We described HIV incidence and mother-to-child transmission (MTCT) among women during pregnancy and lactation. Forty-eight (3.4%) of 1396 women seroconverted during pregnancy or <12 mo after delivery. This group of HIV-exposed children was at 2.3 times higher risk of infection (MTCT 20.5% [8 of 39] vs 9.0% [83 of 925]). An estimated 20% with CD4+ cell counts <350 would have been eligible for antiretroviral therapy (ART), yet all women with incident HIV infections are more likely to transmit HIV to their children. To ensure optimal prevention of MTCT, all women who seroconvert during pregnancy or lactation should be considered for ART for the purpose of prevention of MTCT, and women with CD4+ <350 should continue to receive ART.

The majority of the 430,000 new HIV infections in children younger than 15 y in 2008 are believed to have stemmed from mother-to-child transmission (MTCT) [1]. The World Health Organization (WHO) estimates this to be 18% lower than in 2001; this reduction may be associated with increased (45%) global access to new MTCT reduction strategies [2]. These new strategies, however, omit clinical guidance for a neglected subpopulation of pregnant women who test negative for human immunodeficiency virus (HIV) at their first antenatal visit and remain vulnerable to HIV infection during pregnancy and after delivery [3, 4]. Furthermore, newly acquired HIV infections are characterized by initial high levels of viral replication both in systemic and genital compartments that would typically last for 3–4 mo [5]; hence, such women would potentially pose similar risk factors for MTCT as women with chronic HIV infection [6, 7]. Pregnant and lactating women who acquire HIV infection during pregnancy or during breastfeeding should be considered highly infectious and could potentially increase risk of MTCT antenatally and postnatally during breastfeeding in the absence of antiretroviral therapy (ART).

In the absence of sophisticated techniques to detect new HIV infections in a resource-limited setting, we depended on repeating routine on-site serological HIV testing methods at subsequent antenatal and postnatal visits to identify new HIV infections in a cohort of pregnant women. In this study, we describe the incidence of HIV in women during pregnancy and lactation and attempt to confirm the association between incident maternal HIV infection and MTCT.

**METHODS**

Pregnant women older than 18 y who registered for antenatal care <28 wk of gestation provided a written informed consent to participate in this exploratory cohort study during 2005–2007 in KwaZulu Natal, South Africa. The participants were counseled, screened for HIV by use of on-site HIV-antibody tests (Abbott Determine HIV-1/2, Abbott Laboratories) and confirmed by enzyme-linked immunosorbent assay (ELISA) at a local laboratory. Structured questionnaires included questions relating to relationships, demographic characteristics, and sexual behavior. These questionnaires included unique patient study identifiers and excluded personal identifiers such as name, residential address, and telephone contacts. These questionnaires were administered by the nurse at enrollment and subsequent visits in a manner ensuring privacy and maintaining confidentiality.

Participants were monitored over a period of 18 mo (6 mo during pregnancy and 12 mo after delivery). In addition to routine antenatal tests, laboratory investigations for HIV-positive women (cohort 1) at baseline included a CD4+ count and HIV viral load. As prescribed by the in-country policy during this period, women with a CD4+ count <200 were referred for initiation of ART as lifelong treatment and women
with a CD4\(^+\) count >200 received the HIVNET 012 single-dose nevirapine regimen as prophylaxis to prevent MTCT [8]. The CD4\(^+\) count and viral load were repeated at 3 and 9 mo after delivery. HIV-negative participants (cohort 2) were retested for HIV (Abbott Determine HIV-1 Rapid Tests) during pregnancy (34–40 wk) and 3, 9 and 12 mo after delivery. Women in cohort 2 who seroconverted during the study period followed the same management protocol as that prescribed for HIV-positive pregnant women. All HIV-exposed children were tested for HIV using nucleic acid technology (DNA polymerase chain reaction [PCR]) at birth, 6 wk or 3 mo, 6 mo, and 9 mo. ELISA was performed at 12 mo to confirm HIV status. Children were defined as “definitely infected,” “presumptively infected,” “definitely uninfected,” and “presumptively uninfected” using the Centers for Disease Control and Prevention revised surveillance case definitions (2008) [9]. The timing of MTCT for the HIV-exposed and HIV-infected children was estimated according to a previous and subsequent DNA PCR result defined as in utero (DNA-positive <72 h of birth); intrapartum (DNA-negative <72 h of birth, positive >2 wk and nonbreastfed); postpartum (DNA-negative <6 wk and positive >6 wk and breastfed); or intrapartum/postpartum (DNA-negative <72 h of birth, positive >2 wk and breastfed). Where a DNA-PCR result was unavailable, the timing of MTCT was estimated and defined as peripartum (unknown status at birth, subsequent DNA-positive and nonbreastfed); peripartum/postpartum (unknown status at birth, subsequent DNA-positive and breastfed).

