

Full Length Research Paper

Detection of mycobacterial antibodies in serum samples by enzyme linked immunosorbent assay

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Tuberculosis (TB) is a common and deadly infectious disease caused by *Mycobacterium tuberculosis* and rarely by other subspecies of the *M. tuberculosis* complex, such as *Mycobacterium bovis*. Enzyme-linked immunosorbent assay (ELISA) for diagnosis of tuberculosis has been widely explored over the years. The purpose of this study was to reduce the time duration required for the diagnosis of tuberculosis. We have extracted antigens from the *M. tuberculosis* strain H37Ra. Antibody response was studied using ELISA. A total of 30 samples were analysed for their specificity and sensitivity. Of which, 20 were positive and the sensitivity and specificity of the test appeared to be 89%. The high sensitivities, specificities and promising antigenic combination in detection of TB suggest their potential application in diagnosis of TB.

Key words: Enzyme-linked immunosorbent assay (ELISA), mycobacterial antigens, tuberculosis.

INTRODUCTION

Accurate and early diagnosis of tuberculosis (TB) is crucial for effective patient management and TB control. Moreover, accurate identification of TB is the key to prevent the disease among persons at risk. Despite the enormous global burden caused by TB and overall low rates of case detection, conventional approaches to diagnosis continue to rely on test that have major drawbacks (Sooraj et al., 2011). For example, sputum smear microscopy is insensitive; culture is technically complex and slow; determination of drug susceptibility is even more technically complex and slower; chest radiography is non-specific. Smear microscopy lacks sensitivity and cases of infectious tuberculosis could be

missed (Berean et al., 1988), therefore isolation of *Mycobacterium tuberculosis* through sputum culture either on solid or selective liquid medium remains the gold standard in TB diagnosis (Monteyne and Sindic, 1995). This technique increases both sensitivity and specificity but requires several weeks before the results are known. The main problem is with the patients of extrapulmonary tuberculosis and children, who characteristically do not produce sputum. Moreover, the yield of tubercle bacilli in these patients is poor (Samuel et al., 1984; Watt et al., 1988; Chandramukhi et al., 1989; Zheng et al., 1989; Patil et al., 1996).

Novel methods, such as the radiometric Bactec culture technique and the commercially available deoxyribonucleic acid (DNA) probes have considerably reduced the time needed for culture and identification of mycobacteria. However, these procedures still require up to 1 to 3 weeks to be completed (Lebrun et al., 1997).

In view of these limitations, there is need for less

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Abbreviation: TB, Tuberculosis.

complicated and more accurate tests. Although, truly major advances that would revolutionize TB diagnosis and treatment have not been realized, we are beginning to see the innovations that have been prompted by the recognition of the economic potential of the market for new diagnostic tests and treatments for TB and considerably increased public and private funding.

The purpose of this study is to reduce the time duration required for the diagnosis of tuberculosis. The average incubation period required for the growth of this organism is estimated to be about 6 weeks. During this time period, the infection might be fatal to the patient and will lead to rise in mortality rate. Even though the gold standard diagnostic tool for the organism is culture, the time duration for incubation masks its specificity. In order to overcome this, a variety of diagnostic tools that have sensitivity rate of culture techniques can be developed. We have extracted antigens from the organism and used them for the diagnosis of infection by ELISA technique.

MATERIALS AND METHODS

Sera samples from untreated bacteriologically confirmed sputum positive and sputum negative TB cases were used in this study. The serum samples from tuberculosis patients and healthy individuals with no history of TB were obtained from Microbiological Laboratory, Coimbatore, India. Sera were stored at -20°C after adding sodium azide (0.1%) as preservative.

