

## Evaluation of Serological Tests for Immunodiagnosis of Pulmonary Tuberculosis using A60 and Lipoarabinomannan Antigens

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### ABSTRACT

**Background:** Problems in diagnosis of tuberculosis using smear and culture for acid fast bacilli (AFB) techniques have necessitated the exploration of the utility of serodiagnosis to support the clinical suspicion of tuberculosis. Three serological tests, namely Anti-A60 IgG enzyme-linked immunosorbent assay (ELISA), Anti-A60-IgM (ELISA) using A60 antigen and Mycodot test using lipoarabinomannan (LAM) antigen were evaluated as tools of diagnosing pulmonary tuberculosis against smear and culture methods. **Materials and Methods:** ELISA was used for the detection of IgG & IgM using A60 antigen, while Mycodot test was performed utilizing Lipoarabinomannan (LAM) antigen bound to plastic combs in parallel with other familiar diagnostic methods in 50 patients with pulmonary tuberculosis (Group I), 25 patients with chest diseases other than tuberculosis (Group II) and 25 apparently healthy individuals (Group III). Members of (Group II) & (Group III) participate as control groups. All members of the three groups were examined for Ziehl-Neelsen (ZN) smear stains, culture for acid fast bacilli (AFB) and Tuberculin skin test. **Results:** ELISA IgG results were positive in 42 (84%) tuberculous patients of Group I compared to 10 (40%) non-tuberculous patients in Group II and 8 (32%) individuals in Group III. ELISA IgM results were positive in 30 (60%) tuberculous patients of Group I compared to 3 (12%) non-tuberculous patients of Group II and 2 (8%) individuals of Group III. Mycodot test results were positive in 33 (66%) tuberculous patients in Group I compared to 6 (24%) non-tuberculous patients in Group II and 3 (12%) individuals of Group III. The overall sensitivities and specificities of the three tests (Mycodot, IgG and IgM) were obtained on basis of the receiver operating characteristic (ROC) curve for each test and comparison of (ROC) curves of the three tests and they were (66, 82%) for Mycodot test, (80, 92%) for Anti-A60 IgG and (82, 74%) for Anti-A60 IgM. Positive predictive values and negative predictive values were (78.57, 70.69%) for Mycodot test, (90.91, 82.14%) for IgG and (75.93, 80.43%) for IgM. Results of Ziehl-Neelsen (ZN) smear stains and culture for Acid fast Bacilli (AFB) were positive in all members of group (I), while were negative in all members of control groups { group (II) and group (III) }. Tuberculin skin tests were positive in 46 (92%) tuberculous patients of group (I), 7 of 20 (35%) non-tuberculous patients and 3 of 15 (20%) individuals of group III. **Conclusion:** Anti A60 IgG ELISA was the best serodiagnostic technique compared with Anti 60 IgM ELISA and

*Mycodot test. The high diagnostic performance of Anti 60 IgG makes it a useful, Simple and rapid supporting tool to confirm the clinical judgment of tuberculosis when used as an adjunct to symptoms and signs together with other investigation tools.*

## INTRODUCTION

Tuberculosis (TB), as one of the mankind plagues and a major health concern shows resurgence in prevalence and intensity in both developing and well developed countries. One third of the world's population is infected with *Mycobacterium tuberculosis*. Three million people die every year of the disease with 9 million new cases per year<sup>(1)</sup>. TB has been a cause of significant morbidity and mortality for mankind throughout history<sup>(2)</sup>. Diagnosis of pulmonary tuberculosis depends mainly on the initial clinical suspicion and radiographic findings with subsequent microbiologic confirmation by direct smear microscopy and culture of sputum for acid fast bacilli (AFB). Some of the disadvantages of traditional diagnostic techniques are the lack of sensitivity in smear microscopy and the length of time in case of culture for AFB, sometimes growth takes several weeks<sup>(3,4)</sup>. Therefore, a number of alternative diagnostic tests that use molecular, chromatographic and immunological methods have been developed. Immunological methods use the specific humoral or cellular responses to investigate the presence of infection or disease. Numerous serological tests that use various antigens, such as secreted and heat shock proteins, lipopolysaccharides and peptide have been developed. These tests use various modification

