

Evaluation of enzyme-linked immunosorbent assay (ELISA) in serodiagnosis of pulmonary tuberculosis

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تقييم مقايصة الامتصاص المناعي المرتبط بالإنزيم (إليزا) في مجال التشخيص المصلي للسُّلِّ الرئوي
منى جمال الدين مرسى ورائدا محمود يوسف ويحيى محمد خليل

خلاصة : أجريت لمجموعتين من 45 مريضا بالسُّلِّ (التدرن) الرئوي و45 شخصا صحيحا ، فحوص طبية وشعاعية للصدر ، واختبارات إليزا بحثًا عن الغلوبولين المناعي IgA والغلوبولين المناعي IgG . وتم فحص لطاخات من بلغم جميع المصابين بالسُّلِّ كما زرعت عينات من هذا البلغم . وبمقارنة النتائج مع ما انتهى إليه التشخيص الشعاعي والسريبي ، وجد أن نوعية اختبار إليزا قد تجاوزت 90% في اكتشاف الأضداد النوعية IgA و IgG . كما أن إجراء مقايصة إليزا بالتوازي مع الفحص المجهرى لعينات البلغم ، أسفر عن مستوى حساسية بنسبة 80% بالمقارنة بالفحص السريبي والشعاعي ، وبنسبة 100% بالمقارنة بفحص المزرعة . أما مقايصة إليزا بمفردها فيمكن أن تستعمل لتأكيد عدم وجود التدرن الرئوي وليس لتشخيص هذا المرض . ومن جهة أخرى ، فإن مقايصة إليزا ، يمكن أن تستعمل مصحوبة بالفحص المجهرى ، بدلا من طريقة المزرعة للتوصل إلى تشخيص إيجابي خلال 24 ساعة .

ABSTRACT A total of 45 patients with pulmonary tuberculosis and 45 healthy individuals were subjected to chest examination, radiography and ELISA tests for IgA and IgG antibodies. Sputum smear and culture were performed for all tuberculous patients. Evaluated against clinical and radiological diagnosis, ELISA's specificity exceeded 90% in detecting specific IgA and IgG antibodies. The parallel application of ELISA and microscopic examination of sputum yielded 80% sensitivity compared with clinical and radiological examination and 100% sensitivity compared with culture. ELISA alone can be used in ruling out pulmonary tuberculosis but not in diagnosing the disease. However, coupled with microscopic examination, it can be used instead of culture to provide positive diagnosis within 24 hours.

Evaluation de la méthode ELISA pour le sérodiagnostic de la tuberculose pulmonaire

RESUME Quarante-cinq (45) patients atteints de tuberculose pulmonaire et 45 sujets ont été soumis à un examen et à une radiographie pulmonaires ainsi qu'à des dosages des immunoglobulines A et G par la méthode immunoenzymatique (ELISA). Des frottis et la culture de crachats ont été réalisés pour tous les tuberculeux. Dans l'évaluation effectuée par comparaison avec le diagnostic clinique et radiologique, la spécificité de la méthode ELISA a dépassé 90% pour la détection des anticorps spécifiques IgA et IgG. En appliquant parallèlement la méthode ELISA (IgA et IgG) et l'examen microscopique des échantillons de crachats, on a atteint une sensibilité de 80% en comparaison de l'examen clinique et radiologique et une sensibilité de 100% en comparaison de la culture. La méthode ELISA seule peut être utilisée pour exclure une tuberculose pulmonaire mais pas pour diagnostiquer la maladie. Par contre, si elle est associée à l'examen microscopique, la méthode ELISA peut être utilisée au lieu de la culture pour établir un diagnostic positif dans les 24 heures.

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Introduction

Of the infectious diseases, tuberculosis remains the leading cause of human suffering worldwide [1,2,3]. Bloom and Murray reported that the disease affects nearly one-third of the world's population, resulting in eight million cases of clinical disease and three million deaths each year [1]. In view of the increasing incidence of infection with resistant strains [4,5,6] and the growing problem of human immunodeficiency virus (HIV) infection [1,7], these figures are likely to increase.

