Late postnatal transmission of human immunodeficiency virus type 1 infection from mothers to infants in Dar es Salaam, Tanzania

[Original Studies]

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Abstract

Objective. To study late postnatal transmission of human immunodeficiency virus type 1 in a cohort of children born to HIV-1-seropositive mothers who delivered at Muhimbili Medical Centre in Dar es Salaam, Tanzania.

Materials and methods. Since 1991 a prospective cohort study of mother-to-child transmission of HIV-1 has been conducted at Muhimbili Medical Centre in Dar es Salaam. HIV-1-seropositive mothers and age-matched seronegative controls were recruited into the cohort at delivery together with their newborns. Diagnosis of HIV-1 infection in children was based on polymerase chain reaction, HIV-1 p24 antigen tests and HIV antibody tests. Late postnatal transmission was defined as HIV-1 infection occurring after 6 months of age in a child who was uninfected at 6 months of age and who had an HIV-1-seropositive mother. Children born to HIV-seronegative mothers were used as controls. Breast-feeding was universal in this cohort. CD4 and CD8 T lymphocytes were assayed by flow cytometry in the mothers.
Results. Among 139 children born to HIV-1-seropositive mothers and known to be HIV-uninfected at 6 months of age, 8 children became HIV-1-infected at the end of their first year of life or later. No conversions were observed in children younger than 11 months. The 8 conversions were observed during a follow-up covering 1555 child months between 6 and 27 months of age corresponding to a conversion rate of 6.2 per 100 child years. Among 260 children with HIV-seronegative mothers no child became HIV-infected during the follow-up. The percentage of CD4 T lymphocytes was similar in mothers with early and late transmission but was significantly lower in transmitting than in nontransmitting mothers.

Conclusion. Because no HIV-1 infection occurred in children with HIV-seronegative mothers, we conclude that the observed infections at the end of the first year of life or later among children born to HIV-seropositive women were caused by late transmission from mother to child, most likely through breast-feeding.

INTRODUCTION

Reported rates of mother-to-child transmission of HIV-1 infection vary in different geographical areas and in different population groups. The variation among studies has to some extent been the result of methodologic differences. The overall transmission rates ranged from 13% in Europe to 42% in Africa when a standardized method was used for the calculations. Studies on mother-to-child transmission of HIV-1 in Africa have usually included a majority of breast-feeding mothers, and breast-feeding is probably one of several factors influencing mother-to-child transmission rates. HIV-1 has been isolated from breast milk, and there is evidence that HIV-1 can be transmitted from mother to child through breast-feeding. In a metaanalysis of six prospective studies on mother-to-child transmission of HIV-1, the additional risk for HIV transmission in a breast-feeding population was estimated to be 14%.

Information about the timing of transmission of HIV-1 infection from mother to child through breast-feeding is still sparse. Many studies on mother-to-child transmission have relied on IgG serology to diagnose HIV-1 infection in mothers and children. Because of passive transfer of HIV-1 IgG antibodies from the mother to the child, HIV-1 infection in the child could then not be diagnosed before the age of 15 months. A study from Rwanda showed a seroconversion rate of 5.8 per 100 child years of observation during the age period 20 to 36 months in a breast-fed population. In a study of mother-to-child transmission of HIV-1 in Nairobi, Kenya, where the overall transmission rate was 42.8%, the percentage of HIV-1 infection attributable to breast-feeding >=15 months was reported to be 32%. To allow analyses of what is happening during the first 15 months of life, diagnostic methods other than IgG serology must be used. Results from Rwanda have been reported on late transmission from mother to child using PCR as diagnostic method for the children. Late postnatal transmission, defined as PCR positivity after 3 months of age in that study, was estimated to be 8%, one-third of the overall transmission rate of 25%. Results based on PCR have also been presented from Zaire, where 12% of the overall transmission of HIV-1 observed in a cohort of breast-fed children occurred late postpartum.
Here we report results on late postnatal transmission of HIV-1 from a cohort study on mother-to-child transmission of HIV-1 infection in a breast-feeding population in Dar es Salaam, Tanzania. The children were followed up by PCR for HIV-1 DNA and by HIV serology, including testing for HIV-1 p24 antigen, making it possible to gather information on the timing of late postnatal transmission in the cohort.

