Mother-to-Child Transmission of Human T-Cell Lymphotropic Virus Types I and II (HTLV-I/II) in Gabon: A Prospective Follow-up of 4 Years

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Summary: For 4 years, we determined the mode and risk of mother-to-child transmission of HTLV-I in a prospective cohort of 34 children born to seropositive mothers in Franceville, Gabon. We also determined the prevalence of antibodies to HTLV-I/II in siblings born to seropositive mothers. Antibodies to HTLV-I/II were detected by Western blot, and the proviral DNA was detected by the polymerase chain reaction (PCR). The risk of seroconversion to anti-HTLV-I for the 4 years of follow-up was 17.5%. Anti-HTLV-I/II and proviral DNA were only detected after age 18 months. We observed a seroprevalence rate of 15% among the siblings born to HTLV-I/II seropositive mothers. Furthermore, we report a case of mother-to-child transmission of HTLV-II infection in a population of HTLV-II-infected pregnant women that is emerging in Gabon. The lack of detection of HTLV-I/II proviral DNA in cord blood and amniotic fluid and, furthermore, the late seroconversion observed in the children indirectly indicate that mother-to-child transmission occurred postnatally, probably through breast milk. Key Words: HTLV—Mother-to-child transmission—Prospective cohort—Sibling—Familial clustering—Polymerase chain reaction—Western blot—Amniotic fluid.

The three major routes of transmission of HTLV-I as yet reported include mother-to-child transmission, sexual intercourse, and blood products (1–5). The importance of mother-to-child transmission was confirmed by a prospective cohort study performed in Nagasaki (6): 22% of the infants born to seropositive mothers were found to be HTLV-I carriers, and seroconversion in the infants was observed between the age of 1 and 3 years. Thereafter, several studies in Japan confirmed that mother-to-child transmission is mainly through breast-feeding (7,8), with transmission rates ranging from 15 to 25% (9–12). However, a case of transplacental (intrauterine) transmission has been reported (13), with demonstration of HTLV-I-infected cells in a cord blood sample.

However, in Africa, data on the mode and risk of reversion are lacking, and data from Japan or other industrialized nations may not be extrapolated to be the same in Africa. For instance, mother-to-child transmission rates of the human immunodeficiency virus range from 13 to 32% in in-
The patient samples for this study were part of a follow-up study to determine the impact of HTLV-I infection on the course and outcome of pregnancy (16). The study was initiated in 1987 at the general hospital in Franceville, southern province of Gabon, where most pregnant women in Haut-Ogooue Province are monitored and have given birth. To optimize follow-up, only women living in Franceville and attending the general hospital were recruited after verbal informed consent.

A total of 135 pregnant women were recruited for the study; 45 were seropositive and 90 were seronegative for HTLV-I/II by Western blot. Pregnant women who were indeterminate on Western blot were excluded from the study. The women were matched for age and ethnic group with two seronegative women. We used Western blot as the method of diagnosis, and the polymerase chain reaction (PCR) for detecting the proviral DNA. The PCR also allows diagnosis of infection within the follow-up period.

MATERIALS AND METHODS

Study Population

The patient samples for this study were part of a follow-up study to determine the impact of HTLV-I infection on the course and outcome of pregnancy (16). The study was initiated in 1987 at the general hospital in Franceville, southern province of Gabon, where most pregnant women in Haut-Ogooue Province are monitored and give birth. To optimize follow-up, only women living in Franceville and attending the general hospital were recruited after verbal informed consent.

A total of 135 pregnant women were recruited for the study; 45 were seropositive and 90 were seronegative for HTLV-I/II by Western blot. Pregnant women who were indeterminate on Western blot were excluded from the study. Each seropositive woman was matched for age and ethnic group with two seronegative women who attended the unit for the first time on the same day or the day after the seropositive woman. The women selected for this study were of the lower socioeconomic class, thus belonging to a relatively homogeneous population. The average ages of the women were 25.4 years ± 5.5 for cases and 25.2 years ± 6.2 for controls. Clinical examination of the mothers was performed by a physician once during the pregnancy, at delivery, and 1 year after birth for clinical manifestations of HTLV-I infection. During each bleeding of the infants, they were clinically examined, and information on their blood transfusion status and on breast-feeding was obtained.

To shed light on the mode of transmission, in 1987, blood samples from the mothers and cord blood for PCR were systematically taken at delivery to determine the possibility of intrapartum transmission. Furthermore, amniotic fluid was obtained from 15 seropositive mothers to test by PCR for proviral HTLV-I and HTLV-II in desquamated epithelial cells. Then, every 6 months until 1991 blood was collected from the children for HTLV-I serology and PCR to determine postnatal transmission. The incidence and risk of HTLV-I seroconversion in the first 4 years of life were calculated. Furthermore, a complementary HTLV-I/II seroprevalence survey was performed among 100 siblings, aged 1 to 14 years, living in the same house as the mothers.

