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Early Detection of Human Immunodeficiency Virus Infection among Kenyan Infants Using a Reverse Transcriptase Activity Assay

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Abstract

Two hundred HIV-exposed Kenyan infants were tested for HIV infection at birth and at age 6, 12, 24 and 48 weeks, by DNA PCR and Cavid reverse transcriptase (RT) assays and after age 18 months by HIV antibody test. Eleven (5.5%) infants became HIV infected. In six, positive RT preceded positive DNA PCR. Use of RT assay may facilitate earlier HIV diagnosis in infants.

Keywords

HIV; reverse transcriptase; diagnosis; infant; Africa; resource-limited settings

Most pediatric HIV infections are acquired through mother to child transmission (MTCT). Serologic tests cannot establish HIV infection until maternal HIV antibodies have been cleared at 18 months. In resource-rich countries, the HIV DNA PCR assay is typically used for early infant diagnosis. While the availability of this assay has increased in resource-limited settings, it can be difficult to implement, requiring specialized equipment often located in a centralized laboratory.¹ As an alternative, we evaluated the Cavid ExaVir Reverse Transcriptase (RT) Assay, a commercial assay that can be conducted in a local clinical laboratory.^{2, 3} The RT assay detects enzyme activity of RT on an enzyme-linked immunoassay platform and performs well against multiple HIV clades because it is not a nucleic acid-based assay.⁴ Based on previous cross-sectional studies suggesting the usefulness of the RT assay for infant HIV diagnosis,^{5,6} we conducted a longitudinal study of HIV-exposed Kenyan infants from birth to 18 months to assess the performance of the RT assay compared to that of DNA PCR.

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The authors report no conflict of interest.

Methods

The Institutional Review Board of New York University School of Medicine and the Ethical Review Committees of Kenya Medical Research Institute (KEMRI) and University of Nairobi/Kenyatta National Hospital approved this study.

HIV-exposed infants attending the Bomu Medical Center in Mombasa were enrolled within 48 hours of birth, and seen at 6, 12, 24, and 48 weeks. Approximately 3 ml of whole blood was collected into EDTA tubes at each visit. Dried blood spots (DBS) were used for the DNA PCR assays.¹ Phlebotomists spotted two circles of 50 μ l of whole blood each onto Grade 903 S&S filter paper cards (Whatman, Inc, Florham Park, NJ), which were stored and transported as previously described.¹ Rapid HIV antibody tests on whole blood were conducted at 18 months using two different kits to confirm infection (Determine and SD Bioline tests). We collected data on the mother's pre- antiretroviral therapy (ART) nadir CD4 cell count, history of maternal ART, type of infant feeding and infant gender. At follow-up visits, the interval type of feeding and ART statuses of both mother and infant were recorded. Medical management of infants was conducted according to Kenyan national guidelines.

The HIV DNA PCR tests were done at the KEMRI laboratory that participates in an external quality assurance program of the Centers for Disease Control and Prevention (CDC).¹ Bomu Medical Center laboratory personnel, blinded to the DNA PCR and serologic results, performed the RT assays (Exavir V.3, CaviDi Tech-AB, Uppsala, Sweden). The Bomu Clinic laboratory was enrolled in an external quality assurance program for the RT assay through the National Serology Reference Laboratory, Australia. RT assays were performed according to manufacturer's instructions using 1ml of plasma and as previously described.⁶ Results were quantified as virion-associated RT activity in femtograms (fg)/ ml plasma and as a calculated HIV-1 RNA equivalent copy number (eqc) using a conversion factor derived from a standard curve provided by the kit. A positive RT result was defined as $\geq 10,000$ eqc/ml, based on published guidelines for HIV-1 RNA PCR assays for early infant diagnosis.⁷ In addition, we constructed a receiver operator curve (ROC) curve using the data and 10,000 eqc/ml was within the optimal cutoff range (data not shown).

