Gender Differences in Perinatal HIV Acquisition Among African Infants

Taha E. Taha, MBBS, PhD*; Samah Nour, MBChB*; Newton I. Kumwenda, PhD*; Robin L. Broadhead, MBBS, FRCP‡; Susan A. Fiscus, PhD§; George Kafulafula, MBBS, FCOG‡; Chiwawa Nkhoma, MSc‖; Shu Chen, MSc*; and Donald R. Hoover, PhD¶

ABSTRACT. Objective. We investigated gender-specific risks of mother-to-child transmission (MTCT) at birth and at 6 to 8 weeks among infants born to HIV-infected African women.

Design. Follow-up study of infants enrolled in 2 randomized, phase III, clinical trials to prevent MTCT, conducted in Blantyre, Malawi, in southeast Africa.

Methods. Infants were enrolled at birth and monitored postnatally, and their HIV status was assessed at birth and at 6 to 8 weeks (assessment beyond 6–8 weeks is ongoing). Statistical analyses were stratified according to gender, and comparisons were made with descriptive, univariate, and multivariate statistical tests. MTCT was estimated at birth and at 6 to 8 weeks among infants who were not infected at birth.

Results. Overall, 966 boys and 998 girls were enrolled. The rate of HIV transmission at birth was 9.5% (187 of 1964 infants). However, at birth significantly more girls (12.6%) than boys (6.3%) were infected with HIV. This association remained significant after controlling for maternal viral load and other factors. Among infants who were uninfected at birth, 8.7% (135 of 1554 infants) acquired HIV by 6 to 8 weeks; of these infants, more girls acquired HIV (10.0%), compared with boys (7.4%).

Conclusions. Female infants may be more susceptible to HIV infection before birth and continuing after birth. Alternatively, in utero mortality rates of HIV-infected male infants may be disproportionately higher and thus more HIV-infected female infants are born. In areas of sub-Saharan Africa, where HIV infection rates are high among women of reproductive age, the magnitude of the gender transmission differences observed in this study could have clinical, preventive, and demographic implications.

ABBREVIATIONS. MTCT, mother-to-child transmission; CI, confidence interval; NVP, nevirapine; ZDV, zidovudine.

In developing countries, the risk of mother-to-child transmission (MTCT) of HIV is ~15 to 40%, but varies with antiretroviral treatment and breastfeeding. MTCT of HIV can occur in utero (20–25%), intrapartum (65–70%), or postnatally (10–15%). As with other infectious diseases, the probability of HIV transmission is dependent on the infectiousness of the index case, the mode of spread, and the susceptibility of exposed individuals. For MTCT of HIV, infectiousness is determined by maternal viral load, a risk factor strongly associated with perinatal infection and the potential for HIV transmission through the in utero, intrapartum, and postpartum routes. Susceptibility, however, may be determined by genetic factors and/or acquired resistance.

Among adults, gender differences in heterosexual HIV infection rates have been attributed to various biological (immunologic, genetic, viral, and hormonal), social, and behavioral factors. Among children, gender-specific differences could indicate inherent physiologic factors that influence not only differential progression of the disease but also acquisition of the virus as early as in utero. Few studies have observed more HIV infection among girls than boys. For example, data from the European Collaborative Study showed that the risk of vertical HIV transmission among nonbreastfed infants was significantly higher among girls, compared with boys (odds ratio: 1.49; 95% confidence interval [CI]: 1.04–2.13), after adjustment for maternal CD4 status, elective cesarean section, and use of antiretroviral drugs. The European Collaborative Study also showed that the effect of gender was limited to infants delivered through elective cesarean section, which suggests that girls may have increased risk of in utero transmission, compared with boys. In a small study conducted in the Ivory Coast among children attending a malnutrition clinic during 1994–1995, HIV seroprevalence at 15 months of age was significantly higher among girls (63.2%), 24 38 girls than boys (34.1%, 14 of 41 boys) (P = .01). Other studies, however, including some major clinical trials in the United States (Pediatric AIDS Clinical Trials Group trial 076) and Africa (HIV Network for Prevention Trials trial 012), did not show a significantly increased risk of HIV among girls. More recently, a large individual patient data meta-analysis that included 9 studies from Africa did not show a
statistically significant difference between the proportions of boys and girls with early (by 4 weeks of age) transmission of HIV.20

