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Original Article

Is the risk of mother-to-child transmission of HIV higher among female compared with male infants? A case of Rakai, Uganda

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Abstract. Purpose: To assess gender differences in the risk of mother-to-child transmission (MTCT) of human immunodeficiency virus (HIV). HIV-positive mothers were identified from a population cohort followed from 1994 to 2000. HIV infection in mothers was detected using two independent enzyme immunoassays and infant HIV infection was diagnosed using RNA- polymerase chain reaction. Birth weight was determined by anthropometry. Logistic regression was used to assess the univariate and multivariate risk factors of MTCT. Approximately 16% of 371 infants were HIV-positive in the in-*utero* and intrapartum periods and an additional 16% were infected via breastfeeding. Female infants were significantly more likely to be HIV infected perinatally compared with male infants (20.8% vs. 12.4%, respectively, $P = 0.035$), but there was no significant sex differences in postnatal risk of MTCT. In adjusted analyses, among mothers with higher than median HIV viral loads, there was no significant difference in the risk of MTCT by gender, but among mothers with lower than median HIV viral loads, female infants were significantly more likely to be HIV infected (odds ratio = 4.1, confidence interval = 1.04–16.1). Low birth weight was more frequent in female than male infants born to HIV-positive mothers. Female infants could be more susceptible to HIV infection in the in-*utero* and peripartum period compared with male infants. Alternatively, this sex association could be due to higher in-*utero* mortality rates of male infants or to increased susceptibility of female infants.

Keywords: MTCT, vertical transmission, perinatal transmission, viral load, low birth weight

1. Introduction

Human immunodeficiency virus (HIV) vertical transmission risk in breastfeeding women without prophylaxis is between 16–40% [1] and studies have shown that the major risk factors of mother-to-child transmission (MTCT) of HIV in-*utero* or around delivery include maternal HIV viral load, mode of delivery, pla-

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cental pathology, prolonged membrane rupture, and pre-term or low birth weight [2–5]. However, there is recent debate surrounding the association of infant sex and risk for MTCT.

A study in Nairobi found higher rates of MTCT in females than in males [5]. The European Collaborative Study found a higher risk of MTCT among female infants, but this was limited to babies born by elective caesarean section, suggesting a higher in-*utero* infection of female fetuses [6]. An Italian study also found a higher risk of MTCT at birth for female infants, which was independent of mode of delivery or antiretroviral therapy (ARV) [7]. A more recent study in Zimbabwe found an increased in-*utero* risk of MTCT among female infants, but not in the intrapartum period, or postnatally [8]. In contrast, a meta-analysis of nine African trials found no sex difference in HIV acquisition in the in-*utero* or intrapartum periods, but found that males were at a higher risk of MTCT via breastfeeding [9]. Previous large perinatal trials have not shown sex to be a risk factor for vertical transmission of HIV [10,11].

All African trials reporting sex differences were done on mother-infant pairs recruited in hospital settings in large cities. In order to address the current debate on sex and MTCT risk, we analyze data from a cohort of women in rural Rakai, Uganda, where deliveries take place in a home setting.

2. Materials and methods

Data for this study come from the Maternal Infant Supplementary Study that was nested in the Rakai Sexually Transmitted Diseases Control Study conducted between 1994 to 1998. Detailed methods of this study are provided elsewhere [12,13]. All consenting pregnant women and their infants were followed through 2000 [12,14]. Prevention of Mother to mother-to-child HIV Transmission services such as provision of nevirapine were not available to this cohort until after 2000.

Annual surveys were conducted to collect detailed sociodemographic, behavioral and biomedical data from study participants. Pregnant women were identified from the main cohort and all consenting pregnant women had a prepartum visit (median gestational age was 5 months), where they received routine prenatal care and were asked to provide samples for detection of HIV and sexually transmitted infections. Within a week of delivery, all consenting mothers and their newborn infants were visited by midwives and samples were collected for HIV testing. Infants were examined to determine weight, upper arm and head circumference, and gestational maturity was determined using the Ballard score [15]; or date of last menstrual period was used for children for whom the Ballard score was missing. In this rural setting, most deliveries occur at home, birth weight cannot be assessed at time of delivery, and infants are typically seen within a few days of birth. Infants lose weight after birth due to neonatal diuresis and this makes weight a less reliable means to assess the adequacy of the child's intrauterine growth. Chest circumference of $\leqslant 30$ cm was used as it has been shown to be a reliable proxy measure of birth weight in African populations [16].

