Blocking Vertical Transmission of Human T Cell Lymphotropic Virus Type 1 and 2 Through Breastfeeding Interruption

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Background: Human T cell lymphotropic virus type 1 and 2 (HTLV-1/2) causes serious diseases and is endemic in many parts of the world. It is transmitted from mother to child in 15–25% of the cases, primarily through breastfeeding. Proviral load and duration of breastfeeding are thought to play a role in transmission. This study aimed to detect HTLV-seropositive mothers through testing of neonates, to evaluate maternal HTLV proviral load and to measure the rates of transmission blocking when interruption of breastfeeding was implemented.

Methods: Neonates were screened for HTLV-1/2 IgG by enzyme immunoassay using the neonatal screening program of Minas Gerais State, Brazil. Breastfeeding interruption was recommended to those whose mothers were confirmed HTLV-positive. Children were tested by polymerase chain reaction at birth and at 12 months of age.

Results: Of 55,293 neonates tested, 42 (0.076%) were positive for HTLV-1 or HTLV-2 IgG. All 42 were polymerase chain reaction–negative at birth and 1 of 37 (2.7%) became antibody-positive after 12 months. His mother had delivered him vaginally and was informed of the positive HTLV-1 polymerase chain reaction after 7 days of breastfeeding. The mother’s proviral load was 271 copies/10,000 cells, whereas the average is 109.2 copies/10,000 cells (95% confidence interval: 70.56–147.83).

Conclusions: Maternal HTLV-1 proviral load and the route of delivery may have played a role in the transmission observed. Avoidance of breastfeeding was an effective measure to reduce HTLV transmission. In endemic countries, routine prenatal or neonatal screening combined with formula feeding for mothers confirmed HTLV-positive may be an important strategy to prevent future development of illnesses related to HTLV.

Key Words: human T cell lymphotropic virus type 1 and 2, vertical transmission, breastfeeding, neonatal testing

(Pediatr Infect Dis J 2012;31: 1139–1143)

The Human T cell lymphotropic virus type 1 (HTLV-1) is a human virus considered to be the etiologic agent of adult T cell leukemia, HTLV-1 associated myelopathy/tropical spastic paraparesis, uveitis, infectious dermatitis as well as nonneurologic inflammatory syndromes.HTLV type 2 (HTLV-2) is not clearly associated with disease, but the individuals who harbor this infection may present with myelopathy (HTLV-1 associated myelopathy/ tropical spastic paraparesis), respiratory infections, inflammatory conditions and possibly increased cancer mortality. HTLV-1 and HTLV-2 have distinct geographic distributions: HTLV-1 is endemic in the south of Japan, the Caribbean and Central and South America, whereas HTLV-2 is found in isolated native populations in Africa, North, Central and South America, as well as in high-risk groups such as intravenous drug users in Europe and the United States. Both HTLV types are sexually, parenterally and vertically transmissible. Several studies have shown that HTLV-1 transmission from mother to child occurs essentially through breastfeeding, with rates varying from 15% to 25%. Replacing breastfeeding with bottle-feeding may reduce the risk of transmission to about 3%.

In addition to the mother’s HTLV-1 proviral load, breastfeeding duration has also been described as one of the risk factors associated with vertical transmission. Children who have been breastfed for a period shorter than, or equal to 6 months, have a lower HTLV-1 seropositivity rate, close to 5%, whereas those who have been breastfed for more than 6 months reach seropositivity rates over 20%. The lower rate found in children who have either not been breastfed or who have been breastfed for a short time may be related to the protective effect of maternally derived anti–HTLV-1 IgG antibodies. These antibodies are transferred to the child during pregnancy, are present in higher titers in the first few months of life and generally disappear between 6 and 12 months of age.

Preventing HTLV-1 vertical transmission is an important measure for control of viral spread. It can also help reduce the incidence rate of adult T cell leukemia because vertical transmission has been identified as a risk factor for the development of this neoplasm. To prevent vertical transmission, it is important to diagnose maternal viral infection during the prenatal or neonatal period and to counsel the seropositive mother to avoid breastfeeding. This study evaluated the rate of transmission of HTLV-1 and HTLV-2 in children born to women with HTLV who had been counseled to prevent vertical transmission.