**MATERIALS AND METHODS**

Subjects and collection of specimens. This study on late postnatal transmission of HIV-1 is part of an ongoing prospective study on mother-to-child HIV-1 transmission conducted at Muhimbili Medical Centre, Dar es Salaam, Tanzania, since 1991. After informed consent was given the mothers were enrolled into the study. The study received ethical clearance from the National AIDS Control Programme in Tanzania and from the Karolinska Institute in Stockholm, Sweden. Mothers delivering at Muhimbili Medical Centre were tested for HIV-1 antibodies. The seroprevalence of HIV-1 infection in this group of mothers was steady during the years 1991 to 1994 (12.5, 11.5, 11.3 and 12.8%, respectively). Seropositive mothers and their newborns together with age-matched seronegative controls were recruited into the study. The follow-up contained scheduled visits to the research clinic once a month in the first year and then every third month up to 24 months. Blood samples were collected from the children in EDTA Vacutainer® tubes every third month when possible. Peripheral blood mononuclear cells were used for PCR and plasma was used for HIV-1 serology. The first blood sample was usually collected when the child was 4 to 8 weeks old.

The overall transmission rate in the cohort at the time for this analysis was 30.2% (54 of 179) (95% confidence interval, 23.5 to 36.9%) estimated by the direct method for calculation of transmission rates (intermediate estimate) recommended by the Ghent group. This method is built on a classification of children born to HIV-seropositive mothers according to their probable HIV infection status at 15 months of age. In the original Ghent classification system the laboratory diagnosis of HIV infection was based on serology. However, in our study laboratory diagnosis of HIV infection in children was mainly based on PCR. As a complement p24 antigen tests and HIV antibody tests were used.

In the analysis of late postnatal transmission the following definition of late postnatal transmission was used: HIV infection occurring after 6 months of age in a child uninfected at 6 months of age and who had an HIV-seropositive mother. Conversion from HIV negativity to HIV positivity was considered to have occurred if a child had at least two HIV-negative samples followed by at least two HIV-positive samples or by one HIV-positive sample if the child subsequently died with HIV-related disease.

The study included 139 children born to HIV-1-positive mothers and known to be PCR-negative at 6 months of age. At the time of analysis 78% of these children had been followed for >=12 months, 54% for >=18 months and 27% for 24 to 27 months. Two hundred sixty children born to HIV-seronegative mothers were used as controls. Among children followed after 6 months of age in the control group, 84% had been followed up for >=12 months, 60% for >=18 months and 25% for 24 to 27 months. The analysis forming this report was made when some of the children...
in the cohort had not yet reached the age of 18 to 24 months. Losses to follow-up were mainly caused by change of residence. Very few mothers refused to continue to come to the clinic.

HIV-1 antibody tests. The serum or plasma samples from the mothers were initially screened for HIV antibodies using a rapid test, either HIV Check® (Du Pont de Nemour, Wilmington, DE) or Capillus® (Cambridge Biotech, Worcester, MA). Samples reactive on one of the rapid anti-HIV tests were further tested by enzyme linked immunosorbent assay (ELISA) either Wellcozyme® anti-HIV-1 ELISA (Murex Diagnostics Ltd., Dartford, UK), Micro Trac HIV-EIA® (SYVA) or Enzygnost® anti-HIV-1/HIV-2 (Behringwerke, Marburg, Germany). All samples reactive on both a rapid test and an ELISA were confirmed by a Western blot assay (HIV Blot 2.2®; Diagnostic Biotechnology Ltd., Singapore). Plasma samples from children were tested by Enzygnost® anti-HIV-1/HIV-2 and Wellcozyme® anti-HIV-1 ELISA or Abbott IMx HIV-1/HIV-2 III Plus (Abbott Diagnostics Division, Wiesbaden, Germany). Samples with divergent results were tested by WB.

HIV p24 antigen tests. Testing for p24 antigen was performed on heat-denaturated plasma samples as previously described 14 with the following exceptions: The HIV-1 p24 ELISA-TSA assay (DuPont) was used where an amplification step is included in the kit. All samples were tested in a dilution of 1:6.