The number of HIV-infected women who delivered at Bomu Clinic during the first year of the study limited the number of women enrolled into the study to 200. Sensitivity of the RT assay was calculated as the true positive rate based on the first positive RT result obtained. Specificity was calculated as the true negative rate of children not identified as being infected by the RT assay. The gold standard for HIV infection was defined as having a positive HIV antibody result at 18 months or a positive PCR result at any age. Antepartum transmission was defined as a positive DNA PCR test at birth; intrapartum transmission as a negative DNA PCR result at birth and a positive at 6 weeks; and postpartum transmission as a negative DNA PCR test at birth and at 6 weeks but a positive by week 48. All statistical analyses were done using Intercooled STATA (Version 9.0, Statacorp, College Station, TX) or the R statistical environment. Risk ratios (relative risk) and their 95% confidence intervals were computed with R's "epitools" package.

Results

Two hundred HIV-exposed infants were enrolled within 48 hours of birth. At the time of delivery, 121 (61%) mothers received a triple antiretroviral regimen for prevention of mother-to-child transmission (PMTCT), of whom 114 (94%) received zidovudine (AZT), lamivudine (3TC), and nevirapine (NVP) from 20 weeks gestation plus single dose NVP (sdNVP) administered intrapartum; 7 (6%) women received only intrapartum sdNVP; 74

(37%) mothers received highly active antiretroviral therapy (HAART) for their own health; and five mothers (2%) did not receive any antiretroviral drug pre- or intra-partum. One hundred and eighty-eight (94%) of 200 infants received post-exposure prophylaxis with either 28 days of AZT and sdNVP (183 infants), sdNVP alone (4 infants), or 28 days of AZT alone (1 infant). No infected infant received HAART during the study period. The median prenatal nadir CD4 cell count for 152 mothers for whom data was available was 275 cells/ μ L (Interquartile range [IQR] 132, 456).

Of 200 infants, 11(5.5%) were diagnosed with HIV infection by DNA PCR or HIV antibody tests. Two of 11 infants had a positive DNA PCR but did not have an HIV antibody test conducted at 18 months; one child died and one transferred to another facility. None of the children who were determined to be HIV-uninfected by HIV antibody tests had a positive RT assay. During the course of the study, 10 infants had 11 indeterminate DNA PCR results.

Among 155 infants who had HIV antibody testing conducted at age 18 months, nine were antibody positive. All nine infections were detected by the RT assay for a sensitivity of 100% (9/9) with 95% confidence interval [CI] of 66–100% and a specificity of 100% (146/146) with 95% CI of 97.5–100%. However, on repeat testing after the first positive RT or DNA PCR result used to calculate the sensitivity of these assays, one infant had two false negative RT assay results (weeks 24 and 48) and two infants had a false negative DNA PCR result (both week 24).

Among 11 infants with HIV infection based on DNA PCR and antibody tests, two transmissions occurred antenatally (transmission rate, 1.0%), one peripartum and eight postpartum (the combined peripartum and postpartum transmission rate was 4.5%). Failure to receive any antiretroviral drug before delivery was associated with a relative risk (RR) of overall transmission of 1.80 (95%CI: 0.37,8.71).

Data on feeding practices were complete for all infected and all but one of the uninfected infants. During the study period, 70 (35%) infants were exclusively bottle-fed and 129 (65%) were breast-fed at least once, of which 23 children were exclusively breast-fed. Excluding the 2 cases of antenatal transmission, exposure to breast-feeding was associated with a relative risk of transmission of 4.18 (95%CI, 0.53, 32.7); 8 (6.2%) of 130 breast-fed infants were infected compared to only one (1.4%) of 68 exclusively bottle-fed.

We compared age at first positive RT or DNA PCR assay among the infected infants (see Table, Supplemental Digital Content 1, <http://links.lww.com/INF/B194>). Ten of 11 infants had valid results for both tests at the time of the first positive assay. Four (40%) infants had the first positive RT and DNA PCR assays at the same visit. Six (60%) infants had the first positive RT assay at a median of six weeks earlier than the first positive DNA PCR assay. Of these six, four infants had their first positive RT assay at 6 weeks, one at 12 weeks and one at 24 weeks. The RT assay detected HIV infection significantly earlier than the DNA PCR assay ($p=0.02$). All six infants were breastfed before their first positive RT test result. For the six infants whose positive RT assay preceded the positive DNA PCR assay, the median viral load at the first positive RT assay was 223,494 eqc/mL (range: 44,132, >700,000), substantially higher than the cut-off for a positive RT assay of 10,000 copies/mL.

