

## Human T-Cell Lymphotropic Virus Type 1 Infection Among Pregnant Women in Northeastern Brazil

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**Summary:** An evaluation of human T-cell lymphotropic virus type 1 (HTLV-1) infection among 6754 pregnant women in Salvador, Bahia, Brazil using enzyme-linked immunosorbent assay, Western blot analysis, and polymerase chain reaction assay found a rate of infection of 0.84% (57 of 6754 women). Epidemiologic and obstetric data on the HTLV-1-positive pregnant women were analyzed and compared with data on a control group of HTLV-1-negative pregnant women. The mean age of the HTLV-1-positive women was 26.2 years. All were seronegative for HIV and syphilis, and only 2 reported a past history of sexually transmitted infection and more than 10 sexual partners. Of the HTLV-1-positive women, 88.5% were breast-fed, 4% were bottle fed, and 7.5% did not know. Six women had received blood transfusions, and only 1 reported intravenous drug use. Fifty-two HTLV-1-positive women could be followed: 45 had full-term deliveries, 5 had premature deliveries, and 2 had abortions. Our results indicate that (1) the frequency of HTLV-1 infection among pregnant women is relatively high in Salvador, Bahia, Brazil; (2) maternal infection was probably acquired more frequently through breast-feeding, but the sexual route was certainly the second most important means of transmission; (3) HTLV-1-positive women had a history of eczema-like infections in childhood more frequently than the control group; (4) HTLV-1 infection did not interfere in the course of pregnancy; and (5) no associated congenital infections were observed in the HTLV-1-positive women. **Key Words:** Clinical epidemiologic findings of HTLV-1—HTLV-1-associated infective dermatitis—HTLV-1 transmission—Obstetric aspects of HTLV-1 infection.

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Surveys performed in several Brazilian cities among blood donors showed the highest prevalence rates of human T-cell lymphotropic virus type 1 (HTLV-1) infection in Salvador, Bahia (1.35%) (1). The occurrence of diseases associated with the vertical transmission of HTLV-1 in Salvador such as adult T-cell leukemia/lymphoma (ATL) and infective dermatitis associated with HTLV-1 infection suggests that this route of transmis-

sion may be important in this city (2,3). A recent study that included exclusively lymphoma cases from Bahia demonstrated that 33.3% of the T-cell lymphomas were associated with HTLV-1 infection (4). Furthermore, since September 1997, 18 cases of infective dermatitis associated with HTLV-1 have already been observed in Salvador (Bittencourt A.L., unpublished data).

Considering the importance of vertical transmission as a main route of HTLV-1 infection (5), we decided to evaluate the frequency of this infection among pregnant women in a prenatal care unit in Salvador at which women of low social class are attended. In addition, the clinical, epidemiologic, and obstetric data of the carrier mothers were analyzed, and the epidemiologic data were

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compared with data on a control group of HTLV-1-seronegative pregnant women.

## METHODS

### Study Population

A total of 6754 pregnant women who attended the prenatal care unit of a state maternity hospital (IPERBA) in the city of Salvador (the capital of Bahia) were selected between January 1996 and September 1998 for the HTLV-1/II seroprevalence study. All women who attended the maternity hospital for their first prenatal care visit were invited to participate in the study. During the data collection years 10,809 women attended the prenatal care unit, and 62.5% of them were included in the study. IPERBA attends women of low social economic status. Salvador has a population of 2,274,150 inhabitants, of whom roughly 80% are black or racially mixed. A control group was selected between July and September 1998 for a case-control analysis. Among the daily-screened patients, 2 controls were randomly selected for each HTLV-1-positive mother. Clinical, obstetric, and epidemiologic data were collected for 53 HTLV-1-positive women and 122 HTLV-1/II-negative pregnant controls. Mothers and partners of the HTLV-1 carrier pregnant women who were present at the visit were asked to be screened serologically for this virus. The newborns were classified by gestational age and weight according to Battaglia and Lubchenco (6). Informed consent was obtained from the participants.