of enzyme- Linked immunosorbent assay (ELISA) or immunochromatographic methods to detect different antibody classes<sup>(5,6)</sup>. 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 serodiagnostic techniques have utilized either a mixture of *M. tuberculosis* antigens, such as purified extracted glycolipids, adsorbed mycobacterial sonicates, PPD or more distinct mycobacterial antigens<sup>(5,7-11)</sup>. 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%)<sup>(6)</sup>. The results of other serologic tests studies, including immunoglobulins to diacyltrehaloses, triacyltrehaloses, cord factor and sulpholipid I showed relatively low sensitivity and specificity for cases of tuberculosis infection<sup>(9)</sup>. The use of serological methods to diagnose tuberculosis has been studied since 1898 and A60 IgG, thermostable component of PPD, has been also used in the serodiagnosis of TB. Unfortunately, this molecule is not specific for mycobacteria because it is also present in *Nocardia* and *cornybacterium* species<sup>(5,12-16)</sup>. In the

present study, we aimed to evaluate IgG and IgM using A60 antigen by ELISA technique and Mycodot test using a combination of anti mycobacterial antigens (Lipoarabinomannan) for serodiagnosis of pulmonary tuberculosis in comparison with conventional bacteriological methods for laboratory diagnosis, namely microscopical examination of ZN stained sputum smear, culture and identification for acid fast bacilli (AFB).

## **PATIENTS & METHODS**

From September 2004 to September 2006, a prospective case – control study was performed. The study included 3 groups: 50 adult patients with confirmed diagnosis of tuberculosis (acid fast smear, culture, tuberculin skin test, X-ray), 25 patients with chest diseases other than tuberculosis and 25 apparently healthy individuals. The members of group II & group III participate as control groups.

Blood and sputum samples were collected from all subjects of group I and group II from Giza Chest Hospital, while those of group III were healthy volunteers. Sera were separated from blood samples and stored at - 20°C with 0.1% sodium azide. Sputum samples were submitted for direct microscopic examination for smears stained by ZN, culture for AFB and identification of isolates. Serum samples were submitted for performing the serological tests, Mycodot test which utilizes lipoarabinomannan LAM antigen bound to plastic combs

revealing pink spot when reacting with specific antibody present in serum. ELISA was performed to detect IgG and IgM using A60 antigen. Statistical analysis of results and cutoff values were established by using the receiver operating characteristic (ROC) curve technique.

## **RESULTS**

### **A) Mycodot test Results:**

The results of Mycodot test were positive in 33 cases out of 50 in the first group (confirmed tuberculous cases), while were positive in 6 cases out of 25 in the second group (non-tuberculous pulmonary cases) and were positive in 3 cases out of 25 in the third group (apparently healthy individuals), the ROC curve has been shown in figure (1). From ROC curve of Mycodot test, the sensitivity and specificity were 66% and 82%, while positive predictive value and negative predictive value were 78.57% and 70.69% respectively.

### **B) IgG test results:**

The results of IgG test were positive in 42 cases out of 50 in the first group, 10 cases out of 25 in the second group and 8 cases out of 25 in the third group. From ROC curve of IgG test (figure 2), the sensitivity and specificity were 80% and 92%, while positive predictive value and negative predictive value were 90.91% and 82.14% respectively.

### **C) IgM test results:**

The results of IgM test were positive in 30 cases out of 50 in the first group, 3 cases out of 25 in the second group and 2 cases out of 25 in the third group. From ROC curve of IgM test (figure 3) the sensitivity and

specificity were 82% and 74%, while positive predictive value and negative predictive value were 75.93% and 80.43% respectively.

- The comparison of the three ROC curves of the serological tests has been shown in Figure (4)
- The evaluation parameters of performance of the serological tests are summarized in (Table 1).

