Indian Journal of Clinical Biochemistry, 2003, 18 (2) 1-5

IMMUNODIAGNOSIS OF TUBERCULOSIS: AN UPDATE

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Running Title: Immunodiagnosis of tuberculosis

ABSTRACT

Tuberculosis is still a major health problem in most developing countries and its incidence is rising in many developed countries. This resurgence has been attributed to the HIV epidemic and TB has been declared as a global health emergency by WHO in 1993. The diagnosis of tuberculosis mainly depends upon initial clinical suspicion and radiographic findings with subsequent bacteriological confirmation by sputum smear examination and culture. Lack of sensitivity in smear examination, non specificity of radiological findings, extended turn around time of *Mycobacterium tuberculosis* culture and difficulties in diagnosing paucibacillary, childhood and extrapulmonary tuberculosis has necessitated to explore the utility of immunodiagnosis of tuberculosis as a convenient and cost effective test to supplement clinical information for definite diagnosis. Many commercial tests are available in the market for diagnosis of TB. Most of these tests are based on the detection of IgG, IgA and IgM antibodies to specific mycobacterial antigen or mixture of antigens. Indigenous immunoassay systems have explored excretory-secretory ES-31 mycobacterial antigen for immunodiagnosis of TB. Many a time there is lack of consistent elevation in all the three Ig classes in active infection thus making it more important to determine the ideal antibody isotype assay for reliable diagnosis of tuberculosis and to save the costs of the patient for unnecessary investigations.

KEY WORDS

Mycobacterium tuberculosis, immunodiagnosis, ELISA, antigen, antibody

India accounts for nearly one third of the global burden of tuberculosis and it is a major barrier to socioeconomic development along with being one of India's most important public health problems. In India tuberculosis kills 14 times more people than all tropical diseases combined, 21 times more than malaria and 400 times more than leprosy. Everyday in India more than 20,000 people become infected with tubercle bacillus, more than 5000 develop the disease and more than 1000 die from tuberculosis. Every year another 20 lakh people develop tuberculosis in India. The direct and indirect cost of TB to the country amount to Rs 12000 crore (US $ 3 billion) per year. (1).

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Due to problem in early and unequivocal detection and failure of control programmes for various reasons the TB problem will continue to evade solution.

DIAGNOSIS OF TUBERCULOSIS

Ever since the discovery of Kochs Bacillus, the diagnosis of tuberculosis still largely depends upon the clinical examination, radiographic findings with subsequent laboratory confirmation by bacteriological examination. Smear examination and *in vitro* culture of tubercle bacilli has remained the gold standard. But the sensitivity of AFB smear is compromised because greater than 10^4 bacilli per ml of sputum are required for reliable detection. Though sputum examination is supposed to have 75% sensitivity and 98% specificity but in practice, the sensitivity has been observed to be around 30% for a number of reasons (2). The failure rate being substantially greater in children because they rarely produce sputum specimen adequate for bacteriological examination. The *in vitro* culture methods require an extended turn around time. The

methods using solid media usually takes 3-8 weeks to recover organisms. More recently various techniques which rely upon radio labeled substances in liquid medium (e.g. BACTEC) have been employed in an effort to substantially reduce the time for identification. Rapid species identification can be achieved in these systems by species specific nucleic acid probes or by the identification of specific cell wall mycolic acid. Although sputum digestion, decontamination and concentration have increased the yield of bacilli for *M. tuberculosis,* the organism may be killed if the process is improperly performed resulting in false negative culture methods. To overcome these shortcomings of bacteriological methods, new diagnostic techniques like PCR and other amplification techniques to detect mycobacterium tuberculosis DNA in clinical specimens, have been developed (3). But still these tests are not popular due to lack of sophisticated facilities and training and high cost involved. For all these reasons, the detection of active TB by serological means has attracted the attention of a number of laboratories all over the globe.

TB serology based on antibody and antigen detection for diagnosing and monitoring tubercular infection with low cost and flexibility to adopt to small laboratories may be a boon to the developing countries.