Extraction of *M. tuberculosis* antigens

M. tuberculosis H37Ra (ATCC 25177 from MicroBioLogics, USA) was inoculated onto thymine supplemented Lowenstein-Jensen slants (Hi-Media, Mumbai) and incubated at 37°C for 6 weeks (Pramanik et al., 1997). After incubation, bacilli were inactivated in 5 ml of 5% phenol for 1 h at 4°C. The cells were washed twice in normal saline and then suspended in 4 ml of 0.05 mol/L phosphate-buffered saline (PBS), pH 7.2. The bacilli were sonicated with 30-s bursts (15%, 150W) at 1-min intervals for 30 min. The sonicate was incubated with 2 ml of sodium dodecyl sulfate (SDS) extraction buffer (5% SDS, 5% 2-mercaptoethanol and 8 mol/L urea in 0.01 mol/L PBS, pH 7.2) in boiling water bath for 5 min, followed by incubation at 4°C for 24 h. After centrifugation at 10,000 rpm and 4°C for 30 min, the supernatant was separated, dialyzed against 0.01 mol/L PBS; pH 7.2; for 48 h. The protein content of the antigen was determined by Lowry et al. (1951) method. The antigen was stored at -20°C till future use (Harinath et al., 2006).

Coating of ELISA plates

The ELISA plates were coated as per the procedure described by Kadival et al. (1987) with some modifications. Two 96 well U bottomed polystyrene microtitre plates (Tarsons, India) were coated with the antigens (50 µg/ml in PBS). Fifty microliters of this solution was transferred into the wells of ELISA (Microtitre plates). The plate was kept in the refrigerator overnight. The next day, plate was washed with PBS Tween solution for 3 times, 5 ml of 20x PBS solution was taken and its volume was made into 1x solution with distilled water. To this, 100 ml of solution (2 g) of skimmed milk powder was added. The plates were decanted after overnight incubation and non specific binding sites were blocked by adding 150 µl of 1% PBST milk which was added into Ag coated ELISA plate. Then, the plate was incubated at 37°C for 2 h. The plates were blot dried and stored in the refrigerator.

ELISA assay

The ELISA was performed in 96 well polystyrene microtitre plates as per the procedure described by Chaturvedi et al. (2001) with some modifications. Briefly, 1% PBST milk was prepared (0.5 g of skimmed milk powder was added to 50 ml of PBST solution which is the sample diluent). Four hundred microlitres of PBST milk was added into each dilution tube and one hundred microlitres of sample were added to it. These 1:5 dilutions were mixed well. The microtitre plates were marked as MTSE IgG and MTSE IgM. The first well is the blank and to the second well, MTSE positive control was added. From the dilution tubes, 50 µl was transferred to the ELISA plate wells in duplicates and plates were incubated at 37°C for 1 h and 30 min. After incubation, the plates were washed six times with PBST solution and blot dried. The conjugate IgG HRP and IgM HRP (Sigma, USA) of 5 µl were added to 15 and 5 ml of PBST milk (diluent). Fifty microlitres of IgG conjugate was added to the plate (1:3000 dilution) MTSE IgG and 50 µl of IgM conjugate was added to plate (1:1000 dilution) MTSE IgM. The plates were then incubated at 37°C for 1 h. After the incubation, the plates were washed with the wash buffer (PBST) for 8 times and blot dried. Seventy five microlitres of the substrate (ortho phenylene diamine dihydrochloride in phosphate citrate buffer and hydrogen peroxide) was added into each well of ELISA plates. The plates were then kept in dark place for 30 min for colour development. The colour change was noted and the reaction was stopped using the stop solution 1 N sulphuric acid (50 µl) in each well of ELISA microtitre plates. The plates were read using the ELISA reader at 492 nm.

RESULTS AND DISCUSSION

Tuberculosis is a disease of great antiquity and has probably caused more suffering and death than any other bacterial infection (Bassey et al., 1996). Early diagnosis of this infection is of 'utmost concern' for successful control (Sande et al., 1992).

About 30 clinical samples were analyzed out of which, 20 appeared to be positive. Among the positive samples, 8 showed high IgG positivity; 6 showed high IgM positivity and the remaining 6 showed both IgG and IgM positivity (Figure 1).

IgG positivity showed chronic state of infection and IgM positivity showed acute state of infection. All samples were inoculated into LJ slants for the confirmation of infection. The sensitivity and specificity of the test appeared to be 89% (Figure 2).

Earlier studies demonstrated that the detectable anti-TB antibody was found in cerebrospinal fluid (CSF) (Samuel et al., 1983; Kalish et al., 1983; Chandramukhi et al., 1985). The detection of antibodies correlated well with the disease and was positive in 68 to 80% of the cases. Kadival et al. (1982) and Samuel et al. (1983, 1984) have shown that antigen or antibody detection is useful in the diagnosis of pulmonary, meningeal, pleural and abdominal tuberculosis by radioimmunoassay. Sada et al. (1984) in a limited study of 10 CSF samples from patients with tuberculous meningitis showed the potential usefulness of ELISA in the detection of antigen.

