# Evaluation of Serological Tests Using A60 Antigen for Diagnosis of Tuberculosis

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Abstract- Identification of acid-fast bacilli (AFB) in sputum or tissue samples is among definite diagnostic methods of tuberculosis. However, this method of diagnosis is restricted by certain limitations. Serologic diagnosis of tuberculosis (TB) has been used for a long time. The aim of this study was to determine the sensitivity and, specificity of Antigen 60 (A60) IgG, IgA, IgM test results in TB diagnosis. Mycobacterial A60based ELISA was used to measure specific IgA, IgM and IgG antibodies in the sera of 127 adult TB patients (consisted of 74 pulmonary and 53 extra-pulmonary cases), and 95 controls (46 healthy volunteers and 49 patients with various acute or chronic diseases other than tuberculosis). Data from A60 IgG-based ELISA, chest radiography, AFB culture and pathologic evaluation for AFB were obtained .The cutoff value of A60 IgG, IgA and IgM were chosen according to a receiver operating characteristic (ROC) analysis. The sensitivity, specificity and positive likelihood ratio were determined. The mean levels of IgG, IgA and IgM were significantly higher in patients with pulmonary tuberculosis when compared with control groups. Sensitivity of IgG test was 54.3 %, while the specificity was 84.2%. The IgA test showed a sensitivity of 70.1% with a specificity of 80 %. Combination of the IgG and IgA tests showed a total sensitivity of 45.7 % and a specificity of 94.7% and the positive likelihood ratio of 8.62. Chosen cutoff values of IgG, IgA, and IgM sets were 285,265 and 0.9 ELISA units respectively. Our study results showed a good specificity (94.7%) and a reasonable positive likelihood ratio (8.62) of the test when combined IgA and IgG with new cutoff points were considered on diagnosis of tuberculosis in adult patients. Combined use of both IgG and IgA tests results allows an increased accuracy in diagnostic of tuberculosis.

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**Key words:** Mycobacterial A60 antigen; immunoglobulin G; immunoglobulin A; immunoglobulin M; tubrculosis

# Introduction

Tuberculosis (TB) has been declared a global emergency. The mainstay for its control is the rapid and accurate identification of infected individuals. The simplest rapid method is the detection of acid-fast bacilli by microscopy. However, 40 to 60% of patients with pulmonary disease and ~75% of patients with extrapulmonary disease are smear negative, and in this situation even contemporary culture methods take several weeks to become positive (1-3).

Therefore, a number of alternative diagnostic tests that use molecular, chromatographic and immunological methods have been developed. While molecular methods overcome the insensitivity of the smear method and the time required for culture, they depend upon retrieval of a specimen from the site of infection. Immunological methods use the specific humoral or cellular responses of the host to infer the presence of infection or disease. They do not require a specimen from the site of infection. Numerous serological tests that use various antigens, such as secreted and heat shock proteins, lipopolysaccharides, and peptides, have been developed. These tests use various modifications of enzyme-linked immunosorbent assay (ELISA) or immunochromatographic methods to detect different antibody classes (2-9). Although an enzyme-linked immunosorbent assay (ELI-SA) may not add to the diagnostic yield in those patients in whom sputa examination were available, an accurate serological test to diagnose TB would still have consi-

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derable advantages in those patients who are unable to provide adequate sputum samples (e.g. in children and the very elderly), who are smear- and/or culturenegative, and who have suspected extra-pulmonary TB. In populations where the prevalence of tuberculin skin test positivity is high, earlier detection and treatment of active TB by blood screening may help prevent further transmission of TB. However, serological testing has been confounded by cross reactivity associated with bacillus Calmette Guerin (BCG) vaccination or infection with mycobacteria other than tuberculosis (MOTT). Previous sero assays for TB have utilized either a mixture of M. tuberculosis antigens, such as purified extracted glycolipids, adsorbed mycobacterial sonicates, PPD, or more distinct mycobacterial antigens. Measurement of tuberculostearic acid in clinical specimens was shown to have a high degree of sensitivity and specificity but the assay required considerable expertise to perform (2, 3, 5-8).

A study comparing three different antigen antibodies showed that A60 IgG (sensitivity and specificity, 80.77 and 88.4%) was more antigenic and more effective in its determination than was 38 kDa IgG (sensitivity and specificity, 64.21 and 80.74%) or Kp90 IgA (sensitivity and specificity, 62.58 and 66.3%) (9). The results of other serologic test studies, including immunoglobulin antibody to diacyltrehaloses, triacyltrehaloses, cord factor, and sulfolipid I, showed relatively low sensitivity and specificity for cases of tuberculosis infection (6). The use of serologic methods to diagnose tuberculosis have been studied since 1898 and A60 IgG is the method most frequently used (10-16). The A60 antigen, a thermo stable component of PPD, has also been used in the serodiagnosis of TB. Unfortunately, this molecule is not specific for mycobacteria because it is also present in Nocardia and Corynebacterium species (2, 17-21). In the present study, we have implemented a type of serological assay for detection of IgG, IgA, and IgM antibodies from tuberculous sera, namely a commercially available ERBA LISA (TB IgG) test kit (from Anda Biologicals, Strasbourg, France) which uses A60 antigen complex.