Detection of HTLV-I/II Antibodies

Sera were tested for immunoglobulin G antibodies to HTLV-I/II by enzyme-linked immunosorbent assay (ELISA) (Cambridge Biotech, Worcester, MA, U.S.A.), and the ELISA positives were retested for confirmation by Western blot (Cambridge Biotech). A sample was considered positive for anti-HTLV-I/II when it showed a band at least two gene products (p19 or p24 for gag antigens and gp46 for the envelope antigen) on Western blot.

Detection of HTLV-I and HTLV-II DNA by PCR in Blood Samples

Peripheral blood mononuclear cells were separated by ficoll-hypaque density gradient centrifugation and DNA extracted essentially as previously described (17). The DNA preparation was then subjected to PCR according to the protocol of Saiki et al. (18). Samples were analyzed with two primer couples, one for the HTLV-I gag region (gag 1, gag 2) with the corresponding probe (19), the other for the pol region (SK110, SK111) (20) with specific probe for the HTLV-I and HTLV-II pol region (SK112, SK118) (20). Samples were analyzed in duplicate and under code in Paris and in Antwerp.

Detection of HTLV-I and HTLV-II DNA by PCR in Amniotic Fluid

Desquamated epithelial cells were obtained from amniotic fluid after centrifugation in phosphate-buffered saline. DNA was extracted from cells by lysis with a buffer containing 20 mM Tris (pH 8), 5 mM EDTA (pH 8), 0.4% SDS, 149 µl H2O, and 20 µg/ml proteinase K. The tube content was then vortexed (1 min) and incubated in a water bath at 37°C for 12 to 16 h. Then, 150 µl phenol/chloroform (v/v) was added and vortexed (1 min) followed by centrifugation (15,000 rpm) at room temperature for 5 min. The supernatant was then decanted and 500 µl ether added to the pellet followed by vortexing (5 min) and centrifuging as noted. DNA was precipitated with 3 M ammonium acetate and 2 vol ethanol at 4°C. After centrifugation at 15,000 rpm for 15 min at 4°C, the supernatant was decanted, and the pellet washed twice by centrifuging with 80% ethanol. The pellet was air-dried and resuspended in H2O. The extracted DNA was then subjected to PCR as previously described (18).

Statistical Analysis

For each year after birth the number of person-years at risk was calculated. Seroconversions that occurred within this year were assumed to have occurred in the middle of the year. This was used to calculate the incidence over the 4 years after birth. The annual average incidence over the 4 years after birth was calculated to estimate the risk of seroconversion in the first 4 years of life with the help of the following standard formula:

\[
\text{Risk} = \frac{I_4}{4} \times \frac{1}{N_0} \times 100
\]
RESULTS

Of the 45 HTLV-I/II Western blot positive mothers tested, 43 (95.6%) were positive for HTLV-I and 2 (4.4%) for HTLV-II by PCR. The 43 HTLV-I positive mothers gave birth to 44 infants, of whom 36 (81.8%) and 23 (52.3%) were followed-up for 2 and 4 years, respectively. The 2 HTLV-II positive mothers gave birth to 2 infants who were also followed for 4 years.

During the first year, 2 children from HTLV-I positive mothers and 3 children from seronegative mothers died (2/36 vs. 3/69, X = 0.56 NS). Thus, only 34 children of HTLV-I positive mothers were followed for the first 2 years. No infant had a blood transfusion during the follow-up period. Ville et al. (16) did not observe any impact on fetal outcome by maternal HTLV-I infection in pregnancy. Children were lost for follow-up because of migration to other parts of Gabon. The minimum age of weaning of the infants was 8 months, and the average was 10.1 months. No clinical manifestations of HTLV-I infection were found during pregnancy or 1 year after delivery in both mothers and children. Furthermore, no recurrent infective dermatitis or persistent lymphadenopathic syndrome in infected children was observed.

Serological Follow-up by Western Blot

All 45 samples from the cord blood of babies born to seropositive women, except one, showed the same Western blot pattern as the corresponding seropositive mother. Six months after birth, maternal antibodies in the children had disappeared except for four infants who still showed antibodies to viral core proteins at 9 months of age. By 12 months, all the children were negative in Western blot as in the control group, and no Western blots were indeterminate (Table 1). The first antibodies to disappear were the anti-env (gp46), and the last were the anti-p19.

