

Diagnostic Value Of Adenosine Deaminase in Tuberculous and Malignant Pleural Effusion

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ABSTRACT

Introduction:

Tuberculous pleural effusion (TPE) is a common problem for differential diagnosis from malignant effusion (MPE) in epidemic areas of tuberculosis (TB). Prediction based on adenosine deaminase (ADA) is dependent on age as well as the tuberculosis incidence.

Aim of the work: to estimate the value of cutoff point of ADA in MPE and TPE & to evaluate its role in differential diagnosis in Egypt a country with high incidence of TB.

Subjects & methods: the study was conducted in Kaser El-Aini hospital, Cairo University in the period from April 2011 to January 2012. It was carried on 30 patients. We retrospectively analyzed 30 patients with a definitive diagnosis of TPE (n=19) and MPE (n= 11). The optimal cutoff value of ADA was determined using the receiver operating characteristic (ROC) curve. There was a statistically significant difference according to the levels of pleural fluid ADA between TPE and MPE groups.

Result: confirm that ADA is a very useful parameter for the differential diagnosis of TPE and MPE, specifically in younger with a higher incidence of tuberculosis.

Keywords: Tuberculosis - pleural effusion - adenosine deaminase - malignant effusion

Abbreviations: ADA : adenosine deaminase , TPE: Tuberculous pleural effusion, MPE: malignant pleural effusion , ROC : receiver operating characteristic and TB: Tuberculosis

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Introduction

TPE is the most common form of the extra pulmonary tuberculosis in our country. Its definitive diagnosis is established by determining of the tuberculosis in the pleural fluid or pleural tissue. Acid fast bacilli can be determined by less from 25% with direct examination of the pleural fluid. In TPE, granulomatous pleuritis is determined in 80% of the cases with the pleural biopsy, while the histological examination combined with culture of the biopsy material increases the rate of diagnosis to 90% (1).

The diagnosis cannot be established in 10-20% of the patients with these methods even in the best conditions. Therefore, many studies have been conducted to demonstrate the role of pleural fluid levels of ADA in the differential diagnosis of pleuritis in recent decades. High sensitivity and specificity values reported (2).

These values may vary according to the incidence of the tuberculosis and proportional frequency of the diseases included in the differential diagnosis of the community in which the measurements were done. The level of ADA is used more commonly in the countries with a moderate to high

incidence of tuberculosis in the differential diagnosis of TPE. Because it is a less invasive and more inexpensive method that can be accessed more quickly and accurately specifically in young patients with a high prevalence of TPE(3).

Materials and Methods

Patients:

Total 30 patients presenting with pleural effusions who were admitted to Kasr El Aini Hospital between April 2011 and January 2012 were studied. Informed consent was obtained from the patients. Clinical signs and symptoms, demographic data, and radiologic results were recorded. Of these 14 men and 16 women ranging age from 21 to 70 years old, 19 patients had tuberculous pleurisy, 11 patients had malignant pleuritis, The pleural fluid was exudative with predominantly mononuclear cells (lymphocyte count > 50%) in all patients.

Specimen Collection and Processing

For each subject, at least 40 mL of pleural fluid was collected in a syringe during thoracentesis. A portion of the sample was submitted for acid-fast staining, bacteriologic examination, cytological examination, and measurement of protein, lactate dehydrogenase (LDH), and glucose, ADA.

ADA activity was measured by auto analyzer using commercially available kits. ADA activity was measured by auto analyzer using commercially available kits.

The conditions considered for TPE diagnosis were:

1. Determination of the necrotizing granulomatous inflammation in the

pleural sampling carried out with Thoracoscopic biopsy;

2. While there was not any other reason to explain the pleural fluid with clinical and radiologic appearance suggesting TB with response to anti-TB treatment .

For the diagnosis of MPE, malignancy in cytology of the pleural fluid and/or on histology of the pleural tissue was considered. ADA activity in the pleural fluid was studied and the results were recorded as IU/L in all the patients.

Statistical Analysis

Quantitative data were presented as mean and standard deviation (SD) values. For parametric data, Student's t-test was used for comparisons between mean values of two groups. One way ANOVA (Analysis of Variance) was used to compare between mean values of more than two groups. Tukey's post-hoc test was used for pair-wise comparisons between mean values when ANOVA test is significant.

For non-parametric data, Mann-Whitney U test was used to compare between two groups. This test is the non-parametric alternative to Student's t-test. Kruskal-Wallis test was used to compare between more than two groups. This test is the non-parametric alternative to one-way ANOVA. Mann-Whitney U test was used for pair-wise comparisons between the groups when Kruskal-Wallis test is significant.