In Egypt, it was estimated in 1992 that new cases of smear-positive pulmonary tuberculosis will develop at a rate of 30 to 35 per 100 000 population [8]. With a population of 55.8 million, this corresponds to 16 740 to 19 530 smear-positive cases. Unfortunately, in the same year, only one-third (5608) of the expected cases were reported to the health authorities. It is assumed that another proportion of people with tuberculosis sought private physicians and other health care facilities for diagnosis and treatment. However, many of the cases remain undiagnosed [8]. Indeed, in developing countries, more than half of those with tuberculosis are not in contact with health care facilities [9]. These individuals constitute a pool from which the infection is perpetuated to healthy community members [8,10,11].

Eradication of tuberculosis as a public health problem requires the mobilization of all resources for rapid case-finding and adequate treatment [12,13]. This is the goal of a health department tuberculosis control programme [14]. Case-finding and treatment have the social objective of preventing suffering and premature death and the epidemiological objective of interrupting the chain of transmission, hence reducing the tuberculosis burden in the future. In

fact, the World Health Organization (WHO) recommends that these activities should receive the highest priority in any tuberculosis control programme [10].

A whole century has elapsed since the introduction of the tuberculin skin test and the staining and culture methods for diagnosis of tuberculosis. Tuberculin testing using purified protein derivative has lost its epidemiological value, particularly in areas where BCG vaccination is compulsory, as it fails to distinguish between vaccinated individuals and those who have been infected or developed the disease [15]. Direct microscopic examination of sputum is a simple and rapid means of detecting cases of pulmonary tuberculosis [16]. However, this necessitates the presence of a huge number of bacilli, from 5000 to 10 000 per millilitre of sputum. On the other hand, the culture technique is able to detect as few as 10 to 100 bacilli per millilitre of sputum [11,16]. Definite diagnosis of the disease is based on the identification of the organism by culture; this takes at least 3 to 6 weeks resulting in delay in diagnosis, treatment and contact investigation, in addition to the high cost of the technique, which limits its use in developing countries [11,16]. In Egypt, this method is reserved only for patients with successive negative smears and in whom pulmonary tuberculosis is clinically suspected [8].

Obviously, such conventional methods hinder the rapid and accurate diagnosis of pulmonary tuberculosis, one of the major constraints facing tuberculosis control programmes in developing countries, including the Egyptian National Tuberculosis Control Programme [8,17,18]. In the past, serological tests have been generally unacceptable because of their low specificity. However, now that specific antigens have been identified and recombinant antigens

are more readily obtainable, serological tests are a far more realistic proposition.

This study was undertaken to evaluate the role of enzyme-linked immunosorbent assay (ELISA), a more rapid method for the serodiagnosis of pulmonary tuberculosis than conventional methods.

Subjects and methods

Subject selection

A total of 90 subjects were included in the study since only one kit of ELISA identifying specific IgA antibodies and another for IgG could be afforded. The first 45 cases of pulmonary tuberculosis admitted to Alexandria Main University Hospital and Kom El-Shoqafa Chest Hospital from 1 December 1995 were enrolled in the study. An equal number of controls were selected from patients attending the ophthalmology and dermatology outpatient clinics at Alexandria Main University Hospital.

Cases of pulmonary tuberculosis were identified and enrolled on the basis of meeting the standard case definition of pulmonary tuberculosis [19]. Controls were included according to the following criteria:

- no relevant history of pulmonary tuberculosis
- free from any signs and symptoms suggestive of pulmonary tuberculosis
- chest radiographies revealing no abnormalities consistent with pulmonary tuberculosis.

Methods and data collection

All participants were subjected to:

- Interview questionnaire, to reveal their personal characteristics, including age, sex, marital status, education and occu-

pation as well as their habits, such as smoking, consumption of alcohol and psychoactive substances. Participants were asked about associated health problems and family history of tuberculosis. Detailed history of the illness and the treatment regimen received were obtained from tuberculous patients. The type of regimen and its duration were ascertained by reviewing the hospital files.

- Check for the presence of a scar from BCG vaccination.
- Chest examination and radiography, to accept or reject the diagnosis of tuberculosis.
- Laboratory investigations, including sputum smear, culture and serology for the identification of specific IgA and IgG antibodies.