# **Patients and Methods**

From September 2003 to September 2005, a prospective case-control study was performed. The targeted population consisted of 127 adult patients with confirmed diagnosis of tuberculosis [(acid-fast smear, culture, tuberculin skin test, X-ray), (74 had pulmonary and 53 had extrapulmonary tuberculosis)]. None of the patients with extrapulmonary tuberculosis [lymph nodes (4.7%),

joints (4.7%), skeletal (10.2%), meninges (2.4%), CNS system (2.4%), miliary (7.1%), pleurisy (1.6%)] had clinical or radiological evidence of concurrent active pulmonary tuberculosis. None of the subjects were HIV positive. Control group were divided into two groups; group 1 consisted of 49 patients with various acute or chronic diseases other than tuberculosis and group 2 consisted of 46 healthy volunteers with no signs of clinical impairment. Exclusion criteria were any immuno-suppression condition such as HIV positive or corticosteroid consumption. All patients and control subjects filled informed consent. The study protocol was approved by the Institutional Review Board of Tehran University of Medical Sciences.

Blood samples were collected; sera were separated, and stored at -20° C with 0.1% sodium azide. ELISA was performed on serum obtained from all study cases. The test was performed to detect IgG, IgM, and IgA antibodies against A60 antigen using commercially available kits (Anda Biologicals, Strasbourg, France) according to manufacturer's instructions. Demographic data of subjects from different groups were compared by one-way analysis of variance (one way-ANOVA). The Mann-Whitney rank sum test was used two by two to compare the differences of A60 IgG among and between the groups. The Chi Square test was used to compare the distribution of age among the different groups .Statistical significance was accepted at a level of p<0.05. Cut off values were established by the Receiver Operating Characteristic (ROC) curve technique.

### Results

In the present study no difference was found with regard to the sex and age of the subjects in the different groups.

#### IgG test results

The mean levels of IgG was significantly (Anova test, P < 0.001, CI=0.658-0.79) higher in patients of pulmonary tuberculosis when compared with control groups. The mean levels of IgG was higher in patients of pulmonary tuberculosis when compared with extra pulmonary tuberculosis, but the difference was not statistically significant (P = 0.11). The ROC curve for IgG has been shown in figure1. The value of the area under the curve was 0.725. With a cutoff value set at 285 ELISA units, the sensitivity, specificity and positive likelihood ratio for these groups were 54.3 %, 84.2% and 3.43, respectively.

The mean levels of IgA was significantly (Anova test, p < 0.001, CI=0.733-0.856) higher in patients of pulmonary tuberculosis when compared with control groups. The mean levels of IgA was significantly higher in patients of pulmonary tuberculosis when compared with three groups. The mean levels of IgA was significantly higher in patients of extrapulmonary tuberculosis when compared with healthy individuals (P = 0.01). The mean levels of IgA was significantly higher in patients of extrapulmonary tuberculosis when compared with group of subjects in control group 1, but the difference was not statistically significant (P = 0.23). The ROC curve for IgA has been shown in figure 2. The value of the area under the curve was 0.795. With a cutoff value set at 265 ELISA units, the sensitivity, specificity and positive likelihood ratio for these groups were 70.1 % and 80% and 3.5, respectively.



Figure 2. The ROC Curve for IgA

#### IgM test results

Titres of IgM against A60 were significantly higher in patients with pulmonary tuberculosis than in control subjects (P=0.02). The mean levels of IgM was significantly higher in patients of extrapulmonary tuberculosis when compared with control group, but the difference was not statistically significant. The ROC curve for IgM has been shown in figure 3. The value of the area under the curve was 0.595. With a cut off value was set at 0.9, the sensitivity, specificity and positive likelihood ratio of the test were 43.3 % and 69.5% and 1.42, respectively.

### **Combined tests results**

Sensitivity and specificity of serologic tests has been shown in table 1. The combined implementation of these tests showed an increased specificity more marked than in sensitivity.



Figure 3. The ROC Curve for IgM

Serologic tests	Sensitivity	Specificity	Positive like- lihood ratio
IgA	70.1	80	3.5
IgM	43.3	69.5	1.42
IgG	54.3	84.2	3.43
IgA & IgG	45.7	94.7	8.62
IgA & IgM	31.5	94.7	5.94
IgM & IgG	22	94.7	4.15
IgA or IgG	78.7	69.5	2.58
IgA or IgM	81.9	54.7	1.8
IgM or IgG	75.6	58.9	1.83
IgA & IgM & IgG	18.9	97.8	8.59
IgA or IgM or IgG	87.4	47.4	1.66
IgA or IgG IgA or IgM IgM or IgG IgA & IgM & IgG IgA or IgM or IgG	78.7 81.9 75.6 18.9 87.4	69.5 54.7 58.9 97.8 47.4	2.58 1.8 1.83 8.59 1.66

Table 1. Sensitivity and Specificity of serologic tests

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# Discussion

Detection of Mycobacterium tuberculosis in culture and/or characteristic histological features is generally required to establish the diagnosis. Sensitivity of these methods is moderately good (71% according to Weir and Thornton). However, invasive procedures are often required to obtain samples and the growth time of M. tuberculosis is unacceptably long. Other causes of granuloma formation may cause false positive results on histological examination (5, 6, 22, 23).

