Journal of Rawalpindi Medical College (JRMC); 2007;11(2):

Serological Analysis of Pulmonary and Extra Pulmonary Tuberculosis With Elisa for Anti A60 IgA

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Abstract

Background: Demonstration and evaluation of anti tuberculous IgA antibodies for rapid diagnosis of both pulmonary and extra pulmonary tuberculosis.

Method: ELISA assay based on mycobacterial antigen A60 (Anda Biologicals, France) was used on the sera obtained from 69 cases of tuberculosis and 136 controls in the population of Karachi, Pakistan.

Result: Of 136 controls only 7.3% were positive for IgA. A very good serological response was observed in cases with sputum positive active pulmonary tuberculosis, depicting a positivity of 83.3% for IgA antibodies. Relatively low sero positivity was seen in cases of sputum negative active pulmonary tuberculosis compared to those of sputum active pulmonary tuberculosis. A low positivity of 26.3% for IgA was observed in cases of extra pulmonary tuberculosis. In cases of healed tuberculosis 25% were found positive for IgA. Considering all the cases of active tuberculosis and the controls the global sensitivity of 58.4% and specificity of 92.7% were obtained when IgA antibodies were taken into account.

Conclusion: The estimation of anti tuberculosis antibody IgA against A60 for the rapid diagnosis is clearly demonstrated and therefore recommended.

Key words: ELISA Antigen A60, Anti IgA, Tuberculosis, Serological analysis.

Introduction

Tuberculosis has become an important public health problem in today's world[1]. A recent increase in tuberculosis incidence and complications has been registered in connection with the spread of antibiotic resistance and AIDS[2]. Pakistan is ranked 8th in terms of estimated number of tuberculosis cases by WHO among first 22 high burden countries[3]. Almost 1.5 million people suffer from tuberculosis in a country of 144 million indicating a prevalence exceeding 1% of the total population[4]. Global tuberculosis report by WHO mentions the case notification rate for Pakistan was 23 per 100,000 in the year 2001[5].

The diagnosis of Mycobacterial disease depends upon identifying the infective organisms in secretion or tissues of the diseased individual. However there are several limitations of this method of diagnosis[6]. The rapid detection and identification of Mycobacterium tuberculosis complex in samples is extremely important for optimal diagnosis and effective treatment as well as for prevention and control of tuberculosis transmission[7]. Sero-diagnostic tests based on the presence of antibodies against Mycobacterial antigens in the sera have been identified, purified and tested, with various degrees of success[8].

The present study was designed for the rapid diagnosis of pulmonary and extra pulmonary tuberculosis by measuring the A60 specific IgA antibody levels in the sera.

Patients and Methods

This study was conducted in the Department of Microbiology of BMSI, JPMC Karachi. Patients with tuberculosis and non-tuberculous patients were selected from different medical and surgical wards of JPMC, Karachi. Their ages ranged from 14-70 years.

Serum IgA against antigen A 60 was estimated in 69 patients of tuberculosis and 136 subjects as controls with their age ranging from 13-65 years. The diagnosis of tuberculosis was based on clinical and radiological criteria, histopathology, presence of Acid Fast Bacilli (AFB) and clinical response to anti-

tuberculous treatment.

The laboratory tests included estimation of ESR, Hb%, TLC and DLC by standard techniques, Radiological investigations included X-Ray chest PA view in all patients. Sputum for presence of acid fast bacilli was recorded in all patients with pulmonary disease. Mantoux test was performed in healthy normal subjects.

Serum samples from patients with tuberculosis, non-tuberculous patients and normal healthy subjects were collected and frozen at -20^{o}C after proper labelling.

Concentration of A60 specific IgA in the sera for cases of human tuberculosis and controls was measured by indirect ELISA technique.

Tuberculous Patients: This group of 69 patients was classified as patients with healed tuberculosis (16 cases), patients with sputum positive active pulmonary tuberculosis (18 cases) and patients with sputum negative active pulmonary tuberculosis (16 cases).In the latter the diagnosis was based on clinical and radiological data but AFB were absent in sputum specimens.

The remaining 19 cases had extra pulmonary tuberculosis This group comprised of pleural (4 cases) lymph nodal (5 cases), tuberculous meningitis (2 cases), abdominal (2 cases), osseous (4 cases), miliary (1 case) and psoas abscess (1 case).

The control group (136 persons) was classified into the following categories:

Tuberculosis negative healthy subjects (15 persons) based on a negative response to intradermal injection of 5 IU of PPD.

Tuberculosis positive healthy subjects (9 persons) based on a positive response to intradermal injection of 5IU of PPD. A diameter of induration of >10mm after 72 hours was considered a positive test.

