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Pediatric HIV Testing Challenges in Resource Limited Settings

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1. Introduction

According to UNAIDS, the joint United Nations Program on HIV/AIDS, 2.5 million children younger than 15 years are living with HIV, and during 2008 nearly half a million children were infected with HIV. Most of these children (67%) were from sub-Saharan Africa, and mother to child transmission was the major route of infection (91%) (Vermund 2004). HIV is one of the major causes of infant mortality in developing countries (Marston et al., 2005; Rashid et al., 2005). Prevention of mother to child transmission (PMTCT) of HIV has become an important cornerstone to reduce child mortality in sub-Saharan Africa. The research achievements in PMTCT are remarkable. Unfortunately, it has proved much more difficult to translate these findings into practice in resource-poor settings.

Virology tests are expensive and require sophisticated laboratory facilities. The expense of creating a laboratory with appropriate quality control and assurance to perform virology testing is significant, as well as the recurrent costs of test reagents. Laboratory technicians and scientists are in short supply in many resource-limited countries, and highly trained technicians may have high turnover because they are often sought by researchers and other countries. Venipuncture of infants requires training and supplies that are often unavailable outside large cities. Transport difficulties and distances may prohibit whole blood samples from reaching high-level laboratories in adequate time and condition for testing. Difficulties in returning results quickly to clinical sites may reduce acceptability of testing and cause results to go unclaimed. Effective infant diagnosis programs require a combination of clinical, serologic, and virologic approaches to the question of infant HIV status (Creek et al., 2007).

Diagnosis is an important aspect of providing treatment and care to those children infected. The earlier the diagnosis is made the better the outcome for patients. High mortality in the first few months of life has been reported particularly in resource limited settings (Zijenah et al., 1998, 2004). Approximately 20% can die by 6 months of life, 35-40% by 1 year of age and 50-60% by 2 years (Newell at al., 2004; Obimbo et al., 2004). Currently early detection is very possible with virologic assays which are sensitive and specific. Serologic methods such as HIV- Enzyme Linked Immunosorbent Assay (ELISA), Western blot, and Immunofluorescence Assay can be used to diagnose HIV infection in children older than 18 months. Cheaper rapid diagnostic tests have been introduced which can be used at the point of care (Brambilla et al., 2003).

2. HIV serology

Serology which is appropriate for diagnosing HIV infection in older children can not be used in young infants who are less than 18 months of age, particularly those breast feeding because of interference with maternal antibodies. Maternal antibodies cross the placenta so they can give false positive results in HIV uninfected children. The test does not distinguish maternal from infant antibodies (Lujan-Zilbermann et al., 2006). However when HIV antibodies are not present in HIV exposed children younger than 18 months but older than one year who are not breast feeding or in the window period, a diagnosis of HIV negative status can be made (Alecdort et al., as cited in Creek et al., 2007). Current assays have both a sensitivity and specificity greater than 99% in children older than 18 months. (Palasanthiran et al., 1994). If the initial serologic test is positive, a second ELISA or EIA is performed on the same specimen. If the second test is positive a confirmatory test is indicated, commonly an HIV-1 Western blot or an HIV-1 indirect immunofluorescence antibody assay (Brambillia et al., 2003). These gold standard tests are expensive and require a long waiting period before results can be obtained by clinicians in charge mainly because of the batching of specimens to save reagents.

Rapid tests have become important in resource limited settings mainly because of use by personnel with limited skills, ability to give out results immediately at site which makes counseling easier. Specificity and sensitivity is reasonable and comparable to those of EIAs used for screening (Mylonakis et al., 2000).

In young infants HIV rapid antibody tests can have a role if the maternal HIV infection status is unknown. HIV exposure can be confirmed with a positive result and excluded with a negative one. Infants lose maternal antibodies at different times, as a result young infants who have lost maternal antibodies can be presumed HIV negative at a younger age with rapid tests if they are not breast feeding and not in the window period. This is a reasonable low cost approach of ruling out HIV infection in HIV exposed infants. A large proportion of HIV uninfected children are seronegative by 9 months of age and some as early as 4 months (Blackburn et al., 2006). Errors have been found with some rapid test kits reporting false negative results particularly in severe illness which is associated with hypogammaglobulinaemia hence clinical judgment and appropriate physical examination is necessary at the diagnostic stage.

The testing of saliva for HIV antibody may be useful in infants because oral fluid contains lower concentrations of all antibodies in comparison with blood. Diminishing maternal HIV antibodies in HIV-exposed but uninfected infants likely become undetectable earlier in oral fluids than in blood. Laboratory-based or rapid HIV tests performed on oral fluid can potentially exclude HIV infection earlier in life, and sample collection is less traumatic for the infant and caregiver. Further validation of oral fluid assays is needed to establish the youngest age at which seroreversion can be detected and to determine the sensitivity and specificity of the test at different ages (Sherman et al 2005).

3. Virologic testing

Nucleic acid testing methods include HIV-1 Deoxyribonucleic acid polymerase chain reaction (DNA PCR), HIV Ribonucleic acid polymerase chain reaction (RNA PCR) and HIV-1 p24 Antigen. Polymerase chain reaction (PCR) assays detect HIV-1 DNA within

peripheral blood mononuclear cells (PBMC). Proper specimen collection procedures and processing are important for the validity of the results. Whole blood should be collected in tubes containing edentate calcium disodium (EDTA) or acid citrate dextrose as anticoagulants (Brambilla et al., 2003). Major challenges faced here include the cost of bleeding the patient, namely syringes, needles and appropriate blood tubes. Transportation of specimens and storage are also important where frequent power cuts compromise the quality of results.