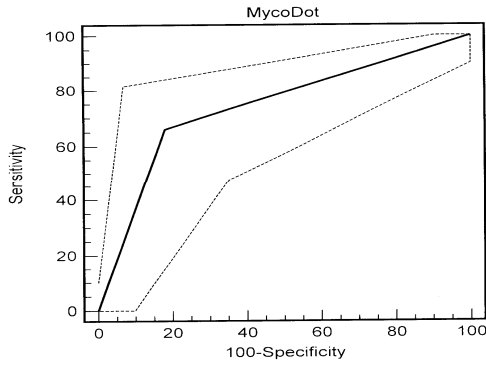


Fig. No. (1): ROC Curve of MycoDot test

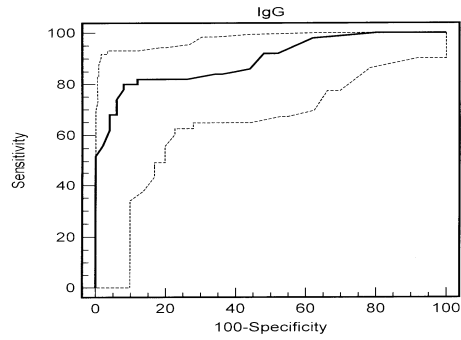


Fig. No. (2): ROC Curve of IgG test

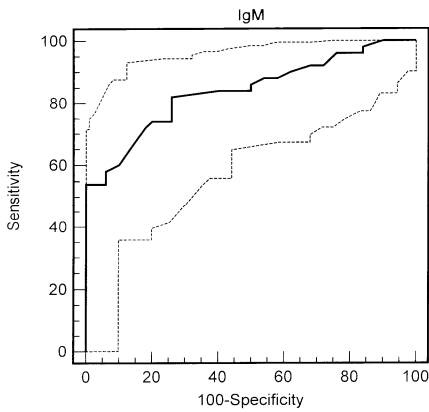


Fig. No. (3): ROC Curve of IgM test

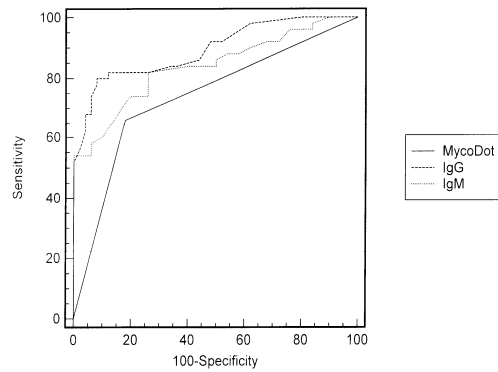


Fig. No. (4): Comparison of ROC curves for the three serological tests

**Table No. (1): Evaluation of Performance of Serological Tests**

Evaluation Parameter	Serological Tests		
	Mycodot	IgG	IgM
Sensitivity	66	80	82
Positive Predicative Value	78.57	90.91	75.93
Specificity	82	92	74
Negative Predicative Value	70.69	82.14	80.43

## DISCUSSION

A tentative diagnosis of TB can be made by observing acid-fast bacilli in smear of sputum, bronchoalveolar lavage, body fluids and gastric washing or urine, etc., while definite diagnosis is established only after the isolation and identification of tubercle bacilli from the patient<sup>(17)</sup> and till now that method is considered to be the gold standard for diagnosing tuberculosis. 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<sup>(8,9,14,18)</sup>.

Despite the increasing development of techniques of rapid identification of mycobacteria by molecular genetic means, there is a hardly need for simple, sensitive and specific test for TB, which would improve or may replace the conventional methods<sup>(19,20)</sup>.

