Artigo / Article

A case-control study of HTLV-infection among blood donors in Salvador, Bahia, Brazil - Associated risk factors and trend towards declining prevalence

Estudo da infecção do HTLV entre doadores de sangue de Salvador, Bahia, Brasil

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Previous data suggest that Salvador, the capital of the State of Bahia, a northeastern state of Brazil, has the highest prevalence of HTLV infection in blood donors among Brazilian cities. The aim of this case-control study was to identify the determinants of risk for HTLV infection among blood donors in the city of Salvador. Between January 2000 and December 2003, 504 blood donors with positive screening tests for HTLV infection (unconfirmed prevalence of 0.48%) were invited to participate in our study. A total of 154 had performed a Western Blot (WB) test, 139 were of which found to be positive (false positive screening rate 9.9%). Using a standardized questionnaire, a single interviewer obtained information on demographic, socio-economical and educational characteristics, as well as sexual behavior from 91 out of the 139 positive by WB and from 194 HTLV-negative blood donors. Prevalence of HTLV infection was 0.48%. Multivariate analysis revealed women (OR 3.79 [1.61-8.88], p=0.002), low family income (OR 3.37 [1.17-9.66], p=0.02), self-reported history of sexual transmitted diseases (OR 6.15 [2.04-18.51], p=0.001), 2 or more sexual partners during life (OR 9.29 [2.16-39.94], p=0.0020) and inconsistent use of condoms (OR 4.73 [1.98-11.26], p=0.0004) as risk factors for HTLV infection. In accordance with previous published data, our results point to an association between low socio-economical level, poor education and unsafe sexual behavior with HTLV infection. We observed a lower prevalence of HLTV infection when compared to previous data. Rev. bras. hematol. hemoter. 2006;**28**(2):120-126.*

Key words: *HTLV; blood donors; risk factors; prevalence.*

Introduction

Despite its relatively low pathogenic potential when compared to the human immunodeficiency virus (HIV), the human T-cell lymphotropic virus type I (HTLV-I) has been linked to an increasing number of diseases[1-5] since it was first described in 1980.[6] The main known transmission routes of HTLV are unprotected sexual contact, exposure to contaminated blood components, and vertically, especially through breastfeeding.[7] It has been estimated that between 15 and 20 million people are infected by HTLV throughout the world,[8] with areas of particularly higher prevalence found in Japan, Melanesia, Africa and South America.[9-11]

Epidemiological studies suggest that Salvador, the capital of Bahia State in northeastern Brazil, has the highest prevalence of HTLV-I infection compared to other large Brazilian cities. The population of Salvador is estimated at 2.5 million according to the most recent survey (www.IBGE.gov.br), 80% of which is composed of blacks or racially mixed African and Portuguese descendents.[12] In

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a recent population-based survey, Dourado *et al*.[14] reported an overall seroprevalence of HTLV-I infection of 1.76%, varying from 1.2% (males) to 2.0% (females). Furthermore, they observed higher rates among men and women above 50 years old (6.3% and 9.0%, respectively). Salvador seems to have the highest prevalence of HTLV-I infection also among blood donors (1.35%) as opposed to estimates of between 0.01 and 0.33 in four other Brazilian cities, as reported in a multi-center, cross-sectional study of blood donors.[15]

In this study we aimed at estimating the rate of HTLV infection among blood donors in the city of Salvador and to analyze the risk factors.

