Point of care testing of HIV in children younger than 18 months with three different HIV virological assays. Experience from a district hospital in a resource-limited setting

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Abstract

Diagnosis of HIV in children younger than 18 months can be challenging in developing countries because requires the use of HIV virological tests. In this study we describe the experience with three commercial assays, HIV-1 DNA polymerase chain reaction (PCR) (Roche Amplicor 1.5) with dried blood spot, HIV-1 RNA PCR (Roche COBAS TaqMan) with plasma and reverse transcriptase activity assay (Cavidi Exavir Load 3) with plasma in a rural setting of India. Sensitivity and specificity were 98.1% (95% confidence interval [CI] 90-100) and 99.3% (95% CI 97.9-99.9) for the HIV-1 RNA PCR assay, 66.7% (95% CI 29.9-92.5) and 100% (95% CI 96.8-100) for the HIV-1 DNA PCR assay, and 100% (95% CI 48-100) and 98.7% (95% CI 92.8-100) for the reverse transcriptase activity assay respectively. The low sensitivity of the HIV-1 DNA PCR assay in this setting is worrisome and warrants further investigations.

Introduction

Prompt diagnosis of HIV and early antiretroviral therapy (ART) can reduce infant mortality by 76%.¹ The 2010 World Health Organization (WHO) guidelines of ART for HIV infection in children recommend early HIV diagnosis and immediate initiation of ART in all infants regardless of the CD4 cell count.² However, diagnosis of HIV in children younger than 18 months can be challenging in resource-limited setting.³ HIV transmission from mother to child can occur in the uterus, during delivery or during breastfeeding.⁴ Because of the passage of maternal antibodies through the placenta to the foetus, HIV sero-

logical testing is not useful for the diagnosis of HIV infection in children younger than 18 months.² According to WHO, diagnosis of HIV in these children requires HIV virological testing with sensitivity of at least 95% and specificity of at least 98%.² However, the evaluation of these assays has been performed in developed countries or in the context of sophisticated experimental studies, so evaluation of these assays in real-life settings from developing countries is lacking.

The aim of this study is to describe the performance of three different HIV virological assays for diagnosing HIV in children younger than 18 months born to HIV positive women in a rural setting of India.

Materials and Methods

The study was performed in the district of Anantapur, Andhra Pradesh, India. India is the third country of the world in terms of HIV infected people⁵ and Andhra Pradesh is the state with largest number of HIV infected people in India.⁶ Rural Development Trust (RDT) is a nongovernmental organization that has three hospitals in the district of Anantapur. In these hospitals, medical care of HIV infected people is given free of cost, including medicines.

The Vicente Ferrer HIV Cohort Study (VFHCS) is an open cohort study of all HIV infected patients who have visited RDT hospitals since June 2006. For this study we selected all HIV exposed children with known HIV status from the VFHCS database who had at least one HIV virological test at age below 18 months. The VFHCS was approved by the ethical committee of the RDT Institutional Review Board.

Following WHO recommendations,² children with a positive HIV serology test after aged 18 months or who had two positive virological tests were considered as HIV infected. Children who were not breastfed for at least 6 weeks and who had one negative HIV serological test or two HIV virological tests were considered as non HIV infected.² Negative HIV virological tests performed during breastfeeding or within six weeks after stopping breastfeeding in children who were finally found to be HIV infected were not considered as false negative results because of the possibility of HIV transmission through breastfeeding.²

We utilized three commercial HIV virological assays, a real time HIV-1 RNA polymerase chain reaction (PCR) assay (COBAS TaqMan HIV-1 test v1.0, Roche Diagnostics) (Method 1),⁷ a qualitative HIV-1 DNA PCR assay (Amplicor HIV-1 DNA assay v1.5, Roche Diagnostics) (Method 2),⁸ and a reverse tran-

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scriptase (RT) activity assay (Exavir load version 3, Cavidi, Sweden) (Method 3).⁹ Methods 1 and 3 were performed in the laboratory of Microbiology of RDT Bathalapalli Hospital with plasma collected from patients. Method 2 was performed with dried blood spots collected from patients and transported to a reference hospital.⁸ For Methods 1 and 3, an HIV viral load cut off of 1000 copies/mL was used for deciding whether the result was positive or negative.^{10,11} All samples were collected from infants older than six weeks.