Qualitative data were presented as frequencies and percentages. Chi-square (χ^2) test was used for studying the comparisons between different qualitative variables.

Spearman's correlation coefficient was used to determine significant

correlations between the different variables.

to establish the optimal cut-off points and the likelihood ratios (LRs) of ADA

ROC (Receiver Operating Characteristic) curve was constructed

Table (1): Mean, standard deviation (SD), frequency, percentage values and results of One-way ANOVA, Tukey's test and Chi-square test for comparison between ages and gender distributions in the studied groups

	TB (n = 19)	Malignancy (n = 11)	P-value
Age (Mean ± SD)	29.2 ± 12.2 ^b	55.2 ± 11.9 ^a	<0.001*
Gender (Frequency, %)			
Male	9 (47.4)	5 (45.5)	0.681
Female	10 (52.6)	6 (54.5)	

*: Significant at $P \leq 0.05$. Different letters are statistically significantly different according to Tukey's test

Table (2): Frequency, percentage values and results of Chi-square test for comparison between clinical features in the studied groups

Features (Frequency, %)	TB (n = 19)	Malignancy (n = 11)	P-value
Fever	12 (63.2)	1 (9.1)	0.032*
Dyspnea	17 (89.5)	11 (100)	0.676
Cough	18 (94.7)	10 (90.9)	0.922
Expectoration	16 (84.2)	8 (72.7)	0.764
Chest pain	8 (42.1)	1 (9.1)	0.186
Loss of weight	6 (31.6)	3 (27.3)	0.413
Night sweat	1 (5.3)	0 (0)	0.889
Anorexia	0 (0)	1 (9.1)	0.609
Sputum			
Negative	16 (84.2)	9 (81.8)	0.898
No sputum	3 (15.8)	2 (18.2)	

*: Significant at $P \leq 0.05$

Table (3): Mean, standard deviation (SD), frequency, percentage values and results of Kruskal-Wallis test, Mann-Whitney U test and Chi-square test for comparison between diagnostic tests in the studied groups

	TB (n = 19)	Malignancy (n = 11)	P-value
ADA (Mean ± SD)	83.5 ± 50.3 ^a	28.7 ± 23.6 ^c	0.002*

Tuberculin test (Frequency, %)			
Positive	16 (84.2)	1 (9.1)	<0.001*
Negative	3 (15.8)	10 (90.9)	

*: Significant at $P \leq 0.05$, Different letters are statistically significantly different according to Mann-Whitney U test

Diagnostic accuracy of ADA

ROC curve analysis

ROC (Receiver Operating Characteristic) curve was constructed to establish the optimal cut-off points and the likelihood ratios (LRs) of ADA and Interferon.

ADA

ROC curve analysis of ADA values for the diagnosis of TB in the present study showed that the optimal cut-off point was determined at 30 IU/L. The likelihood ratios (LRs) were 3.76 and 0.14 for values above or below this cut-off point.

Table (4): Results of ROC curve analysis for ADA in diagnosis of TB

Area under the ROC curve (AUC)	0.838
Standard error	0.066
95% Confidence interval	0.688 to 0.935
P-value	0.0001*

*: Significant at $P \leq 0.05$

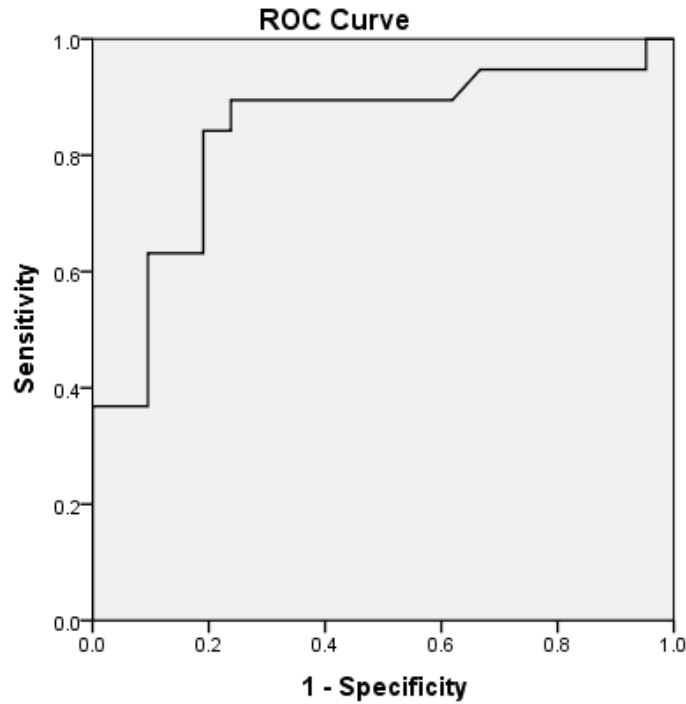


Figure (): ROC curve for ADA

Table (5): Sensitivity, specificity, predictive values and diagnostic accuracy of ADA in detecting TB