The comparison of the median RT viral loads and HIV DNA PCR result by study week is shown in Figure 1. We did not include children with indeterminate DNA PCR test results in the figure. Of note, one infant had concordant positive assay results at birth, week six and week 12 but discordant results (a positive DNA PCR assay but negative RT assay) at weeks 24 and 48. Factors that may have accounted for a falsely negative RT assay were not identified. Specifically, the infant was not started on HAART during the study period, lab

error was unlikely as samples from all visit dates for each infant were assayed in the same run, and the full one milliliter of plasma was used in the assay.

Discussion

Compared to the 18-month HIV antibody tests, the RT assay was 100% sensitive and specific when comparing the first positive RT result and children consistently testing negative by the RT assay, respectively. Because we conducted repeat RT and DNA PCR testing from birth we found that the RT assay detected infection earlier than the DNA PCR assay, especially at 6 weeks of age, when early infant diagnostic testing is recommended in resource-limited settings.⁸ Several reasons may explain this finding. First, breastfeeding infants may have been in the acute phase of infection when testing was done,⁹ and levels of HIV RNA and virion-associated RT can be higher than levels of HIV DNA during acute HIV infection¹⁰. Second, the DNA PCR assay may be more prone to false negative results than the RT assay due to laboratory or logistical errors. We minimized this possibility by conducting both assays in laboratories that were proficiency certified by external quality assurance programs. However, the current Kenyan protocol for DNA PCR assay utilizes 6mm² punch of dried whole blood spot which represent approximately 5 µl of whole blood¹¹ compared to 1 ml of plasma used in the RT assay; this difference in tested specimen volume may increase the risk of a false negative DNA PCR result relative to the RT assay. Finally, we believe it unlikely that the RT assays represented false positive results because the HIV viral loads were more than 4-fold higher than the 10,000 copies/mL threshold for a positive RT assay with the majority over 100,000 eqc/mL.

Our finding that a significant number of HIV infections are undiagnosed using DNA PCR testing at 6 weeks of age is important for several reasons. First, in resource-limited settings where access to medical care can be intermittent, accurate and early diagnosis of HIV when the child *does* present for care (during birth or routine immunization visits at 6 weeks) is especially critical. In a recent study conducted in Kenya, 65% of HIV-exposed infants were lost to follow-up by age 18 months, of whom 43% were lost to care by age 4 months.¹² Therefore if a 6-week DNA PCR test result is falsely negative, it is very likely that the child will not return for a repeat DNA PCR test or a confirmatory antibody test. An assay, such as the RT assay, that could detect infection soon after transmission occurs, such as at birth or 6 weeks could dramatically increase the number of infected children initiated on ART. Second, repeat HIV testing is indicated 6 weeks after cessation of breastfeeding for infants who continue to be breastfed after the initial negative DNA PCR. We found that if transmission occurred within 6 weeks of testing, the DNA PCR may be falsely negative. Although larger studies are needed to confirm this, it may be prudent to delay confirmatory DNA PCR testing until 3–6 months after breastfeeding cessation in asymptomatic infants or improve the sensitivity of the current DNA PCR assays. To avoid delays in diagnosis, the RT assay could be used as soon as 6 weeks after the end of breastfeeding to rule in infection. The Cavi RT assay is currently being used in several countries in Africa, for viral load monitoring.^{2, 13} By expanding the application of the RT assay for early infant HIV diagnosis, laboratories could achieve economies of scale through the purchase of larger quantities of kits.

In summary, we demonstrate that the RT assay detected HIV infection earlier than the DNA PCR test in HIV-exposed infants, especially at 6 weeks. Our study raises concerns that a significant number of HIV-exposed infants may be misdiagnosed as uninfected at 6 weeks of age using DNA PCR testing alone. Larger studies should explore the use of the RT assay to provide earlier detection of HIV infection in breast-feeding infants living in resource-limited settings.

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Figure 1.

Results of early infant HIV testing by DNA PCR and RT assay by week of test. Each marker on the graph represents data from specimens with valid data for both assays. The dotted horizontal line represents a level of 10,000 equivalent copies/ml, the cutoff between a positive and negative RT assay. The shaded box and line represent the median and quartiles of the group. **Infants with indeterminate DNA PCR results are not included in the figure.**