Early morning sputum samples (at least two samples) were aseptically collected from tuberculous patients for smear and culture. Smears were stained with Ziehl-Neelsen (ZN) stain for acid-fast bacilli. Culture was performed in two Löwenstein-Jensen glycerol tubes (Biolife commercial basic product) after Petroff's decontamination procedure [16]. Culture tubes were incubated at 37 °C for 12 weeks and not discarded before then. This was followed by ZN-stained film even if there was no apparent growth. Healthy subjects could not be tested because sputum cannot be provided, only saliva. Hence, they were considered negative for the organism by both techniques.

A 2 ml sample of venous blood was drawn from all participants. Serum was then separated and stored at -20 °C until tested. IgA antibodies to Kp-90 Im-CRAC characteristic for active tuberculosis were assayed by ELISA (Pharmeupe Medical and Diagnostic, Amsterdam, Netherlands).

For the detection of IgG antibodies to *Mycobacterium tuberculosis* complex, an ELISA using a highly specific recombinant 38 kDa antigen (Omega Diagnostics Limited, Alloa, Scotland) was used. Both tests were applied according to the manufacturers' instructions.

Data analysis

Data were analysed using *SPSS* (version 6). The kappa coefficient was computed to test the agreement between the two diagnostic tools relying on the strength of agreement rather than the *P* value. Accordingly, the strength of agreement was classified as poor ($\kappa < 0$), slight ($\kappa = 0-0.20$), fair ($\kappa = 0.21-0.40$), moderate ($\kappa = 0.41-0.60$), substantial ($\kappa = 0.61-0.80$) and almost perfect ($\kappa = 0.81-1.00$) [20]. In addition, the likelihood ratio (LR) of a positive test, and the validity and predictive values of the performed serological tests were computed. The Chi-square test and the Student *t*-test were used to test the significance at the 5% level.

Results

The majority of the participants studied were men—women constituted less than one-third of the sample. The mean age of tuberculous patients (39.4 ± 16.53 years) was significantly higher than that of the controls (28.6 ± 10.64 years) ($t = 3.67$, $P = 0.0004$). The composition of the two groups by marital status did not differ significantly. Similarly, they did not differ significantly regarding their educational level or occupation (Table 1).

The patients had been suffering from pulmonary tuberculosis for a mean duration of 2.5 ± 2.76 years with a minimum of 2 weeks and a maximum of 14 years. Less than half of the patients (46.7%) reported

that they had previously received antituberculous chemotherapy and only 52.4% of these had completed the regimen as prescribed by the treating physician. At the time of the study, 40 patients (88.9%) had been under specific treatment for a mean duration of 2.53 ± 4.09 months (minimum 2 weeks, maximum 18 months). Out of these 40 cases, isoniazid was prescribed to 90.0% of the patients followed by rifampicin (85.0%), ethambutol (67.50%), pyrazinamide (57.0%) and streptomycin (52.50%). The majority of patients were receiving a combination of three (42.50%) or four (40.0%) antituberculous drugs, while a small percentage were receiving a combination of two (7.5%) and five drugs (10.0%).

Only 20.0% of the healthy subjects were regular smokers and none of them reported the consumption of alcohol or psychoactive substances. On the other hand, smoking and the consumption of alcohol and psychoactive substances were encountered in a significantly higher percentage (64.4%) of tuberculous patients ($\chi^2_1 = 55.27$, $P = 0.00001$).

In conjunction with pulmonary tuberculosis, other chronic health problems were reported by 20.0% of the patients, including diabetes ($n = 7$) and bronchial asthma ($n = 2$). The majority of healthy participants (91.1%) were totally free of any chronic health problem, but rheumatic heart disease was encountered in 8.9% of the cases. However, no significant difference was detected between the two groups, ($\chi^2_1 = 2.25$, $P = 0.1338$).