Methods

Study design and population

This is a case-control study conducted in the largest blood bank of Bahia State, known as Hemoba (Bahia's Hematology and Blood Bank Foundation), where an average of 25,000 blood donations are made each year. Data were collected from individuals who donated blood between January 1st 2000 and December 31st 2003. Blood donors were screened for HTLV infection according to the recommended protocol of the Brazilian Ministry of Health, namely an Elisa Test, followed by a repeated Elisa test in duplicate and a Western Blot test if the Elisa tests were repeatedly positive. All blood units from patients infected by HTLV were discarded. All donors with a positive screening test for HTLV infection by EIA (Elisa, HTLV-I rp21, enhanced, EIA, Cambridge Biotech Corporation, Worcester, MA) were asked to return to the blood bank by letter and by phone contact (for those who did not answer the letters). Those who returned had another blood sample drawn for a new Elisa test (performed in duplicate), which was followed, if repeatedly positive, by a confirmatory Western Blot test (HTLV Blot 2.4, Genelabs Diagnostics, Science Park Drive, Singapore). HTLV-positive blood donors by WB were defined as cases. Controls were randomly selected on the day of the donation while they were in the waiting room before donation, and the information obtained through the questionnaire was included in the analysis as controls only after the final results of the screening tests for HTLV were reported as being negative. All interviews, both of cases and controls, were performed between June 2003 and December 2004. We invited a number of individuals sufficient to give a ratio of 2 controls to each case. The study protocol was approved by the Ethics Committee of the Fundação Oswaldo Cruz (Fiocruz).

Data collection procedures

A standardized questionnaire was applied to all participating individuals by a single interviewer (A. M.), after the candidates had signed a consent form. The questionnaire was structured to allow the systematic collection of the following data: current and previous residential addresses, previous blood donation, personal and family monthly incomes, years of education, marital status, number of children, number of brothers and sisters, religion, smoking and alcohol habits, age at first sexual relationship, frequency of use of condoms during sexual intercourse (never, rarely, frequently or always), number of sexual partners (in the last six months, in the last two years and since initiation of sexual life), frequency of homosexual relationships (same as for condom use), frequency of oral and anal sex (same as for condom use), history of breastfeeding (less than six months, more than six months, unknown), previous self-reported history of sexually transmitted diseases (STD) and history of blood transfusions. Finally, each individual was asked if he or she had ever donated blood with the sole purpose of having his or her blood tested for HIV infection. Race was not considered as a variable given the difficulty to characterize and allocate individuals into different ethnic groups in our country, and in particular in the state of Bahia.[12,13]

Statistical analysis

The variables age, personal and family income, years of education, number of children, number of brothers and sisters, age at first sexual relationship and number of sexual partners were all obtained as continuous variables. All other variable were registered as categorical. The Kolmogorov-Smirnof test was performed for all continuous variables to attest their normal distribution, allowing the use of parametric tests. Continuous variables are reported as means and standard deviations, and the Student's T test was used for comparisons. The chi-squared tests (or Fisher's exact test when appropriate) was used to compare the categorical variables. Odds ratios and respective 95% confidence interval were used to estimate the association of study variables with HTLV infection. All variables with statistical significance (considered as two-tailed p-value $\leq$ 0.05) were entered in the multivariate model. All tests were run in the statistical packages SPSS (version 9.0) and EpiInfo (version 6.0 - CDC, Atlanta, GA, USA).

Results

Between January 1st 2000 and December 31st 2003 there were 104,835 blood donations at Hemoba's blood bank. Among these blood donors, 504 had a positive screening test for HTLV-I/II infection, which gives a crude prevalence of 0.48%. All 504 individuals were contacted by letter (2 times) and phone, but only 154 (30.5%) returned to draw a blood sample for additional tests. Fifteen samples were found to be negative upon retesting with Elisa (false positive rate of 9.1% among those who were retested), giving a total of 139 confirmed HTLV-infected subjects. Ninety-one out of

the 139 HTLV-infected subjects consented in participating in the case-control study. The vast majority of the 48 individuals who did not want to participate simply "were not interested". No information is available about the individuals who did not return for retesting. There was no difference between the participating and non-participating infected subjects with regard to mean age, sex and place of origin (data not shown). One hundred and ninety four blood donors with no evidence of HTLV infection consented in participating as controls.

Table 1 depicts the baseline characteristics of the participating individuals.

