CASE REPORT

HIV sero-conversion during late pregnancy – when to retest

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The South African National Prevention of Mother-to-Child Transmission of HIV programme has resulted in significant reductions in vertical transmission, but new infant HIV infections continue to occur. We present two cases of HIV seroconversion during late pregnancy, demonstrating the limitations of the current programme. These could be mitigated by expanding the programme to include maternal testing at delivery and at immunisation clinic visits as we pursue the elimination of mother-to-child transmission.

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In order to identify HIV-infected women and offer antiretroviral (ARV) prophylaxis, the South African National Prevention of Mother-To-Child Transmission (PMTCT) of HIV programme recommends 2 HIV tests during pregnancy: at the first antenatal visit and at 32 weeks of gestation.[1] We present two cases that suggest that additional HIV testing strategies are needed to help eliminate the mother-to-child transmission of HIV.

In a longitudinal study on HIV-exposed infants, we recruited infants whose mothers were known to be HIV-infected post partum along with a control group of infants born to HIV-negative women. All women delivering at the Kraaifontein Midwife Obstetric Unit were eligible for enrolment. HIV-exposed infants were matched with HIV-unexposed controls within one month of birth. Mother-infant pairs were recruited within three days of delivery and a $CD4^+$ T-cell count was performed on all women regardless of HIV status. In accordance with the study protocol, the infants were reviewed at 2 weeks of age and regularly thereafter. At the 2-week visit, the HIV status of the uninfected mothers was confirmed with an HIV rapid assay using finger-prick blood (Alere Determine HIV 1/2).

Recently, HIV infection was identified on the rapid tests at the 2-week visit in 2 women (Table 1). According to antenatal clinic documentation, both tested negative during pregnancy: at booking (at 21 weeks and 28 weeks of gestation, respectively) and at 32 weeks of gestation, as recommended in the national guideline.[1] Neither infant was born before 38 weeks of gestation. The HIV rapid assay in use in the antenatal clinic at the time was the First Response HIV1-2-0 Card Test (Premier Medical Corporation Ltd, India). According to policy, only a single test is required to screen for HIV. Positive screening tests are confirmed with a second rapid assay (ABON HIV 1/2/0 Tri-Line HIV Rapid Test Device).

Both women elected to breastfeed, although one mother switched to infant formula after one week owing to poor feeding. Her $CD4^+$ T-cell count at delivery was 680 x 10^6 cells/l. Her infant was symptomatic at age 2 weeks and was immediately hospitalised, requiring transfer to the intensive care unit. An HIV DNA polymerase chain reaction (PCR) test (Amplicor HIV-1 DNA prototype assay 1.5) at 2 weeks was positive.

The $CD4^+$ count of the second woman was 157 x 10^6 cells/l at delivery. Her baby was well and the HIV DNA PCR at 2 weeks was negative. Daily nevirapine (NVP) for the infant was initiated and the mother was referred for combination antiretroviral therapy (cART) which was commenced within 2 weeks.

Discussion

These two cases raise concerns about antenatal HIV screening and the implications for vertical transmission. As expected, neither woman received any ARV prophylaxis.

A recent Medical Research Council (MRC) report on the effectiveness of the national PMTCT programme in South Africa[2] demonstrated that, among mothers who reported being HIV-negative, 4.1% had infants who were HIV-exposed at 4 - 8 weeks (measured by the presence of HIV antibodies in the infants' blood). A 2007 surveillance study in KwaZulu-Natal (KZN) found that 6.9% of infants whose mothers reported a negative HIV status had similar evidence of exposure (i.e. the

Table 1. Patient characteristics

Patient	Maternal age (years)	Gestational age, first ANC (weeks)	Gestational age, first HIV test (weeks)	Gestational age, second HIV test (weeks)	Infant date of birth	Maternal CD4$^+$ count at birth (x10^{6}cells/l)	Feeding choice	Infant PCR test at 2 weeks
1	29	21	21	32	31/10/2012	680	Breast	Positive
2	23	28	28	32	05/11/2012	157	Breast	Negative

ANC = antenatal clinic visit; PCR = polymerase chain reaction.

presence of HIV antibodies in the infants' blood).[3] Of concern is that the vertical transmission rate in this group was high (31% v. an overall rate of 20.2% at that time).