Methods based on Molecular biology are costly and complicated, so that they are not useful for the routine diagnosis in low-income countries<sup>(7,8,20,21)</sup>. Serological tests may be especially useful for a rapid diagnosis of TB in these countries, which may be over 90% of the global

burden of TB cases<sup>(20,21)</sup>. The aim of the present study was to evaluate the diagnostic usefulness of three serological tests, namely Mycodot test using LAM antigen, IgG& IgM tests by ELISA using A60 antigen in pulmonary tuberculosis in adults.

Diagnostic accuracy of a test depends 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 may be have been resulted from the cross reactivity of native antigens with environmental mycobacteria<sup>(9)</sup>. In most of the studies, it has been shown that IgG holds the great promise in diagnosing an active disease in both children as well as adults, when compared with IgM or Ig A classes. IgG is also found to be a useful antibody for monitoring the response of antituberculous treatment. IgG levels have been found to be increased in active TB. Increased IgM levels have also been reported in two studies, while several studies revealed no significant change. 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 stage

of the disease, which may not hold diagnostic promise because the delay on the part of the patients in visiting qualified doctors<sup>(3,14-16&18,22-25)</sup>.

In the present study, patients of group I with pulmonary tuberculosis showed high reactivity rate in the three serological tests compared with those of group II (non-tuberculous pulmonary patients) and those of group III (healthy volunteers). The sensitivity and specificity of IgG against A60 were 80% and 92% which agreed with other results, where they were found to be (36, 91%) and (68, 98%) respectively<sup>(26,27)</sup>.

These results are similar or mildly lower than those of other authors where the positivity rate ranges from 36% to 85%<sup>(26,28)</sup>. This may be because of the population chosen or the criteria that the authors used for diagnosis. The result of IgG in the present study agreed with that of **Alifano et al.**<sup>(14)</sup> which showed sensitivity and specificity of IgG 73.8% and 96.1% respectively and disagreed with that of **Kochak et al.**<sup>(36)</sup> which showed sensitivity and specificity 54.3% and 84.2% and that of **Turner and vonNerom**<sup>(29)</sup> which showed positivity level of 29%. The measurement of IgM A60 in the present study differed with that of **Yüce et al.**<sup>(30)</sup> which showed sensitivity and specificity of 43.8% and 97.04% respectively. The sensitivity value of IgM was previously reported to be between 24% and 60%<sup>(26,27,31-33)</sup>. The results of Mycodot test may agreed with that of **Abebe et al.**<sup>(34)</sup> that showed sensitivity and specificity of 89.3% and 100% and disagreed with that of<sup>(35)</sup> which showed sensitivity and

specificity of 18.5% and 100% and it was found that the concentration of antibodies against LAM antigen were elevated in relapse tuberculosis than that of newly acquired TB. In the present study, the detection of IgG by ELISA using A60 was the best method in diagnosing pulmonary tuberculosis as a sensitive and rapid serological tool of investigation compared with IgM A60 by ELISA and Mycodot test using LAM antigen where their sensitivities and specificities were (80,92%), (82,74%) and (66,82%) respectively.

So, it is recommended that, a development of serodiagnostic techniques for TB should be undertaken to define the target antigen(s) of *Mycobacterium tuberculosis* and the isotype(s) of antibody classes which is responsible for immune response against that target antigen(s).

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### تقييم اختبارات مصلية للتشخيص المناعي للدرن الرئوي باستخدام (A60 and Lipoarabinomannan antigens)

