Problems in Diagnosis of HIV Infection in Babies

Col AK Praharaj*

Abstract

Serological diagnosis of human immunodeficiency virus (HIV) infection in babies born to HIV infected mothers is difficult because of presence of maternal anti-HIV antibody up to 18 months. Conventional enzyme-linked immunosorbent assay (ELISA) and western blot may be positive in un-infected cases. Various other modalities which have been adopted include detection of HIV specific IgA, IgM, IgE, detection of p24 antigen, viral culture and detection of HIV nucleic acid by polymerase chain reaction (PCR). Viral culture or PCR positivity within first 48 hours of life indicates intrauterine infection. An early diagnosis of HIV infection in babies born to HIV infected mothers is essential as definite antiretroviral therapy (ART) can be instituted and unnecessary toxicity of drug therapy avoided if found negative. Though viral culture and DNA-PCR has sensitivity of >95% after one month of age, some cases can not be diagnosed during this period. Other tests like viral RNA detection by reverse transcription polymerase chain reaction (RT-PCR) and combination of tests will be required.

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Introduction

Across the placenta of human immunodeficiency virus (HIV) infected mothers and their presence in the neonate poses a diagnostic challenge. Conventional enzyme linked immunosorbent assay (ELISA) and western blot assay cannot be used for definitive diagnosis. Since an early diagnosis is mandatory for instituting anti retro viral therapy (ART), exclusion of infection, will spare the babies of avoidable toxicity of the antiretroviral drugs. Though all infants born to HIV positive mothers are initially seropositive, only 13-40% develop HIV infection [1]. Out of the available tests, none can provide 100% accurate diagnosis in infected infants at an early stage. Hence various tests including polymerase chain reaction (PCR), viral culture, p24 antigen detection may be required to obtain a diagnosis.

The methods for diagnosis of HIV infection include detection of antibody (Ab) to HIV, detection of p24 antigen (Ag), HIV culture, deoxy ribonucleic acid (DNA) and reverse transcription polymerase chain reaction (RT-PCR) and immunological tests.

Antibody detection

Presence of anti-HIV IgG Ab in < 18 months of age indicates transfer of maternal Ab or infection whereas positivity at > 18 months of age indicates infection. A false negative can be seen in cases of hypogammaglobulinemia. Ab testing should be done at

birth, 3,6,12,18 and 24 months of age. Two different ELISA tests followed by western blot (WB), indirect immunofluorescence assay (IFA) and if required HIV-2 confirmatory test should be carried out [2].

Detection of antibody isotypes

As IgM, IgA and IgE class of Ab cannot cross placenta, their presence in the new born suggests infection. Anti-HIV IgM lacks sensitivity and specificity and its presence is transient, hence not preferred. Several studies have evaluated HIV specific IgA as a possible diagnostic test. Despite excellent specificity (> 99%) and several performance enhancing procedures (i.e. IgA specific western blot, IgG depletion) its sensitivity ranges from only 40-80% during the 1st month of life. Without performance enhancing method the sensitivity is 17% at 1 month and 67% at 3 months of age. Thereafter the sensitivity increases to 80-90% at 4 months, > 94% at 6 months and 100% at 9 months of age. A negative assay does not exclude infection in exposed neonates. IgA immunoblot has shown more specificity as compared to IgA ELISA [3,4]. The test has advantage of being simple ELISA based, cost effective and helpful after 4 months of age if PCR/HIV culture are not available [3].

A sensitive assay to detect HIV specific IgE has been reported and such an assay could provide an infant-specific diagnostic signal. This test has not been fully evaluated [5,6].

*Senior Advisor (Pathology & Microbiology), Command Hospital (SC), Pune.

Invitro production of HIV specific antibody by peripheral blood mononuclear cells

In-vitro production of HIV specific antibody by peripheral blood mononuclear cells (PBMC) has been evaluated, as positivity indicates infection. In this test PBMC from the patient is co-cultivated with donor T lymphocytes in the presence of phytohaemagglutinin (PHA) or *tat* protein. HIV specific antibody produced is detected by ELISA. But this test is more sensitive and specific only in infants >6 months of age. It is not widely available, difficult to standardise and will not detect 10% of infected infants who do not produce Ab [1].