Data management and statistical analysis were conducted with SPSS software version 15.0 (SPSS Inc). Statistical testing for differences between medians was performed using a non-parametric 2-sample test, and Pearson \(\chi^2\) test or Fisher exact test as appropriate was used to compare proportions between groups. Linear trend was assessed using \(\chi^2\) test. Kaplan-Meier survival analysis was used to analyze HIV survival rates and these were compared between groups using the log rank test. \(P < .05\) was considered significant for all analyses.

RESULTS

The mean age of the 2793 participants was 24 y (range, 18–40 y), 10% (95% confidence interval [CI], 9.1–10.8) were married and 37% (95% CI, 29.5–32.9) were cohabiting. Thirty-nine percent (n = 1090) were primigravid, and median gestation at booking was 25 wk (5–41 wk). Approximately 53% registered for antenatal care between 20 and 28 wk; 811 (29%) registered >28 wk of gestation.

A total of 1206 (43%) and 1587 women tested positive and negative, respectively, for HIV at their first antenatal visit. Of the HIV-positive and HIV-negative cohorts, 995 (83%) and 1396 (88%) maternal-child pairs, respectively, completed study follow-up. The HIV-negative cohort (n = 1396) was followed over a cumulative period of 1946 y during pregnancy and 12 mo after delivery, during which 48 women seroconverted. HIV incidence was estimated at 3.4% (48 of 1396 women), and HIV incidence rate was 2.5 infections per 100 person-years (95% CI, 1.8–3.2).

Among all reported behavioral and demographic characteristics stratified by final HIV status, education level (Pearson \(\chi^2\), 13.7; \(P = .033\)), marital status (Pearson \(\chi^2\), 92.1; \(P < .001\)), and financial dependency (Pearson \(\chi^2\), 7.5; \(P = .024\)), were strong correlates of HIV infection. Condom use was lowest (3.2%) among women who seroconverted (Pearson \(\chi^2\), 20.7; \(P < .001\)) and was more common in the HIV-negative (53.4%) as opposed to the HIV-positive women (43.5%). Women who used condoms previously used them for the sole purpose of contraception. None of the women reported condom use during this pregnancy. The frequency of reported sexual intercourse was higher among the HIV-negative group (80.9%).

The median CD4+ count among the HIV-positive women (n = 1052) including the HIV seroconverters was 322 cells/mL (interquartile range [IQR], 212–459). The median count was significantly lower among the 1032 women with established HIV infection (320 cells/mL; IQR, 210–455) compared with 20 women who seroconverted (498 cells/mL; IQR, 367–743). Of the women with established HIV infection, 231 (22.4%) had a CD4+ <200 and met the eligibility criteria for ART. Considering current WHO recommendations for pregnant women, 576 (55.8%) of the HIV-positive women and 4 (20%) of the women with incident HIV infections had a CD4+ <350 and would have been eligible for ART.

