PROFORMA AND PROTOCOL
INTRODUCTION

Tuberculosis (TB) is a major public health problem especially in the third world countries. In India, it is estimated that at present there may be approximately 10 million persons suffering from radiologically evident pulmonary TB of whom about 2.5 million would be sputum positive. The number of deaths due to tuberculosis is estimated to be approximately 5 lacs per year (Park and Park, 1989).
Definitive diagnosis of tuberculosis depend on smear examination and culture of appropriate biological fluids. However only upto 50% of the pulmonary and 25% of extrapulmonary TB are diagnosed by smear examination (Bothamley et al, 1988). Further, the sputum usually remains negative till the development of the cavities, which may be late in the course of the disease (Kim et al, 1984).

Traditional culture methods take around 6 weeks before the diagnosis can be established. It is not only slow but also lacks sensitivity (Daniel, 1990). The disadvantage of tuberculin testing as a diagnostic tool are well known. Attempts have been made to improve the sensitivity and the speed of the detection of the tubercular bacilli or their components by techniques such as radiometric determination of the bacterial growth, gas chromatography, DNA hybridization and polymerase chain reaction. However, all of these have met with the problems of either sensitivity or specificity or have proven too technically demanding (Grange and Laszo, 1990).

Chemotherapy for tuberculosis is prolonged, expensive and can have serious side effects. If the diagnosis of TB is delayed, it leads to increased morbidity and in some cases mortality. Often the clinicians have to start the therapy empirically. There is a great need, therefore, for
tests which are highly sensitive, specific as well as rapid (Gupta et al, 1993).

The introduction of serological identification of Mycobacterium tuberculosis was a major breakthrough in the detection of tuberculosis. An Enzyme Linked Immunosorbent Assay (ELISA) based on mycobacterial antigen A60 for the estimation of mycobacterium specific immunoglobulins in the serum has been now used successfully for the rapid diagnosis of tuberculosis (Gupta et al, 1995).

The first report of the serodiagnosis of tuberculosis was probably that of Arloing which was published in 1898, only 16 years after Koch's identification of the tubercle bacillus. He developed an agglutination test and reported that serum specimens from 57% of the patients with pulmonary tuberculosis and 11% of healthy control subjects were positive (Daniel and Debanne, 1987).

With the development of the ELISA technique in 1972 by Engvall and Perlmann and the continued refinement and purification of the antigen used in the detection of the mycobacterial antibodies, the sensitivity and the specificity and the rapidity of this test in the detection of tuberculosis had greatly improved (Gupta et al, 1993).
Antigen A60 is a highly purified, major mycobacterial antigen. It is isolated by exclusion chromatography and affinity chromatography (Cocito and Vaniinden, 1986).

Using the antigen A60 the humoral response occurring during mycobacterial infection is analyzed. IgM antibodies indicate a primo-infection or a process of reactivation while IgG determinations allow an evaluation of the intensity of the infectious process. This test is also applicable to extrapulmonary tuberculosis (Maes, 1991).

There are few studies that have analyzed the sensitivity and specificity of the detection of mycobacterial antibodies using the ELISA test based on the A60 antigen.

Charpin et al (1990) in a study using the ELISA test for the diagnosis of tuberculosis, found that by combining the result of IgG and IgM measurement, the sensitivity, specificity and positive predictive value of the test were 68, 100 and 100% respectively. Similar results were obtained by Garlo (1991) who demonstrate high titers of IgG in 100% patients of both pulmonary and extrapulmonary tuberculosis.

Indian studies have been few and on a very limited number of patients (Munshi et al, 1993). In a study on 337 patient of tuberculosis and 131
controls, using ELISA assay based on antigen A60, Gupta et al (1993) reported a sensitivity of 91% and specificity of 90% in adult human tuberculosis. No other study of similar magnitude or nature has been done in India.

It has been demonstrated that the cut off values of IgG and IgM antibodies against mycobacterial antigens, that may be taken as diagnostic of tuberculosis, vary among different countries and different population groups (Gupta et al, 1995). Hence the cut off values demonstrated by other studies may not be applicable to our population.

This study is thus being undertaken to evaluate the role of mycobacterial antibodies (IgG and IgM) in the diagnosis of tuberculosis based on antigen A60 using ELISA and the cut off values that may be taken as diagnostic of tubercular infection in our population.

**AIMS AND OBJECTIVES**

1. To evaluate the role of A60 antigen in serological diagnosis of tuberculosis in adults using ELISA.

2. To find out the cut off value of the IgG and IgM antibodies that may be taken as positive in the population of this area.
MATERIAL AND METHODS

The study will be conducted at the Christian Medical College and Hospital, Ludhiana.

150 consecutive patients with microbiologically or histologically proven tuberculosis (pulmonary or extra pulmonary) will be entered into the study. The patients' demographical profile, history, results of PPD, ESR, and other mycobacterial and histological tests for tuberculosis and X-ray findings will be noted as mentioned in the protocol. The anti-tubercular therapy given and the response to therapy will also be ascertained. Venous blood will be drawn in each case, before the patient is given PPD, and analyzed for antitubercular IgG and IgM.

100 healthy patients with no apparent clinical suspicion of tuberculosis will be taken as the controls. The control group will include 75 healthy blood donors and 25 doctors and staff of the microbiology department who are frequently exposed to patients with tuberculosis or frequently handle tuberculous body fluids and tissues for analysis.

In both the patient as well as the control group mycobacterial antibodies IgG and IgM will be analyzed against the A60 antigen using the ELISA technique.
Appropriate quality control measures will be incorporated at each step during the estimation to enhance reproducibility of the serological tests.

The diagnostic utility of antitubercular IgG and IgM in the serological diagnosis of tuberculosis will be determined by statistically comparing the values of IgG and IgM in patients with microbiologically or histologically proven tuberculous infection against those in healthy controls, in using appropriate statistical methods. The false positives, false negatives, sensitivity, specificity and the predictive value of the tests will be calculated. Further, the above comparison will also be used to determine the cut off value of the IgG and IgM antibodies that may be taken as positive, at different confidence levels, in the test population.
1. Unit no.  2. Study serial no.

3. Name  4. Age

5. Sex

6. Address

7. Occupation

8. Previous history of tuberculosis (pulmonary / extrapulmonary)

9. No. of times ATT started  10. Drugs used

Drugs lost

11. Presence of other risk factors

   Diabetes (yrs)
   Smoking (pack/day x yrs)
   Alcohol (ml/week)
   Chronic renal failure
   Malignancy
   Immunosuppression / steroid intake

12. Family history of tuberculosis or contact

13. Presenting complaints with duration

14. Examination findings

   Weight

15. Investigations

   Haemoglobin
   ESR
   PPD
   Radiological findings
   ADA
16. Body fluid examination
   Type of fluid
   Site
   Cytology
   Protein  Sugar  LDH  ADA

AFB (Smear & Culture)
   Day 1
   Day 2
   Day 3

17. Biopsy
   Site and tissue
   Report

18. Serum anti-TB IgG  IgM

19. Treatment regimen
   Drugs  Dosage  Duration

20. Therapeutic response
   Adequate / Inadequate / Nil
REFERENCES