Equal percentages (40%) of participants in the index and control groups had received the BCG vaccine as indicated by the presence of the characteristic scar. However, family history of tuberculosis was encountered in a significantly higher percentage of tuberculous patients (20.0%)

Table 1 General characteristics of tuberculous patients and their controls

Characteristic	Cases (n = 45)		Controls (n = 45)		Test of significance
	No.	%	No.	%	
<i>Sex</i>					
Male	32	71.1	33	73.3	$\chi^2_1 = 0.06$ $P = 0.813$
Female	13	28.9	12	26.7	
<i>Marital status</i>					
Married	23	51.1	16	35.6	$\chi^2_2 = 2.48$ $P = 0.289$
Single	20	44.4	25	55.6	
Widowed/divorced/separated	2	4.4	4	8.9	
<i>Education</i>					
Illiterate/read and write	28	62.2	21	46.7	$\chi^2_3 = 2.36$ $P = 0.502$
Primary/preparatory	8	17.8	10	22.2	
Secondary	8	17.8	12	26.7	
University	1	2.2	2	4.4	
<i>Occupation</i>					
Professional	1	2.2	2	4.4	$\chi^2_5 = 9.99$ $P = 0.075$
Skilled/semiskilled	6	13.3	6	13.3	
Manual	21	46.7	32	71.1	
Housewife	10	22.2	4	8.9	
Student	3	6.7	-	-	
Not attending school nor working	4	8.9	1	2.2	
<i>Age (years)</i>					
Mean \pm s	39.36 \pm 16.532		28.60 \pm 10.641		$t = 3.671$
Min-Max	15-72		15-50		$P = 0.0004$

s = standard deviation

compared to 2.2% among controls ($\chi^2_1 = 4.60$, $P = 0.031$).

Table 2 shows the results of the microscopic examination and culture of sputum specimens collected from the 45 tuberculous patients. *M. tuberculosis* was detected in 42.2% of the patients by smear examination and in an equal percentage (42.2%) by culture. However, discordant results were observed in six patients.

Serum examination for specific IgG antibodies denoting activity yielded a positive result in 37.8% of tuberculous patients compared to only 4.4% of the controls. Moreover, 53.3% of the patients were

Table 2 Results of smear examination and culture of sputum specimens collected from tuberculous patients

Smear examination	Culture		Total
	Positive	Negative	
Positive	16	3	19
Negative	3	23	26
Total	19	26	45

found to have specific IgA antibodies (compared to 6.7% among the controls). These differences were statistically highly significant (Table 3). Among tuberculous

Table 3 Distribution of the participants according to the specific *Mycobacterium* antibodies detected by serodiagnosis

Type of antibodies detected	Cases (n = 45)		Controls (n = 45)		Test of significance
	No.	%	No.	%	
<i>IgG</i>					
Positive	17	37.8	2	4.4	$\chi^2 = 15.01$ $P = 0.00010$
Negative	28	62.2	43	95.6	
<i>IgA</i>					
Positive	24	53.3	3	6.7	$\chi^2 = 23.33$ $P = 0.00000$
Negative	21	46.7	42	93.3	

patients, discordant results were observed in 17 patients (Table 4).

A fair agreement was observed between the specific IgA antibodies detected by the ELISA technique and the results of the microscopic examination of sputum specimens ($\kappa = 0.36$), culture ($\kappa = 0.24$) and either technique ($\kappa = 0.35$), while moderate agreement was observed with clinical and radiological examination ($\kappa = 0.46$) (Table 5). The sensitivity of ELISA in detecting specific IgA antibodies just exceeded 50% evaluated against the clinical and radiological diagnosis (53%) and the detection of the organism by culture (53%) and by either smear or culture (59%). However, it was 63.2% compared to the microscopic examination of sputum. A positive test was about eight times more likely to be obtained among patients identified by clinical and radiological diagnosis (LR = 7.99), while it was two to three times more likely among those identified bacteriologically by smear (LR = 2.98), culture (LR = 2.19) or either technique (LR = 2.87). The specificity of this serological test was high (76%–93%), being highest with the clinical and radiological diagnosis and lowest with culture. The predictive value positive of the test was lowest compared with the detection of

Table 4 Distribution of tuberculous patients according to the specific antibodies detected by the two types of ELISA

IgG antibodies	IgA antibodies		Total
	Positive	Negative	
Positive	12	5	17
Negative	12	16	28
Total	24	21	45

the organism by culture (37%) and highest (89%) compared with the clinical and radiological diagnosis. With the latter, the lowest predictive value negative (67%) was observed while a higher predictive value negative (89%) was obtained compared with the smear examination of sputum (Table 5).