Despite the large number of perinatal studies conducted to date, it appears that uncertainty remains about the role of gender in MTCT of HIV. Most studies did not explicitly stratify MTCT analyses to show the independent contributions of in utero and postnatal transmission, and the lack of association with infant gender was made on the basis of overall MTCT rates at ≥4 weeks. Earlier perinatal studies, especially in Africa, that used serologic HIV testing after 12 months of age were not able to distinguish between in utero and postnatal infections. In the current study, we use data from 2 perinatal clinical trials conducted in Malawi, Africa, to investigate gender differences in MTCT of HIV at the time of birth and at age 6 to 8 weeks.

METHODS

Study Population

Infants originally enrolled in 2 concurrent clinical trials at 6 clinics in Blantyre, Malawi, southeast Africa, during the period of April 2000 to March 2003 constituted the study population. The purpose of these trials was to determine whether short regimens of infant postexposure antiretroviral prophylaxis could reduce MTCT of HIV. The mothers of these infants were described as either early or late presenters on the basis of their time of attendance at the labor room for delivery. For early presenters, there was adequate time (estimated time from admission to delivery of ≥4 hours) to obtain consent, perform HIV counseling, and perform HIV testing to establish the HIV status of the woman. HIV-infected, early-presenting women were provided intrapartum nevirapine (NVP) at the standard dose of 200 mg, as a single oral dose, and the infants were provided NVP or NVP plus zidovudine (ZDV) regimens starting immediately after birth.5 Maternal HIV status was not known at the time of admission for the late presenters, and time was inadequate to counsel and perform HIV testing for administration of the drug NVP before delivery. Late presenters were therefore counseled and tested for HIV postnatally. For late presenters found to be HIV infected, the infants were provided either NVP alone or NVP plus ZDV regimens starting immediately after birth.5 In Malawi, ~70% of women arrive late in the labor ward.

The results of these trials were previously published,21 and assessment of samples to measure late postnatal transmission of HIV (ie, after 6–8 weeks of age) and other outcomes over time is now in progress. Among infants born to late presenters in cases in which the mothers did not receive intrapartum NVP, the risk of MTCT at 6 to 8 weeks for infants not infected at birth was significantly less if the infants received NVP plus ZDV prophylaxis (7.7%), compared with NVP alone (12.1%) (P = 0.03).5 Among infants born to early presenters (who received intrapartum NVP), there were no differences in MTCT at 6 to 8 weeks (14.1% if the infant received NVP alone and 16.3% if the infant received NVP plus ZDV, P = 0.36).21

All mothers who participated in this study provided written informed consent for HIV testing and enrollment in the study. This research was approved by the Johns Hopkins University Committee on Human Research and the Malawi College of Medicine Research and Ethics Committee.

Study Design, Enrollment, and Follow-Up Monitoring

A combined prospective study of infants enrolled in 2 randomized, open-label, controlled, phase III, clinical trials was conducted. Mother/infant pairs were eligible for enrollment into these clinical trials if the mother was HIV positive and signed an informed consent form, and the infant was singleton, was not preterm, and did not have other disorders requiring admission to the intensive care unit. The infants of both early and late presenters (as described above) were randomized at birth to receive either NVP alone (single oral dose of 2 mg/kg weight) or NVP (same single dose) plus ZDV (4 mg/kg weight administered orally twice daily for 7 days). Mothers and their infants were usually discharged from the clinic within 6 to 48 hours after delivery. None of the women or infants included in this analysis was participating in other trials.

