DIAGNOSIS OF TUBERCULOSIS CURRENT STATUS

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Tuberculosis is a major cause of morbidity and mortality in developing countries. Out of an estimated global prevalence of 30 million cases, India alone accounts for 10 million cases, with an estimated half a million deaths(l). One of the principle reasons for the failure of tuberculosis control programs in developing countries is the in-ability to detect infectious case early enough(2).

Despite huge amount of research, there is no simple, reliable and rapid test for the diagnosis of active tuberculosis. The only sure way of diagnosing tuberculosis at present is to find the tubercle bacillus. But the culture methods are slow and insensitive in multibacillary pulmonary tuberculosis. The difficulties are magnified in the case of paucibacilliary pulmonary and non-pulmonary disease(3,4).

Many workers have attempted various techniques to diagnose tuberculosis rapidly and reliably. The approaches are excellent in principle but many problems have arisen in practice, *viz.,* lack of sensitivity and specificity, inability to differentiate between active disease from past infection, BCG vaccination and environmental sensitization, high cost of equipments and reagents, financial and technical difficulties in training of personnel.

The various tests available are briefly discussed below:

(1) Smear Examination for Acid Fast Bacilli

The conventional method for rapid diagnosis of tuberculosis is by smear examination for acid-fast bacillus. It has serious limitations since smear identification requires a minimum of 5,000 to 10,000 organisms/ml of specimen(5). Moreover, it does not differentiate between pathogenic and contaminant mycobacteria. The test is not much helpful in the paucibacillary tuberculosis of childhood. The demonstration of the organism in the CSF of tuberculous meningitis patients by smear examination is possible in less than 5 % of cases. Fluorescein microscopy using auromine increases positivity. Centrifugation also increases positivity but there are more chances of contamination by saprophytes.

(2) Culture Identification

The 'Gold standard' for the diagnosis of tuberculosis is the identification of the mycobacteria by culture method. However, the identification on Lowenstein-Jensen medium

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takes 3-4 weeks. Isolation of the organism has become important with emergence of drug resistant strains. Because conventional tests are relatively slow, there has been great interest in using radiometric systems(6). The principle behind such systems involves the incorporation of a radiolabelled 14 C palmitic acid by the growing mycobacterial **in** column with the consequent release of 14 CO_2. It is the detection of this gas that allows the early identification of mycobacterial growth prior to the presence of visible growth. It is possible to identify the growing mycobacteria within a week. Drug susceptibility tests can also be adopted to such a system with additional savings in time(7).

(3) **Tuberculin Skin Test**

It was the first immunodiagnostic test introduced for diagnosing tuberculosis. It is still widely used to determine the annual infection rate of tuberculosis in a country, to assess the effectiveness of control measures, to indicate those requiring BCG vaccination or chemoprophylaxis. The test can not distinguish active tuberculosis from past infection or sensitization by BCG/environmental mycobacteria. Moreover, the test will be negative in a malnourished child, intrinsically non-reactive or in advanced cases of tuberculosis. It is possible to improve the diagnostic potential of the skin testing by sophisticated physiological studies of the reaction(8).

(4) Chest **Radiography**

The radiological signs suggestive of tuberculosis are well described but most of these signs are not very specific. The diagnosis of primary complex in children is so complex that one physician starts treatment only to be stopped by another(9).

(5) Acute Phase Reactants

The acute phase reactants like erythrocyte sedimentation rate (ESR), C-reactive protein are of little value in the diagnosis and can help only as **a** guide to resolution or reactivation of tuberculosis(10).

(6) **Precipitation Test**

This could **be a** simple test for the diagnosis of tuberculous meningitis. The modified Lavinson's precipitation test consists of taking equal amount of CSF samples in two same sized test tubes. An equal amount of 3 % sulfosalicylic acid is added to the first test tube and 6% mercuric chloride to the second test tube. The tubes are incubated at 37°C for half an hour and 4°C for 2 hours. If the height of the precipitation in the test tube with mercuric chloride is 3 times more than that with sulfosalicylic acid then the test is positive. The test is more sensitive than cobweb formation in CSF but not specific(11).

(7) **Adenosine Deaminase Estimation**

Adenosine deaminase is an enzyme produced by* T-lymphocytes. It catalyses the deamination of adenosine into inosine and ammonia. The enzyme activity is inversely proportional to the degree of cell differentiation. Adenosine deaminase activity was increased in tuberculous infection of the pleural, pericardial, peritoneal and meningeal spaces. However, some authors have observed false positive results in 16% of bacterial meningitis cases(12-14).

(8) **Bromide Partition Test**

The partition of bromide ion between serum and CSF after a loading dose reflects the integrity of the blood-brain barrier. The simultaneous estimation of bromide from blood and CSF is done 24 hours after **a** loading dose of sodium bromide intravenously. A ratio of less than 1.6 is obtained in cases of tuberculous meningitis. The

sensitivity and specificity of the test is around 9096(15,16).