Overall, a seroconversion risk of 17.5% (95% CI, 2.4-30.2%) was observed after a 4-year follow-up (Table 2). The first seroconversion was observed 18 months after birth. By the age of 2 years, a total of 4 of the 34 children had seroconverted. By the age of 3 years, one more child had seroconverted, making a total of 5 seroconverters (Tables 1 and 2). The annual average incidence of seroconversion was 4.8% during a 4-year follow-up, and the incidence varied after age 1 year but before age 3 years (Table 2). But no other child seroconverted in the fourth year of follow-up (Tables 1 and 2). The pattern of appearance of antibodies was the same for all the children who seroconverted, with anti-p19 and anti-p24 appearing first and anti-gp46 later. Among the 66 negative controls followed, no children from initially seronegative mothers seroconverted for HTLV-I.

PCR Analysis of Mother-To-Child Transmission

Among the children born to HTLV-positive mothers, it is interesting that none of the cord blood lymphocytes (46 samples) nor the amniotic fluid (15 samples) were positive for HTLV-I or HTLV-II, thus suggesting that transmission was not intrauterine or perinatal. All cord blood samples from chil-

<p>| Table 1. Prevalence of HTLV-I seropositivity on sequential blood samples from children born to seropositive mothers |</p>
<table>
<thead>
<tr>
<th>Age (mo)</th>
<th>No. tested</th>
<th>No. pos.</th>
<th>Western blot</th>
<th>No. tested</th>
<th>No. pos.</th>
<th>PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>0”</td>
<td>34</td>
<td>34</td>
<td>34</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>34</td>
<td>4</td>
<td>21</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>34</td>
<td>6</td>
<td>NT</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>34</td>
<td>1</td>
<td>NT</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>34</td>
<td>4</td>
<td>16</td>
<td>2</td>
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<td></td>
</tr>
<tr>
<td>30</td>
<td>23</td>
<td>5</td>
<td>NT</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>36</td>
<td>23</td>
<td>5</td>
<td>NT</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>42</td>
<td>23</td>
<td>5</td>
<td>NT</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>48</td>
<td>23</td>
<td>5</td>
<td>NT</td>
<td>0</td>
<td>0</td>
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</tr>
</tbody>
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PCR, polymerase chain reaction; no. pos., number positive; NT, not tested.

* Thirty-four infants’ cord blood samples were taken at birth (0).

** Two of 16, and 2 of 14 were positive on Western blot and PCR; the rest were negative. The samples tested by PCR at 6, 24, and 36 months were randomly selected.

<p>| Table 2. Incidence of HTLV-I seroconversion by age among children born from HTLV-I seropositive mothers |</p>
<table>
<thead>
<tr>
<th>Age</th>
<th>No. person years at risk</th>
<th>No. seroconversions</th>
<th>Rate per 100 person years (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-1</td>
<td>34</td>
<td>0</td>
<td>0.0 (95% CI)</td>
</tr>
<tr>
<td>1-2</td>
<td>32.5</td>
<td>4</td>
<td>12.3 (9.2-15.4)</td>
</tr>
<tr>
<td>2-3</td>
<td>20.25</td>
<td>1</td>
<td>4.9 (2.8-7.0)</td>
</tr>
<tr>
<td>3-4</td>
<td>18</td>
<td>0</td>
<td>0.0 (95% CI)</td>
</tr>
<tr>
<td>Total</td>
<td>104.75</td>
<td>5</td>
<td>4.6 (3.6-5.6)</td>
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</table>
dren born to seronegative mothers were negative in PCR.

Among the samples available for PCR analysis, we selected 21 samples at 6 months, 16 at 2 years, and 14 at 3 years after birth, while including at least 2 Western blot positive samples at each stage of testing. Of these children tested, 2 of 16 were positive by 2 years after birth and 2 of 14 more became positive for HTLV-I by 3 years after birth (Table 1). The children positive on PCR were positive on Western blot, while the others negative on PCR were negative on WB as well. All the 21 samples tested at age 6 months were negative, thus confirming that these children were not infected. Furthermore, 1 child with indeterminate Western blot pattern at age 1.5 years was negative by PCR. This same child was also negative on WB and on PCR at 3 years of age.

HTLV-I/II Seroprevalence Among Siblings

A total of 100 siblings, aged 1 to 14 years, born to seropositive mothers (group I) and 175 born to seronegative mothers (group II) were tested for anti-HTLV-I/II by ELISA and Western blot. In group I, 15 of 100 (15%) were positive versus 1 of 175 (0.6%) in group II (Table 3). The child positive in group II was multitransfused for sickle cell anemia.

Mother-To-Child Transmission of HTLV-II

Of the two HTLV-II PCR positive mothers' children consecutively followed for the 4-year study period, one child was confirmed by PCR and Western blot to have been infected and seroconverted; the other child stayed negative throughout follow-up.