The mean viral load in all HIV-positive participants at baseline was 51,397 ± 167,093 copies/mL (ranged between <50 to 2,476,997 copies/mL) and was higher among women with established HIV infections compared with women with incident HIV infections (52,059 ± 168,876 vs 22,995 ± 39,944 copies/mL). High viral loads (>10,000) were reported in 55% of women with established HIV infections and 61% of the women with incident HIV infections. This comparison was not statistically significant (\(\chi^2\) linear trend, .135; \(P = .71\)).

Ninety-one of the 964 HIV-exposed children were confirmed to be HIV-infected by age 12 mo (overall MTCT rate 9.4% [95% CI, 7.7–11.5]). A larger proportion of infants born to HIV seroconverters were also infected (20.5% [8 of 39] vs 9.0% [83 of 925]). Children born to HIV seroconverters were at 2.3 times higher risk of also being infected (odds ratio, 2.29; 95% CI, 1.19–4.38; \(P = .024\)). Overall, 64 of the 91 (70%) perinatal HIV infections were seemingly acquired during pregnancy and labor/ delivery. Among these, 44% (n = 28) occurred in utero, 39% during labor/delivery, and the remaining 17% that were not known whether they were acquired either in utero or during labor/delivery. Due to the small number of perinatal HIV infections and incident HIV infections among women, the timing of perinatal HIV transmission in these subgroups could not be compared. Infants born to women with a CD4+ count <350
had a higher risk of vertical HIV infection, although this relationship was not statistically significant (Table 1). HIV-1-free survival rates for infants born to women with established HIV infection and women who seroconverted during pregnancy or postnatally were 87.3% and 77.5%, respectively. The difference in HIV-1-free survival at 12 mo after delivery is estimated to be 10% ($P = 0.077$). The MTCT rate was significantly higher among women with established HIV infection (47 of 451; 10.4%) with CD4 < 1,350 and among the HIV seroconverters with CD41.350 (3 of 16; 18.7%; $P = 0.049$) (Table 2). Neither of the 2 seroconverting women with a CD4 < 1,350 transmitted HIV to their infants.

**DISCUSSION**

In our cohort of pregnant women, 3.4% seroconverted between their first antenatal visit and 12 mo after delivery, and children born to women with incident HIV infections were at 2.3 times higher risk of being infected.

Several studies have reported low but relatively significant incident HIV infections among pregnant and breastfeeding women [3, 4, 10]. These studies provide adequate evidence for the need of enhanced prevention strategies among women who remain vulnerable during pregnancy and postnatally in settings where social norms and economic conditions encourage short-term relationships and multiple partners.

The MTCT rate in our study among women who seroconverted sometime during pregnancy and breastfeeding was 20.5% as opposed to 9.0% among women with established HIV infection. In an early cohort study of breastfeeding women in Rwanda, Van de Perre et al identified 15 women who seroconverted after delivery and found 9 of the infants (53%) infected, 8 of whom presumably acquired HIV infection during breastfeeding [11]. A larger cohort study of breastfeeding women in Zimbabwe reported one-third of the 336 children to be infected subsequent to maternal seroconversion during this breastfeeding period [12]. There is therefore increasing evidence to support the current belief that acute HIV infection associated with proliferative viral replication may predispose to a higher risk of vertical transmission during pregnancy and breastfeeding.

**Table 1. Maternal Laboratory Investigations as Risk Factors for Mother-to-Child Transmission**

<table>
<thead>
<tr>
<th>CD4+ count (n = 834)</th>
<th>Transmitting Mothers</th>
<th>Nontransmitting Mothers</th>
<th>Overall Population</th>
<th>Odds Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;350</td>
<td>47 (10.4%)</td>
<td>406 (89.6%)</td>
<td>453 (54.3%)</td>
<td>1.79</td>
</tr>
<tr>
<td>350-500</td>
<td>18 (8.3%)</td>
<td>198 (91.7%)</td>
<td>216 (25.9%)</td>
<td>1.41</td>
</tr>
<tr>
<td>&gt;500</td>
<td>10 (6.1%)</td>
<td>155 (93.9%)</td>
<td>165 (19.8%)</td>
<td>1</td>
</tr>
<tr>
<td>$\chi^2$ for linear trend</td>
<td>2.900, $P = 0.089$</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 2. CD4 and Virological Determinants of Mother-to-Child Transmission in Women With Established and Recent HIV Infections**