### Serologic Testing

All sera were screened for the presence of antibodies against HTLV-1/II by enzyme-linked immunosorbent assay (ELISA) (rp21e-enhanced enzyme immunoassay; Cambridge Biotech Corporation, Worcester, MA, U.S.A.). Repeated reactive samples were then submitted for serologic confirmation and discrimination between HTLV-1 and HTLV-II using Western blot analysis (HTLV Blot 2.4; Genelabs, Singapore). Western blot results were interpreted according to the manufacturer's instructions as follows: (1) HTLV-1-positive reactivity to GAG p19 with or without p24 and two ENV (GD21 and rgp 46-I; (2) HTLV-II-positive reactivity to GAG p24 with or without p19 and two ENV (GD21 and rgp46-II0); (3) HTLV-positive reactivity to GAG p19 and p24 and ENV GD21; (4) indeterminate reactivity to HTLV specific bands detected but did not meet the criteria for HTLV-1, HTLV-II, or HTLV seropositivity; and (5) HTLV-negative reactivity to HTLV specific bands. In all seropositive women, the tests were repeated in a second sample obtained on another day. Serologic screening for HTLV-1 was also performed in 10 mothers of the HTLV-1-positive pregnant women. Sera were also screened for *Treponema pallidum* antibodies and evaluated by serum reactivity to nontreponemal cardiolipin antigen by the Venereal Disease Research Laboratory (test) (Laborclin, Paraná, Brazil), and *Trypanosoma cruzi* antibodies were evaluated by a passive hemagglutination test (Biolab, Rio de Janeiro, Brazil). *Toxoplasma gondii* antibodies (IgG and IgM), HIV-1/HIV-2 antibodies, rubella virus antibodies (IgG and IgM), cytomegalovirus (CMV) antibodies (IgG and IgM), hepatitis C virus antibodies, hepatitis B core antibodies, and hepatitis B surface antigen were detected by ELISA (Roche, Basel, Switzerland).

### Polymerase Chain Reaction in Peripheral Blood Mononuclear Cells

Polymerase chain reaction (PCR) analyses were performed in the serologically indeterminate pregnant women and in 35 serologically

HTLV-1-positive pregnant women. Genomic DNA was extracted from samples of peripheral mononuclear cells by sodium dodecyl sulfate/proteinase K treatment followed by phenol/chloroform extraction. Nested PCR was performed targeting the pol region of the HTLV. One microgram of genomic DNA was used to amplify a fragment of 398 base pairs (bp) in the *pol* gene with the following primers: 12P1 (5'GCCTTCATGTATGGGTAGAACACTT3') and SK111 (5'GTG-GTGGATTGGCCATCGGGTTTT3') corresponding to nt 4525-4549 and nt 4899-4922, respectively. A second round was carried out using the nested primers 12P5 (5'TGGTTGATTGTCCATAGGGCT3'), 1P1 (5'AGCCATCTCAGCTACCCAAAAGAGA3'), and 2P3 (5'CGCAT-CAAGCATTCTACCCA3') corresponding to nt 4853-4873 for HTLV-1/II, nt 4556-4580 for HTLV-1, and nt 4711-4730 for HTLV-II, respectively. The inner primers amplify a fragment of 318 bp for HTLV-1 and 161 bp for HTLV-II. Reactions were performed in reaction mixtures containing 1.5 µg of DNA, 2.5 U of Taq DNA polymerase (Perkin Elmer-Cetus, Norwalk, CT, U.S.A.), 50 mmol of KCl, 10 mmol of Tris-HCl (pH 8.3), 1.5 mmol of MgCl<sub>2</sub>, 0.2 mmol of each deoxynucleotide triphosphate, and 10 pmol of each primer. PCR cycling conditions used were the same described previously (7). The amplified products were analyzed by 3% agarose gel electrophoresis followed by ethidium bromide staining.

### Histopathologic Findings

The placentas and adnexa of the seropositive mothers were fixed in buffered formol. Sections of paraffin-embedded tissue were stained by hematoxylin-eosin. Autopsy was performed on the aborted fetuses.

### Data Analysis

Frequency distributions were determined for each variable, including the mean and SD for continuous variables. Crude odds ratios (ORs) and 95% confidence intervals (CI) measuring the association of selected variables and HTLV-1 infection were calculated from 2 × 2 tables (8).

## RESULTS

Sixty-one samples were repeatedly reactive by ELISA. When analyzed by Western blot, these samples yielded the following results: 53 (0.78%) were seropositive for HTLV-1, 2 (0.03%) were seropositive for HTLV-II, and 6 (0.08%) were indeterminate. The PCR analysis of these samples showed that 4 were positive and 2 were negative. Thus, the overall prevalence of HTLV-1 carrier mothers was 0.84%. Thirty-five (67.3%) serologically positive women for HTLV-1 were also positive by PCR.