MATERIALS AND METHODS

Study Outline, Population and Period

The Interdisciplinary Research Group on HTLV (GIPH) tested 55,293 live newborns, who had been screened by the Newborn Screening Program of Minas Gerais State and identified 42 HTLV-1 and HTLV-2 seropositive women through a prevalence study conducted from September to November 2007 and described by Ribeiro et al. After identification of HTLV-1/2 positivity, the mothers followed the current interventional prospective study that followed their 42 children up to 1 year of age.
Description of the Study and Data Collection

Whole blood samples were collected from newborns and peripheral blood mononuclear cells were separated to perform real-time polymerase chain reaction (rPCR). Simultaneously, the mothers’ blood samples were also collected for laboratory confirmation of the presence of HTLV-1 and HTLV-2 antibodies. For mothers who were confirmed to be HTLV-1–infected by Western blot (WB) and PCR, the HTLV-1 proviral load was quantified by rPCR. No proviral load was done for the HTLV-2–positive mothers. The mothers who were identified as HTLV-1/2 seropositive were instructed by medical staff, verbally and in writing (written instructions in a flyer prepared specially for this research), to stop breastfeeding and were given lactation inhibitors (Cabergoline, Dostinex, Pfizer, New York City, NY) along with their results of serologic HTLV testing. To replace breast milk, infant milk formula (NAN, Nestle, São Paulo, Brazil; Aptamil, Danone, São Paulo, Brazil) was provided to all newborns in the study for a minimum period of 6 months. Careful instruction to prepare the formula in a hygienic manner was provided to the mother by the local health team, according to the Ministry of Health guidelines. After the children reached 12 months of age, new blood samples were required to test them with enzyme immunoassay (EIA), WB and PCR for HTLV-1/2. During the GIPH medical follow-up, the mothers provided information on gender, route of delivery and breastfeeding duration.

Laboratory Tests

All blood samples, which had been collected from the children at birth and at the age of 12 months, were submitted to rPCR, using TaqMan system (Roche Molecular Systems, Indianapolis IN) for diagnosis confirmation of HTLV-1 and HTLV-2 infection, as described by Andrade et al. Peripheral blood DNA samples were obtained through purifying column (QLAamp DNA Blood kit, QIAGEN, Hilden, Germany), according to the manufacturer’s recommendations. A multiplex reaction was performed for HTLV-1 and human albumin, and a second reaction was performed for HTLV-2. MT2 DNA, an HTLV-1 viral, particle-producing, cellular model, was used as positive controls. As negative controls, we used the DNA of 2 seronegative individuals, besides the mix control, with no DNA addition. These techniques have been standardized by the Research Laboratory of Hemonomas Foundation, and they have presented high sensitivity (99.4%) and specificity (98.5%). The samples collected from 12-month-old children were initially subjected to the immunoenzymatic test, the enzyme-linked immunosorbent assay (HTLV-1/2 Ab-Capture ELISA Test System, Ortho Clinical Diagnostic Inc, NJ). The samples that returned positive when tested with the enzyme-linked immunosorbent assay, were also WB tested (HTLV Blot 2.4, MP Diagnostics, Singapore) for diagnostic confirmation. The results of both tests were interpreted according to the manufacturer’s specifications.

rPCR was performed, using the SYBR Green system (Life Technologies Corporation, Karlsruhe, Germany), in the HTLV-1 seropositive mothers’ samples to quantify the HTLV-1 proviral load. Separate reactions were performed for amplification of the HTLV pol sequence, to measure the number of proviral copies and the sequence of the human albumin gene, thereby enabling the measurement of the number of cells in the sample. The calibration curves for determining the number of proviral copies and of the number of cells were obtained, respectively, by serial dilution of MT2 DNA cells. The PCR for pol and albumin of the same sample was always held on the same plate in duplicate and each plate had calibration curves.

