Efficacy of HIV PCR Techniques to Diagnose HIV in Infants Born To HIV Infected Mothers – An Indian Perspective

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Abstract
Aim : To determine the efficiency of HIV DNA PCR (qualitative) and HIV RNA PCR (quantitative) to detect or rule out HIV infection in infants born to HIV infected mothers.
Setting : Pediatric and perinatal HIV clinic in a tertiary pediatric hospital.
Study Design : Prospective study
Methods and Materials : 52 infants born to HIV positive mother were tested for HIV infection by HIV DNA PCR or HIV-1 viral load from 1.5 to 7 months of age. Their HIV status was confirmed by an HIV ELISA test at 18 months of age.
Results : Of the 36 patients tested by HIV DNA PCR test, 15 patients (41.8%) had a positive test and 21 patients (58.2%) had a negative test. 12 patients (80%) had a false positive test (80%) and no patient had a false negative test. Sensitivity of HIV DNA test 100% with specificity of 53.9%. Of the 18 patients tested by HIV viral load, 17 patients (94.4%) had a negative test and 1 patient (5.6%) had a positive test. No patient had a false negative or false positive test. Sensitivity and specificity of HIV viral load was 100%, which was statistically significant (p=0.0004).
Conclusion : HIV-RNA PCR (quantitative) is highly specific and sensitive test for diagnosing and excluding perinatal HIV infection in children in India.

INTRODUCTION

The diagnosis of human immunodeficiency virus (HIV) infection in infants born to HIV-infected mothers by ELISA method is problematic due to the presence of passively acquired maternal antibodies. Virologic assays including HIV DNA PCR and HIV RNA PCR and viral cultures have been used to detect or rule out infection in infants less than 18 months of age. ELISA tests for HIV are used to diagnose infection in infants more than 18 months of age, when transplacental antibodies have disappeared. Both HIV RNA PCR and HIV DNA PCR have been found to be highly sensitive and specific for early diagnosis of pediatric HIV infection with a higher sensitivity of viral load technique (RNA PCR) as compared to proviral DNA PCR in younger infants.

There have been no studies to determine the efficacy of the various HIV PCR techniques to diagnose HIV infection in infants born to HIV infected mothers in the Indian scenario. This study was undertaken to determine the reliability of HIV DNA PCR (qualitative) and HIV RNA PCR (quantitative) in diagnosing or ruling out HIV infection in these infants.

METHODS AND MATERIALS

This prospective analysis was carried out over a period of 3 years from December 2001 to December 2004. 52 infants born to HIV infected pregnant women were enrolled in the study after verbal informed consent. All the infants were monitored clinically and virologically to determine their HIV status. HIV DNA PCR (qualitative) was offered at 1 ½, 3, 5 ½ and/or 7 months of age. HIV RNA PCR (HIV viral load) was done between 5-7 months of age. HIV viral load less than 20 copies/ml was considered as uninfected. All the children were followed upto 18 months of age and their HIV status was confirmed by HIV ELISA test. A positive ELISA was reconfirmed by another ELISA test using a different kit or by a Western blot test.

Laboratory analysis: HIV DNA PCR was determined by the Proviral DNA PCR technique with primers against
the gag region and HIV viral load was determined by real time PCR using the ARTUS kit. HIV ELISA was done using the DETECT-MC and HIV-CheX kits.

Statistical Analysis: The sensitivity and specificity of both the PCR techniques was determined by the ANALYSE-IT SOFTWARE (Version 1.7) and the reliability of each test was determined to detect or rule out infection in infants born to HIV infected mothers.

RESULTS

Of the 52 patients, 36 (63.2%) underwent an HIV DNA PCR analysis whereas 18 (34.6%) were tested by HIV viral load of which 2 (3.8%) were tested by both HIV DNA PCR and HIV viral load. HIV DNA PCR was positive in 15 patients (41.8%) and negative in 21 patients (58.2%) whereas HIV viral load was negative in 17 patients (94.4%) and positive in 1 patient (5.6%). Total 3 patients (5.8%) were HIV infected of which 2 died due to AIDS at a mean age of 5 months. 14 patients (27%) still have to complete 18 months of age and have not been tested by HIV ELISA test.

Of the 15 patients who tested positive for HIV DNA PCR, 3 patients (20%) were HIV infected of which 2 died of AIDS and 1 patient had hepatosplenomegaly with failure to thrive and lymphadenopathy with a positive HIV ELISA test at 18 months of age and also HIV viral load of 5,00,000 copies/ml. Remaining 12 patients (80%) tested negative for HIV by ELISA at 18 months of age and were clinically asymptomatic of which 1 patient also had a negative HIV viral load.

Of the 21 patients who tested negative by HIV DNA PCR, 7 patients had not completed 18 months of age and had not been tested for HIV ELISA. However, they were clinically asymptomatic. Remaining 14 patients (100%) were HIV uninfected and had a negative HIV ELISA test at 18 months of age.