HIV infection in mothers was detected during pregnancy and postpartum using two independent enzyme immunoassays (Vironostika HIV-1; Organon Teknika, Charlotte, NC, and Cambridge Biotech, Worcester, Mass), with Western blot confirmation of discordant enzyme immunoassay results or seroconverters. For infant HIV determination, heel stick filter paper samples or serum were obtained postpartum, at or around birth to 4–6 weeks ($n = 371$) and later during breastfeeding ($n = 94$). Lack of funding and loss to follow-up due to maternal out-migration made it challenging to re-test all HIV-negative infants for postnatal HIV infection. Infant HIV infection was detected by reverse transcriptase RNA-polymerase chain reaction (PCR) (RT-PCR) using the Amplicor HIV-1 Monitor 1.5 Assay (Roche Molecular Systems, Branchburg, NJ) on dried blood spots or sera. A serum aliquot was frozen at $-70\,^{\circ}\mathrm{C}$ and shipped to Johns Hopkins, Baltimore, Maryland for viral quantification (Roche Amplicor HIV-1 MONITOR Test, version 1.5). With home deliveries in this rural setting, it was difficult to visit all women on the day of birth, so it was not always possible to distinguish between in-*utero* and intrapartum transmission. Therefore, perinatal HIV transmission (i.e., during the in-*utero* and intrapartum periods) was defined as a positive HIV PCR at birth up to 6 weeks, and postnatal transmission was defined as a negative HIV PCR at 6 weeks followed by a subsequent positive HIV PCR. Antiretroviral therapy for HIV-infected mothers or nevirapine for the prevention of mother to child HIV transmission was not available in Uganda at the time this study was conducted. The study was approved by ethical committee of Johns Hopkins University IRB and local Ugandan IRBs

2.1. Statistical analyses

Data were analyzed using Intercooled STATA statistical software for Windows, version 8 (STATA Corpo-

ration, College Station, Texas, USA). MTCT rates were calculated overall and by infant sex, and Fisher's exact tests were used to assess the statistical significance of associations between covariates and MTCT rates. Univariate and multivariate odds ratio (OR) of MTCT were calculated using logistic regression. The multivariate models adjusted for maternal viral load above or below the median (4.2 log cps/mL), and for low birth weight and sexually transmitted infections, which were found to be associated with MTCT in the univariate analyses.

3. Results

The median gestation age of children at birth was 40.28 weeks. PCR results between birth to 6 weeks were available for 371 infants born to HIV-positive mothers. The overall proportion of infants infected with HIV in the perinatal period (birth to 6 weeks) was 16.4% (61/371, 95% confidence interval (CI): 13%–21%). During the perinatal period, there was an excess of female HIV infections (MTCT in female 20.8% vs. male infants 12.4%, $P = 0.035$). The average CD4 count for mothers in this study was 617 cells/mm^3 and the median HIV $\log_{10}$ viral load was 4.15. To assess whether maternal viral load had a differential impact on MTCT rates by sex, we assessed MTCT risk by infant sex, stratified by maternal HIV viral loads above or below the median log viral load. At maternal HIV viral loads above the median, there was no significant difference in MTCT rates by sex (females 17.2% vs. males 24.0%, $P = 0.4$). However, at maternal HIV viral loads below the median, female infants were significantly more liked to be HIV infected compared with males (20.3% vs. 4.8%, respectively, $P = 0.014$). The sex ratio at birth for children born to HIV-positive mothers was 1.08 (193 males and 178 females). When stratified by maternal HIV viral load, the sex ratio at birth was 1.17 (75 males and 64 females) for infants born to mothers with viral loads above the median, and 0.98 (63 males and 64 females) for infants born to mothers with viral loads below the median. This difference was not statistically significant ($P = 0.96$) among mothers who ever had an sexually transmitted diseases, MTCT rates were significantly higher in female vs. male infants (24.7% vs. 10.6%, $P = 0.02$).

Female infants were more likely to be born with low birth weight (females, 40/177: 22.6% vs. males, 25/186: 13.4%, $P = 0.02$), and low birth weight infants were significantly more likely to be infected with HIV compared to infants born at normal birth weight (MTCT 30.8% in low birth weight vs. 13.8% in normal birth weight, $P = 0.001$).

There was no significant difference in maternal HIV viral loads by infant sex ($\log_{10}$ viral load was 4.15 cps/mL for mothers of both females and males, $P = 0.7$). Infant HIV viral loads obtained at the postpartum visit were 4.75 $\log_{10}$ cps/mL in females and 5.13 cps/mL for males ($P = 0.5$).