Statistical analysis was performed using Stata Statistical Software (Stata Corporation, Release 11, College Station, Texas, USA).

Results

The performance of the three methods is described in Table 1. Method 1 had good sensitivity and specificity. The only false negative result was seen in a non-breastfed one year old child who had a viral load of 338 copies/mL. Method 2 had lower sensitivity for detecting HIV infection. Two false negative cases had a

Table 1. Sensitivity and specificity of the three virological assays.

	Method 1 n = 473	Method 2 n = 121	Method 3 n = 80
False positives	3	0	1
False negatives	1	3	0
True positives	52	6	5
True negatives	417	112	74
Sensitivity (95% CI)	98.1% (90-100)	66.7% (29.9-92.5)	100% (48-100)
Specificity (95% CI)	99.3% (97.9-99.9)	100% (96.8-100)	98.7% (92.8-100)

Method 1, COBAS TaqMan HIV-1 test v1.0 with plasma; Method 2, Amplicor HIV-1 DNA assay v1.5 with dried blood spot; Method 3, Exavir load 3 with plasma. CI, confidence interval.

positive RNA PCR test from samples collected before or at the same time that DNA PCR sample was collected. In the other false negative case, the DNA PCR sample was collected two months after stopping breastfeeding in a child who was found to be HIV infected later. Method 3 had only one false positive case. With Methods 1 and 3, results were known within one week in most of the cases, whereas with Method 2 the average turnaround time was 4 weeks.

Discussion

The low sensitivity of the HIV DNA PCR assay with dried blood spot is quite worrisome because this is the most common method used for screening of HIV infection in infants from developing countries.³ In fact, previous studies have reported false negative HIV DNA PCR tests in children infected by non-B HIV-1 subtypes.¹²⁻¹⁵ In American guidelines for ART in paediatric HIV infection,¹⁶ it is recommended to use HIV RNA PCR assays for confirming negative HIV DNA PCR tests when a non-B HIV-1 subtype is suspected because of the improved sensitivity of the RNA PCR assays for these subtypes.¹⁷ Although HIV DNA PCR tests are able to detect HIV even in patients who are taking ART,² they may not be the best option for diagnosing HIV in children younger than 18 months in countries with predominant non-B HIV subtype such as India.¹⁸ The results of this and previous studies suggest that HIV RNA PCR assays may be preferable, especially if they are able to process dried blood spots.¹⁹ False negative results can falsely reassure health workers and parents and, therefore, avert the initiation of HIV treatment for reducing the risk of death during the first years of life.¹

Plasma HIV RNA PCR and RT activity assays had good sensitivity and specificity and had shorter turnaround time. Although we had four false positive cases with these methods, these children were correctly identified as non HIV

infected after repeating the HIV virological test. The RT activity assay is a very interesting option for developing countries because of the low cost of the consumables and it does not require expensive and sophisticated equipment. This method could be suitable for district hospitals with experienced laboratory technicians.²⁰ The main drawback of this assay is the incapability of processing dried blood spot samples which makes more difficult the transportation of samples from patients living in remote areas.

The study has some limitations. The low number of HIV infected children included in the study warrants to be cautious about the conclusions on the sensitivities of the assays. New studies with larger number of HIV infected children are needed to confirm these results.

In conclusion, the study suggests that dried blood spot DNA PCR has lower sensitivity than plasma RNA PCR and RT activity assays for detecting HIV infection in HIV exposed children younger than 18 months in India. Because of the good results in terms of sensitivity and specificity, the short turnaround time and its low cost, the RT activity assay is an interesting option for implementing HIV diagnosis of children aged below 18 months in district hospitals from resource-limited settings.

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