Table 6 presents the sensitivity, specificity and predictive values of serodiagnosis detecting specific IgG antibodies in relation to the different diagnostic techniques adopted. A fair to moderate agreement existed between ELISA and the other diagnostic tools as revealed by the value of the kappa coefficient (0.33–0.46). The sensitivity of ELISA was the lowest (38%) compared with the clinical and radiological

Table 5 Validity and predictive values of ELISA (IgA) in relation to the different diagnostic methods used

Diagnostic method	ELISA (IgA)		Kappa coefficient	Sensitivity (%)	Specificity (%)	Predictive value	
	Positive (n = 27)	Negative (n = 63)				Positive (%)	Negative (%)
<i>Clinical and radiological</i>							
Positive	24	21	0.46	53.3	93.3	88.9	66.7
Negative	3	42					
<i>Sputum smear</i>							
Positive	12	7	0.36	63.2	78.9	44.4	88.9
Negative	15	56					
<i>Sputum culture</i>							
Positive	10	9	0.24	53.6	76.1	37.0	85.7
Negative	17	54					
<i>Either smear or culture</i>							
Either positive	13	9	0.35	59.1	79.4	48.1	85.7
Both negative	14	54					

Table 6 Validity and predictive values of ELISA (IgG) in relation to the different diagnostic methods used

Diagnostic method	ELISA (IgG)		Kappa coefficient	Sensitivity (%)	Specificity (%)	Predictive value	
	Positive (n = 19)	Negative (n = 71)				Positive (%)	Negative (%)
<i>Clinical and radiological</i>							
Positive	17	28	0.33	37.8	95.6	89.5	60.6
Negative	2	43					
<i>Sputum smear</i>							
Positive	10	9	0.39	52.6	87.3	52.6	87.3
Negative	9	62					
<i>Sputum culture</i>							
Positive	10	9	0.39	52.6	87.3	52.6	87.3
Negative	9	62					
<i>Either smear or culture</i>							
Either positive	12	10	0.46	54.5	89.7	63.2	85.9
Both negative	7	61					

diagnosis and increased to 53%–55% compared with the detection of the organism by microscopic examination of sputum, culture or either method. The possibility of a positive test was nearly eight times higher

among patients identified clinically and radiologically (LR = 8.49), four times higher among those identified by smear (LR = 4.15) or culture (LR = 4.15) and five times higher among patients identified by

Table 7 Validity and predictive values of ELISA (IgG and IgA) in relation to the different diagnostic methods used

Diagnostic method	ELISA (IgG and IgA)		Kappa coefficient	Sensitivity (%)	Specificity (%)	Predictive value	
	Either positive (n = 34)	Both negative (n = 56)				Positive (%)	Negative (%)
<i>Clinical and radiological</i>							
Positive	29	16	0.53	64.4	88.9	85.3	71.4
Negative	5	40					
<i>Sputum smear</i>							
Positive	12	7	0.24	63.2	69.0	35.3	87.5
Negative	22	49					
<i>Sputum culture</i>							
Positive	12	7	0.24	63.2	69.0	35.3	87.5
Negative	22	49					
<i>Either smear or culture</i>							
Either positive	15	7	0.33	68.2	72.1	44.1	87.5
Both negative	19	49					

either technique (LR = 5.29) compared with those who were not identified by these methods. The test's specificity was high (87%–96%) with all diagnostic methods used. The predictive value negative of ELISA exceeded 85% with all diagnostic tools except with the clinical and radiological diagnosis where it was 61%. A reverse trend was observed for the predictive value positive of the test.

The validity and predictive values of ELISA in detecting both specific IgA and IgG antibodies combined (classified as either positive or both negative) compared with the other diagnostic methods are presented in Table 7. The kappa coefficient denotes a moderate agreement between the serodiagnosis and the clinical and radiological one ($\kappa = 0.53$), but a fair agreement with the diagnosis by smear ($\kappa = 0.24$), culture ($\kappa = 0.24$) or either technique ($\kappa = 0.33$). Evaluated against the different diag-

nostic methods, the sensitivity of ELISA was 63%–68%. The probability of a positive test was two times higher among patients identified bacteriologically by smear (LR = 2.03), culture (LR = 2.03) or either technique (LR = 2.44), while nearly six times higher among those identified clinically and radiologically (LR = 5.79). The highest specificity of the test (89%) was obtained compared with the clinical and radiological diagnosis, while the lowest (69%) was when compared with the smear and culture results. The highest predictive value positive of the test observed was 85% when compared with the clinical and radiological diagnosis and it was the lowest (35.3%) when compared with the smear and culture techniques. Compared with the different diagnostic tools, the predictive value negative of the test exceeded 85%, except with the clinical and radiological diagnosis where it was 71%.

