

## EDITORIAL

### LABORATORY DIAGNOSIS OF CHILDHOOD TUBERCULOSIS

Tuberculosis in childhood occurs with different manifestations. All these forms of tuberculosis, except when cavitation occurs in pulmonary tuberculosis, are paucibacillary in nature. For this reason, even though at the present time bacteriological confirmation is still the final proof of tuberculous disease, it is difficult to obtain. Depending on the form of disease manifestation, several specimens like sputum and/or gastric lavage, as children are often unable to produce sputum, lymphnodes and other biopsy specimens, pus, ascitic fluid, pleural or cerebrospinal fluid (CSF) need to be collected. If delay is anticipated, biopsy specimens may be collected in suitable transport medium for sending it to laboratory.

There are two ways to address diagnosis of tuberculosis. The direct approach is concerned with the detection of the bacteria by microscopy or culture, detection of tuberculostearic acid, detection and identification of mycobacterial antigen by the use of polyclonal or monoclonal antibodies, analysis of lipid composition by chromatography, and the detection of DNA or RNA of mycobacterial origin by hybridization with a DNA probe with or without amplification of nucleic acids. The indirect approach relates to measurement of host immune response against the mycobacteria. This includes humoral immunity via the detection of antibodies against the bacteria and cellular response via skin tests<sup>1</sup>.

#### Direct approach

##### Microscopy

Traditionally the detection of tubercle

bacilli in sputum and other body fluids is done by acid fast staining method. Although it is a highly specific technique, it is a less sensitive method in paucibacillary conditions like childhood tuberculosis.

##### Culture

For conventional culture, while sputum after processing is inoculated onto two slopes of LJ medium, all other specimens are inoculated into multiple media consisting of LJ, LJ with pyruvate (LJP), selective Middlebrook 7H11 oleic acid albumin agar and selective Kirschner's liquid medium (SKLM) in order to increase the yield. By using microscopy and culture in extrapulmonary situations like tuberculous meningitis and tuberculous lymphadenitis bacteriological confirmation was obtained in 58% and 68% respectively in studies reported by the Tuberculosis Research Centre, Chennai. Similarly in a study reported on 50 pediatric pulmonary tuberculosis, the overall bacteriological yield with broncho alveolar lavage (BAL) and gastric lavage (GL) was 34%, while GL alone was positive in 32% indicating that GL is better than BAL<sup>2</sup>.

Conventional culture methods are slow and not very sensitive in paucibacillary forms of tuberculosis and delayed bacteriological confirmation will not be of help in the management of sick children. Hence more rapid and sensitive methods must be explored for their application in the diagnosis of childhood tuberculosis. Today several rapid methods are available for culture of mycobacteria. Of them, detection of mycobacteria using the microcolony method is relatively inexpensive. It requires only half the time needed for conventional culture. However, it is labour

intensive and less efficient than conventional method. Besides, its usage in paucibacillary situation has not been looked into. Others include the radiometric Bactec method, the Septi Check AFB method, and the microbial growth indicator test (MGIT) system. These tests are specific, excellent, and offer earlier diagnosis even in the paucibacillary situation<sup>3</sup>. However, they are not available indigenously, expensive and not within the reach of the laboratory of developing countries.

### **Tuberculostearic acid (TBSA)**

TBSA is a cell wall fatty acid of mycobacteria. It can be detected with the gas chromatography - mass spectrometry (GC-MS) combined with selective monitoring. The detection of TBSA in CSF samples results in the rapid diagnosis of tuberculous meningitis (TBM). However, it is a specialised technique and impractical for the average routine laboratory.

### **Antigen detection**

Enzyme linked immunosorbent assays (ELISA), immunoperoxidase staining techniques using polyclonal and monoclonal antibodies (MABS) have been reported for antigen detection. Likewise, agglutination of sheep RBC sensitized with MABS directed against lipid arabinomannan (LAM) was used to detect antigens in TBM patients. Similar tests have been evolved using staphylococcus and latex particles bound with rabbit antibodies against LAM. None of these tests have been used on a large scale and their clinical use yet to be proved.

### **High performance liquid chromatography (HPLC)**

In this technique, the pattern of high molecular weight fatty acids unique to mycobacteria and related genera, namely the mycolic acids, is generated and compared

using an automated chromatography after extraction of the lipid component of the mycobacterial cells using standardised protocols. These patterns have been found to be species - specific allowing rapid identification of mycobacterial cultures. This method is reliable, easy to perform and cheaper than nucleic acid based identification methods. However, this technique requires considerable expertise.

### **Identification**

The conventional identification procedures include tests such as determination of growth rate, growth at different temperatures, colony morphology, pigment production and susceptibility to para-nitro benzoic acid (PNB). The main disadvantage here is that species identification takes an additional 4 weeks using these methods.

Other rapid techniques include DNA probes, nucleic acid amplification including polymerase chain reaction (PCR), strand displacement assay (SDA), line probe assay (LIPA), restricted fragment length polymorphism (RFLP), pulsed field gel electrophoresis (PFGE) and phage reporter assay. These methods have been found to be helpful in the detection of cases, identification and differentiation of mycobacteria, in measuring drug resistance and epidemiological finger printing of mycobacteria obtained in different clinical setting.

### **Indirect approach**

#### **Skin tests**

Delayed type hypersensitivity (DTH) reaction following intradermal injection of a predetermined amount of tuberculin or purified protein derivative (PPD) remains the simplest and frequently used method of inferring tuberculous infection. Although skin test does not discriminate between active

tuberculosis, past infection with mycobacteria or BCG vaccination, in the diagnosis of childhood tuberculosis it plays an important role especially in association with contact history<sup>4</sup>.

### **Antibody detection**

In this technique, antibodies against mycobacterial antigens in sera from patients by enzyme linked immunosorbent assay (ELISA) is done using polyclonal or monoclonal antibodies. When crude mycobacterial preparation are used for these tests, the test has low specificity. The most promising purified antigens among those available so far are the 38KDa antigen and A60 antigen from *M. tuberculosis*. However, it appears unlikely that all tuberculosis patients will recognise a single antigen which may prove to be a handicap for antibody based detection systems for mycobacteria. Hence, diagnosis cannot be made solely based on currently available serological tests.

### **Conclusions**

Today many new techniques are available for the detection and identification of *M. tuberculosis*. However, detection of AFB by direct microscopy and identification of cultured mycobacteria by biochemical methods are still the recommended methods for developing countries. In addition, direct drug susceptibility testing with smear positive sputum deposits for rifampicin and isoniazid to the extent possible might help in the management of patients with drug resistant organisms. Faster culture methods using radiometric systems such as Bactec are being recommended mainly because they reduce the time of culture to about two weeks. Nucleic acid amplification techniques are mainly employed for cases where there is a chance that the infection may be due to a mycobacte-

rium other than *M. tuberculosis*. It is also to be remembered that most of the new techniques described involve prohibitive expenditure in terms of instrumentation, expertise and reagents and they are beyond the means of most public health laboratories in the developing countries such as India. Hence, in the diagnosis of childhood tuberculosis, a thorough evaluation of clinical condition, eliciting history of patient's contacts, skin test finding and radiological diagnosis may still remain as the most important tools in the years to come. Other laboratory investigations may only play an adjunct role as of now.

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