The overall mean age was 33.6 years, differing significantly between cases and controls: 39.3 and 31.1 years, respectively (p<0.0001). While 66.6% of all participants were males, the prevalence of HTLV infection was significantly higher among the women: 43.1% versus 26.3% (p=0.004). The proportion of "new donors" (individuals who donated blood for the very first time) between cases and controls was, respectively, 55.6% and 31.6% (p < 0.0001). Considering all participants in the study, the average numbers of years in school was 10, with significant differences between cases and controls: 8.6 versus 10.7 years, respectively (p<0.0001). There were also significant differences in mean personal and family incomes (in Brazilian minimum wages*) between cases and controls: respectively 1.6 versus 2.6 (p=0.03) and 3.0 versus 6.4 (p<0.0001). The mean age at first sexual encounter did not differ between groups: 16.4 years old among the cases and 16.5 years old among the controls (p = 0.84). Likewise, no difference was observed between groups regarding the mean number of different sexual partners during life: 8.9 among the cases and 8.1 among the controls (p = 0.33). However, the proportion of individuals who had 2 or more as opposed to 0-1 sexual partners during life significantly differed between cases and controls: 92% and 81%, respectively, p = 0.013).

Table 1
General characteristics of the participating blood donors.

Variable	HTLV-positive N (%)		HTLV-negative N (%)	
	Men 50 (100)	Women 41 (100)	Men 140 (100)	Women 54 (100)
Age				
18-29	11 (22.0)	11 (26.8)	77 (55.0)	30 (55.6)
30-39	13 (26.0)	10 (24.4)	37 (26.4)	8 (14.8)
40-49	18 (36.0)	12 (29.3)	18 (12.9)	15 (27.8)
50 or more	8 (16.0)	8 (19.5)	8 (5.7)	1 (1.9
Education grade				
Fundamental	15 (30.0)	6 (14.6)	15 (10.7)	6 (11.1)
First grade	13 (26.0)	13 (31.7)	30 (21.4)	13 (24.1)
Second grade	20 (40.0)	20 (48.8)	67 (47.9)	24 (44.4)
High School/College	2 (4.0)	2 (4.9)	28 (20)	11 (20.4)
Use of condoms				
Never	16 (32.7)	15 (37.5)	8 (5.8)	7 (13.2)
Rarely	18 (36.7)	17 (42.5)	34 (24.8)	23 (43.4)
Frequently	10 (20.4)	5 (12.5)	59 (43.1)	12 (22.6)
Always	5 (10.2)	3 (7.5)	36 (26.3)	11 (20.8)
Breastfeeding				
Yes (< 6 months)	8 (16.0)	5 (12.2)	22 (15.7)	11 (20.4)
Yes (= 6 months)	25 (50.0)	19 (46.3)	93 (66.4)	33 (61.1)
No	0	3 (7.3)	5 (3.6)	3 (5.6)
Unknown	17 (34.0)	14 (34.2)	20 (14.3)	7 (12.9)
Cigarette smoking				
Yes	19 (38.0)	8 (19.5)	18 (12.9)	6 (11.1)
No	31 (62.0)	33 (80.5)	122 (87.1)	48 (88.9)
Previous blood transfusion				
Yes	4 (8.0)	5 (12.2)	4 (2.9)	0
\no	46 (92.0)	36 (87.8)	136 (97.1)	54 (100)
Previous blood donation				
Yes	30 (61.2)	10 (24.4)	103 (74.1)	29 (53.7)
No	19 (38.8)	31 (75.6)	36 (25.9)	25 (46.3)

Table 2 depicts the results of both univariate and multivariate analysis. When compared to the control group, HTLV-infected individuals were more likely to be older than 51, have a lower education level, lower monthly personal and family incomes, as well as more children. Additionally, they more frequently reported inconsistent use of condoms during sexual relationships, had more frequently history of previous STD (self reported) and blood transfusions, as well as a more frequent history of cigarette smoking. In addition, infected individuals were more likely to have had more than one sexual partner during life when compared to controls. Being single was found to be protective to HTLV infection, and age at first sexual encounter did not act as a risk factor for HTLV infection. Likewise, breastfeeding was not found to be a risk factor for HTLV infection in this study population.

