HUMAN T LYMPHOTROPIC VIRUS TYPE I INFECTION IN PREGNANT WOMEN
IN RIO DE JANEIRO, BRAZIL

Janaina Chaves Câmara¹, José Pascoal Simonetti¹, Sandra Regina Rodrigues Simonetti¹, Rosemary Suely Ribeiro², Maria Célia de Freitas Leite Costa², Claudia Marques Barquinha Lopes², Hermann Gonçalves Schatzmayr³, Sílvia Maia Farias de Carvalho⁴

¹Laboratório de Morfologia e Morfogênese Viral (formerly: Laboratório de Ultra-estrutura Viral, Departamento de Virologia), Instituto Oswaldo Cruz, Fiocruz, Rio de Janeiro, RJ, Brasil.
²Instituto Fernandes Figueira, Fiocruz, Rio de Janeiro, RJ, Brasil.
³Laboratório de Flavivirus, Instituto Oswaldo Cruz, Fiocruz, Rio de Janeiro, RJ, Brasil.
⁴Instituto Estadual de Hematologia, HEMORIO, Rio de Janeiro, RJ, Brasil.

Corresponding author:
Tel.: + 55 21 2332-8611
Fax: + 55 21 2224-7030
E-mail: silvia@hemorio.rj.gov.br

Key words: HTLV-I, pregnant women, Brazil
ABSTRACT

Human T lymphotropic virus type I (HTLV-I) is a retrovirus and the transmission occurs from mother to child, by sexual contact or by blood transfusion. This study was performed to evaluate the prevalence ratio of HTLV infection in a group of 389 pregnant women attending at Fernandes Figueira Institute, Fiocruz, in Rio de Janeiro, Brazil. The specimens were screened for HTLV antibodies by ELISA and reactive samples were submitted to a Western Blot (WB) for confirmation. Detection of HTLV-I provirus in DNA from peripheral blood mononuclear cells (PBMC) and breast milk mononuclear cells (BMMC) from the seropositive women, was performed by PCR using primers for LTR, tax, pol regions. Of the 389 pregnant women tested, four (1%) were HTLV-I seropositive and three were indeterminate in the WB. PCR was done in three of the seropositive cases and HTLV-I provirus was detected in PBMC and BMMC from two of them. Three blood samples (0.8%) that presented WB indeterminate patterns, were negative by PCR. Our data indicate that the frequency of HTLV-I infection among pregnant women is relatively high in Rio de Janeiro and that preventive measures against the vertical transmission of the virus must be adopted in Brazil.

INTRODUCTION


Transmission of this virus occurs from mother to child, primarily through breast-feeding from an infected mother (Hino et al. 1985, Ando et al. 2003a) by sexual contact (Kakuda et al. 2002, Kaplan et al. 1996) or by the transfusion of contaminated cellular blood components (Manns et al. 1992).

Children born to HTLV-I infected mothers acquire infection predominantly from breast-feeding. A longer duration of breast-feeding has been associated with an increased risk of infection (Wiktor et al. 1997, Takezaki et al. 1997). It was demonstrated that the maternal HTLV-I provirus in peripheral blood mononuclear cells (PBMC) is an independent predictor of mother-to-child transmission (Hisada et al. 2002). The vertical transmission from mother-to-child has an
efficiency varying from 10 to 20% and it is thought to occur by ingestion of lymphocytes in breast milk containing the HTLV-I provirus, after the decline of the protective IgG maternal antibodies (Hino et al. 1990). Vertical transmission is an important route of transmission, because infection early in life is associated with a subsequent risk of ATL (Pombo-de-Oliveira et al. 2001).

Although many persons infected with HTLV-I can remain asymptomatic for decades, others suffer serious consequences of this infection. It is likely that almost all children who develop infective dermatitis, virtually all patients with ATL and a high proportion of individuals with HAM/TSP, acquire HTLV-I infection early in life (LaGrenade et al. 1990, Pombo-de-Oliveira et al. 2001, Osame et al. 1987).

Studies performed in Brazil demonstrated that perinatal infection is thought to be associated with a heightened risk of developing ATL after a long latency period (Carvalho et al. 1997, Pombo-de-Oliveira et al. 2001).

Considering the importance of vertical transmission of the HTLV-I and that few data are available in Brazil, we evaluated the frequency of this infection among pregnant women in a prenatal care unit in Rio de Janeiro.

MATERIAL AND METHODS

Study population.