HIV culture and PCR

Part of controversy surrounding the various diagnostic assays and the difficulty in establishing HIV diagnosis in newborn relates to the timing of HIV infection. When HIV infected pregnant women were assessed by ultrasound guided foetal blood sampling, all 28 foetuses (mean 22 weeks gestation) had negative cultures, PCR and immune complex dissociation (ICD) p24 Ag assay [7]. Most transmissions appear to occur late in pregnancy and early foetal blood sample testing is not recommended. Vertical transmission can be intrauterine (13-40%), peri-partum (60-75%) or during breastfeeding (10-15%) [8]. Vertical transmission is defined as intrauterine if HIV culture or DNA PCR is positive within the first 48 hours of life. Transmission is defined as being intrapartum if these studies are negative during the first week of life but positive within one month [9].

The use of HIV culture in neonates is largely determined by the timing of infection. Several studies have found that PBMC culture positivity ranged from 20-50% at birth and rose to 75-90% at one month indicating that most infants are infected during the intrapartum period. The overall sensitivity also depends on immunological status i.e. >95% in infants with CD4 count of < 1500, and lower with high CD4 counts [10]. Plasma culture is less sensitive than PBMC culture. The disadvantage of culture is high cost, time consuming (2 - 4 weeks), limited availability and the requirement of a large volume of sample.

Detection of HIV by PCR

Like viral culture the sensitivity of PCR also depends on the age of the infants. The sensitivity is 40-50% in 1st 48 hours of life, 90 % at 14 days of age, >95% at 1-2 months and 99- 100% at 6 months. DNA PCR has been the method of choice for diagnosis in neonates. Ten or more copies per sample achieves 99% sensitivity and virtually all infants are diagnosed by 6 months of age. Studies of vertical transmission indicate that infected infants have a rapid rise of HIV plasma RNA over the first 1 to 2 months of life, followed by a slow

decline over the next 22 months. The mean values throughout the first year of life are usually more than 10⁵ copies per millilitre [11,12]. The median RNA levels at birth and at one month are much higher, for infants infected in utero compared to peripartum (10,000 vs 400 and 716,000 vs 100,000 copies per millilitre at one month respectively) [13,14]. In contrast to most adults, whose levels decline by 1 to 3 log copies per millilitre after seroconversion, perinatally infected children have levels that remain exceedingly high. The plasma RNA assay appears to be more sensitive than DNA PCR for diagnosis within 4 weeks of birth, although the two methods appear equally sensitive by 1 - 2 months [10,15]. The advantage of DNA PCR is that, it is not affected by the mode of delivery or maternal or neonatal anti-retro viral treatment, while that of RT-PCR lies in assessing prognosis and monitoring ART. Methods used for RT-PCR for detection of viral RNA include Amplicor assay, branch DNA signal system and Nucleic Acid Sequence Based Analysis (NASBA). Amplicor assay has been found to be most acceptable.

The HIV PCR should be performed within first 48 hours of life, at 14 days (for modification of ART), at 1 to 2 months of age and at 4 to 6 months of age (in those previously negative). Cord blood sample should not be used to prevent contamination of maternal blood. If one sample is positive then another sample is to be tested as soon as possible and a positive result given only if both samples are positive [1].

Detection of p24 Ag

Regular p24 Ag testing has been found to be highly specific but lacked sensitivity in HIV diagnosis. False positive cases are common at < 1 month of age [16]. Sensitivity in infants > 6 months of age is 50-75%. Immune complex dissociation p24 Ag assay has increased the sensitivity to 50-100% at > 1 month age. p24 Ag is undetectable in 50% cases with CD4 count > 1500 and absence of p24 Ag does not exclude infection. It is clearly inferior to HIV culture/PCR [17], though ultra sensitive, p24 antigen assay has been proposed as an substitute [18].

Immunological tests

Though various immunological tests co-relate with HIV infection, these tests are carried out to classify the cases into various groups and for monitoring prognosis/ART. They do not provide a definite diagnosis. The lymphocyte subset CD4, CD8 and CD4/CD8 ratio is to be carried out at 1, 3 and 6 months and then every three months until HIV status is known. Monitoring is carried out thereafter every 3 to 6 months in HIV infected babies[2]. The quantitative estimation of immunoglobulins of various Ig isotypes has been carried out, but they are

not useful for prognosis. Total IgG level >1700 mg/dl has co-related well with HIV infection in neonate [1]. Skin tests (candida, mumps, and tuberculin) can be carried out to detect the status of cell mediated immunity (CMI) in the babies and for use in vaccine protocol.