TB SEROLOGY

About sixteen years after Kochs identification of *M.tb,* Arloing in the year 1898, described an agglutination test for the diagnosis of tuberculosis. But he did not provide details about actual procedures involved which hindered the further research in the field for number of years. Then in 1918 Brown and Petroff reported the use of CFT for diagnosis of TB in 540 patients. But no useful information on the specificity of the test was provided. After these beginnings, many serological procedures were evaluated for diagnosing tuberculosis. The situation changed dramatically with the introduction of ELISA by Engvall and Perlman in 1972. Nassau and coworkers applied ELISA to the serodiagnosis of tuberculosis in 1976 achieving a sensitivity of 57% and a specificity of 98% in 46 patients and 48 controls (4). Many semipurified, purified and immunodominant mycobacterial antigens are being used in commonly available commercial kits in India, like A-60 antigen (Anda-TB, Anda biologicals Strasbourg France and Erba Lisa Transasia India), 38 kDa and 16 kDa recombinant antigens (Pathozyme TB Complex plus), 38 kDa with another antigen purified form *M. tb.* present in all members of the genus mycobacterium (Pathozyme Myco, Omega Diagnostics UK).

Various studies which have measured the levels of different antibodies against these antigens are briefly reviewed here.

A60 ANTIGEN

The A 60 antigen used in ERBA LISA kit is a thermostable component of PPD and has been used in the serodiagnosis of TB. Unfortunately this molecule is not specific for mycobacteria, because it is also present in the Nocordia and Corney bacterium species. In a large study conducted (5) two ELISA tests (IgG and IgM) for serodiagnosis of TB, were applied to 1,644 controls and patients to analyse the immune response in different forms of this infectious disease. Out of 200 healthy individuals, 148 being tuberculin positive and BCG vaccinated, only 10 contacts, (Nurses, Lab Technicians) were found positive for Anti A 60 IgG. Out of 344 cases of primary Pulmonary TB 88% were positive for IgG class and 75% for the corresponding IgM class. Among 97 cases of primary EPTB 94% were found to be IgG positive and only 33% IgM positive. About 100% of both Pulmonary and EPTB (367 cases) had high titres of IgG levels, but IgM positivity was observed in only 15% of the cases whereas in inactive and quiescent non cavitatory TB (442 cases) 57% of the patients were weakly positive for IgG class and none was positive for IgM. Anti A 60 IgM marks the initial stages of the disease whereas IgG lasts longer than IgM and provide an evaluation of the intensity of the infectious process. In another study on infected children with or without disease, high IgG level was observed in disease cases (6). Measurement of IgM levels from control and diseased children overlapped leading to low sensitivity (19%) in children with clinically active TB.

Like wise many other studies (7, 8) have evaluated the diagnostic potential of different classes of immunoglobulins against A-60 antigen and IgG is found to be quite sensitive and specific. It has been seen that tuberculosis patients were usually positive for IgG antibody than IgM.

38 KDa ANTIGEN

The 38 kDa antigen was originally described as a

component of antigen 5 (9). Since then it has been proved to be specific (9,10,11,12) but the sensitivity of the test even with the addition of 16-kDa antigen was low. Detection of IgG against 38 kDa antigen has revealed a sensitivity of 64% and specificity of 81% (13,14). The evaluation of Pathozyme TB complex and pathozyme myco was undertaken along with other serological tests for detection of IgG, IgA and IgM antibodies. Combining IgA or IgM as an adjunct test increased the sensitivity but at the cost of specificity (15). In comparative analytical study using 5 different commercial tests(16) (Pathozyme TB complex, Pathozyme Myco, Detect TB, Tuberculosis IgG ELISA and Myco Dot) to evaluate the use of mycobacterial antibody detection in an endemic area, the sensitivity of individual kits varied from 46% to 68%. In serodiagnosis of EPTB, using 38 kDa for IgG antibody detection, it was found to be 73% sensitive and 98% specific (17). Antibody IgG titres were also found to be related to the extent of the disease.

SEVA TB ES-31 ANTIGEN

In an elegant study (18) using secretory proteins, ES-31 antigen for detecting IgM, IgA and IgG immunoglobulins in clinically and bacteriologically confirmed PTB cases, it was found that detection of IgG antibody against ES-31 Ag showed better sensitivity and specificity. The IgM positivity was observed in increased number of healthy normals compared to IgG or IgA, and may have been due to contact with environmental mycobacteria or prepatent infection. On analyzing immune response in TB cases with different grades of sputum positivity or bacillary load, the IgG response was found to be predominant in all grades. The predominance of IgG antibody response may be due to the establishment of infection and continuous antigenic stimuli secreted from live bacilli. It was observed in this study that amongst SEVA TB ES-31 antigen specific Ig class, IgG alone with considerable sensitivity and specificity is more useful for detection of TB infection compared to IgM or IgA alone or in combination in patients who are coming with the signs and symptoms of disease attending the hospital.