Non-tuberculous patients with pulmonary pathology (15 cases). They included 3 cases of lung abscesses, 2 cases of COPD, 3 cases of bronchogenic carcinomas and one case each of chronic bronchitis, hydro-pneumothorax, nephritic syndrome with pleural effusion and eosinophilic pneumonia.

Non-tuberculous patients with extra pulmonary pathology (7 cases) included 5 cases of cervical lymph-adenopathy and one case of each nephritic syndrome and benign thyroid Disease.

Contact of cases of tuberculosis (36 Persons) included members of the staff serving in the wards like doctors, nurses, ward boys, aides, dieticians, waiters, dressers, liftmen, sweepers etc.

Subjects handling mycobacteria (54 persons) included technicians from different laboratories in the town.

Specimens of blood were taken with appropriate consent. Five ml of blood was drawn from superficial vein from each subject with the help of disposable syringe under aseptic conditions. It was transferred to a sterile cup and allowed to clot at room temperature. Then it was centrifuged and serum was separated and transferred with the help of disposable Pasteur pipette to a sterile cup and stored in a refrigerator at -20o C until processed for analysis.

In the present study the measurement of IgA antibodies against A60, strain BCG of Mycobacterium bovis was done in serum of our study population with an ELISA KIT (Anda TB biological, Strasbourg France). Stored serum samples of our study population were taken out from the freezer one-hour prior to the test. Anti A60 IgA was estimated in the sera of the subjects under study employing indirect ELISA technique as per recommendations of the manufacturer. Each time the positive as well as the negative reference sera provided with diagnostic kit were included in the test along with the test sera. For IgA determination, the curves were constructed by plotting the OD values of different reference curve.

Results

In serological analysis of tuberculous cases with regard to antigen A60 specific antibodies of IgA class it was observed that 83.3 % (15/18) were positive in 18 cases of sputum positive active pulmonary tuberculosis. However, in 16 cases of sputum negative active pulmonary tuberculosis serological positivity was seen in 68.7 % for IgA antibodies. Very low serological positivity was observed (26.3 %) for IgA in 19 cases of extra pulmonary tuberculosis. Similarly 16 cases of inactive tuberculosis depicted a low positivity (25 %) for IgA antibodies. Considering the overall picture of the cases of active tuberculosis 58.4 % were positive for IgA antibodies (Table 1). The corresponding mean titer for the class of IgA antibodies in active tuberculous cases were 1.216 OD (Table 2)

In serological analysis of controls, 15 tuberculin negative healthy subjects were negative for anti A60 IgA indicating 100 % sero-negativity in this group. This specificity was observed at 1:100 serum dilutions. Among the tuberculin positive healthy controls, none were positive for IgA antibodies. Overall among healthy controls no subjects yielded a positive serology and 100 % (0/24) specificity was

observed for IgA antibodies. There was no serological difference between tuberculin positive and tuberculin negative controls. However, when the 54 laboratory technicians were analyzed 5.5% (3/54) were positive for IgA antibodies. Among 36 contact cases , only 2.7 % (1/36) were positive for IgA antibodies. Among the 22 diseased controls 27.2% (6/22) were positive of IgA antibodies. Taking the overall picture of 136 controls 7.3% (10/136) were positive for IgA antibodies (Table 3). The corresponding mean titer for IgA antibodies in control subjects were 0.516 OD (Table 4).

Discussion

A cut off point which is essential for the interpretation of serological data, is based on large surveys of control subjects (healthy persons with no history of a given disease and patients with other diseases) and varies according to environmental conditions. Our approach was to apply the cut off recommended by the manufacture i.e, IgA absorbance was negative if < 200 sero units, dubious between200 – 300 sero units and positive if > 350 sero units

In our study a cut off value of 350 sero units/ml gave a global specificity 92.7% (Table 3).

In the present work we studied several control groups, both healthy and pathological and evaluated separately patients suffering from different forms of tuberculosis. The usefulness of studying several control groups consists both in defining the cut off point to use for tuberculous patients from our region and consequently, evaluating the specificity of the test.

In the healthy control groups very few subjects yielded a positive serology against A60 antigen. In tuberculin negative and positive healthy subjects, all were serologically negative for IgA antibodies with 100% specificity. In human contacts with cases of tuberculosis a few subjects showed a positive A60 serology. In the group of laboratory staff who had been routinely handling the Mycobacterial cultures showed surprisingly low serological positivity. The IgA levels in the control subjects in this study were appreciably lower compared to those in case of active and healed tuberculosis. Similar results were shown in India[9]. The findings are more or less in the agreement with earlier reports [10-13].

In this study the sero negativity of the tests was 66.6% in non-tuberculous patients with pulmonary pathology and 85.7% in non tuberculous patients with extra pulmonary pathology, but some patients analyzed departed strikingly from this norm and yielded different pattern of sero positivity. Other authors reported similar events[10,11,13,-,15].