Information on demographic features and pregnancy, intrapartum, and delivery histories was collected at birth. Enrolled infants were scheduled for follow-up visits at 1 week, 6 to 8 weeks, and 3 months. Subsequent quarterly visits are planned to 2 years of age. At each visit, clinical history and breastfeeding questionnaires were completed, and physical examinations of the infant and the mother were conducted. Routine clinical care was provided in the clinic, and all HIV samples were reviewed with the HIV counseling and testing center at the local clinic at least once a week. Infants were provided pneumocystis pneumonia prophylactic cotrimoxazole at no cost up to 6 months of age, as recommended in Malawi. Referral for specialized care was available for mothers and children.

Laboratory Tests

We tested maternal venous blood of early presenters or cord blood of late presenters to determine the HIV status of women at the time of presentation to the labor ward. Trained study nurses used a rapid HIV test (Determine HIV-1/2; Abbott Laboratories, Tokyo, Japan) to screen for HIV infection. The results were available in ~30 minutes. All HIV-positive samples were confirmed with a Wellcozyme HIV test (Murex Biotech, Dartford, United Kingdom), and the results were available either before discharge from the hospital or at the 1-week postnatal follow-up visit. After enrollment and before discharge from the hospital, maternal venous blood was obtained for syphilis testing, baseline measurement of viral load, and complete blood count measurement. Women who demonstrated reactivity for syphilis were provided appropriate treatment at no cost. Maternal viral load testing was performed in the United States (University of North Carolina at Chapel Hill, Chapel Hill, NC) with a Roche Amplicor Monitor HIV RNA assay, version 1.5 (Roche Diagnostics, Indianapolis, IN). Complete blood count measurements were performed locally with a Coulter ACT Diff hematology analyzer (Coulter, Miami, FL).

Heel-prick infant blood samples were blotted on filter paper cards (Guthrie cards), and dried blood spots were used to perform HIV-1 RNA testing at birth and at subsequent visits, with a nucleic acid sequence-based amplification assay (NucliSens HIV-1 RNA QL assay; BioMerieux, Durham, NC). Testing was conducted in the United States (University of North Carolina at Chapel Hill) by laboratory personnel who were not aware of the study treatment allocation. Mothers were counseled regarding the HIV status of their infants as soon as the results were available. We used the following testing strategy: all 6- to 8-week samples were tested for HIV RNA, except for infants found to be positive at 6 to 8 weeks, dried blood spot samples collected at birth were tested. If the dried blood spot sample obtained at birth was negative, then the 3-month sample was tested to confirm the 6- to 8-week HIV RNA result. For confirmation, all HIV RNA-positive tests were repeated on the same samples. Discrepant results were resolved by performing additional tests on serial samples whenever possible. Dried blood spot samples collected at birth were also tested for infants who died or were lost to follow-up monitoring between birth and the 6- to 8-week visit. An infant was identified as HIV infected at birth if the birth sample tested positive and a later sample did not test negative. An infant was classified as infected between birth and 6 to 8 weeks if the birth sample tested negative, the 6- to 8-week sample tested positive, and a later sample did not test negative.

Statistical Analyses

Study forms and laboratory results were checked for completeness and consistency and were double-entered at the study site. There were no statistically significant differences between early- and late-presenting women with respect to demographic or biological characteristics; therefore, data from the 2 clinical trials were combined for this analysis. Statistical analyses were stratified according to gender groups and compared for binary characteristics with χ2 tests and for continuous characteristics with means and t tests. Risks of MTCT of HIV were estimated at birth and also at 6 to 8 weeks for those not infected at birth; risks were compared between male and female infants with exact tests (2-sided probability). Multivariate logistic regression analyses for
comparisons of HIV infection and gender were performed to adjust for maternal HIV viral load and other potential confounders. Viral load was log_{10} transformed to obtain a more symmetrical distribution without outliers.