Diagnosis in children

The criteria for diagnosis in children less than 18 months of age include positive results on two separate determinations (excluding cord blood) from one or more of the following tests, namely HIV culture, HIV-DNA-PCR, and HIV-p24 Ag or meets criteria for AIDS diagnosis based on 1987 AIDS surveillance definition, >18 months of age who is HIV antibody positive by repeated enzyme immuno assay (EIA) and confirmatory test (WB or IFA) or meets any of the above criteria.

Diagnosis of sero-reverter

A child is born to an HIV infected mother and who has been documented as HIV Ab negative (i.e two or more negative EIA tests performed at 6-18 months of age or one negative EIA test after 18 months of age). There has been no other laboratory evidence of infection (has not had two positive viral detection tests, if performed), and has not had an AIDS defining condition.

Diagnosis of perinatally exposed

A child who does not meet these criteria, who is HIV seropositive by EIA, confirmatory test (WB/ IFA) and is <18 months of age at the time test or has unknown antibody status, but was born to a mother known to be infected with HIV.

Exclusion of HIV infection

PCR is negative at 1-2 months of age and at 4-6 months of age, HIV antibodies performed at > 6 months of age are negative on at least two samples collected one month apart, HIV Ab performed after 18 months of age are negative in the absence of hypogammaglobulinaemia and clinical signs of HIV infection.

Once a diagnosis of HIV infection is made, depending on the clinical presentation and immunological status of the baby, the cases should be classified into various category as per CDC criteria [2].

Clinical category A consists of children with two or more of the conditions listed below, but none listed in categories B and C namely lymphadenopathy (0.5 cm at more than two sites; bilateral = one site), hepatomegaly, splenomegaly, dermatitis, parotitis and recurrent or persistent upper respiratory infection, sinusitis or otitis media.

Clinical category B consists of children who have symptomatic conditions or other than those listed in

categories A and C that are attributed to HIV infection namely anaemia (<8 gm/dl), neutropenia (<1000/ cmm), or thrombocytopenia (<100000/ cmm) persisting for 30 days, bacterial meningitis, pneumonia, or sepsis (single episode), candidiasis (oropharyngeal thrush, persisting for >2 months in children >6 months of age), cardiomyopathy, cytomegalovirus infection with onset at <1 month of age, diarrhoea (recurrent or chronic), hepatitis, herpes simplex virus (HSV) stomatitis (recurrent with more than 2 episodes within one year), HSV bronchitis, pneumonitis, or oesophagitis with onset before one month of age, herpes zoster (shingles involving at least 2 distinct episodes or more than one dermatome), leiomyosarcoma, lymphoid interstitial pneumonia (LIP) or pulmonary lymphoid hyperplasia complex, nephropathy, nocardiosis, persistent fever (lasting > 1 month), toxoplasmosis (onset before 1 month of age) and varicella, (disseminated complicated chickenpox).

Clinical category C consists of children who have any condition listed in the 1987 surveillance case definition for AIDS, with the exception of lymphocytic interstitial pneumonia (LIP).

In babies born to HIV infected mothers a virological diagnosis is preferred. HIV DNA PCR remains the test of choice. DNA PCR should be carried out within first 48 hours of life, at 14 days (for modification of ART), at 1 to 2 months of age and at 4 to 6 months of age if found negative previously. Simultaneously antibody testing should be carried out at birth, 3,6,12,18 and 24 months of age to have a diagnosis or to rule out HIV infection. Absence of virological evidence during first six months and absence of Ab after six months will exclude HIV infection in neonates.

Children who are born to HIV infected women should undergo monitoring of the CD4⁺ lymphocyte count and percentage, total blood count, differential leucocyte count, and platelet count during the first 6 months of life. Monitoring should continue in children found to be infected and in those whose infection status is unclear at 6 months of age. The CD4⁺ lymphocyte count and percentage should be monitored at 1 and 3 months of age in all HIV-exposed infants. Infants identified as infected or whose infection status is unclear should continue to undergo monitoring of the CD4⁺ lymphocyte count and percentage at 3 month intervals (at 6, 9 and 12 months of age) or more frequently if the CD4⁺ lymphocyte count or percentage declines rapidly. Quantitative immunoglobulins should also be measured by the time the infant is 4 to 6 months of age. Haematological abnormalities, hypergammaglobulinemia, and an abnormally low CD4⁺ lymphocyte count and percentage (below the age-related normal levels) are frequently seen in HIV infected children. The CD4⁺

lymphocyte count and percentage are no longer used as guidelines for prophylaxis for *P carinii* pneumonia (PCP) during the first year of life, but results obtained during that first year are used to guide prophylaxis for HIV-infected children during the second year of life.

Conflicts of Interest

None identified

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