Europe in approximately 90% of the infected individuals, including Brazil. HIV-1 F is associated with blood transmission through the use of injected illicit drugs[6 19]. In a study performed among 108 AIDS patients at the Hospital da Fundação, University of Rio Grande, in the State of Rio Grande do Sul, in 2002, subtype C was found in 58% of them, subtype B was found in 32% and subtype F in 3%, which differs from other regions in Brazil[18]. A study carried out in 2001 among 95 users of illicit injected drugs in the cities of Santos and São Paulo, in the State of São Paulo, showed subtype B in 88% of the infected individuals, F in 9.6% and C in 2.4%, in 83 out of the 95 samples[19]. Both of the above two studies discuss the possibility that the appearance of subtype C may have occurred later than that of subtype B, thus explaining the distribution mentioned above[20]. A study performed in Brasilia among 19 infected individuals found subtype B prevalence in 17 (89.5%) of the samples[3]. Another study performed among 150 sex workers in Addis Ababa, Ethiopia, found serum reactivity in 45% (67 samples) in the anti-HIV test, such that HIV-1 subtype C was present in 66 (98.5%) women, and subtype D in one woman[7].

In the present study, it can be suggested that the introduction of subtype C must have occurred as a result of the arrival of foreign ships. According to the Maritime Traffic Sector of the Port Management Department, there is great variation in the movements in the port, in relation to the origin and destination of foreign ships. The routes of both the Brazilian and foreign ships change all the time, as does the kind of cargo transported. According to an assessment made within that department, the first semester of 2003 registered ships that had stopped at ports in Saudi Arabia, Argentina, Nigeria, Chile, Poland, United States, Italy, Ukraine, Yemen and Brazil. Highway BR 101 goes through the City of Imbituba, an important road that connects Brazil and other Mercosul countries. Thus, infection by HIV subtype C can disseminate to other regions both through the port and along the BR 101 highway. The sex workers in that city have sex with sailors and truck drivers. The City of Imbituba may be a critical site in Brazil for the transmission and dissemination of distinct viral stocks in that region, in Brazil and in the other Mercosul countries. Furthermore, it was found that 36.7% of the studied women were transitory workers in the region, which shows that this population is in constant movement from one city to another. Since they do not have a fixed residence, they migrate frequently to other port cities, such as Itajaí, Camboriú and São Francisco do Sul, in Santa Catarina, and Paranaguá, in Paraná. Moreover, even those who stated that they had a fixed residence in Imbituba said that they also worked in other prostitution areas, particularly in Tubarão, Florianópolis and Balneário Camboriú, and returned occasionally to their home cities, especially when few ships were in the docks.

Another situation that must be taken into consideration in relation to the transmission and dissemination of subtype C is that Imbituba is also a place that attracts many tourists from other regions of Brazil and from abroad, due to its many beaches, traditional fairs and festivities like carnival and the Shrimp Fair. On those occasions there is increased use of alcohol and illicit drugs and an increase in sexual encounters, thus increasing the risk of contamination of the sex workers and these tourists, as well as disseminating HIV.

Considering that the predominant HIV type found in this study was HIV-1 subtype C, rather than subtype B, which predominates in the rest of Brazil, governmental and non-governmental programs should pay more attention to the Port of Imbituba, while concentrating their efforts on better examination of the viral dynamics in this location, as well as developing preventive action.

Further studies on genotyping are recommended for this region, including a greater number of volunteers who are not necessarily sex workers.

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