Despite the increasing development of techniques of rapid identification of mycobacteria by molecular genetic means, there is a hardly need for a simple, sensitive, and specific test for TB, which would improve or replace the sputum smear (1, 24).

Methods based on molecular biology are costly and complicated, so that they are not useful for the routine diagnosis in low-income countries (3-5, 24). Serological tests may be especially useful for a rapid diagnosis of TB in these countries, which shoulder over 90% of the global burden of TB cases (1, 24).

The goal of the present investigation was to evaluate diagnostic usefulness of serological tests in pulmonary and extrapulmonary tuberculosis in adults.

Diagnostic accuracy of a test depended on the type of antigens used and on the population examined. For both adults and children, specificity of IgG assays based on recombinant antigens was very high (97-99%). The specificity of assays based on native antigens were lower, which maybe have been resulted from the cross reactivity of native antigens with environmental mycobacteria (6). In most of the studies, it has been showed that IgG holds the great promise in diagnosing an active disease in both children as well as adults, when compared with IgM or IgA class. IgG is also found to be a useful antibody for monitoring the response of anti tubercular treatment.

In a cross sectional study, investigating changes in IgG, IgA and IgM levels along with other serum proteins in treated pulmonary TB, 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, increased IgM levels have also been reported to be in two studies while several studies revealed no significant change. IgA levels have also been reported to be increased in several studies 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 (20-23, 25-29).

In this study, the selected cutoff value of IgG, IgA and IgM were set at 285, 265 and 0.9 ELISA units, respectively. In different studies, cutoff values of these tests were different (Table 2).

	Table	2. Cut off va	lues of serologic tes	sts in different s	studies	
Test	Kit Instruc- tion	Iran	India 1995	Taiwan	India 2003	Italy
IgA	350	265	150	-	-	320
IgM	1.0	0.9	1.5	-	1.1	-
IgG	225	285	200	261.5	400	370

<b>Table 3.</b> Sensitivity and Specificity of combination IgG and IgA
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	Iran	Poland	India	Italy	
		2002	1995		
Sensitivity	45.7	56	91.6	80.9	
Specificity	94.7	85	90	92.3	

In the present study, patients with pulmonary and extrapulmonary tuberculosis showed significantly higher titres of IgG and IgA and IgM against A60 compared with control groups. Detection of anti A60 IgG is characterized by good sensitivity (54.3%) and specificity (84.2 %) with positive likelihood ratio 3.43 in pulmonary tuberculosis. Our study results were similar to those of other investigators (4,23).

Wu and et al have recently reported a sensitivity of 49.4 % and specificity 68.4% with positive likelihood ratio 4.2. In another study anti A60 IgG levels in Taiwanese patients had the cut off value of 340 ELISA units which defined the sensitivity and specificity for his tests of 80.77% and 88.40%. Thus, A60 IgG in combination with chest radiography could help to diagnose tuberculosis (6). In different studies, searching for IgG against A60 is considered a useful diagnostic tool in pulmonary tuberculosis, with a reported sensitivity ranging between 78% and 94% by different authors and the combined use of the IgG and the IgA test increases overall diagnostic accuracy (2,5,6,10,22,23,26-28). In this study, the results revealed that humoral response differences depend on immunoglobulin subtypes. Measurements of IgG, IgA or both IgG and IgA as most sensitive against A60 are potentially more useful serological tests in clinical practice. Similar results have also been reported by Zielonka and et al (28). Gupta and et al reported that the percentage of positive anti-A60 IgG and IgA titres in patients with inactive pulmonary tuberculosis were 58.3% and 46.6%, respectively (10) (Table 3). Results of IgM sensitivity and specificity of our study were different from other studies. Specificity and sensitivity of IgM was higher in another studies (2, 5, 6, 22, 29). In a study, measurement of both IgM and IgG in an ELISA revealed a sensitivity of 68% and specificity of 100% (2) but in our study sensitivity of 22% and specificity of 94.7% was achieved. Although measurement of IgA, IgG and IgM against A60 may be considered a useful diagnostic tool in pulmonary and extrapulmonary tuberculosis, especially if molecular techniques are not available, combined use of IgA and IgG tests allows an increased diagnostic accuracy of tuberculosis. Longitudinal studies are required to establish the diagnostic usefulness of A60 based serological testing in cases with negative results of microbiological and/or histological examinations.

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