In the multivariate analysis, five variables remained as independent risk factors for HTLV infection: being a woman (OR=3.79; 95% CI 1.61-8.88), family monthly income lower than 5 minimum wages (OR = 3.37, 95% CI 1.17-9.66), inconsistent use of condoms (OR = 4.73, 95% CI 1.98-11.26), two or more sexual partners during life (OR = 9.29, 95% CI 2.16-39.94) and previous self-reported history of STD (OR = 6.15, 95% CI 2.04-18.51) (Table 2).

** 1 minimal wage = US ~100.00*

Table 2
Univariate and multivariate analysis of HTLV infection and different variables

Variable	Univariate analysis				Multivariate analysis	
	Cases N (%)	Controls N (%)	P value	OR (95% CI)	P value	OR (95% CI)
Age (years)						
≥ 51	13 (14.2)	7 (3.6)				
≤ 50	78 (85.8)	187 (96.4)	0.001	4.45 (1.71 - 11.58)	0.38	2.10 (0.39 - 11.28)
Gender						
Female	41 (45.1)	54 (27.8)	0.004	2.12 (1.27 - 3.57)	0.002	3.79 (1.61 - 8.88)
Male	50 (54.9)	140 (72.2)				
Years of education						
≤ 5	21 (23.1)	21 (10.8)	0.007	2.47 (1.27 - 4.81)	0.55	1.35 (0.49 - 3.68)
≥ 6	70 (76.9)	173 (89.2)				
Personal monthly income*						
≤ 5	89 (97.8)	174 (89.7)	0.016	5.11 (1.17 - 22.38)	0.31	3.43 (0.31 - 37.69)
> 5	2 (2.2)	20 (11.3)				
Family monthly income*						
≤ 5	81 (89.0)	119 (61.3)	< 0.0001	5.10 (2.49 - 10.46)	0.02	3.37 (1.17 - 9.66)
> 5	10 (11.0)	75 (39.7)				
Marital status						
Single	26 (28.6)	87 (44.8)	0.009	0.49 (0.28 - 0.84)	0.20	1.97 (0.69 - 5.63)
Other	65 (71.4)	107 (55.2)				
Offspring						
≥ 1	69 (75.8)	105 (54.1)	<0.0001	2.65 (1.52 - 4.63)	0.44	1.51 (0.52 - 4.36)
0	22 (24.2)	89 (45.9)				
Use of condoms						
Never/rarely	66 (74.2)	72 (37.9)	< 0.0001	4.70 (2.69 - 8.21)	0.0004	4.73 (1.98 - 11.26)
Frequently/always	23 (25.8)	118 (62.1)				
Previous history of STD						
Yes	31 (34.1)	15 (7.7)	< 0.0001	6.16 (3.11 - 12.19)	0.001	6.15 (2.04 - 18.51)
No	60 (65.9)	179 (92.3)				
Total number of sexual partners in life						
≥ 2	84 (92.3)	157 (80.9)	0.013	2.82 (1.20 - 6.61)	0.002	9.29 (2.16 - 39.94)
0 - 1	7 (7.7)	37 (19.1)				
Cigarette smoking	27 (29.7)	24 (12.4)	< 0.0001	2.98 (1.60 - 5.55)	0.95	1.02 (0.37 - 2.82)
Yes	64 (70.3)	170 (87.6)				
No						
Previous blood transfusion						
Yes	9 (9.9)	4 (2.1)	0.003	5.21 (1.56 - 17.41)	0.08	12.16 (0.70 - 209.98)
No	82 (90.1)	190 (97.9)				

* - in Brazilian minimum monthly wage (~ U$100.00)
STD - Sexually Transmitted Diseases

Since several other blood tests are performed to screen blood donors, we also looked for differences between HTLV-infected and HTLV-non-infected individuals regarding the results of other serological tests. Twenty out of 97 HTLV-infected individuals had at least one additional abnormal screening test, as opposed to 9 out of 194 HTLV-non-infected individuals (20.2% versus 4.6% - $p < 0.0001$). The most frequent second abnormal test among the cases was anti-HBc (11/91), followed by anti-HCV (6/91), VDRL (4/91), anti-HIV (1/91), and Chagas' disease (1/91). Among the controls, the most frequent abnormal screening tests were AgHbs (3/194) and Anti-Hbc (3/194), HIV (1/194), VDRL (1/194) and Chagas' disease (1/194).