Table 8 Validity and predictive values of ELISA (IgG and IgA) and sputum smear in relation to the clinical and radiological diagnosis and culture results

Diagnostic method	ELISA (IgG and IgA) and sputum		Kappa coefficient	Sensitivity (%)	Specificity (%)	Predictive value	
	Either positive (n = 41)	Both negative (n = 49)				Positive (%)	Negative (%)
<i>Clinical and radiological</i>							
Positive	36	9	0.68	80.0	88.9	87.8	81.6
Negative	5	40					
<i>Sputum culture</i>							
Positive	19	-	0.48	100.0	69.0	46.3	100.0
Negative	22	49					

Combining the results of ELISA (both IgA and IgG) and that of the smear classified as either positive or both negative (Table 8) yielded a substantial agreement ($\kappa = 0.68$) with an 80% sensitivity, 89% specificity, a predictive value positive of 88% and negative of 82% when evaluated against the clinical and radiological diagnosis. Either positive smear or positive ELISA were seven times more likely to be obtained among patients diagnosed clinically and radiologically (LR = 7.20). A moderate agreement was observed between this combined technique and the culture result ($\kappa = 0.48$). Compared with the culture, it yielded a 100% sensitivity and predictive value negative with a low specificity (69%) and predictive value positive (46%). Either positive smear or positive ELISA were about three times more likely to be obtained among patients identified by culture compared with those who were not identified by this method (LR = 3.22).

Discussion

Tuberculous patients enrolled in the present study were comparable to their con-

trols except for being significantly older. An identical proportion of patients and healthy participants had received BCG vaccine. However, smoking and the consumption of alcohol as well as narcotics were overrepresented among tuberculous patients. These habits are known to increase a person's vulnerability to the disease. The presence of the disease among family members is an additional factor that increases the probability of contracting the infection and developing the disease.

In the present study, less than half of the patients had been previously diagnosed and prescribed antituberculous drugs. However, nearly half of these had not complied with the treatment regimen. Generally, incompleteness of the treatment is associated with a substantial increase in the number of chronic patients who can infect others. Moreover, they are more likely to harbour strains of the bacilli that are resistant to one or more antituberculous drugs [8]. This is only one of the factors that has contributed to the re-emergence of tuberculosis as a major public health problem [21].

The reason for the failure of tuberculosis control programmes in developing countries is not only the inability to cure

those who are diagnosed, but also the inability to detect a sufficient number of tuberculosis cases, who are the prime source of infection [17]. Among the countries of the Eastern Mediterranean Region, Egypt is classified as a country of intermediate tuberculosis incidence ranging from 2 to 100 per 100 000 population with a low detection rate of less than 40% [22]. In the present study, bacteriological diagnosis revealed the presence of the organism in less than half the patients. This could be attributed to the fact that the majority of patients had been under specific treatment with a combination of powerful drugs for a mean duration of 2.5 months. In three cases, the tubercle bacilli were detected in the sputum specimen by culture but not detected by smear. This is an advantage of the culture over microscopic examination. In the other three microscopy positive, culture negative patients, the organism appeared fragmented indicating dead bacilli secondary to treatment effect. Consequently, they failed to show any growth.

It is widely accepted that infection with *M. tuberculosis* often induces a humoral response that can be detected and measured by specific antigen [23]. Indeed, the availability of new, more sensitive antibody assays, particularly ELISA, has stimulated a resurgence of interest in the serology of tuberculosis as a rapid method of diagnosis. Since the mid-seventies, several antigens have been evaluated for their serodiagnostic potential in detecting antibodies using this technique with considerable variation in their sensitivity and specificity [24-28].