From the 57 HTLV-1 carrier women, 5 (8.4%) could not be contacted and were excluded from the case-control analysis. All the HTLV-1 carrier mothers except 2 were from the State of Bahia, and 59% were from Salvador. Of the HTLV-1-positive women, 71.7% were black or mulatto, and the mean age was 26.2 years. Only 2 reported a past history of sexually transmitted infections. Forty-six (88.5%) reported being breast-fed as a baby, 2 (4.0%) were not breast-fed, and 4 (7.5%) did not

**TABLE 1.** Univariate analysis for HTLV-1-positive and HTLV-1-negative pregnant women in Salvador, Bahia, Brazil

Variable	HTLV-positive (%)	HTLV-negative (%)	Odds ratio	95% confidence interval
Age (years)*				
14-20	13.4	22.9	1.00	—
21-30	59.6	54.9	1.85	0.68-5.24
31-42	26.9	22.1	2.07	0.65-6.78
Total (N)	52	122		
Literacy				
Illiterate	69.2	56.7	1.72	0.86-3.43
Literate	30.8	43.3	—	—
Total (N)	52	120		
Marital status				
Married	49.0	31.9	2.21	1.14-4.32
Single	51.0	68.0	—	—
Total (N)	51	122		
Income**				
<1 mw-1 mw	41.2	24.6	3.27	1.04-10.64
2 mw-5 mw	47.1	52.5	1.75	0.59-5.39
+5 mw	11.8	23.0	1.00	—
Total (N)	51	122		
Age at first sexual encounter (years)				
11-15	30.8	24.2	1.39	0.64-3.05
16-20+	69.2	75.8	—	—
Total (N)	52	120		
Number of lifetime sexual partners				
>2	50.0	27.8	2.58	1.32-5.07
1-2	50.0	72.1	—	—
Total (N)	52	122		
Reported eczema-like lesion				
Yes	15.6	0.85	21.4	2.54-179.3
No	84.4	99.2	—	—
Total (N)	45	117		
History of blood transfusion				
Yes	11.5	3.3	3.85	1.03-14.3
No	88.5	96.7	—	—
Total (N)	52	122		

\*  $p = .19$  ( $\chi^2$  for linear trend).

\*\*  $p = .016$  ( $\chi^2$  for linear trend).

HTLV, human T-cell lymphotropic virus; mw, minimum wage ( $\approx$ \$60).

know. Six had received blood transfusions, and 1 was an intravenous drug user (IDU). Ninety percent of the women had 1 to 4 sexual partners, and only 2 had more than 10 sexual partners. Only 10 mothers and 5 partners of the HTLV-1-positive pregnant women agreed to serologic screening for this virus. Six mothers (60%) were seropositive, and all the partners were seronegative. The pregnant women whose mothers were seronegative (4) had no history of blood transfusions or use of intravenous drugs. Aside from 1 patient with ATL, none of the other pregnant women had symptoms of HTLV-1-associated diseases. Two women presented with symptoms of sickle-cell anemia, and 1 had hypertension during gestation.

All the 57 HTLV-1-positive women were serologically negative for syphilis and HIV. Only 1 demonstrated antibodies against *T. cruzi* (1.7%). Aside from 1 woman, all the others were screened for toxoplasmosis, rubella,

CMV infection and hepatitis B and C. IgG antibodies against toxoplasma, CMV, and rubella were found, respectively, in 62.5%, 84%, and 71.4% of the screened women, but no IgM antibodies against these agents were observed. There was no serologic evidence of hepatitis B or hepatitis C.

Fifty-two HTLV-1-positive women were followed: 45 had full-term deliveries, 5 experienced preterm deliveries, and 2 had abortions. Of the 45 full-term newborns, 42 were adequate to gestational age and 3 were small for date. Three of the preterm births were stillbirths beyond 30 weeks of gestation. The fetal deaths were caused by complications of sickle-cell anemia and hypertension. The 2 preterm newborns had attained 35 and 36 weeks of intrauterine age. One abortion at 6 weeks of gestation was spontaneous, and the other, at 12 weeks of gestation, was therapeutic. The mother had ATL and had to be treated with chemotherapy and interferon- $\gamma$ .

The histopathologic study of 42 placentas of the carrier women showed mild and focal chronic villitis in 12%. Furthermore, ascending infection was not seen in any of these placentas. Autopsy performed in the 2 aborted fetuses showed no evidence of infection.

### Case-Control Analysis

The mean age at enrollment and age at first intercourse were 27.4 years (SD:  $\pm 6.11$ ) and 17.8 years (SD:  $\pm 3.60$ ), respectively, for the seropositive women and 25.6 years (SD:  $\pm 5.77$ ) and 18.1 years (SD:  $\pm 3.58$ ), respectively, for the controls. These differences were not statistically significant. Most (70%) seropositive women and controls were black or mulatto. Table 1 presents a univariate analysis for the association of HTLV-1 infection with demographic, social, and biologic variables. A greater proportion of seropositive women than controls were married, had less income (<1 minimum wage–1 minimum wage), had more than 2 lifetime sexual partners, and had a history of blood transfusion (OR = 2.21; CI: 1.14–4.32), (OR = 3.27; CI: 1.04–10.64), (OR = 2.58; CI: 1.32–5.07), (OR = 3.85; CI: 1.03–14.34), respectively. The risk of infection did not vary much as age increased. The OR for the association of HTLV and age was 2-fold greater for those women 21 to 30 years old and 31 to 42 years old compared with the younger group (14–20 years old). This association was not statistically significant, however. There was a statistically significant positive trend in the ORs as income decreased ( $p = .016$ ). The ORs for all the other variables in the tables were in the direction of a positive association with HTLV-1 infection, but the CIs were wide and not precise.