PCR. Peripheral blood mononuclear cells were separated from EDTA blood by Ficoll-Isopaque® (Pharmacia, Uppsala, Sweden) density gradient centrifugation. Remaining erythrocytes were lysed using fluorescence-activated cell sorter lysing solution (Becton Dickinson, San Jose, CA). Cellular DNA was prepared as described.15 The PCR testing was done at the Swedish Institute for Infectious Disease Control. A nested PCR technique was used including primer sets OG 33-57 (gag), OG 154-197 (pol), OG 462-502 (vif) and OG 722-749 (env), as previously described.16 To control the quality of the DNA preparations, a human beta-globinspecific PCR was performed on all samples. From October, 1995, the following modifications of the PCR methodology were used as recently described.17 The PCR preparations were made according to the Amplicor® whole blood preparation method (Roche Diagnostic Systems, Inc., Nutley, NJ). Primarily the primer sets OG 462-502 (vif) and OG 7253-7541 (env) were used in nested PCR. In case of divergent results a PCR with a third primer set, OG 154-197 (pol), was done.

T lymphocyte subsets. The number of peripheral white blood cells was determined in a cell counter (Coulter Electronics, North West Drive Luton, London, UK). The T lymphocyte subsets were determined by use of a FACScan® flow cytometer, SimulSET® software, and Simultest® reagents (Becton Dickinson, Immunocytometry Systems, San Jose, CA). The following panel of monoclonal antibodies was used: CD45-fluorescein isothiocyanate (FITC)-conjugated/CD14-phycocerythrin (PE)-conjugated; isotype control-FITC/isotype control-PE; CD3-FITC/CD4-PE and CD3-FITC/CD4-PE according to CDC guidelines.18

RESULTS
Among the 139 children in the cohort born to HIV-1-seropositive mothers and known to be PCR-negative at 6 months of age, 8 children turned HIV-positive as demonstrated by PCR and p24 antigen assay during the follow-up covering 1555 child months between 6 and 27 months of
age. This represents a conversion rate of 6.2 per 100 child years of observation in this age range. Seven of these conversions were confirmed by a positive PCR test on a second sample. Two of the children died, one of whom was the child with a positive PCR result on only the last sample. Laboratory results available for the 8 children who became HIV-1-infected later than 6 months after birth are shown in Figure 1.

Among 260 children with HIV-seronegative mothers no child turned HIV-positive during the follow-up.

Results of determination of CD4 T lymphocytes done on mothers within 10 weeks after delivery were available on six of the eight mothers transmitting HIV-1 infection to their children later than 6 months after delivery (late transmitters). In these six mothers the percentage of CD4+ T cells was <=25. The mean percentage of CD4+ T cells and the proportion of mothers with a low percentage of CD4 T cells (<=21%) among early and late transmitters and among nontransmitters are shown in Table 1. The cutoff value for the CD4 T cell percentage was arbitrarily set at 21 to coincide with the mean value in transmitting mothers. The mean percentage of CD4 T lymphocytes did not differ between early and late transmitters but was significantly lower in transmitters than in nontransmitters. Similarly the proportion of mothers with a low percentage of CD4 T cells was significantly higher among early and late transmitters than among nontransmitters.

<table>
<thead>
<tr>
<th>Type of Transmitter</th>
<th>No. of Cases</th>
<th>CD4 % Mean (SD)</th>
<th>Proportion of Mothers with CD4 % Values &lt;=21</th>
<th>p (proportion)</th>
<th>p (mean)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Late transmitters</td>
<td>6</td>
<td>21.0 (5.1)</td>
<td>49/64 (75)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Early transmitters</td>
<td>23</td>
<td>21.2 (9.7)</td>
<td>14/23 (61)</td>
<td>0.002</td>
<td>0.004</td>
</tr>
<tr>
<td>Transmitters</td>
<td>29</td>
<td>21.0 (8.9)</td>
<td>16/29 (55)</td>
<td>0.002</td>
<td>0.004</td>
</tr>
<tr>
<td>Nontransmitters</td>
<td>41</td>
<td>27.2 (10.0)</td>
<td>7/41 (17)</td>
<td>0.002</td>
<td>0.004</td>
</tr>
</tbody>
</table>

* p test for equality of means.
* Chi-square exact test.