3.1 HIV deoxyribonucleic acid (DNA) PCR

HIV DNA PCR is the standard method for virologic diagnosis of HIV in infants in the developed world. It has been used for many years, is the diagnostic test of choice recommended by the WHO, and has acceptable sensitivity and specificity (Bremer et al., 1996; Sherman et al., 2005). For HIV -1 subtype B, the sensitivity and specificity of HIV-1 DNA PCR assays approach 96% and 99% respectively, by 28 days of age. They are less sensitive for detection of non-B subtype infection (Lujan-Zilbermann et al, 2006). HIV infection can often be detected at birth, and essentially all perinatal infections are detectable by 4 weeks of age (Sherman et al., 2004). Infections acquired postpartum (i.e., through breast-feeding) can be detected by 4-6 weeks after the last exposure. Different PCR tests exist world wide and not all tests are equally accurate with all HIV subtypes. The cost can range from $8 USD to $16USD per test (Creek et al., 2007).

3.2 Ribonucleic acid (RNA) PCR

An HIV RNA PCR, quantitative or qualitative, is also an accurate method of diagnosing HIV in young infants, with sensitivity and specificity comparable with DNA PCR testing. However, this test is more expensive and requires the use of plasma, which is difficult to obtain from infants and transport intact. HIV RNA PCR is routinely used to monitor disease progression and response to HAART. Whole blood specimens collected should be processed within 6 hours for accurate results which poses a challenge in resource poor settings (Brambilla et al 2003).

3.3 Real-time PCR

Real-time PCR allows the technician to view the increase in the amount of DNA or RNA when it is amplified. Real-time PCR as a new approach is gaining acceptability because of its improved rapidity, sensitivity, reproducibility, and the reduced risk of carry-over contamination, and it may reduce the cost of nucleic acid testing (Katsoulidou et al., 2006 & Zhao et al., 2002 as cited in Creek et al., 2007).This method is in use in numerous research settings and performs very well. However, at present the only commercial kits available are for quantitative and not qualitative detection of HIV, and large-scale use of these assays for public health programs has not been attempted (Creek et al., 2007).

3.4 Ultrasensitive (US) p24 antigen assay

The US p24 antigen assay is slightly less sensitive than HIV PCR in identifying HIV infection in infants across various subtypes and has a specificity similar to that of HIV PCR

(Patton et al., 2006; Sherman et al., 2004;) quantitative viral protein detection assay utilizes simpler technology than is required for detection of viral nucleic acids, but it is still relatively complex with multiple processing steps. US p24 is not in general use because studies validating it for infant diagnosis are recent, and achieving valid results in field settings has been challenging. US p24 may provide a useful alternative where PCR is not available. It can be used as a marker of disease progression and therapeutic response (Keenan et al., 2005).

3.5 Dried blood spots (DBS)

The HIV-1-DNA PCR on dried blood spots have made a significant difference in giving young HIV infected infants a chance for survival by early diagnosis. Spotting of whole blood onto filter paper offers technical and economic advantages over conventional venipuncture methods since it simplifies sample collection and transport to reference laboratories for diagnostic testing and viral load quantification (Zhang et al., 2002). Because infant blood for testing can be taken by simply pricking a heel, toe, or finger and dried cards are stable for relatively long periods without refrigeration; many logistical barriers to infant testing can be overcome using this simple technique. However proper training of staff is required to improve validity of results. PCR performed on DBS is as accurate as PCR performed on whole blood but has higher reagent cost and some increase in processing time. DNA and RNA PCR, both standard and real time, have been successfully performed on DBS in many settings and HIV subtypes with no loss of accuracy (Cassol et al., 1996; Lyamuya et al., 2000; Sherman et al., 2004; 19, 26-27). Routine collection of DBS for early infant diagnosis is being implemented in many countries. Dried blood spots have been successfully used to measure HIV-1 RNA in patients and to diagnose perinatal HIV infection. Some studies have reported low stability of nucleic acids in dried blood spots particularly when stored under inappropriate conditions.

Dried blood spots may also be used for HIV-1 sub typing and genotypic resistance testing (Fiscus et al., 2006). Although HIV-1 viral load can be successfully enumerated using dried blood spots there are limitations posed by volume of blood or plasma especially if sensitive nucleic acid testing is done or multiple tests are done on the same sample. The Vivest (formerly referred to as SampleTanker) is an economical dried specimen storage transportation system where significantly increased plasma volumes can be shipped at optimum temperatures (Zanoni et al., 2010). Its use was validated in Brazil, it was demonstrated that DNA HIV-1 viral load results from dried plasma eluted from Vivest are generally comparable to that of fresh plasma. Its use present a cost effective way of transporting specimens in resource poor settings where the logistics associated with shipping frozen plasma are expensive(Zanoni et al., 2010).

4. Conclusion

Clinicians looking after children should be aware of the different tests available to diagnose HIV infection in children which are useful in resource limited settings. All the different tests have different applications in these settings and accuracy should not be compromised so that the goal of reducing child mortality is met.

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It can be said that now is the best time for everyone infected to become aware of their own HIV status. The state of the art in HIV management progressively reveals that antiretroviral treatment can prevent transmission, as well as chronic damage in the human body, if started early. Unfortunately, antiretrovirals are not widely available in many places, especially in developing countries. In these parts of the world, diagnosis of HIV infection must be kept in the agenda as a priority, in order to understand specific details of local epidemics and as an effort to interrupt the chain of HIV transmission.

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