Prevalence Ratio of HTLV-1 in Nursing Mothers From the State of Paraíba, Northeastern Brazil

Flávia C. F. Pimenta, MD, Simone Kashima Haddad, PhD, João G. de Medeiros Filho, MD, PhD, Maria José C. Costa, PhD, Margareth F. M. Diniz, PhD, Melina P. Fernandes, MS, Lenisio B. de Araújo, MD, and Maria S. Pombo-de-Oliveira, MD, PhD

Abstract
The human T-cell lymphotropic virus type 1 (HTLV-1) was the first human retrovirus known as direct causal agent of a malignant disease. The vertical route of HTLV transmission is the most frequent pathway of the virus contamination. This study was performed to determine the prevalence ratio of HTLV-1 infection among nursing women. From January 2004 to January 2005, blood samples from 1033 nursing mothers from Paraíba, Brazil were evaluated for HTLV antibodies by ELISA and HTLV-1 viral particles confirmed by polymerase chain reaction (PCR). HTLV antibodies were detected in 7 women. The overall seroprevalence ratio was 0.68% and HTLV-1 viral sequences were confirmed by PCR in 2 women. These preliminary data suggest that HTLV screening should be introduced as mandatory test before breastfeeding and breast milk donation in Paraíba, Brazil. Additionally, counseling programs would help reduce the prevalence ratio of HTLV-1 infected individuals in this Brazilian region. J Hum Lact. XX(X):xx-xx.

Keywords: HTLV-1/II infection; breastfeeding infants; nursing mothers

Received for review Month X, XXXX; revised manuscript accepted for publication Month X, XXXX

Flávia C. F. Pimenta is working in the area of hematology-oncology and is leader of the research concerning HTLV-1 infection and leukemia/lymphoma at the Department of Pediatrics, Sciences of Nutrition, and Department of Pharmaceutical Sciences, Federal University of Paraíba, João Pessoa, Brazil. Simone Kashima Haddad is Head of the Molecular Laboratory of Hemocentro, Ribeirão Preto, São Paulo, Brazil. Her main project is related to HTLV-1 infection. João G. de Medeiros Filho is the Head of the Pediatric Department, Federal University of Paraíba, João Pessoa, Brazil. Maria José C. Costa is chief of the Serological Laboratory, Department of Pharmaceutical Sciences, Federal University of Paraíba, João Pessoa, Brazil. Margareth F. M. Diniz is the Head of the Department of Pharmaceutical Sciences, Federal University of Paraíba, João Pessoa, Brazil. Melina P. Fernandes is a medicine student at the Federal University of Paraíba, Brazil, training on epidemiological studies and working in collection of cases. Lenisio B. de Araújo is a medical doctor and collaborator of the Network of Acute Leukemia Study group in Brazil. He is at the Department of Surgery, CCS, Federal University of Paraíba, João Pessoa, Brazil. Maria S. Pombo-de-Oliveira is the Head of the Pediatric Leukemia and Lymphoma Group at the Divisão de Medicina Experimental, CPq, Instituto Nacional do Cancer, Rio de Janeiro, Brazil. The main projects are related to environmental factors and childhood leukemia in Brazil.

Address correspondence to: Maria S. Pombo-de-Oliveira, MD, PhD, Instituto Nacional de Cancer, CPq-Experimental Medicine, Rua André Cavalcanti, 37 CEP 20 231 050-Rio de Janeiro, Brazil; e-mail: mpombo@inca.gov.br, mspoliveira@hotmail.com

J Hum Lact XX(X), XXXX
DOI: 10.1177/0890334408316084
© Copyright 2008 International Lactation Consultant Association

Introduction

Human T-cell lymphotropic virus, type 1 (HTLV-1) associated to adult T-cell leukemia/lymphoma (ATLL) and HTLV-1 associated myelopathy (HAM/TSP) share a common etiology with distinct pathogenic mechanisms. HTLV-1/2 infections are life-long and can be transmitted in 3 ways: (1) from mother to child through breast milk; (2) by the exchange of sexual fluids, particularly but not exclusively, from men to women; and (3) via HTLV-1 contaminated blood transfusions and intravenous drug users. The first surveys indicated that HTLV infected people from endemic areas were restricted by sociodemographic features and associated with the pathways of HTLV transmission. Later, maternal level of infected cells and the titer of anti-HTLV-1 antibodies have been assigned as risk factors for HTLV-1 transmission to children born to HTLV-1 carrier mothers.