Data Analysis

Descriptive analysis of children with respect to gender, route of delivery and duration of breastfeeding was done. The rate of vertical transmission was calculated based on the number of 12-month-old, HTLV-1 and HTLV-2 seropositive children divided by the total number of children tested at 12 months of age.

Ethical Aspects

The project was approved by the Ethics Research Committee of Universidade Federal de Minas Gerais/COEP-UFMG (Protocol no. 482/06) and by the Ethics Research Committee of the City Health Secretary of Belo Horizonte (Protocol no. 082/2007).

RESULTS

Of 55,293 neonates tested, 40 (0.072%) had their blood reactive to HTLV-1 and 2 (0.004%) to HTLV-2 in the EIA screening (Fig. 1). The mothers of all 42 seropositive infants had confirmation of HTLV infection: 40 confirmed positive for HTLV-1 (95%) and 2 (5%) for HTLV-2. Of the 40 children born to HTLV-1 seropositive women, 18 (45%) were male. Twenty children (50%) had been delivered vaginally, 14 (35%) by cesarean delivery and in 6 (15%) cases the information on the delivery route was not obtained. The delivery route was similar by infant gender.

Infant blood samples were collected between 16 and 75 days of life (mean, 34 days) for proviral detection by PCR (Fig. 1). One sample was not processed due to insufficient material for the test. Of 39 (97.5%) samples subjected to PCR for HTLV-1, 38 (97.4%) were negative and 1 was positive. Due to the possibility of contamination, or exchange of one infant’s specimen for another, the test was repeated with a new blood sample collected from the child at 4 months of age, which was negative for HTLV-1. This child was not retested later.

After reaching 1 year of age, 35 (87.5%) of the children underwent PCR and serology for HTLV-1. Of the 35 tested, 34 (97.2%) were negative and 1 (2.8%) was positive for HTLV-1. Five mothers positive for HTLV-1 did not allow the collection of blood samples of their children at 12 months of age, which included the child with positive results at birth that became negative at 4 months of age.

The 2 infants born to HTLV-2 seropositive mothers, 1 female and 1 male, were subjected to PCR tests for HTLV-2 from samples collected at 35 and 42 days of age, respectively. The 2 mothers belonged to ethnically pure native Brazilian population, and received orientation for replacement of breastfeeding by infant formula, but 1 continued to breastfeed her baby. Due to language barriers, it was not possible to determine how long these mothers breastfed their children because the interpreter at the time of enrollment was no longer present. The laboratory tests (EIA and PCR) after the 2 children completed 1 year of age were negative for HTLV-2, consistent with no vertical transmission of HTLV-2 in both cases during the study period.

Thirty-five (87.5%) mothers reported breastfeeding duration, which varied from 0 to 60 days (mean, 27 days). Among HTLV-1–infected mothers, the proviral load was undetectable in 3 and ranged from 0.05 to 376 copies/10,000 cells, an average of 109.2 copies/10,000 cells (95% confidence interval: 70.56–147.83) in the rest. The child who was positive for HTLV-1 was a boy who had been delivered vaginally, nursed by his mother for 7 days, and had a negative HTLV-1 PCR test at age 26 days. His results for HTLV-1 by EIA, WB and PCR at 12 months of age were positive. The mother of this child has categorically denied breastfeeding him after 7 days of life and confirmed that he never received breast milk from anyone else. The proviral load in the mother of the HTLV-1 vertically infected child was 271 copies/10,000 cells.
The cost of each test was: US$ 1.25 (EIA, n = 55,293 tests performed), 88.00 (WB, 84 tests performed), 30.00 (PCR, 84 tests) and the milk formula was about US$ 270/child (n = 42 children), for the 6 months in which it was distributed. Summing up all the expenditures (~US$ 90,000) and dividing by the theoretical maximum number of infections avoided (n = 9), we found that the cost per transmission prevented was approximately US$ 10,000.

FIGURE 1. Human T cell lymphotropic virus (HTLV) type 1 and 2 testing algorithm in neonates and infected mothers, using enzyme immunoassay (EIA), polymerase chain reaction (PCR) and Western blot (WB). GIPH Cohort Study, Belo Horizonte, Brazil.