RESULTS

Of 21,824 women screened in the 2 clinical trials, 19,860 were excluded for various reasons, the majority for being HIV negative (8878 were HIV negative, 5508 refused consent for HIV testing, 1229 refused to participate, 4196 infants met exclusion criteria [eg, admitted to the special care infant unit], and 49 enrolled but data on gender were not available). Therefore, 1964 infants were enrolled at birth in the 2 studies; 966 (49%) were boys and 998 (51%) were girls (Fig 1). Of the infants enrolled, approximately one half of each gender received either NVP and ZDV or NVP alone, on the basis of our randomization scheme (Fig 1). The overall MTCT rate at birth among these infants was 9.5% (187 of 1964 children), and the MTCT rate at 6 to 8 weeks among all infants not infected at birth was 8.7% (135 of 1554 children).

The characteristics of these infants according to gender were mostly similar, with some interesting exceptions (Table 1). Of the main variables that were significantly different between boys and girls, mothers of female infants had higher mean viral loads at the time of delivery, compared with mothers of male infants. Additional analyses of MTCT with different maternal viral load cutoff values (<10,000, 10,000–49,999, 50,000–99,999, and ≥100,000 copies/mL), stratified according to infant gender, also showed that the risk of MTCT was consistently higher among girls, compared with boys; with the exception of transmission at <10,000 copies/mL, these differences were statistically significant (data not shown).

The mean birth weight for boys was higher than that for girls, and the mean Apgar score at 1 minute for girls was higher than that for boys. It is worth noting that >99% of the infants were breastfed immediately after birth and at 6 to 8 weeks of age. There were no differences among mothers of boys and girls enrolled in this study regarding maternal syphilis (tested at the time of delivery), history of other sexually transmitted diseases during this pregnancy, or past history of reproductive losses such as miscarriages and stillbirths (data not shown).

The risk of HIV infection at birth was significantly higher among female infants, compared with male infants (12.6% [126 of 998 infants] among girls vs 6.3% [61 of 966 infants] among boys; P < .0001) (Table 2). At 6 to 8 weeks of age among infants not infected at birth, the rate of infection was higher among girls (10.0%, 77 of 770 infants) than among boys (7.4%, 58 of 784 infants) (P = .07). The numbers of infants who were lost to follow-up monitoring or missed visits (105 boys and 90 girls) (Fig 1) were not...
significantly different (P = .40). In multiple logistic regression models that adjusted for the most important variables in Table 1 that were statistically significant, the odds of infection at the time of birth were more than twofold higher among girls, compared with boys. Most importantly, the association of female gender and transmission was highly significant whether maternal viral load (a strong predictor of transmission by itself) was not included (adjusted odds ratio: 2.14; 95% CI: 1.55–2.94) or included (adjusted odds ratio: 2.06; 95% CI: 1.49–2.85) in these models (Table 3).

Among infants who acquired HIV infection between birth and 6 to 8 weeks of age (ie, excluding those infected at birth), the association between female gender and HIV infection was weaker. The odds of infection among girls were 1.39 (95% CI: 0.97–1.98) in a model that adjusted for other variables excluding maternal viral load at delivery, and the odds were 1.37 (95% CI: 0.95–1.98) in a model that included maternal viral load as well as other variables (Table 3). Maternal viral load was strongly associated with HIV acquisition both at the time of birth and at 6 to 8 weeks of age (Table 3). There were no differences according to gender, either at birth or at 6 to 8 weeks of age, in hematologic indices, including white blood cell count, lymphocyte count, hemoglobin level, hematocrit level, and platelet count (data not shown). In this study, there were no differences in mortality rates by 6 to 8 weeks of age between male and female infants, either overall or among HIV-infected and uninfected infants.

**DISCUSSION**

These data indicate that the risk of acquisition of HIV is higher for female infants than for male infants. This association of gender and HIV infection was substantial (adjusted odds ratio: >2.0) and statistically very significant (P < .0001) at birth, which suggests in utero acquisition and indicates that this association is unlikely to be attributable to chance. Female infants continued to acquire infections postnatally more than boys up to 6 to 8 weeks of age, but the difference in risk of acquisition between girls and boys was less substantial and was not statistically significant.

