REVIEW ARTICLES

UTILITY OF PCR TESTS FOR DIAGNOSIS OF HIV IN INFANTS EXPOSED TO HIV VIRUS - PROBLEMS IN INDIA

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Abstract

Correct diagnosis of human immunodeficiency virus (HIV) is essential for proper management. A confirmed HIV infection needs monitoring and adequate treatment to prevent progression to AIDS. However, false positive and false negative results can have both therapeutic as well as social and emotional implications. Diagnosis of HIV is especially difficult in infants born to HIV infected mothers due to presence of transplacentally acquired maternal HIV antibodies that may persist for upto 18 months of age. Virologic assays including HIV DNA PCR and HIV RNA PCR as well as viral cultures have been used to detect or rule out infection in HIV exposed infants less than 18 months of age (1). However these tests have to be interpreted with caution taking into account clinical presentation, method of doing the test and quality control of the test. The problems of diagnosis of HIV in infants in India with PCR techniques are highlighted in this article.

Keywords: PCR, HIV, diagnosis of HIV in infants

Introduction

In developing countries such as India and sub-Saharan Africa, HIV in children is still rampant. Children represent 6% of all people infected with HIV/AIDS globally as of December 2005 but accounted for 18% of AIDS death in 2005. Thus early diagnosis of HIV for adequate treatment is essential. The predominant mode transmission of HIV in children is through the vertical route (2). Without intervention, the mother to child transmission of HIV ranges from 15 to 40% (3, 4). Infants infected with HIV must be diagnosed as early as possible to ensure the early institution of therapy to limit HIV related morbidity. For children below 18 months of age, standard serological tests such as ELISA and Western blot are not useful due to presence of transplacentally acquired maternal antibody against HIV which may confound the result. In such a case, HIV cultures and HIV PCR tests have been used for early diagnosis.

HIV Culture

HIV culture is done from peripheral blood mononuclear cells (PBMCs) but is technically difficult and time consuming. It is expensive and done in research institutes. Positive results are available by 1-2 weeks but negative results are not reported till there is no evidence of HIV replication for 30 days. Sensitivity for detecting infection has been reported to be 50% at birth and 90% by 3 months of HIV (5). However a single test is not conclusive of the diagnosis and should be confirmed by a repeat test or PCR test.

HIV PCR

Polymerase chain reaction (PCR) is a method of in-vitro replication of target nucleic acid sequences. PCR amplifies a short nucleic acid sequence that is specific to the organism being detected. DNA from the PBMCs is released by enzyme lysis and mixed with a mixture consisting of DNA polymerase enzyme, deoxynucleoside triphosphates and primers that bind to specific areas of HIV gene. The deoxynucleoside triphosphates are the building blocks for new strands of DNA (6). There are various primers available that bind to gag region (SK 19 or SK-102) or envelope region (SK-70) of the HIV gene (7). The DNA to be tested will bind to new matching bases in PCR solution. Binding requires the help of primers to start the process. Primers are small sequences of DNA complementary to the target DNA sequences and will bond only to their matching target sequences. If no sequences match, PCR will not occur and a negative result will be recorded (8). If binding occurs, the deoxynucleoside triphosphates bind to the primer and extend the DNA strand till a complete copy of the target sequence is produced resulting in one copy of original. Multiple PCR cycles occur to yield millions of copies. This material is enough to detect presence of HIV DNA. The product is then subjected to agarose gel electrophoresis or tested by colorimetric ELISA like assay. On agarose gel, the amplified positive HIV DNA will be visualized after ultraviolet light illumination. With colorimetric assay, positive tests will give a colour to solution. In a quantitative assay, the intensity of the colour is related to the amount of DNA copies present and thus the original viral load can be calculated.

There are several types of HIV PCR tests available. However, qualitative PCR is used for diagnosis of HIV infection and quantitative PCR is used for monitoring an HIV infected child.

Qualitative tests that are used are HIV DNA PCR and HIV RNA PCR. HIV DNA PCR is available as standard kit (Roche Amplicor HIV-1 DNA test or branched DNA test) or may be developed by the laboratory as an in-house technique using different primers for various areas of the HIV gene. Qualitative HIV RNA PCR used is nucleic acid sequence based amplification assay (NASBA).

Quantitative HIV PCR (HIV viral load) is usually done by the COBAS Ampiclor system, branched DNA assay, NASBA test and real time RT PCR assay. The newer realtime RT-PCR assays offer several advantages, they are very sensitive (40 to 50 copies/mL) with a broad linear range (6log10), and they pose a lower risk of carry over contamination than standard PCR assays. Microwell PlateThe AMPLICOR HIV-1 MONITOR™ is a semi automated system which includes the HIV PCR process. From a small amount of viral RNA extracted from plasma, the AMPLICOR system will perform reverse transcription and PCR, measure the number of copies of the viral genetic code produced and calculate the original amount of RNA present ie. the amount of viral RNA, and therefore virus, in the plasma.

Blood that is used for PCR test may be collected from dried blood sample on filter paper or from whole blood.

Efficacy of HIV PCR tests:

Pediatric AIDS collaborative Transmission study demonstrated the sensitivity of NASBA assay to be 38% at < 7 days, 97% at 7-41 days and 95% at 42-93 days of life with specificity of 99% (9). HIV DNA PCR has found to have sensitivity ranging from 90%

to 100% with a specificity 53.9% to 99% (10, 11) with efficiency improving to 100% by 7 months of age (10). Thus, there is always a risk of false positive and false negative results. Thus, it is always recommended to confirm the diagnosis with a second test. However, there have been instances where repeated tests may also be fallacious (10, 12) and thus, one may need to correlate the results with clinical symptoms and may be even confirm with an ELISA test and Western blot test after 18 months of age. 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 (13). There have also been reports of clearance of HIV infection in perinatally infected children who tested positive by PCR (14) suggestive that though seroconversion may be a possibility, chance of false positive PCR is likely.

Data regarding use of HIV RNA quantitative PCR for diagnosis of HIV infection is limited and sensitivity was found to range from > 90% to 100% after 1 month of age and specificity was 100% (11, 16). HIV RNA may be falsely positive in low copy number in exposed uninfected infants and not be undetectable. In such case, one should not assume infection. Similarly, undetectable copies may occur in infected infants who have received antiretroviral drugs in utero or postnatally (17).

Why false positive and false negative results with HIV PCR?

The problem of false reports with PCR may be due to contamination, suboptimal PCR conditions, non-inclusion of control samples, improper sample collection, choice of primers and cross-reaction with other micro organisms (7). A multi centre study in France demonstrated that false positive and false negative results were observed in all laboratories who participated in the study when they tested coded samples from HIV-1 seropositive individuals and HIV-1 seronegative individuals (15). Thus, this false positive and negative results are not restricted only to the infants but also the adults.

Conclusion

Diagnosis of HIV infection in infants is difficult and current HIV diagnostic tools need to be interpreted with caution and in association with clinical presentation. Many of the HIV PCR assays currently in use need validation and optimization to prevent false positive and false negative results.

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