### DISCUSSION

The prevalence of HTLV-1 carriers among pregnant women in the current study was relatively high (0.84%), and only 2 cases of HTLV-II infection were found (0.03%). The prevalence of HTLV-1 infection is similar to that found by Santos et al. (9), who evaluated 1024 pregnant women in Salvador (0.88%). It was lower than the prevalence of HTLV-1 found among blood donors in that city (1.34%) (1), however, which can possibly be explained by the lower age of our study population. It is known that the prevalence of HTLV-1 infection increases with age (5,10). The OR for the association between HTLV and age was greater for older women compared with the younger age group. Even though it is not statistically significant, this association probably reflects the accumulation of exposure to HTLV-1 through time (11).

The epidemiologic data of the carrier mothers suggest that the vertical route was certainly the most frequent mode of HTLV-1 transmission. A total of 88.5% of the women gave information that they were breast-fed, and few women had received blood transfusions or had more than 10 partners during their lives. Half of the carrier women referred to only 1 or 2 partners. In addition, serologic screening of these women did not demonstrate sexually acquired infections such as HIV infection, syphilis, and hepatitis B. Nevertheless, sexual transmission may have occurred in some of the carrier women. Four mothers of 10 pregnant women who were submitted to serologic screening for HTLV-1 were seronegative, demonstrating that HTLV-1 transmission had also occurred by the sexual route (these pregnant women had received no blood transfusions and were not IDUs). Furthermore, it was observed that a higher proportion of the seropositive women than the controls reported having more than 2 partners and a sexual life initiated at an age ranging from 11 to 15 years old. We cannot rule out blood as a possible source of infection among a few patients with a history of transfusion.

The only IDU observed in this study was HTLV-1-positive. Unlike findings in the United States and Europe, in Bahia as well as in São Paulo, HTLV-1 infection is more prevalent than HTLV-II infection among IDUs (12,13).

It is interesting to point out that 15% of the seropositive women had a past history of eczema-like lesions in childhood, which was probably HTLV-1-associated infective dermatitis, a condition associated with vertically acquired infection (3). Contrasting with these data, only 0.8% of the seronegative pregnant women inferred the same past history. This observation indicates that HTLV-1 is certainly an important cause of childhood dermatitis in Salvador.

With the exception of the case of ATL, no clinical manifestations associated with HTLV infection were observed in the carrier pregnant women. HTLV-1 infection did not interfere with the course of pregnancy. The three perinatal deaths observed were caused by hypertension and sickle cell anemia, which are conditions not associated with HTLV-1 infection. Six percent of the full-term births resulted in small-for-date babies without other abnormalities, but this frequency is not higher than that observed in the literature (14).

There is a great deal of evidence that HTLV-1 carriers present a significant alteration of the immune function, with an increased risk for acute and chronic infections by several pathogens. An increased prevalence of strongyloidiasis, leprosy, mycosis, and tuberculosis has been referred to in the literature (15). For this reason and

through serologic testing, we evaluated in the carrier pregnant women the occurrence of infections that may be transmitted transplacentally, but the results were negative for syphilis, hepatitis B and C, and HIV infection. In addition, none of the mothers screened presented serologic evidence of acute rubella, acute toxoplasmosis, or acute CMV infection. The frequency of positivity for IgG antibodies against rubella virus, CMV, toxoplasma, and Chagas' disease (1.7%) was similar to or lower than the frequency referred to in other serologic investigations among pregnant women in Brazil (9,16). A mild and focal villitis of unknown origin was observed in 12% of the placentas studied, but the frequency of villitis of unknown origin in normal-term placentas varies from 6% to 34% (17). No case of ascending infection was observed in the placentas studied. These data suggest that HTLV-1-positive pregnant women are no more susceptible to infections than other pregnant women.

The prevalence of 0.84% of HTLV-1 infection among pregnant women indicates the necessity of serologic screening for this infection as part of the prenatal care services offered in Salvador so as to advise the carrier mothers to refrain from breast-feeding. Considering that most of these carrier mothers are of low social status, it is necessary to provide an alternative nutritional supply to their offspring.

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