We do not know the biological mechanisms involved in gender differences influencing transmission. Although viral loads were modestly (P = .02) higher among mothers of girls (Table 1), we controlled for viral load as a measure of infectiousness and the association with gender remained strongly significant. Similarly, we adjusted for birth weight, which was significantly lower among female infants. The mode of spread of HIV in this study was uniformly perinatal and, for infections detected at the time of birth, the major determinants that confound studies of adult HIV transmission, such as behavioral and social factors, were avoided. We are not aware of cultural interventions that are gender-specific before delivery in this setting; in addition, women do not know prenatally the gender of their offspring in Malawi. After birth, different rates of breastfeeding and antiretroviral prophylaxis did not influence the slightly elevated risk of HIV infection for girls between birth and 6 to 8 weeks of age, because our data showed that breastfeeding was universally practiced in this population (Table 1) and equal proportions of male and female infants were allocated to receive postexposure prophylaxis (Fig 1).

If the associations we observed were not attributable to chance, then 2 explanations are possible. The
first is that female infants are more susceptible than male infants to HIV infection before birth. We speculate that in utero (from conception to birth) HIV infection may preferentially target female offspring. The factors involved may be genetic, immunologic, hormonal, or environmental. In Malawi, the major subtype of HIV-1 is C (>90%) and that of the coreceptor CCR5 is the non–syncytia-inducing phenotype. Preferential targeting of one gender over the other has been postulated for other adverse reproductive outcomes, and it is possible that female infants could be infected with HIV in utero more often than male infants. For example, couples’ smoking around the time of conception was suspected to result in female offspring, presumably because the sperm carrying the male Y chromosome might be more susceptible than the sperm with the female X chromosome to the effects of tobacco and thus less likely to fertilize the egg. Differential susceptibility of boys and girls has been observed for some childhood infectious diseases, such as whooping cough and measles; these 2 airborne diseases appear to have higher morbidity and mortality rates among female children than male children, mainly because of increased susceptibility among girls. Furthermore, this difference has been associated with Th1 and Th2 cytokine production, and a balance between these 2 responses could determine susceptibility, resistance, survival, or death.

The second explanation is that female and male infants are equally susceptible to HIV infection but infected boys are more likely to die before birth than are infected girls. This alternative explanation assumes that male mortality rates in utero among HIV-infected infants are disproportionately higher, resulting in more HIV-infected female infants than male infants being born. Pertinently, we observed a small excess of girls (32 more girls than boys). The male to female gender ratio at birth we observed in this study, ie, 0.98:1.00, is lower than the typical male to female ratio of 1.03:1.00 expected in Africa and elsewhere. A low gender ratio, however, is not surprising for countries in eastern and southern African populations of Bantu origin, including Malawi. We are not aware of any major recent adverse demographic or environmental factors that could affect the gender ratio at birth in this population. Generally, situations of stress, such as viral infections, wars, and famine, have been reported to increase the gender ratio. During the first 6 to 8 weeks, the mortality rate among HIV-infected male infants was not significantly higher than that among infected girls, to account for the slightly higher HIV infection risk among girls.

Our findings of increased in utero HIV infection among female infants in Malawi is in agreement with the results of the European Collaborative Study. However, the recent individual patient data meta-analysis from 9 African studies reported no differences according to gender with early HIV transmission. There were some differences in the definition of early transmission in these studies; in our study we considered HIV infection before and after birth separately, whereas the meta-analysis study combined in utero, intrapartum, and neonatal infections. The increased trend of transmission among male infants between birth and 6 to 8 weeks in our study was not statistically significant, and the differences could be explained on the basis of chance alone. The meta-analysis study also showed that girls were significantly less HIV infected than boys in the late postnatal period (infections after 4 weeks of age; adjusted hazard ratio: 0.6; 95% CI: 0.4–0.9). We do not have data to estimate the risk of late postnatal HIV transmission. As stated by the authors of the meta-analysis study, the lower risk of HIV acquisition among girls late postnatally might be attributable to the greater vulnerability of girls to HIV infection early (in utero, intrapartum, or neonatally), reducing the number of available female infants, compared with...
male infants, who are susceptible to be infected late postnatally. 20

