



RESEARCH ARTICLE

IMMUNOLOGY

## DETECTION OF MYCOBACTERIAL INFECTION USING FILTRATE ANTIGEN

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**ABSTRACT**

Among infectious diseases, TB remains a leading cause of morbidity and mortality worldwide. Identification of tuberculosis infected individuals is essential in order to control the disease. Therefore there is a need for newer methods for rapid, sensitive and accurate diagnosis. In the present study, we have made use of antigens for the organism extracted from *Mycobacterium tuberculosis* H<sub>37</sub>Ra (ATCC 25177). ELISA technique performed gave sensitivity and specificity of 90% of TB in *M. tuberculosis* infected patients confirmed by culture. We conclude that the performance of extracted antigens is valuable in the diagnosis of TB.

## KEYWORDS

Tuberculosis, ELISA, Filtrate antigens, Diagnosis.

## INTRODUCTION

Tuberculosis continues to pose a diagnostic problem especially in developing countries. The diagnosis of pleural and peritoneal tuberculosis still remains problematic, as evidenced by reports indicating the absence of acid-fast bacilli on microscopic examination in more than 60% of patients<sup>1,2,3,4</sup>.

Histological examination of pleural biopsy specimens can be unhelpful for diagnosis for over 20% of patients with pleural tuberculosis<sup>4,5</sup> whereas peritoneal biopsy specimens are diagnostic for less than 40% of patients<sup>1,3,6</sup>. Direct examination of ascitic fluid reveals acid-fast bacilli only when large volumes of fluid are concentrated<sup>7</sup>. In addition, many patients have negative results on skin tests for tuberculosis, and chest X rays are often normal<sup>8</sup>.

Cultures of cerebrospinal fluid (CSF) specimens are not always helpful in the diagnosis of tuberculous meningitis. Cultures may show no growth or take up to 6 weeks to become positive for *Mycobacterium tuberculosis*<sup>6,9</sup>. Failure to diagnose the disease promptly in its early stages results in an increase in mortality. Sensitive tests such as radioimmunoassay and enzyme-linked immunosorbent assay (ELISA) for the detection of *Mycobacterium tuberculosis* antigen<sup>6</sup> or antibody<sup>9</sup> appear to be promising procedures for the early diagnosis of tuberculosis.

In the present study, 30 serum specimens from patients with diagnosed tuberculosis were examined for the presence of *M. tuberculosis* antibody by a sensitive and specific ELISA technique.

## MATERIALS AND METHODS

### Serum samples

Sera samples from untreated bacteriologically confirmed sputum positive

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

### ATCC Strain

*M. tuberculosis* H<sub>37</sub>Ra (ATCC 25177 supplied from MicroBioLogics, USA) was used.

### *M. tuberculosis* culture filtrate antigen preparation

*M. tuberculosis* culture filtrate antigen was prepared as per by Collins *et al.* (1988) with some modifications<sup>11</sup>. *M. tuberculosis* H<sub>37</sub>Ra was cultured in synthetic Sauton's medium (Hi-Media, Mumbai) enriched with 0.5% glucose, 0.5% sodium pyruvate and 0.05% tween 80 for 3 weeks (35 °C; 5%-10% CO<sub>2</sub>) and was centrifuged at 12,000 x g for 30 min at 4°C, and the supernatant fluid was sterilized by filtration through a 0.22 µm-pore-size membrane (Sartorius Stedim biotech). The proteins were precipitated with 80% ammonium sulfate in cold overnight, dissolved in sterile phosphate-buffered saline (PBS) and dialyzed at 4°C until free of ammonium ions. The protein content was determined by the method of Lowry against a bovine serum albumin (BSA) standard<sup>12</sup> and adjusted to 5mg/ml<sup>13</sup>. The protease inhibitor phenylmethylsulfonyl fluoride was added to a concentration of 10 mM. The culture filtrate preparations were stored at -20°C until required.