DISCUSSION

Several modes of HTLV-I transmission from mother-to-child have been suggested, including intrauterine, perinatal, or postnatal. Our findings of lack of detection of HTLV-I or -II by PCR in cord blood or amniotic fluid support those of Hino et al. (6), in a series of 227 cord blood samples from HTLV-I seropositive mothers, did not find any case of HTLV-I—containing cells or antibody activity in the IgM class. This suggests that intrauterine infection is less likely to be the major route of maternal HTLV-I transmission. If, therefore, perinatal transmission plays a major role in maternal HTLV-I transmission, HTLV-I or -II proviral DNA would have been detected ≤6 months after birth, but this was not the case in our study (Table 1). Furthermore, this possibility of perinatal transmission may be excluded since IgG antibodies gradually disappeared ≤1 year, and seroconversions were only observed at age >1 year. Because ~20% of breast-fed infants compared to 3% bottle-fed infants or HTLV-I carrier mothers are infected (10,21) and because all the cord blood samples in our study were HTLV-I/II negative on PCR, it is likely that the at-risk period during which transmission could occur would be during the breast-feeding period. We note that lymphocytes bearing HTLV-I in breast milk from carrier mothers (8,22,23) and the oral transmission of HTLV-I in a monkey model (24,25) have been reported, thus suggesting postnatal transmission. Our results of late seroconversion and detection of HTLV-I by PCR after age 1 year are evidence supporting postnatal transmission. However, our sample size may limit a firm conclusion.

In our study, the annual average incidence of anti-HTLV-I antibodies in infants born to seropositive mothers with 4 years of follow-up was 4.8%, and the risk of seroconversion was 17.5%. No other child seroconverted after age 3 years. Similar to our findings, Kusuhara et al. (10) also observed there was no seroconversion in children after age 3 years in a 15-year follow-up study in Japan. Despite the diverging life-style and environmental factors in Japan compared to Gabon, reports have indicated that ≤15 to 22% of children born to HTLV-I seropositive mothers seroconvert by age 3 years (6,10,26). Fifteen percent of children born to HTLV-I seropositive mothers in Jamaica seroconvert (27), results that are similar to ours.

The seroconversion rate of 15% (±8%) of HTLV-I/II among the siblings aged 1 to 14 years is a useful index of this infection in a young generation of an HTLV-I/II endemic population in Gabon. Seropositivity observed for HTLV-I in children and young adolescents is probably due to mother-to-child transmission (10). However, in our study, we can-

<table>
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<th>TABLE 3. Seroprevalence of HTLV-III in siblings (aged 1 to 14 years) born to seropositive and seronegative mothers</th>
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<tr>
<td>Group</td>
</tr>
<tr>
<td>-------</td>
</tr>
<tr>
<td>Group I</td>
</tr>
<tr>
<td>Group II</td>
</tr>
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</table>

No. pos, number positive in enzyme-linked immunosorbent assay (ELISA) and Western blot.

* Groups I and II are siblings born to seropositive and seronegative mothers, respectively.
not confirm whether the siblings' mothers were infected before their first births. This high seroprevalence rate calls for future prevention strategies and points to the need for an effective testing of blood for transfusion.

Since the first description of HTLV-II infection in Gabon (28), different reports have confirmed the presence of this virus in other African countries (29-31). However, reports on the modes of transmission in these countries are lacking. To our knowledge, we report the first case of mother-to-child transmission of HTLV-II in Gabon. More investigation on the modes of transmission of this virus in Africa are warranted.

We and others (32) have shown that children born to seropositive carrier mothers passively acquire maternal antibodies prenatally that decline and disappear over time. High levels of maternal anti-HTLV-I have been suggested to correlate with high levels of HTLV-I-positive lymphocytes in breast milk and are associated with transmission in long-term (≥6 months) breast-fed children (27,33). Therefore, babies of carrier mothers who are breast-fed for a long time (≥8 months in our study) would continue to ingest HTLV-I-infected lymphocytes from the breast milk as the passively acquired high-titered and anti-env (anti-HTLV-I) antibodies gradually disappear from circulation, thus resulting in HTLV-I infection. We note it has recently been shown that maternal antibody may inhibit HTLV-I infection by short-term breast-feeding (3 months), but would not inhibit HTLV-I infection by long-term breast-feeding after the decay of the maternal antibody (32). However, this may not be applicable in the developing world because the advantages of breast-feeding outweigh the risk of HTLV-I-associated morbidity. Alternatively, it is conceivable that immunization of seropositive pregnant women, as has been demonstrated in newborn rabbits (34), with high-titered anti-env antibodies might be an effective means of boosting passively acquired antibodies in infants to protect against HTLV-I infection.

In conclusion, the lack of detection of HTLV by PCR in cord blood and amniotic fluid and the late seroconversions are arguments for postnatal transmission in which maternal milk plays an important role, because none of the children had blood transfusion during the follow-up period. This is the first report of a prospective study on mother-to-child transmission of HTLV-I in Africa. More studies in other regions of Africa are warranted.

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REFERENCES