 Confirmation of these findings in other settings would be important. We are currently monitoring these children to estimate the risk of late postnatal HIV transmission (infections after 6–8 weeks) and death, to determine whether these trends continue. This study was based on data from 2 clinical trials conducted among infants and mothers who met specific enrollment criteria, and some infants were excluded (eg, to receive immediate clinical care). We have no gender-specific data on these infants. These inevitable exclusions might have introduced bias or limited the generalizability of these results. However, the magnitude of the association between in utero HIV infection and female gender was substantial, and not all excluded infants were expected to have been born to HIV-infected mothers. We have no data on genetic polymorphisms in this population or similar African populations to explain these gender differences (other studies in progress in Malawi might provide such information). We do not have explanations for some of the interesting gender-specific observations in this study, such as differences in maternal viral loads, birth weights, and Apgar scores; these merit additional investigation. A prospective follow-up study among HIV-infected women that monitors reproductive losses from early pregnancy according to gender could also address our assumption that male HIV-infected infants may die disproportionately more in utero.

CONCLUSIONS

We observed a strong association of infant HIV infection and gender in Malawi, with 12.6% of girls, compared with only 6.3% of boys, being infected at birth. Although it is surprising that similar associations would not have been discovered earlier, the low probability of finding a difference this extreme ($P < .0001$), as well as previous studies in Europe and Africa 16,17 with similar results, supports our findings. In some parts of Africa, including Malawi, 20–30% of pregnant women are infected with HIV. 28 The risk of MTCT of HIV remains high, and successful prevention programs are not yet universal. Therefore, these gender differences might have important clinical (eg, antiretroviral treatment), preventive, and demographic implications.

ACKNOWLEDGMENTS

This study was funded by the Fogarty International Center, National Institutes of Health (AIDS Fogarty International Research Collaboration Award 5U01TW01199 and supplement), and the Doris Duke Charitable Foundation. We are indebted to scientists in Malawi and the United States who continuously guided us throughout the performance of these studies. We thank the study team of the Johns Hopkins University-Malawi College of Medicine Research Project for their excellent collaboration. We are indebted to the mothers and children who participated in this study.

REFERENCES


Gender Differences in Perinatal HIV Acquisition Among African Infants
Taha E. Taha, Samah Nour, Newton I. Kumwenda, Robin L. Broadhead, Susan A. Fiscus, George Kafalula, Chiwawa Nkhoma, Shu Chen and Donald R. Hoover
Pediatrics 2005;115;e167
DOI: 10.1542/peds.2004-1590

Updated Information & Services
including high resolution figures, can be found at:
http://pediatrics.aappublications.org/content/115/2/e167

References
This article cites 27 articles, 2 of which you can access for free at:
http://pediatrics.aappublications.org/content/115/2/e167.full#ref-list-1

Subspecialty Collections
This article, along with others on similar topics, appears in the following collection(s):
Fetus/Newborn Infant
http://classic.pediatrics.aappublications.org/cgi/collection/fetus:newborn_infant_sub
Infectious Disease
http://classic.pediatrics.aappublications.org/cgi/collection/infectious_diseases_sub
International Child Health
http://classic.pediatrics.aappublications.org/cgi/collection/international_child_health_sub

Permissions & Licensing
Information about reproducing this article in parts (figures, tables) or in its entirety can be found online at:
https://shop.aap.org/licensing-permissions/

Reprints
Information about ordering reprints can be found online:
http://classic.pediatrics.aappublications.org/content/reprints
Gender Differences in Perinatal HIV Acquisition Among African Infants
Taha E. Taha, Samah Nour, Newton I. Kumwenda, Robin L. Broadhead, Susan A. Fiscus, George Kafulafula, Chiwawa Nkhoma, Shu Chen and Donald R. Hoover

Pediatrics 2005;115;e167
DOI: 10.1542/peds.2004-1590

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://pediatrics.aappublications.org/content/115/2/e167