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الخلفية: إن مشكلات تشخيص مرض الدرن باستخدام طرق فحص وزراعة الميكروب المسبب للمرض (AFB) تتطلب اكتشاف طرق مصلية تدعم التشخيص الاكلينيكي للمرض. لذا تم تقييم ثلاثة اختبارات مصلية لتشخيص الدرن الرئوي مقارنة بطرق فحص وزراعة الميكروب المسبب للمرض وهي:  
 باستخدام A60 antigen وكذلك Mycodot test باستخدام Lipoarabinomannan (LAM) antigen بطريقة (Anti-A60 IgG, Anti- A60 IgM) Enzyne-Linked immunosorbent assay (ELISA) بطريقة (Anti-A60 IgG, Anti- A60 IgM) باستخدام antigen A60 للكشف عن IgG and IgM باستخدام antigen A60 في حين تم استخدام antigen (LAM) مثبت فيه أمشاط بلاستيكية عند اجراء Mycodot test وقد تم اجراء هذه الاختبارات بالاضافة الي الاختبارات التقليدية الاخرى لتشخيص المرض علي ٥٠ مريضا مصابا بالدرن (المجموعة الاولى) و ٢٥ مريضا مصابا بأمراض صدرية غير الدرن (المجموعة الثانية) و ٢٥ شخصا سليما تماما ( المجموعة الثالثة) وقد اعتبر افراد المجموعة الثانية والثالثة كمجموعتين ضابطين. وقد تم اجراء اختبارات فحص افلام الدرن باستخدام صبغة Ziehl- Neelsen (ZN) ومزرعة ميكروب AFB والاختبار الجلدي Tuberculin test علي افراد المجموعات الثلاثة.  
 النتائج :

في ELISA-IgG كانت النتائج ايجابية في ٤٢ حاله (٨٤%) في المجموعة الاولى (مرضي الدرن) وكانت ايجابية في ١٠ حالات (٤٠%) في المجموعة الثانية (مرضي صدر غير الدرن) في حين كانت ايجابية في ٨ حالات (٣٢%) في افراد المجموعة الثالثة. وفي اختبار (ELISA) IgM كانت النتائج ايجابية في ٣٠ حاله (٦٠%) في افراد المجموعة الاولى وكانت ايجابية في ٣ حالات (١٢%) في افراد المجموعة الثانية في حين كانت ايجابية في حالتين (٨%) من افراد المجموعة الثالثة.

وفي اختبار Mycodot test كانت النتائج ايجابية في ٣٣ حاله (٦٦%) في افراد المجموعة الاولى وكانت ايجابية في ٦ حالات (٢٤%) في افراد المجموعة الثانية في حين كانت ايجابية في ٣ حالات (١٢%) في افراد المجموعة الثالثة. وقد تم تحديد حساسية وخصوصية الاختبارات الثلاثة (IgG and IgM, Mycodot test) علي اساس الطريقة الاحصائية منحنى Receiver operating characteristic (ROC) لكل اختبار ومقارنة منحنيات (ROC) للاختبارات الثلاثة وكانت ٨٢,٦٦% لاختبار Mycodot و ٩٢,٨٠% لاختبار IgG وكانت ٧٤,٨٢% لاختبار IgM وكانت قيم التوقع الايجابية والسلبية في الاختبارات الثلاثة كالتالي :-

٧٨,٥٧% - ٧٠,٦٩% لاختبار Mycodot و ٩٠,٩٨% - ٨٢,١٤% لاختبار IgG وكانت ٨٠,٤٣% - ٧٥,٩٣% لاختبار IgM. أما اختبارات الفحص باستخدام صبغة ZN والمزرعة لميكروب AFB فقد كانت النتائج ايجابية في كل افراد المجموعة الاولى (مرضي الدرن) وكانت سلبية في كل افراد المجموعتين الضابطين (المجموعتين الثانية والثالثة) أما نتائج اختبار Tuberculin test فكانت ايجابية في ٤٦ حاله (٩٢%) من افراد المجموعة الاولى، ٧ حالات من ٢٠ حاله (٣٥%) من افراد المجموعة الثانية وكانت ٣ حالات من ١٥ حاله (٢٠%) من افراد المجموعة الثالثة.

**الاستنتاج:** تبين من الدراسة أن A60 IgG هي افضل طريقة مصلية لتشخيص الدرن الرئوي مقارنة بـ A60 IgM و Mycodot test وهذه القيمة التشخيصية العالية لـ A60IgG تجعلها مفيدة في تشخيص الدرن حيث انها طريقة سريعة وقليلة التكلفة وعامل مدعم للتشخيص الاكلينيكي بالاضافة لطرق التشخيص الاخرى.