(9) Biochemical Identification of Mycobacterial Products

It is tempting **to** think that recognizing mycobacterial products or constituents by biochemical means should be highly specific. There are reports of detection of 3-2-ketohexy indoline by electron-capture gas chromatography and detection of tuberculostearic acid by gas chromatography or mass spectroscopy in the CSF of patients with tuberculous meningitis. Patients receiving amikacin therapy give false positive results for estimation of tuberculostearic acid. These tests require complex instrumentation which may not be available in developing countries(17,18).

(10) **Antibody to Mycobacterial Antigens**

In view of the problem encountered with the tuberculin and other tests for cell-mediated immunity much attention has been paid to the serodiagnosis of tuberculosis. There are many methods but none has yet found a place in regular clinical use. Agglutination, precipitation and complement fixation tests have been tried initially. During the 1960's the immunofluroscent technique was introduced in the mycobacterial field. Conventional indirect fluorescent antibody test by placing the patients sera on a smear prepared from mycobacterial culture was later replaced by the soluble antigen fluorescent antibody (SAFA) test. The test is modified using soluble antigen fixed on an artificial matrix and measuring the fluorescence objectively using a fluorometer(19,20). Enzyme linked immunosorbent assay (ELISA) and radioimmunoassay have been used with increased sensitivity for detecting the antibodies(21-23).

The major limiting factor in the serodiagnosis of tuberculosis is that no single specific antibody against mycobacterium tuberculosis has been shown to be significantly elevated in all cases of tuberculosis. Most of the antibody response in tuberculosis is directed towards those antigens common to all mycobacteria and to some other genera. The antibody response in tuberculosis is a weak one. Sensitive assays show that virtually all individuals have such antibodies as a result of exposure to environmental mycobacteria. The chances of overlap of levels is greater in patients with paucibacillary tuberculosis and tuberculous like infections. Thus the available tests fail to help in the very circumstances where they should have been most useful.

With the modern immunoassay methods, sensitivity is not a problem but specificity is. This is due to the fact that the pattern of antibodies varies from person to person probably due to genetic factors. Antibody assay to a single species antigen by monoclonal antibody based tests are negative in a substantial number of patients with tuberculosis. This problem can be partially overcome by assaying antibodies to a variety of antigens. But such methods are costly and cumbersome for routine use(18,24).

(11) Detecting Mycobacterial Antigens

The tests based on the detection of mycobacterial antigens rather than antibody response to them is likely to prove more successful. A number of antigen-capture assays have been described usually based on enzyme-linked immunosorbent assays (ELISA) or agglutination of antibody coated latex particles. ELISA with anti-BCG antibody has been successfully used by several workers. They are suitable for detection of antigens in 'clean' materials such as CSF, pleural or peritoneal fluid. It is likely that

specimens such as sputum will contain many interfering substances and may, therefore, require some form of pretreatment. An alternate approach would be to apply an antigen detection lest to a liquid culture after a few days of incubation.

Primary tuberculosis is a systemic disease and antigen may be detected in the blood. Alkana *et al.* in studies in childhood tuberculosis found a heat stable polysaccharide antigen in the serum at an early stage of the disease and antibody to the antigen at a later date. However, there was an intermediate stage when neither antigen nor antibodies were detected unless the immune complex was dissociated by heating. There is an inverse relationship between antigen and antibody so that estimation of both greatly increased the diagnostic efficiency(25-28).

(12) Nucleic Acid Probes

The genome of any micro-organism contains DNA base sequences that are specific for genus, species and strain. The tuberculous bacillus has specific DNA sequence which can be detected on clinical specimens after suitable preparation by hybridization with specific cloned DNA probes. In principle this appears to be a sensitive diagnostic technique. However, in practice, at its present state of development this is not true. The level of detection by such probes is about 50 picograms of DNA which is the amount present in about 10,000 mycobacterial cells. The sensitivity of smear microscopy is about 5,000 to 10,000 bacilli/ml of sputum(29-31).

(13) Amplification of Mycobacterial DNA and Polymerase Chain Reaction (PCR)

This newer diagnostic approach is based on artificially increasing the number of markers of the bacteria present in a given sample using the polymerase chain reaction(32-35). The sensitivity can be further increased by the amplification of DNA sequence present in multiple rather than single copies in each genome(36). The specificity of the test depends on the precise DNA sequence chosen for amplification. It can allow the detection of as little as one genome in the given sample. The extreme sensitivity of the technique could be a problem when there is contamination during laboratory methods(37).

In conclusion, the definitive diagnosis of tuberculosis other than by smear examination or culturing the organism is still difficult. The search for simple, cheap and reliable immunological and chemical detection techniques has been fraught with disappointment. There has been slow and erratic progress. The rapidly advancing discipline of molecular biology and mycobacterial immunology may open up new approaches.

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