The study enrolled 389 pregnant women who attended at the prenatal care unit, Fernandes Figueira Institute (IFF-Fiocruz) in the city of Rio de Janeiro. They were selected at two periods, between November and December 2000 and March and June 2002, according to random access to the Hospital Service. The mean age was 22 years (range 12 - 45 years) and they had previously been tested for HIV infection. The study was approved by the Ethics Committee of IFF-Fiocruz under n° 102/2000 and informed consent was obtained from all study participants.

HTLV serological assays.

The peripheral blood samples collected from all pregnant women were screened for the presence of antibodies to HTLV-I and II using an enzyme immunoassay (ELISA - Vironostika HTLV-I/II Organon Teknika, Boxtel, Holland), according to the manufacturer's instructions. The specimens repeatedly reactive on ELISA were further submitted to a Western Blot assay (HTLV
2.4 Genelabs Diagnostics Pte. Ltd. Singapore), for confirmation and type differentiation according to the manufacturer's instructions. This assay defines as HTLV-positivity when the sample is reactive to at least two different HTLV structural gene products: $gag$ p24 and/or p19, and $env$ gD21 and/or gp46; viral type was defined as HTLV-I or HTLV-II when reactive to recombinant gp46-I or gp46-II, respectively; as HTLV-indeterminate when the pattern does not meet positivity criteria and as negative in complete absence of reactivity.

An other blood sample from the pregnant women presenting positive and indeterminate HTLV Western Blot (WB) were specifically collected for polymerase chain reaction (PCR) analysis, as well breast-milk samples from three of the four pregnant women HTLV-I seropositive (cases 4, 6 and 8) were collected in the day of the delivery. In one of the seropositive, the breast-milk sample was not available.

**HTLV-I molecular assays.**

Peripheral blood mononuclear cells (PBMC) were separated by ficoll-hypaque density gradient centrifugation and mononuclear cells from breast milk (BMMC) were prepared by centrifugation at 400g for 30 min, followed by a wash with PBS (GIBCO BRL). DNA was extracted from PBMC and BMMC using the Capture Column™ kit (Gentra Systems) according to the manufacturer’s instructions. Detection of HTLV-I provirus in DNA from PBMC and BMMC was performed by PCR using specific primers for three different HTLV-I genomic regions ($LTR$, $tax$, $pol$) as described elsewhere (Ehrlich et al. 1990, Nagamine et al. 1991).

**RESULTS**

Among the 389 pregnant women tested for anti-HTLV, ten were repeatedly reactive by ELISA. When the reactive samples were submitted to the confirmatory WB assay, four (1%) were HTLV-I positive, three (0.8%) were indeterminate, presenting only p19 and p28 bands and three (0.8%) were negative (Table 1). The seropositive women were 24, 26, 28 and 33 years old. HTLV-I and HIV-1 co-infection was found in one case (Table 1).
Table 1. HTLV serological test results in blood samples from pregnant women

<table>
<thead>
<tr>
<th>Results</th>
<th>ELISA</th>
<th>Western Blot</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>%</td>
</tr>
<tr>
<td>Positive</td>
<td>10</td>
<td>2.57</td>
</tr>
<tr>
<td>Indeterminate</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Negative</td>
<td>379</td>
<td>97.43</td>
</tr>
</tbody>
</table>

HTLV: Human T lymphotropic virus; ELISA: enzyme-linked immunosorbent assay

We performed PCR using PBMC from three HTLV-I seropositive and three indeterminate pregnant women. One seropositive pregnant woman was not available to collect a second sample. Among the three HTLV-I seropositive cases, two had HTLV-I provirus detected in PBMC by PCR and in one case that presented low reactivity to HTLV-antibody in the ELISA (DO=0.474 / CO=0.352), the PCR was negative in PBMC, despite the positivity observed in the WB. In the three WB-indeterminate cases, the PCR was negative in PBMC, showing that these women were not infected (Table 2).

When the BMMC from the three seropositive mothers were submitted to the PCR, the HTLV-I provirus were detected in two of them (cases 6 and 8) and in one (case 4) the PCR was negative in BMMC and PBMC (Table 2).