DISCUSSION

The negative results of molecular tests performed on newborns corroborate the fact that the main route of HTLV-1 and HTLV-2 vertical transmissions is through breastfeeding. The observed rate of vertical transmission of the virus (2.8%) was similar to the rates previously described in which natural breastfeeding is substituted by infant formulas, probably reflecting the interruption of virus passage to the newborn. In the absence of policies for breastfeeding interruption, previous studies have shown HTLV-1 vertical transmission rates between 15% and 25%. Data from our cohort study (GIPH) prior to the present neonate survey, showed that HTLV-1 vertical transmission was approximately 25%, preceding the entry of the index case (female blood donor) in the study group (GIPH cohort, unpublished data), and dropped considerably for the subsequent offspring after counseling. Considering these figures, if there had been no breastfeeding interruption, we would have expected to find about 5–9 infected children by the end of the follow-up study. The evaluation period for the identification of children born to HTLV-infected mothers lasted 3 months. If HTLV screening had been incorporated into the Minas Gerais State routine prenatal screening, vertical infection could have been avoided in 20–36 children in 1 year and 200–360 children in 10 years in that state alone. HTLV-1/2 prevalence studies in pregnant women in Brazil (Fig. 2) show that the rates vary from 0.076% to 1.3%, with a national average of 0.5%. Considering these figures, and knowing that in 2007 Brazil had 2,891,328 births, a total of 2169–3614 HTLV-1/2 vertical infections could be circumvented each year, with an estimated cost of US$ 10,000.00 per infection avoided (see Results section). These calculations refer to research costs and could drop considerably if the procedures became routine. Considering the diseases and morbidity associated with HTLV-1 infection, it is important that endemic countries perform detailed cost-effectiveness analysis to take adequate preventive measures.

The hypotheses for the single child transmission observed in the study, assuming that the mother’s information is reliable, are: 1) transmission occurred during pregnancy or childbirth, or 2) transmission occurred via breastfeeding during the 7 days in which the mother reported having breastfed the infant. In these 2 situations, the PCR test performed on the 26th day of the child’s life was negative, which can be explained by a provirus load below the limit of detection by that assay. It is possible that the mother’s proviral load and the type of delivery may have contributed to the
virus’ s vertical transmission. The proviral load based on the mother’ s sample collected on the 26th day after delivery is considered high (271 copies/10,000 cells = 2.71%) when compared with the average found among other mothers, as well as when compared with data from HTLV-I–infected individuals’ proviral loads from GIPH’ s cohort.20

One of the limitations of the present study was the fact that there were 5 subjects lost to follow-up who were not tested at the age of 12 months, which could lead to underestimation or overestimation of the number of transmissions. Nonetheless, the results indicate that avoidance of breastfeeding is an effective measure to block the transmission of the virus.

One constraint when applying the results of this study in a larger scale is the cost of the infant formula and the necessary hygiene to avoid gastrointestinal disease in the infant due to water and recipients contamination with pathogenic bacteria. The Ministry of Health in Brazil has issued guidelines to orient the population on how to adequately prepare the infant diet, to avoid infection and consequent dehydration and malnourishment.13 This is publicized in the unified health system of Brazil (SUS) and has improved newborn health outcomes in other programs such as vertical HIV transmission prevention programs. Nevertheless, we believe that in countries endemic for HTLV, it is appropriate to test all pregnant women in the prenatal care setting or newborns through neonatal screening, provide counseling to avoid breastfeeding and provide for safe replacement feeding to prevent transmission of the virus and future development of illnesses related to HTLV.

FIGURE 2. Seropositivity for HTLV-1/2 in pregnant women, nursing women and newborns in Brazil. GIPH Cohort Study, Belo Horizonte, Brazil.

ACKNOWLEDGMENTS

The present research was performed as part of the Interdisciplinary HTLV Research Group (GIPH) studies. CT and MLM are recipients of FAPEMIG’ s fellowships. ABFC-P and FAP are recipients of CNPq fellowships. The authors thank Cissa Nunes Soares for reviewing the manuscript.

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