Table: 1 Serological analysis of cases of Tuberculosis

Subjects	No of Cases	A60 ELISA Sensitivity IgA No (%)
Patients with sputum positive active pulmonary tuberculosis	18	15(83.3)
Patients with sputum negative active pulmonary tuberculosis	16	11(68.7)
Patients with extra pulmonary tuberculosis	19	05(26.3)
Patients with healed tuberculosis	16	04(25.0)
Total	69	31(58.4)

A very good serological response was observed in cases with sputum positive active pulmonary tuberculosis, especially with regards to IgA antibodies depicting a sensitivity of 83.3%. The mean levels of IgA antibodies were appreciably higher than in the controls. In studies carried out earlier an IgA positivity ranging from 68-75% has been documented[9,11,12]. These wide variations could be due to different age groups studied, geographical areas and severity of disease in different studies as well as variety in cut -off limit used.

Table: 2 A 60 specific antibody titres in cases of Tuberculosis

Subjects	No of Cases	Mean antibody titres (Range) IgA
Patients with sputum positive active pulmonary tuberculosis	18	1.766 (0.407-3.371)
Patients with sputum negative active pulmonary tuberculosis	16	1.295 (0.139-2.612)
Patients with extra pulmonary tuberculosis	19	0.588 (0.248-1.936)
Patients with healed tuberculosis	16	0.729 (0.232-2.367)

In the present study, relatively low sero positivity was seen in cases of sputum negative active pulmonary tuberculosis compared to those of sputum positive active pulmonary tuberculosis as shown in a previous study[11]. As far as serology in patients with extra pulmonary tuberculosis is concerned a sensitivity of 26.3% was observed. The mean antibody levels were lower than the active pulmonary tuberculosis.

Table 3 Serological analysis of nontuberculous individuals (controls)

Subjects	No of Cases	A 60 ELISA Specificity IgA No. (%)
Control Groups		
Tuberculin negative healthy subjects	15	0(100)
Tuberculin positive healthy subjects	09	0(100)
Non-tuberculous patients with Pulmonary pathology	15	05(33.3)
Non-tuberculous patients with extra pulmonary pathology	07	01(83.8)
Contact of cases of tuberculosis	36	01(97.3)
Subjects handling Mycobacteria	54	03(94.5)
Total	136	10(92.7)

In one Indian study a 68.4%IgA positivity was observed in cases of extra pulmonary tuberculosis[8,9]. Again wide variations were observed which may be due to different clinical type of extra pulmonary tuberculosis, variety in cut-off limit used, different age group studied, and demographic areas as well as severity of diseases.

On analysis of cases of healed tuberculosis (treated) a large number were found positive for IgA . The results observed in cases of healed tuberculosis compared to those documented by a previous study. Moreover, the mean antibody levels were also higher than those of control, although the levels were lower than those in active pulmonary tuberculosis. Substantial lowering of anti A60 immunoglobulins observed by different authors is probably due to the varying time between the inclusion in the study and the end of anti-tuberculous therapy.

Table 4
A 60 Specific antibody titres in controls

Subjects	No of Cases	Mean antibody titres (Range) IgA
Tuberculin negative healthy subjects	15	0.386 (0.117-0.600)
Tuberculin positive healthy subjects	09	0.563 (0.300-0.946)
Non-tuberculous patients with Pulmonary pathology	15	0.753 (0.156-1.618)
Non-tuberculous patients with extra pulmonary pathology	07	0.613 (0.255-1.337)
Contact of cases of tuberculosis	36	0.385 (0.113-1.221)
Subjects handling Mycobacteria	54	0.401 (0.161-1.231)

In our study detection of Anti A60 antibodies in patients with pulmonary and extra pulmonary tuberculosis was negative in a very small percentage of cases. This may be a result of immuno-depression due to disease as well as presence of immune complexes[15].

A small percentage of our healthy subjects and patients with non tuberculous disease showed sero-positivity. This might be due either to sub clinical infection of environmental non tuberculous Mycobacteria that also express A60 or to the presence in the host of commensal non-pathogenic Mycobacteria. The disregulation of the hormonal immune response that occurs frequently in several

diseases might be another cause of positive results in patients with non tuberculous disease.

Considering all the cases of active tuberculosis and the controls the global sensitivity of 58.4% and specificity of 92.7% were obtained when IgA antibodies estimation were taken into account. A previous study done in India showed a test sensitivity of 79.8% and specificity 95.4% in post primary tuberculosis by estimating A60 specific IgA antibodies[9]. Similar results were shown in France[11].

We conclude that the estimation of IgA antibodies against A60 antigen for the rapid diagnosis of tuberculosis is clearly demonstrated and therefore recommended.

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