	TB		Total
	+ ve	- ve	
ADA			
+ ve	16 (True +ve)	3 (False +ve)	19
- ve	2 (False -ve)	9 (True -ve)	11

$$\text{Sensitivity (\%)} = \frac{16}{16 + 2} \times 100 = \mathbf{80\%}$$

$$\text{Specificity (\%)} = \frac{9}{3 + 9} \times 100 = \mathbf{85\%}$$

$$\text{Positive predictive value (PV}^+) \text{ (\%)} = \frac{16}{16 + 3} \times 100 = \mathbf{84.2\%}$$

$$\text{Negative predictive value (PV}^-) \text{ (\%)} = \frac{9}{2 + 9} \times 100 = \mathbf{81\%}$$

$$\text{Diagnostic accuracy (\%)} = \frac{16 + 9}{30} \times 100 = \mathbf{83.3\%}$$

A total of 30 patients (14 male and 16 female) were included to the study.

TPE group consisted of 9 male and 10 female; the mean (SD) age was 29.2 ±

12.2 years..2 ± MPE group consisted of 5 men and 6 women; the mean (SD) age was 55.2 ± 11.9 years. Table 1.

Mean age of the MPE group was significantly higher than the TPE group ($p < 0.0001$). There was no statistically significant difference between the two groups in the gender distribution ($p > 0.05$).

The mean level (SD) of ADA was 83.5 ± 50.3 U/L in TPE and 28.7 ± 23.6 U/L in MPE. ADA level of the pleural fluid was significantly higher in TPE ($p < 0.001$).

Mean levels of ADA with minimum and maximum values were given in Table () Considering all patients, a significant negative correlation was defined between the fluid's ADA activity and age ($p < 0.0001$).

The cutoff value of ADA for diagnosing TPE was 30 U/L, the sensitivity of ADA was (80%), specificity was (85%) and the diagnostic accuracy was 83.3%. positive predictive value (PPV) = 84.2%, negative predictive value (NPV) = 81% When the level of ADA and age were considered together, diagnostic value of the test was remarkably increased. Sensitivity, specificity, PPV, NPV and accuracy for the critical ADA

Discussion

Tuberculosis is common in our country, TPE is the most common extrapulmonary form of the tuberculosis in our country. Although it is usually seen in the young ages, yet it may be seen also in the advanced ages and its differential diagnosis with MPE might be a problem (4)

Histopathological and microbiological analysis of the pleural fluid or tissue

may seem as the most ideal method, but definitive diagnosis cannot be reached in approximately 20% of the patients (4,5).

Hence many markers that may be helpful in the differential diagnosis were studied in the pleural fluid. Two of these, ADA and interferon gamma are the most widely used and currently the most accepted tests (6).

Especially ADA has been more commonly preferred for the diagnostic algorithms in the countries with a moderate to the high incidence of tuberculosis because it is a more inexpensive method that can be accessed more quickly (7).

ADA is an enzyme catalyzing the conversion of the adenosine and deoxyadenosine to the inosine and deoxyinosine in the purine degradation pathway. Its quantity increases in the immature and non-differentiated T-lymphocytes following mitogenic and antigenic stimulation (8).

While the increase of ADA activity in the MPE has been associated with the predominance of CD8, prominent rise observed in TPE has been tried to be explained with the presence of gradually increasing CD4 blastogenesis after the mycobacterial antigenic stimulus (9).

There are numerous studies in the literature indicating the increase of pleural fluid levels of ADA in the TPE. High values of the sensitivity and specificity have been reported in the countries with the high prevalence of TPE, particularly in the young patients (10).

In our study we retrospectively evaluated 30 patients (14 male and 16 female), TPE group consisted of 9 male and 10 female; the mean (SD) age was 29.2 ± 12.2 years..2 ± MPE group consisted of 5 men and 6 women; the mean (SD) age was 55.2 ± 11.9 years. Table (1).

Mean age of the MPE group was significantly higher than the TPE group ($p < 0.0001$). There was no statistically significant difference between the two groups in the gender distribution ($p > 0.05$).

These result was agreed with **Valdes et al** that found that the mean age of tuberculous group was 33.9 ± 13.2 years that of malignant group was 45.5 ± 16.8 years(10) .

In the current study the mean level (SD) of ADA was 83.5 ± 50.3 U/L in TPE and 28.7 ± 23.6 U/L in MPE. ADA level of the pleural fluid was significantly higher in TPE ($p < 0.001$) .