Discussion

In line with previous reports, our results show an association between HTLV infection and lower socio-economical and educational levels, as well as unsafe sexual practices.[13,16-18] In the univariate analysis the following were identified as risk factors for HTLV infection: an older age, being female, lower education level, lower personal and family incomes, higher number of children and inconsistent condom use. Likewise, previous self-reported history of STD, previous blood transfusion and smoking were also found to be risk factors. However, only five of the above variables remained as independent risk factors for HTLV infection in the multivariate model: being female, family income, frequency of condom use, number of sexual partners during life and previous self-reported history of STD.

A relatively unexpected finding of our study was the lack of association between age at first sexual relationship and risk for HTLV infection, not even in the univariate analysis. In the study by Rouet *et al*. in Guadalupe,[16] in which age at first sexual relationship was found to be a risk factor for HTLV infection, mean ages of initiation of sexual relationships for cases and controls were, respectively, 17.2 and 19.3 years old. While the mean age of first sexual contact among HTLV-infected individuals was quite similar in both studies, in our control population the initiation of the sexual life occurred almost three years earlier. Studies undertaken in our country reveal numbers for the initiation of sexual relationships similar to ours.[19-21] It is possible therefore that differences in sexual behavior between these two countries may explain the lack of association for this specific variable with HTLV infection in this study. Similarly to what was observed for age at first sexual encounter, the mean number of sexual partners during life was not found to be a risk factor for HTLV infection. Similar to what was observed for age at first sexual relationship, behavioral differences may explain this observation. For instance, while 23.9% of controls in Guadalupe had had two or more different sexual partners in the three years that anteceded the interview, 44.3% of our controls had had two or more different sexual partners in the two years that preceded our study. However, when this variable was categorized as 1 versus 2 or more sexual partners during life, it did act as a risk factor for HTLV infection both in the univariate and multivariate analysis (Table 2).

Another interesting finding of our study was the fact that single individuals were at lower risk for HTLV infection when compared to individuals of other marital statuses. A closer look at this subgroup revealed that they were younger than the individuals of other marital statuses (mean age 27.4 years versus 37.8 years, $p < 0.0001$), had higher education (11.3 years in school versus 9.3, $p < 0.0001$), and reported using condoms more frequently than the individuals in the other groups (used condoms always or frequently: 59.7% versus 16.8%, $p < 0.0001$). This profile is known to be associated with lower risk for HTLV infection. There was no difference between singles and individuals in the other marital status with regard to age at first sexual intercourse (16.4 versus 16.7 years old, $p = 0.49$), number of different sexual partners during life (7.9 versus 8.7, $p = 0.33$), and family income (6.1 minimum wage versus 4.8 minimum wage, $p = 0.08$). Unfortunately we were not provided with details of this population in the Guadeloupean study, where single marital status was found to be a risk factor for HTLV infection, yet without statistical significance.[16]

Another unexpected and also interesting finding of the present study was a lower prevalence of HTLV infection among blood donors when compared to data published in 1993.[14] We have observed a reduction in the prevalence of HTLV infection among blood donors in Salvador from 1.35% to 0.48% in the span of one decade. There may be more than one reason for this finding. Firstly, a nation-wide program, known as COAS (Centro de Orientação e Apoio Sorológico – Serologic Testing and Orientation Center) was launched in 1994 with the objective of centralizing the serological testing and health care (counseling, follow-up and treatment) of people with sexually transmitted diseases, with particular focus on HIV infection. As this program gained rapid popular acceptance, it is possible that there has been a migration from blood banks to the COAS program of individuals willing to know their serological status for several sexually transmitted diseases, including HTLV and HIV. Secondly, improvements in clinical screening of blood donors may have prevented, throughout the recent years, an increasing number of higher risk individuals from donating blood and consequently being detected as infected individuals through serological screening. Thirdly, as previously suggested,[22] a diluting effect over time of the screening process might have played some role, in the sense that number of infected individuals detected in the screening process (and indefinitely exclude thereafter) may be not be replaced over time at the same rate in which they are removed from the blood donor pool. Lastly, and perhaps less likely, the prevalence of HTLV infection may have truly decreased