Human milk, besides nutritious components, contains a complexity of cells, membranes, and molecules in its composition that act protecting the newborn. The Brazilian health system recommends that even with complementary foods mothers should breastfeeding at least for 12 months (www.saude.gov.br). However, infection diseases involving the mothers may represent a hindrance for breastfeeding.
In Brazil, several studies have demonstrated the HTLV-1/2 infections prevalence ratio among individuals with HTLV-1 associated diseases, or individuals exposed to blood transfusions, and/or with sexually transmitted diseases (STDs). However, very few data are available for the route of HTLV-1 transmission among nursing mothers. Recently, several cases of ATLL have been identified in the state of Paraíba. This disease has been directly associated with the vertical transmission of HTLV-1. Thus considering that serological prevalence rates in blood bank screening tests and also in sexual transmitted disease programs has a heterogeneous geographic distribution in Brazil, this study was performed in puerperal women from the state of Paraíba, Brazil, to determine the prevalence of HTLV infection among nursing mothers.

**Material and Methods**

**Subjects**

This is a prevalence study of HTLV infection among women, exclusively. Nursing mothers with their newborns hospitalized in the Hospital Cândida Vargas, João Pessoa, Paraíba, Brazil, entered consecutively in the study from January 2004 to January 2005.

Nursing mothers with adequate amount of milk were also invited to participate in the milk donation program. They were asked to participate and a questionnaire was applied in mothers breastfeeding their babies.

**Criteria**

The criterion of inclusion in this study was basically healthy nursing mothers willing to donate breast milk. The exclusion criteria were HIV-infected mothers and women who refused to participate and/or sign a consent form after an easy-to-understand explanation of the study.

**Questionnaire**

The questionnaire was structured with the following questions: demographic features, including marital status, place of living, age of onset of sexual activity, number of partners, number of bearing children, number of breast feeding children, previous blood transfusion, exposure to drugs intravenous use, history of previous STDs, and medical care during pregnancy.

All procedures were performed according to technical norms proposed by the Brazilian Ministry of Health (www.saude.gov.br). Researcher and Ethics Committees of the Federal University of Paraíba approved the project according to the procedures followed in accordance with the ethical standards of Helsinki Declaration of 1975, as revised in 1983.

**HTLV Serological Assays**

Peripheral blood (PB) samples were collected through venous puncture in dry tube for serological test (~4 mL of PB). For confirmatory results, a second PB sample was collected to perform further tests, if necessary.

Serum samples were tested by enzyme-linked immunosorbent assay (ELISA) for HTLV-1/2 (ELISA kit, label ABOTT-MUREX, lot H840310). All positive or grey zone results were tested twice. The criterion of positivity was applied when the ratio between the cutoff/optical density (OD) were superior to 20% of the cutoff value established in the beginning of each ELISA analysis as a standard procedure. Then, a second sample of reagent case was tested by a confirmatory assay. Different cutoff values can be found in different reagents plates.

**Molecular Assays for HTLV-1**

The DNA of the samples was extracted from the buff coat, using the Super Quick Gene DNA Isolation extraction kit (Analytical Genetic Testing Center-AGTC, Denver, CO) following the manufacturer’s instructions. After homogenization and complete dissolution of the DNA extracted, quantification was performed in dilutions of 1:200 water and reading in spectrophotometer at wavelengths of 260 and 280 nm were performed with the objective of obtaining the purity degree of the samples.

HTLV-1 gene sequences for the LTR and tax regions were detected according to standard procedures. The HTLV-1 primers used in this study were from a highly conserved region of HTLV-1 proviral sequence as reported elsewhere. For each sample, 5 to 10 μL of DNA was amplified with Taq polymerase in MgCl₂ (25 mM), dNTPs (1.25 mM) and 10× buffers. Samples were subjected to 94°C for 5 minutes and followed by 35 cycles using the following parameters: 94°C for 1 minute; 50°C to 55°C for 1 minute, and 72°C for 1 minute. The polymerase chain reactions (PCR) were initially optimized using 100 to 500 ng of genomic DNA, buffer 10× (500 mM KCl, 100 mM Tris-HCl pH 8.5, 15 mM of MgCl₂), 0.2 mM of each deoxyribonucleic dNTPs (Applied Biosystems, Boston, MA), 10 pmol of each set of primers at 10 pmol/μL and 2.0 U of Taq DNA polymerase (Amersham Pharmacia Biotech, Sweden).