It is possible that some women knew, but did not admit their HIV-positive status. However, the KZN study was anonymous, and the MRC report demonstrated a high uptake of HIV testing and disclosure, making it unlikely that this scenario contributed significantly to the observations.[2,3] Moreover, the two subjects described here each had HIV-negative results for rapid tests on two different occasions.

Alternatively, there may have been a problem with the HIV rapid antibody assay, including that the tests were conducted within the window period. Antibodies to HIV can be detected at 2 - 3 weeks after infection by fourth-generation laboratory enzyme-linked immunosorbent assay (ELISA) tests, which detect both antibody to HIV and p24Ag (Fiebig stage II and III).[4] The third-generation rapid tests have a window period of three to four weeks post infection (Fiebig stage III).[4,5] Testing during this time will yield a false-negative result. The reported sensitivity and specificity of the First Response HIV 1-2-0 Card Test are 100% and 98.8% when used correctly[5] within the WHO recommended range of >98%.[6] In our subjects, this explanation could only apply to the second assay at 32 weeks, and would indicate recent acquisition of infection. The rapid assay may have recorded a false-negative result for the second test. All batches of rapid tests are validated by the National Institute for Communicable Diseases (NICD) on a panel of laboratory samples, but they have not been validated in pregnant women specifically or in the field. Assay sensitivities have been reported between 87% to 95% in clinics depending on the product.[2,7] More data are therefore required to assess and validate HIV rapid assays in pregnant women, and to ensure the quality of testing at clinic level. Importantly, there is no quality-control procedure for negative rapid tests.

The most likely explanation for our findings is true acquisition of HIV during pregnancy and breastfeeding. Pregnancy poses an increased risk for HIV acquisition by women, even after adjustment for behavioural and other factors; it is possible that the hormonal and other biological changes associated with pregnancy play a role.[8] High viral loads during primary HIV infection increase the risk of vertical transmission *in utero*, peri partum and post partum,[9,10] especially in the absence of ARV prophylaxis. In studies in Botswana and SA, new mothers with negative HIV test results or of unknown HIV status were tested immediately post partum or at infant immunisation visits. The results demonstrated a seroconversion rate of 2.4 - 7.9% during pregnancy and post partum.[2,7,11,12] These women are at high risk of vertical transmission.[13-15] In addition, they are more likely to use mixed feeding practices, placing their infants at greater risk for HIV infection.[2,16-18] 'Mixed feeding' refers to the use of breast milk in addition to other fluids (infant formula, water, tea) for infant feeding. The increased incidence of mixed feeding observed in these women is presumably because, having tested HIV-negative, they perceive no risk.

Repeat HIV testing of mothers during late pregnancy, at delivery or at the clinic immunisation visits, would identify women who acquire HIV during pregnancy and in the early post-partum period. The HIV diagnosis of infants whose mothers tested negative during pregnancy is often delayed,[18] with significant implications for morbidity and mortality.[19] Most SA women deliver at a healthcare facility[20] and 99% attend the 6-week vaccination visit.[2] Moreover, testing at these time-points shows high uptake,[11,21,22] while offering HIV tests to both partners may identify discordant couples and allow counselling on HIV prevention.[2] A proviso to this is increasing evidence that, even within discordant partnerships, a significant number of new HIV infections arise from extra-couple transmission.[23]

Conclusion

While the elimination of mother-to-child transmission of HIV is feasible, it will require a modification of current protocols/guidelines to include repeat HIV testing of women at delivery and/or post partum, a quality-control strategy for laboratory testing of a small percentage of negative rapid tests, involvement of male partners in testing and counselling, and an emphasis on exclusive feeding practices, regardless of HIV status.

Ethics approval. The Mother Infant Health Study (MIHS) is approved by the Ethics Committees of Stellenbosch University and the University of British Columbia.

Conflict of interest. None.

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