### Coating of ELISA plates

The ELISA plates were coated as per the procedure described by Kadival *et al.* (1987) with some modifications<sup>14</sup>. Two 96 well 'U' bottomed polystyrene microtitre plates (Tarsons, India) were coated with the



antigens (50µg/ml in PBS). 50 µl of this solution was transferred into the wells of ELISA (Microtitre plates). The plate was kept in the refrigerator for 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 100ml of solution 2g 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 was added into Ag coated ELISA plate. Then the plate was incubated at 37 °C for 2 hours. The plates were blot dried and were 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<sup>15</sup>. Briefly, 1% PBST Milk was prepared (0.5g of skimmed milk powder was added to 50ml of PBST solution. This is the sample diluent). 400µl of PBST milk was added into each dilution tube. 100µl of sample was 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 50µl was transferred to the ELISA plate wells in duplicates and plates were incubated at 37 °C for 1hr and 30 minutes. After incubation, the plates were washed for six times with PBST solution and it were blot dried. The conjugate IgG HRP and IgM HRP (Sigma, USA) of 5µl were added to 15ml and 5ml of PBST milk (diluent). 50µl of IgG conjugate was added to the plate (1:3000 dilution) MTSE IgG and 50µl of IgM conjugate was added to plate (1:1000 dilution) MTSE IgM. The plates were then incubated at 37 °C for 1 hour. After the incubation the plates were washed with the wash buffer (PBST) for 8 times and were blot

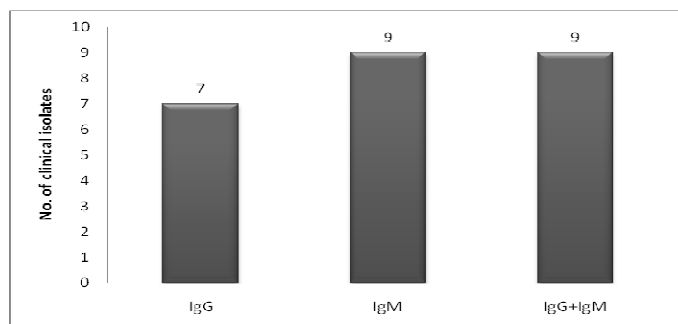
dried. 75µl of the substrate (ortho phenylene diamine dihydrochloride in phosphate citrate buffer and hydrogen peroxide) were added into each well of ELISA plates. The plates were then kept in a dark for 30 minutes for colour development. The colour change was noted and the reaction was stopped using the stop solution 1N sulphuric acid (50µl) into each well of ELISA microtitre plates. The plates were read using the ELISA reader at 492 nm.

## **RESULTS AND DISCUSSION**

Tuberculosis and leprosy are caused by mycobacteria and remain major diseases in developing countries. Laboratory diagnosis of tuberculosis has been difficult: microscopic examination gives a low positive yield, whereas culture techniques require complex media and sometimes fail to detect the disease sufficiently early<sup>16</sup>.

The development of the enzyme-linked immunosorbent assay<sup>17</sup> and of the dot-immunobinding assay<sup>18</sup> using mycobacterial antigens has stimulated interest in serological tests for the diagnosis of mycobacterial infections. In a limited study of 10 CSF samples from patients with tuberculous meningitis, Sada *et al*. (1984) showed the potential usefulness of ELISA in the detection of antigen<sup>6</sup>.

About 30 clinical samples were analyzed out of which 25 appeared to be positive. Among the positive samples, 7 samples showed high IgG positivity; 9 samples showed high IgM positivity and remaining 9 samples showed both IgG and IgM positivity. IgG positivity showed chronic state of infection and IgM positivity showed acute state of infection. All samples were inoculated onto LJ slants for confirmation of infection. The sensitivity and specificity of the test appeared to be 90%.