<table>
<thead>
<tr>
<th>CD4+ Count (n = 834)</th>
<th>Transmitting Mothers (n= 75)</th>
<th>Nontransmitting Mothers (n = 759)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Est HIV</td>
<td>SCV</td>
</tr>
<tr>
<td>&lt;350</td>
<td>47 (65.3%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>350-500</td>
<td>25 (34.7%)</td>
<td>3 (100%)</td>
</tr>
<tr>
<td>&gt;500</td>
<td>12 (16.0%)</td>
<td>3 (100%)</td>
</tr>
<tr>
<td>Fisher exact test*</td>
<td>$P = 0.049$</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Viral load (n = 478)</th>
<th>Transmitting Mothers (n = 51)</th>
<th>Nontransmitting Mothers (n = 427)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;10,000</td>
<td>19 (39.6%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>&gt;10,000</td>
<td>29 (60.4%)</td>
<td>3 (100%)</td>
</tr>
<tr>
<td>Fisher exact test**</td>
<td>$P = 0.285$</td>
<td></td>
</tr>
</tbody>
</table>

**NOTE.** Est HIV = established HIV infection; SCV = maternal incident HIV infection.

*The first comparison is a stratified analysis of CD4+ group by maternal incident HIV infection or established infection among transmitting and nontransmitting women.

**The second comparison is of viral load by maternal incident HIV infection status also stratified by transmitting mothers and nontransmitting women.
MTCT risk is lacking due to the limited diagnostic capacity to detect acute HIV infections in resource-limited settings. Hence, the baseline viral load among women who seroconverted in this study does not provide an accurate measure of risk for MTCT among women who have seroconverted.

Studies have reported benefits of initiating ART during acute HIV infection in nonpregnant adults in viral load suppression [13, 14]. These studies also emphasize the increased benefits of initiating ART during acute HIV infection as opposed to delaying ART to between 2 and 6 mo after seroconversion.

If we had to apply WHO recommendations of initiating ART in women with CD4+ <350, only 20% of women (1 in 5) who seroconvert during pregnancy or breastfeeding would have been eligible for ART. Yet the vast majority (80%) of these women are still immunocompetent but could pose a high risk of MTCT due to the underlying viremia during acute/primary HIV infection. To ensure optimal protection against MTCT, all women who seroconvert during pregnancy or breastfeeding should be considered for ART independent of a CD4+ count, for the sole purpose of optimizing prevention of MTCT.

Likewise, since a third of MTCT (34.7%) occurred among women who had a CD4+ >350 and therefore were not eligible according to the WHO recommendations, all pregnant women who test positive for HIV should receive ART independent of their CD4+ count. However, stopping or continuing ART in HIV-positive women would depend on available country resources and issues of clinical safety that are yet to be addressed in planned studies. To ensure optimal reduction of MTCT worldwide, our study findings suggest that all pregnant HIV-positive women should receive ART for the primary purpose of prevention of MTCT. Thereafter, in resource-limited settings and until more safety information is available, ART should be discontinued in women with a CD4+ >350 at delivery (in nonbreastfeeding women) or until cessation of breastfeeding (breastfeeding women).

Our findings also highlight the need for retesting of women during and after pregnancy to allow for drug interventions and modifications in infant feeding. Recently acquired HIV infection is therefore commonly missed among women who test negative for HIV once during their pregnancy unless alternative diagnostic or screening strategies are used [15].

Acknowledgments

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References