Of the 17 patients who had a negative HIV viral load estimation, 7 patients had not completed 18 months of age and had not been tested for HIV ELISA. However, they were clinically asymptomatic. Remaining 10 patients (100%) were HIV uninfected and had a negative HIV ELISA test at 18 months of age.

One patient had a viral load of 5,00,000 copies/ml and was also HIV ELISA positive and thus infected.

The sensitivity and specificity of HIV DNA PCR and HIV RNA PCR is depicted in Table 1. HIV RNA PCR has better efficiency (p=0.0004) as compared to HIV DNA PCR (p=0.13) when correlated with HIV ELISA test.

DISCUSSION

Transmission of HIV virus from an infected mother to her child perinatally is the leading cause of spread of HIV infection in the pediatric population. PCR techniques, viral cultures have been the gold standards to detect or exclude HIV infection in infants born to infected mothers. Accurate and timely diagnosis of infection status is of utmost importance. Although PCR is one of the best available tests for diagnosis of HIV infection in neonates and infants, it is not definitive. A meta-analysis has revealed that the sensitivity and specificity of PCR in neonates is lower than in older infants, which results in a low positive predictive value; however, negative tests are informative. Similar results were seen in our study. Thus, a negative test may finally predict an uninfected child but a positive test may not still predict HIV infection in an infant. Repeating the PCR on independent samples may be required to reduce the test errors. Infact Busch MP et al have reported a very high 18.5% rate of false positive detection by HIV DNA PCR in uninfected individuals. There have also been reports of clearance of HIV infection in perinatally infected children. Roques PA et al have reported that almost 6.7% of the children born to HIV-seropositive mothers cleared HIV infection during the first year of life. Infact 5 of these 12 children were tested positive only by PCR suggesting that though seroconversion may be a possibility, chances of false positive PCR are very high. Similar troublesome diagnosis has been reported by Liberatore D et al. One of the reasons stated for the false positive results is contamination. Optimal PCR conditions, inclusion of control samples, strict rules on sample preparation, pre- and post-PCR handling, repetition of results, confirmation of specificity by hybridization, choice of material from which HIV-1 is amplified and the primers used for amplification will all predict the reliability of HIV DNA PCR.

In our study, we found that HIV RNA PCR (viral load) had no false positive or false negative result suggestive of a good reliability while interpreting the test in infants. Infact, a Spanish study has reported 100% sensitivity of the RNA viral load in newborns after 2 months of age, and the viral load technique was found to have a higher sensitivity than proviral DNA-PCR and viral culture in

| Table 1: Sensitivity and specificity of HIV DNA PCR and HIV viral load |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| HIV DNA PCR     | True negative  | 14              | True positive  | 3               | False negative | 0(0%)           | False positive | 12(80%)         | To Follow up    | 7               | Sensitivity     | 100%            | Specificity     | 53.9%           |
| HIV RNA PCR     | True negative  | 10              | True positive  | 1               | False negative | 0(0%)           | False positive | 0(0%)           | To Follow up    | 7               | Sensitivity     | 100%            | Specificity     | 100%            |
infants younger than 2 months. We also found that HIV DNA PCR had a high percentage of false positive tests and reliability of this test to diagnose HIV infection in the Indian scenario is problematic with a specificity of only 53.8%. This could be due to the reason that HIV DNA PCR technique was an in-house developed technique whereas HIV viral load was done by a standard kit. HIV being a social stigma, such high incidence of false positive result would create a lot of emotional as well as mental trauma to the parents which itself would question the utility of this test.

Thus, if one would like to have an early determination of HIV status in infants born to HIV infected mothers, HIV RNA PCR would be a better option as compared to HIV DNA PCR. The only deterrent would be the cost as HIV RNA PCR is 3 times costlier as compared to HIV DNA PCR. In such a case, HIV ELISA at 18 months of age would be the gold standard to determine the HIV status of such children.

Thus in conclusion, HIV RNA PCR (quantitative) is more reliable than HIV DNA PCR (qualitative) to diagnose or exclude HIV infection in infants born to HIV infected mothers. A positive HIV DNA PCR in an asymptomatic infant should be interpreted with caution. HIV ELISA test at 18 month is still the gold standard to determine the HIV status in infants.

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REFERENCES


Book Review

Textbook of Environmental Emergencies

Environment related illness as we commonly understand, includes problems likely to be encountered in the air, on the ground, or in the water, like altitude illness, envenomation, smoke inhalation, poisonous plant ingestion, cold injuries, heat stroke and scores of the other potentially serious disorders. In recent years, outdoor sports have become increasingly popular and humans are stretching their limits of endurance, with many taking parts in adventure sports. As the venturesome from many nations share the excitement of testing their limits, so will many thousand experience injury and illness related to adventure sports and trekking in the wild. The primary care physician is often confronted with unpredictable adventure activity related injuries and needs access to description and therapeutic guidelines and uncommon disorders.

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