The age of the seropositive pregnant women vary from 24 to 33 years old and they were informed of the test results and received orientation about the risk of virus transmission to the children.
**Table 2.** HTLV tests results performed in blood and breast milk samples from ELISA serum-reactive pregnant women

<table>
<thead>
<tr>
<th>Samples (N(^2))</th>
<th>Serum Western Blot</th>
<th>Peripheral blood HTLV-I PCR</th>
<th>Breast milk HTLV-I PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>LTR</td>
<td>Tax</td>
</tr>
<tr>
<td>4</td>
<td>HTLV-I (+)</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td>6</td>
<td>HTLV-I (+)</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>8</td>
<td>HTLV-I (+)</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>21</td>
<td>HTLV-I (+)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>14</td>
<td>Indeterminate</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td>15</td>
<td>Indeterminate</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td>19</td>
<td>Indeterminate</td>
<td>(-)</td>
<td>(-)</td>
</tr>
</tbody>
</table>

HTLV: Human T lymphotropic virus; PCR: polymerase chain reaction; ND: Not done.

**DISCUSSION**

The prevalence rate of HTLV-I infection reported here in pregnant women was relatively high (1%), with three cases (0.8%) presenting indeterminate results in the Western Blot. This prevalence is higher than those reported in pregnant women from two other regions of Brazil: Salvador, where the HTLV-I prevalence rate was 0.84% (Bittencourt et al. 2001) and Paraíba, where the rate was 0.68% (Pimenta et al. 2008).
Concerning to the three indeterminate cases that presented reactivity to p19 and p28 in the WB and PCR negative, it was observed that individuals just showing antibodies only directed to the gag gene antigens, do not seem to be infected, in the majority of the cases (Rouet et al. 2001).

The HTLV-I provirus was detected by PCR in both peripheral blood and breast milk from all the seropositive cases tested, except one that tested positive for HTLV-I antibody but did not have provirus detected by PCR analyses. This case could be explained as newly infected woman with low viral load as previously reported (Kaplan et al. 1996, Pimenta et al. 2008).

There are many data showing that HTLV-I prevalence rate increase with age and we can observe this in our study, once the positive women were 24, 26, 28 and 33 years old.

Breast milk is known to be the primary vehicle for the vertical transmission of HTLV-I and the presence of the provirus in breast-milk from infected mothers remained a strong predictor of HTLV-I transmission to children (Bittencourt et al. 2002, Hisada et al. 2002, Maloney et al. 2006, Ureta-Vidal et al. 1999). In the present study, we detected HTLV-I provirus in breast-milk samples from two of the three seropositive mothers tested. In a prospective study of 101 mother-child pairs in Jamaica, 22 of 23 transmissions occurred in children breast-fed by mothers who had provirus detected in their breast milk (Li et al. 2004).

There are increasing epidemiological data showing that HTLV-I infection in early life poses a high risk for associated diseases, particularly ATL. In a previous report in Brazil, the prevalence rate of HTLV-I infection in family members of ATL index cases was 36%, and the high rate was mainly due to maternal positivity. Twenty-seven ATL patients were born to HTLV-I positive mothers (Pombo-de-Oliveira et al. 2001).

Studies performed in endemic areas have indicated that a reduction in the number of mothers breast-feeding and a shortening of the breast-feeding period decrease the mother-to-child HTLV-I transmission (Ando et al. 1987, Ando et al. 2003a, Ando et al. 2003b, Hino et al 1987).

In Japan, where there were high HTLV-I vertical transmission rates, the seropositive mothers were advised that should refrain from breast-feeding and a decline in HTLV-I prevalence has been observed. However, in countries like Brazil the restriction of breast-feeding may be not feasible, considering nutritional issues and prevention of infectious disease in the newborns. In some places, mainly in developing countries, it is recommended short-term breast-feeding (< 6 months) instead of completely quitting, as a measure to avoid vertical transmission of this virus (Hino et al. 1997, Takezaki et al. 1997).
Based on high HTLV-I vertical transmission rate worldwide, there is no doubt that preventive measures against the vertical transmission of the virus must be adopted in Brazil, as introduction of HTLV screening tests in the prenatal care routine and education programs for the seropositive mothers.

ACKNOWLEDGEMENTS

We thank Andreia de Oliveira Santos, Laboratory of Immunology, HEMORIO, for the technical assistance in the HTLV serological tests. Financial support: CNPq (HGS).

REFERENCES


Carvalho SMF, Pombo de Oliveira MS, Thuler LC, Rios M, Coelho RC, Rubim LC, Silva EM, Reis AM, Catovsky D 1997. HTLV-I and HTLV-II infections in hematologic disorder patients,