The odds of MTCT are presented in Table 1. The unadjusted odds of MTCT in females relative to males was OR = 1.85, CI: 1.1–3.2, and higher maternal HIV viral load and low birth weight were significant risk factors for MTCT. In the adjusted analyses, female infants were not at a significantly higher risk of MTCT than males (OR = 1.11, CI: 0.56–2.21). However, among mothers with HIV viral loads below the median, the adjusted OR of MTCT in female vs. male infants was 4.1, CI: 1.04–16.1). There was no significant difference in the sex-specific risk of MTCT among infants born to mothers with viral loads above the median (OR = 0.47, CI: 0.18–1.2).

There were no significant differences between HIV+ and HIV- infants for the following outcomes: eyes infected by gonorrhea or chlamydia (1.9% vs. 0%, respectively, $P = 1.0$), or prematurity (2% vs. 13%, respectively, $P = 1.0$).

4. Discussion

This study was conducted in Rakai, Uganda, where the female HIV prevalence was approximately 17 percent. There was a higher risk of MTCT during the in-*utero* and/or intrapartum periods in female relative to male infants. The multivariate adjusted risk of MTCT was significantly higher for female infants born to mothers whose HIV viral loads were lower than the median. At higher maternal HIV viral loads, female and male infants were at equal risk of acquiring HIV. This suggests that male infants may be less susceptible to HIV at lower maternal HIV viral loads, compared with female infants, or that infected male infants are more likely to die in-*utero* and thus are not observable at birth. However, the overall sex ratio at birth among children born to HIV-positive mothers and children born to mothers with viral loads lower than median were close to one, which does not suggest an increased male fetal loss.

One hypothesis to explain this increase in vertical transmission risk is that maternal HIV viral loads are higher for mothers of female vs. male infants [17].

Table 1
Adjusted odds ratio of mother-to-child transmission of human immunodeficiency virus

Parameters	Adjusted odds ratio ($n = 249$)		
	Overall	Maternal human immunodeficiency virus viral load (<50%)	Maternal human immunodeficiency virus viral load (>50%)
Female sex	1.11 (0.56–2.21)	4.1 (1.04–16.1)	0.47 (0.18–1.2)
Log viral load	2.80 (1.69–4.66)	4.91 (1.1–22.5)	11.4 (3.4–38.6)
3 Ever had a sexually transmitted infection	1.02 (0.51–2.05)	1.22 (0.37–4.05)	1.31 (0.51–3.38)
Low birth weight (reference normal weight)	1.96 (0.90–4.25)	3.4 (0.91–12.8)	1.44 (0.51–4.1)

In Malawi the risk of MTCT was increased for female infants around birth, with this sex association remaining for children who became infected by 6 to 8 weeks, and maternal HIV viral load was higher for mothers of female infants [17]. More recently, these Malawian investigators reported a higher in-*utero* risk of MTCT for females among singleton births and higher in-*utero* and perinatal risks of MTCT for girls among twin births [18]. However, we found no significant difference in maternal viral loads by sex of the infant.

The consistent findings of increased female vulnerability to in-*utero* and intrapartum MTCT is contrary to other adverse pregnancy outcomes, which tend to be more common in males [19–22]. However, it is noteworthy that there is increased female susceptibility to HCV infection, irrespective of mode of delivery [23, 24] and it is hypothesized that hormonal or genetic factors may make females more susceptible to acquiring HCV in-*utero* [23].

We found an excess of low birth weight among females, and low birth weight infants are more likely to be infected with HIV. It may be that a combination of genetic and hormonal factors, risk during vaginal delivery, as well as increased likelihood of being low birth weight that predisposes females to higher vulnerability acquiring HIV in-*utero*.

Our study has limitations because we were unable to disaggregate in-*utero* and intrapartum risks, and the small sample size constrained the power to detect gender differentials in MTCT. Also, we were not able to assess and control for all known risk factors of perinatal transmission (other concurrent infections, viral load levels at different times during pregnancy, etc.).

In conclusion, female infants are at higher risk of HIV infection than males during the in-*utero* and intrapartum periods, if their mothers' viral load was below the median. Further studies are needed to understand this increased vulnerability female infants have to acquiring HIV. This study has demographic implications and gender differences need to be considered in preventive as well as medical management of HIV-infected children.

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