In the present study, the technique detected specific antibodies of the IgA class in nearly half of the cases and of the IgG class in just over one-third of them. Generally, the ability of either test to detect those who truly had the disease based on clinical and radiological as well as bacteriological

diagnosis was low. This was expected since the spectrum of the illness was extremely wide ranging from two weeks to 14 years. Some of these patients had active tuberculosis associated only with a rise in the characteristic IgA antibodies; in others, inactive or improving tuberculosis set in with a decrease in IgA antibodies to an undetectable level, coupled with an increase in the level of IgG antibodies. In spite of this low sensitivity, the likelihood ratio of a positive test was high. This is attributed to the small number of patients classified as false positive by either test rather than to an acceptable sensitivity. In fact, both tests were able to identify more than 90% of subjects classified as not having the disease by clinical and radiological examination with a low predictive value negative of just over 60%. Evaluated against the bacteriological diagnosis, the specificity of ELISA in detecting specific IgG antibodies exceeded 85%, while it was more than 75% for the test detecting specific IgA antibodies. Both tests had a high predictive value negative.

Combining the results of the two tests classified as either positive or both negative was associated with a slight improvement in sensitivity at the expense of specificity. Evaluated against the culture, the yielded sensitivity compares favourably with that reported by previous studies [29,30], however with a much higher predictive value positive. A much higher sensitivity exceeding 85% was reported by Wang et al. [31] and Krambovitis [32]. Moreover, some of the studies [29,32] reported a much higher specificity than that obtained in the present one, although with a comparable predictive value negative. This variation in the validity and predictive values of the tests may be attributed to differences in the characteristics of the patients, as well as the types of antigens used.

It is true that a perfect test would always be positive in the presence of the disease and negative in its absence. However, false negative and false positive results commonly mar the capacity of a test to achieve these goals [33]. False negative results were viewed by Krambovitis [32] to be related to the pathogenesis of the illness. It is possible that the constant release of organisms or antigens from the site of infection leads to chronic stimulation of antibody synthesis and the production of low avidity antibody synthesis. This may result in the formation of circulating immune complexes of the more reactive antibodies with the released antigen and a considerable reduction in detectable antibodies. This hypothesis is consistent with two observations: the first is that tuberculosis causes an increase in the overall concentration of circulating IgG and IgA [34,35], not reflected in the specific antibody measured by ELISA with mycobacterium antigens [36]; secondly, the presence of circulating immune complexes has been demonstrated [37,38] with an inverse relation between the concentration of immune complexes and antimicrobial antibodies [38]. An alternative explanation has been proposed by Daniel et al. [26] based on the immunological spectrum. This concept suggests that at one extreme of the spectrum, tuberculosis is confined to localized foci with well developed cell mediated immunity, but little or no detectable antibodies.

Since a single perfect test does not exist, a combination of two or more tests can be used in series or in parallel to enhance specificity or sensitivity. Evaluated against the bacteriological diagnosis, the sensitivity of ELISA was still low. The fair association obtained indicated by the kappa coefficient denotes that ELISA *per se* is unlikely to replace the bacteriological diagnosis. Parallel application of ELISA and the

microscopic examination of sputum specimens was associated with a sensitivity of 80% compared with the clinical and radiological diagnosis and 100% compared with the culture. The latter figure was much higher than that reported by Daniel et al. [29] and Zeiss et al. [30] who combined the results of smear and ELISA. The predictive value positive of this combination of tests was comparable to that reported by Zeiss et al. [30] but much lower than that reported by Daniel et al. [29]. The low specificity of the parallel application of these two tests reflects the ability of either method to detect cases which have not been identified by culture rather than an associated excess of false positive results.

The present study has its limitations. Enrolled patients presented great heterogeneity regarding the spectrum of illness. In addition, the small number selected, governed by the affordability of the test, did not allow the stratification of the sample and consideration of the validity of the test in relation to the spectrum of illness. However, the results indicate that the tests may be promising in ruling out the disease, particularly in patients with lung lesions which mimic pulmonary tuberculosis clinically and radiologically. Furthermore, ELISA can be a useful adjunct to sputum smear in ruling in tuberculosis within 24 hours. In monetary terms, the purchase price in Egypt is 7 Egyptian pounds for IgG (US\$ 1 = LE 3.37) and 15 Egyptian pounds for IgA, much less than the cost of examination, radiography and culture; hence, diagnosis can be achieved at a fraction of the usual cost. An additional advantage is that ELISA can be performed under field conditions typical of those found in developing countries as its application does not require major equipment or the training and experience mandatory for the culture.

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