**Fig 1**  
**Enzyme linked immunosorbent assay**

## CONCLUSION

A specific and definitive test for the diagnosis of the tuberculosis is a prerequisite to early institution of treatment. The demonstration of *Mycobacterium tuberculosis* by culture though specific is time consuming and therefore delays the diagnosis. In this study, we have developed a specific and sensitive 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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## REFERENCES

- Jain SC, Misra SM, Misra NP and Tandon RL, Peritoneal biopsy in ascites. J. Indian Med. Assoc. 43: 219-224 (1964).
- MacGregor RR, A year's experience with tuberculosis in a private urban teaching hospital in the post sanatorium era. Am. J. Med. 58:221-228 (1975).
- Baig MME, Pettengell KE, SimJee AE, Sathar MA and Vorster BJ, Diagnosis of tuberculosis by detection of mycobacterial antigen in pleural effusions and ascites. S. Afr. Med. J. 69:101-102 (1986).
- Strankings W F, Nauta JJ, Straub JP and Stam J, Adenosine deaminase activity in tuberculous pleural effusions: a diagnostic test. Tubercle 68:137-140 (1987).
- Yanez MA, Coppola MP, Russo DA, Delaha E, Chaparas SD and Yeager H, Determination of mycobacterial antigens in sputum by enzyme immunoassay. J. Clin. Microbiol. 23:822-825 (1986).
- Sada E, Ruiz-Palacios GM, Lopezvidal Y and Ponce De Leon S, Detection of mycobacterial antigens in CSF of patients with tuberculous meningitis by ELISA. Lancet ii: 651-652 (1984).
- Taylor BA, Tuberculous peritonitis presenting in a hernial sac. J. R. Coll. Surg. Edinburgh 31:313-315 (1986).
- Kalish SB, Radin RC, Phair JP, Levitz D, Zeiss CR and Metzger E, Use of an enzyme-linked immunosorbent assay technique in the differential diagnosis of active pulmonary tuberculosis in humans. J. Infect. Dis. 147:523-531 (1983).
- Daniel TM and Debanne SM, The serodiagnosis of tuberculosis and other mycobacterial diseases by enzymelinked



- immunosorbent assay. *Am. Rev. Respir. Dis.* 135: 1137-1151 (1987).
10. Samuel AM, Kadival GV, Irani S, Pandya SK and Ganatra RD, A sensitive and specific method for the diagnosis of tuberculous meningitis. *Indian J. Med. Res.* 77:752-757 (1983).
  11. Collins FM, Jonathan RL and Douglas BY, Biological activity of protein antigens isolated from mycobacterium tuberculosis culture filtrate. *Infection and immunity.* 56(5): 1260-1266 (1988).
  12. Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ, Protein measurement with folin-phenol reagent. *J. Biol. Biochem.* 193, 265–275 (1951).
  13. Andersen AB, Yuan ZL, Haslov K, Vergmann B and Bennedsen J, Interspecies reactivity of five monoclonal antibodies to mycobacterium tuberculosis as Examined by immunoblotting and enzyme-linked immunosorbent assay. *J. Clin. Microbiol.* 23(3): 446-451 (1986).
  14. Kadival GV and Chaparas SD, Production, characterization, and species specificity of five monoclonal antibodies to mycobacterium tuberculosis. *J. Clin. Microbiol.* 25(1): 76-80 (1987).
  15. Chaturvedi V and Gupta HP, Evaluation of integral membrane antigens of Mycobacterium Habana for serodiagnosis of extrapulmonary tuberculosis: association between levels of antibodies and mycobacterium tuberculosis antigens. *Immun. Med. Microbiol.* 33:1-7 (2001).
  16. Lowell AM, Tuberculosis: its social and economic impact and some thoughts on epidemiology, p. 1021-1056. In P. Kubica and L. G. Wayne (ed.), *The mycobacteria. Part B.* Marcel Dekker, New York (1984).
  17. Van Vooren JP, Turneer M, Yernault JC, De Bruyn J, Burton E, Legros F and Farber CM, A multidot immunobinding assay for the serodiagnosis of tuberculosis, comparison with an enzyme linked immunosorbent assay. *J. Immunol. Methods* 113:45-49 (1988).