**TABLE 1. Mean percentage of CD4 T lymphocytes and proportion of mothers with a percentage of CD4 T lymphocytes <=21**

The general policy for breast-feeding in Tanzania is exclusive breast-feeding for 4 to 6 months and then complemented breast-feeding for 2 years and older. All women in our cohort were
breast-feeding and they followed the breast-feeding policy formulated for the country. Seven of the eight mothers with children diagnosed as infected after 6 months of age were breast-feeding at the time for diagnosis and one mother had stopped breast-feeding between the last negative sample and the first positive sample. None of the eight children had had a blood transfusion. Because we have not found any HIV-1 infection in children with HIV-seronegative mothers, we conclude that the observed infections at the end of the first year of life or later among children born to HIV-1-positive women were caused by late transmission from mother to child, most likely through breast-feeding. None of the late transmitting mothers had reported any breast-feeding complications such as mastitis or cracked nipples that might have increased the risk for HIV-1 transmission through breast-feeding.

No conversions were diagnosed before the age of 11 months, but we do not know when the conversions occurred between the last negative sample and the first positive sample. The general impression of low HIV-1 transmission between 6 and 9 to 10 months of age in this cohort is further underlined by the fact that this is the time period that is best covered in the follow-up. There are many possible explanations to this observation. Breast-feeding as the main source of nutrition for the baby might be connected with a lower transmission rate than partial breast-feeding because of protective immunologic components in the breast milk, or properties of breast milk could change over time in a way that leads to an increasing risk for HIV-1 transmission. Alternatively if passively transferred maternal antibodies present in the blood of the child are protective, the gradual loss of these antibodies with time could make the child less resistant to HIV. In a study in South Africa it was found that 94.5% of the children studied had lost their maternal HIV-1 antibodies at 12 months of age (100% at 15 months of age).19 Another possible explanation of increased risk for postnatal transmission of HIV infection through prolonged breast-feeding could be that infants who have developed teeth are at increased risk of becoming infected if they bite their mothers' nipples so that bleeding occurs.

The HIV-1 conversion rate per 100 child years after the age of 6 months in the children of HIV-1-seropositive mothers in the present study is similar to that reported from a study in Rwanda based on HIV-1 seroconversion during the age period 20 to 36 months.10

In the present study as well as in a previously reported study of predictive markers for mother-to-child transmission of HIV-1 in Dar es Salaam,20 we found an association between immunodeficiency in the mother as reflected by decreased CD4 T lymphocytes and transmission of HIV-1 to the child. This finding is also consistent with results from other previous studies.21, 22 We found no difference in CD4 T lymphocytes between mothers with early and late transmission, but the number of tested mothers with late transmission was small.

It is generally accepted that breast-feeding is important for the well-being of a child. The fact that HIV-1 infection can be transmitted from mother to child through breast-feeding has therefore led to a dilemma when counselling HIV-infected women about breast-feeding, especially in developing countries, where a reasonably safe alternative to breast-feeding is not available in many settings.12, 23 This dilemma was clearly expressed in the recommendations concerning breast-feeding and HIV from WHO/UNICEF 1992 24 and is still reflected in the interim statement from the Joint United Nations Programme on HIV/AIDS Concerning HIV and Infant Feeding 1996.25
Several studies are under way to evaluate the effect of various interventions to prevent mother-to-child transmission of HIV-1 infection in developing countries, for example through the use of antiretroviral therapy. Even if these studies give promising results, it will still be a long way to go before mothers and children living under poor conditions in developing countries can benefit for economic and practical reasons. Finding the optimal time for stopping breast-feeding in different settings may be the most important way of preventing mother-to-child transmission of HIV-1 in the nearest future in communities in poor socioeconomic conditions. Our results suggest that 6 to 9 months might be the age after which the advantages with breast-feeding no longer exceed the risk for HIV-1 transmission, even in surroundings with high mortality in children because of malnutrition and infectious diseases in countries like Tanzania. Ongoing studies especially designed to add information on breast-feeding and HIV-1 transmission will hopefully soon make it possible to formulate new recommendations on breast-feeding in developing countries.

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