Swati Banerjee *et al* (19) analysed tubercular antibody, circulating free and immune complexed antigen (CIC-Ag) in confirmed pulmonary tuberculosis sera by ELISA, using ES-31 antigen and affinity purified anti ES-31 antibody out of 25 confirmed tuberculosis sera 92% were observed to be positive for IgG antibody to ES-31 antigen. Using anti ES-31 antibody free tubercular antigen could be detected in 80% cases whereas circulating immune complexed antigen (CIC-Ag) in 72% cases by Sandwich ELISA. Of the two sera showing absence of antibody, one showed presence of free and CIC-Ag whereas the other showed the presence of free antigen. They concluded that antigen assay may be used as an adjunct tool for confirmation of pulmonary tuberculosis along with antibody assay.

CHOICE OF IMMUNOGLOBULIN CLASS IN TB SEROLOGY

Many studies have included measurement of antibody to mycobacterial antigens separately for immunoglobulin class i.e. IgG, IgM, IgA for which necessary reagents are readily available from many commercial sources. The worried patient and a doubtful clinician gets all the classes of immunoglobulins (i.e. IgG, IgA, IgM) done and finally cannot come to definitive conclusion due to inconsistency of different antibody assays. It is important that the serological tests should be unequivocal and should have the credibility for the clinicians to rely on. Though a number of commercial serodiagnostic ELISA based on antibody to TB antigen tests entered the market, more commonly available test kit in use are that of ANDA Biologicals (France) and Omega Diagnostic (UK). These kit inserts do quote literature to support the usefulness of the tests for serodiagnosis of TB. While ANDA TB has extensively quoted to show usefulness of IgA, IgG and IgM. Pathozyme-myco has an insert in 1995 for IgG assay while in 1988 it shows IgG and IgA assays with the same literature. Self serving and aggressive manufacturer's supported research and commerce have further complicated the understanding of usefulness and reliability of the different tests in particular based on detection of different classes of immunoglobulins.

In most of the studies, it has been seen that Immunoglobulin G holds the great promise in diagnosing an active disease in both children as well as adults, as compared to IgM or IgA class. IgG is also found to be an useful antibody for monitoring the response of antitubercular treatment. In a cross sectional study (20), on changes in IgG, IgA and IgM levels along with other serum proteins in Pulmonary TB during therapy, serum IgG and IgA levels have mostly reported to be increased while most of the authors have reported unchanged IgM levels. IgG has been found to be much higher ($p<0.001$) at 0 month compared to control population

while no significant difference was found in IgA class. IgM levels at 0 month were also higher when compared to control groups. In general IgG levels have been found to be increased in active TB (21-27), increased IgM levels have also been reported to be in two studies (22, 25) while several studies revealed no significant change (21, 23, 24, 26). IgA levels have also been reported to be increased in several studies (21, 23, 24, 25, 27) but more or less in extensive, advanced diseases which are unlikely to be missed on clinical examination. Measurement of different classes of immunoglobulins using different antigenic preparations have shown that IgM antibody levels have been found to be so low that their reliable measurement has been difficult. IgA levels have generally paralleled IgG class, but also have tended to be low and more difficult to measure reliably. IgM is found to be the initial antibody produced. This feature suggests that the presence of IgM antibody to TB protein antigen might be characteristic of early disease, which may not hold much diagnostic promise because of the delay on the part of the patients in visiting qualified doctors.

It is also high time for the practising clinician to rethink on the unnecessary financial burden put on poor patients by long list of diagnostic tests. Regarding low cost and relative simplicity of the technique, ELISA will be a boon for the poor patients. At present, considering the delay of patients in getting consultation from qualified person, we have to decide whether it is useful to go for all the classes of immunoglobulins against mycobacterial antigens? As various studies have revealed, it will be better to go only for 'IgG' estimation assay as an adjunct test for confirming clinical suspicion. Assay of IgM and IgA will not be of much help in eliciting any more information. Further in tuberculosis cases negative for IgG antibody, antigen detection may be more useful as observed with 31 kDa antigen(19) in confirming active tuberculosis infection.

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