over the years. Behavioral changes, specially among the youth (e.g. more frequent condom use), may account for this third possibility. For instance, a recent, nation-wide population based survey revealed that 79.5% of individuals who had different sexual partners during the preceding 12 months reported using condoms during their "last sexual contact", compared to 64% in 1998 (www.aids.gov.br). Likewise, the proportion of individuals who reported having only one sexual partner in the 12 months preceding the survey was 85% in 2003, as opposed to 81% in 1998 (www.aids.gov.br). Yet these figures may not be irrefutable evidence to justify the observed lower prevalence of HTLV infection among our blood donors, they suggest that behavioral changes may be in course towards a safer profile for sexually transmitted diseases.

Finally, our data also give some clues with regard to the main transmission routes for HTLV among blood donors in Bahia. It is interesting to note that there was no relationship between breastfeeding and HTLV-infection (not even in the univariate analysis) in this study population. Furthermore, people who were HTLV-infected were more likely to have another abnormal screening test. Since the vast majority of additional abnormal tests are related to other STD's, it is reasonable to state that sexual transmission is perhaps the main route of HTLV transmission among blood donors.

It is important to highlight some of the limitations of the present study. Firstly, the fact that we studied a blood donor population, the results are not to be extrapolated to the general population. Blood donors are known to be healthier than the general population, which implies that the prevalence in the general population is expected to be higher.[14] Secondly, there are methodological limitations proper to case-control design, particularly selection bias and recollection bias, which should be considered for the appropriate interpretation of the data. For instance, there was a relatively low response rate of participation among the HTLV-infected individuals (among all HTLV-infected individuals identified by the screening test, only 1/4 actually participated as cases). Misreported address may have significantly contributed to this low rate of response, as this problem has been described in other Brazilian blood banks.[20] In addition, the fact that the interview was not blinded to the serologic status could have introduced selection bias in the present study. However, the inexistence of significant differences between participating and non participating infected individuals attenuates the probability of this bias to some extent.

Resumo

*Estudos anteriores sugerem que Salvador, capital da Bahia, um estado do Nordeste do Brasil, tem a maior prevalência de infecção por HTLV em doadores de sangue entre as cidades brasileiras. O objetivo deste estudo caso-controle foi identificar os determinantes de risco para a infecção por HTLV entre doadores de sangue em Salvador. Entre janeiro 2000 e dezembro 2003, 504 doadores de sangue positivos para a infecção por HTLV em teste de triagem (prevalência não confirmada de 0,48%) foram convidados para participar deste estudo. O Western Blot foi realizado em 154 destes doadores dos quais 139 foram confirmados como positivos (taxa de triagem falso positivo de 9,9%). A partir de um questionário padronizado, informações demográficas, socioeconômicas e sobre as características educacionais assim como sobre o comportamento sexual foram obtidas de 91 participantes, entre os 139 Western Blot positivos e de 194 participantes HTLV-negativos. A prevalência da infecção de HTLV foi de 0,48%. A análise multivariada revelou os seguintes fatores de risco para a infecção de HTLV: sexo feminino (OR 3.79 [1.61-8.88], p=0.002), baixa renda familiar (OR 3.37 [1.17-9.66], p=0.02), relato espontâneo de DST (OR 6.15 [2.04-18.51], p=0.001), dois ou mais parceiros sexuais durante a vida sexual, (OR 9.29 [2.16-39.94], p=0.0020), utilização inconstante de camisinhas (OR 4.73 [1.98-11.26], p=0.0004). Conforme dados da literatura, nossos resultados indicam uma associação entre o baixo nível socioeconômico, a baixa escolaridade e o comportamento sexual de risco com a infecção por HTLV. Rev. bras. hematol. hemoter. 2006;**28**(2):120-126.*

Palavras-chave: *HTLV; doadores de sangue; fatores de risco; prevalência.*

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