The nested PCR was performed with 1 μL of the product obtained in the first amplification using the internal pair of primers at the same conditions established
Positive and negative controls were running in each step of the technique.

Statistical Analysis
Statistical analysis was performed using the SPSS program; version 13.5 was used to perform most statistical analysis from a database bank generated during the study. Descriptive statistics include mean, range, and standard error deviation as appropriate. The following data were analyzed: HTLV-1 mother’s status, and demographic features such as ethnicity, marital status, previous blood transfusion, history of STDs, and so on.

Results
The demographic characteristics and main results of interviews of nursing mothers are shown in Table 1. The mean age was $23 \pm 5.8$ years (range 13-42 years). The onset of sexual life was $17.1 \pm 3.86$ years (range 10-41 years). The vast majority of women were single (75.7%), but maintaining stable marital life with one partner. A total of 20 women referred to have only occasional sexual activities. The size of family was $2 \pm 0.8$ children (range 1-6).

Among 1033 samples submitted to serological HTLV screening test, 7 had a reagent pattern with sera HTLV antibodies title superior to the cutoff value established of each ELISA test, as shown in Table 2. The overall prevalence ratio was 0.68%. Samples from 2 nursing mothers were tested twice in different time setting points with concordant positively HTLV results. Seropositive nursing mothers were called to collect a new sample for molecular analysis, and in 2 samples the PCR confirmed the presence of HTLV-1 viral gene sequences ($\text{LTR}$ and $\text{tax}$).

The characteristics of the 7 women with a positive ELISA test were as follows: mean age = 18 years (range 15-25 years), ethnicity (blacks, $n = 4$; mullatos, $n = 2$; and native Brazilian, $n = 1$). They began sexual activities at the mean age of 13 years, with an average of 2 partners. None of them received blood transfusion. Only one of them reported the use of illicit drugs use. A total of 4 out of 5 have already breastfed their previous offspring. There were no particularly significant facts regarding place of residence of the women; nor was there a distinct cluster of HTLV positive cases in the state of Paraíba.

Discussion
There are increasing epidemiological data showing that HTLV-1 infection in early life poses a high risk for associated diseases. Particularly in ATLL, additional cofactors are long duration of breastfeeding (> 18 months), mother’s age (> 30 years), and high HTLV-1 proviral load. In previous reports, we described the clinical diversity of ATLL and the mode of HTLV-1 transmission in familial clusters, justifying the current research study.

The seroprevalence ratio of the HTLV antibodies reported here in nursing women (0.68%) is higher than that described among blood donors in some regions of Brazil. Usually, the HTLV-1 infection is more frequent in women and the prevalence ratio increase with age (mean age, 20-30 years of age), coincident with childbearing age. This study confirms these previous
findings, and 2 women 20 years of age, tested positive for HTLV antibodies but did not have viral sequence by PCR analysis. These cases could be explained as newly infected women with small viral load as previously reported.3

Serologic tests became a mandatory procedure in blood donors with additional counseling program for infected individuals since 1993. These procedures efficiently reduced the prevalence rate of antibody to HTLV-1 in individuals exposed to multiple blood transfusions (Carvalho SMF, personal communication).

Based on high HTLV-1 vertical transmission rates, Japanese health authorities advised that HTLV-1 seropositive mothers should refrain from breastfeeding and a dramatic decline in HTLV-1 prevalence has been observed.18-20 Therefore, based on these evidences, we recommend that prophylactic measurements regarding HTLV-1 infection transmission from mother to child should also be adopted in Brazil. In this matter, we are facing a dilemma. In the northeast region of Brazil, the restriction of breastfeeding may be dramatic with consequent high rate of infant mortality. The reason is that Brazilian health authorities have been encouraging nursing women to participate in a nationwide human milk bank network. There is no doubt about the positive effects of breastfeeding on improvement in pediatric weight gains as well as protection against pulmonary and gastrointestinal infections diseases.21,22 On the other hand, as very well pointed out by Proietti et al,23 the social and financial cost of HTLV-1 associated disease for the health system is immense. Therefore, to prevent the dissemination of these viruses, we suggest that HTLV-1/2 screening test should be performed regularly in prenatal care centre in Brazil, with counseling and educating programs for high-risk HTLV-1 positive mothers.

References