Mean levels of ADA with minimum and maximum values were given in Table (3) Considering all patients, a significant negative correlation was defined between the fluid's ADA activity and age ($p < 0.0001$).

The cutoff value of ADA for diagnosing TPE was 30 U/L, the sensitivity of ADA was (80%), specificity was (85%) and the diagnostic accuracy was 83.3%. positive predictive value (PPV) = 84.2%, negative predictive value (NPV) = 81% When the level of ADA and age were considered together, diagnostic value of the test was remarkably increased. Sensitivity, specificity, PPV, NPV and accuracy critical for ADA .

In a study conducted by Castro et al. evaluating consecutive 410 nontuberculous lymphocytic pleural fluid samples, were identified the level of ADA above 40U/L only in seven cases (1.71%). They can accurately use ADA levels < 40 U/L to rule out the tuberculosis (11).

In another study was conducted in Japan, reported that the ADA level < 50 U/L was used to rule out the tuberculosis, but they performed the thoracoscopic pleural biopsy on 50

patients with ADA level under 50U/L and defined tuberculosis in 6 (12%) of these patients (12).

This high rate of the false negative results may be due to extremely higher cutoff value they used in this study.

In a study conducted in Serbia in which the tuberculosis is moderately common, 54 TPE and 67 MPE cases were prospectively evaluated. On the TPE diagnosis, sensitivity was found as 89.2%, specificity 70.4%, PPV 84.4% and NPV 78.4% for the cutoff value of ADA 49 U/L. Furthermore, they reported the ADA level of 16 U/L that was even lower than in our study (30 U/L) could be used to rule out the tuberculosis. All patients underwent pleuroscopy-guided pleural biopsy to confirm the diagnosis in the same study. Sensitivity of this process was calculated as 66.7%, specificity 100%, PPV 100% and NPV as 78.8% (13).

In another retrospective study, Chen et al., evaluated 63 TPE and 147 nontuberculous pleurisy cases and reported higher diagnostic values like the sensitivity of 87.3%, specificity 91.8%, PPV 82.1% and NPV 94.4% for the cutoff value similar to ours (55.8 U/L) in the TPE diagnosis. They concluded total ADA value of the pleural fluid is an appropriate and fast diagnostic tool for the diagnosis of tuberculosis(14).

Age is used most commonly along the ADA in the diagnostic algorithms. Diagnostic value of the pleural fluid levels of the ADA increases when it was used together with age. Since the mean age in the MPE group of our study was high, higher diagnostic values were obtained for the same cutoff value (30 U/L) in the TPE diagnosis of the patients under 50 years old in the presence of increased risk of TPE.

Out of the tuberculosis, high levels of ADA in the lymphocytic pleural effusion have been also reported in the

fungal infections such as coccidioidomycosis and histoplasmosis. However, our country is not among the regions that have endemic mycosis and this is seen very rarely and usually in the immunosuppressive patients.

Among the noninfectious cases, high levels of the ADA are seen in the malignancies and collagen vascular diseases (e.g. rheumatoid arthritis and systemic lupus erythematosus (15).

The conditions other than malignancy are more easily differentiated than TPE with clinic, laboratory and fluid profile. However, differentiation of the MPE and TPE is more difficult, since they present similar clinical features and pleural fluid profiles.

This study has some limitations. First, we included only the patients with TPE and MPE in the differential diagnosis. However, as mentioned above, most problems occur in the differential diagnosis of these two diseases in the exudative lymphocytic effusions.

Second, since our study was retrospective, ADA was not studied again in the tuberculous effusions with low levels of ADA. Querol et al. found the level of ADA below the threshold value (43 U/L) in 9 patients with TPE(16).

They reported that the ADA level measured in 5 of these patients after a few days, raised above the threshold value (16).

Also ADA isoenzymes in the MPE group with a high level of ADA could not be studied. While some studies reported this contributes to the differential diagnosis (17), the others stated isoenzymes did not provide an important contribution to the total level of ADA, and that was more expensive and not readily available (15). On the other hand, the advantage of our study being retrospective is the both groups to consist of the sufficient number of the patients with a definitive diagnosis.

In conclusion, ADA level of the pleural fluid is a non invasive, inexpensive and repeatable test that provides the results quickly. This study indicates that the ADA levels of the pleural fluid can be used with high diagnostic rates in the diagnosis and exclusion of the tuberculosis in the patients whom the pleural tissue could not be obtained with various causes in the differential diagnosis of TPE and MPE and those with waiting for the laboratory outcomes of the pleural tissue.

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