Conclusion

In this study, we have developed a specific and sensitive

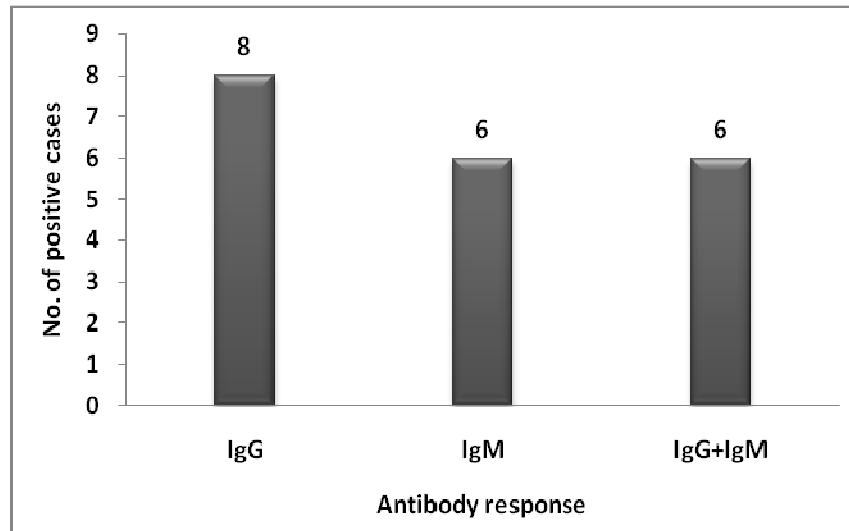


Figure 1. Graphical representation of results.

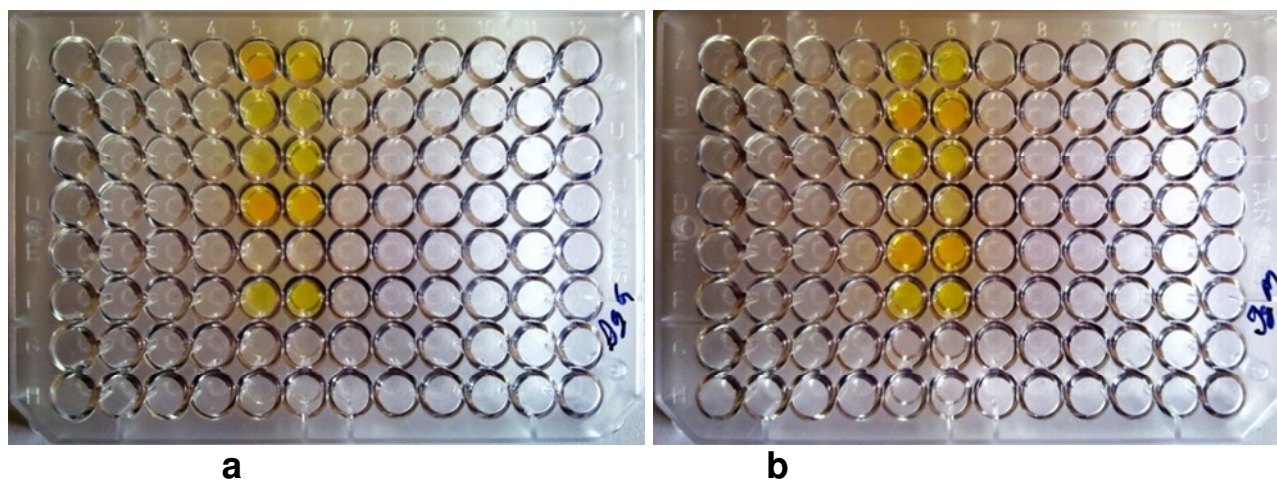


Figure 2. Microtitre plates showing (a) IgG and (b) IgM response.

assay for the detection of anti-TB antibody which can be used for the diagnosis of tuberculosis. This can be adopted as a method of choice for the diagnosis of mycobacterial infections in cases where suspicion is high in combination with other clinical criteria and could be an alternative to other more expensive sophisticated techniques.

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