Diagnostic value of pleural fluid adenosine deaminase among the patients with pleural tuberculosis

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Abstract

Purpose

Extra-pulmonary tuberculosis occurs in about 10-20% of patients most commonly as tuberculous lymphadenitis or pleural effusion. Pleural fluid Adenosine deaminase (ADA) activity considered as a useful biomarker for detecting pleural tuberculosis. The purpose of this study was to evaluate the diagnostic accuracy of pleural fluid adenosine deaminase level in patients with pleural tuberculosis.

Methods

In this cross-sectional study, 113 patients with exudative pleural effusion with unknown underlying diagnosis, were enrolled. Physical examination, chest CT, measurement of ADA level of pleural fluid, direct thoracoscopic examination, and biopsy of pleura were performed for all individuals.

Results

The diagnosis of tuberculous pleurisy was established in 40 individuals regarding the pathology report of biopsy samples. The mean ADA level of the TB and the non-TB group was 39.90±22.93 IU/L and 30.74±38.27 IU/L respectively, which was not statistically significant (P-value=0.167). Sensitivity, specificity, positive predictive value, and negative predictive value of ADA test were 35%, 86.30%, 58.33%, and 70.79%, respectively.

Conclusion

Based on low sensitivity and specificity of ADA test, in patients with unexplained exudative pleural effusion especially in those who were suspicious for tuberculous pleurisy, despite the low level of ADA, direct thoracoscopic pleural observation and multiple biopsies of pleura is highly recommended.

Background

Tuberculosis is a chronic bacterial infection caused by Mycobacterium tuberculosis. It remains as a disease with a high rate of mortality in the world especially in developing and low-income countries (1). Extra-pulmonary tuberculosis occurs in about 10% -20% of patients and the most common forms of involvement are tuberculous Lymphadenitis and tuberculous pleural effusion (2). Pleural tuberculosis (TB) which is the topic of this study is characterized by symptoms such as chest pain, cough, and fever. Chest Radiography of these patients shows a small to moderate unilateral
pleural effusion which is lymphocyte dominant in serologic evaluations. The condition also could be bilateral within the minority of cases (1, 3).

Based on epidemiologic studies in our region, the incidence of TB is differing from 16.7 to 95 cases per 100,000 with an average age of 38 years. The prevalence of tuberculosis among all patients with pleural effusion is between 4-22%, and pleura is involved in 3-23% of patients with tuberculosis. While in the developed areas, such as the United States, the overall incidence of tuberculosis is only 9.3 cases per 100,000 with a pleural involvement within less than 5% of patients (4, 5).

Different diagnostic methods have been used to diagnose pleural tuberculosis, including thoracentesis, measurement of serum and pleural fluid adenosine deaminase (ADA) level, pleural biopsy, and thoracoscopy assisted pleural examination and biopsy (3).

Measuring ADA activity in pleural fluid is an easy, inexpensive, fast and useful way for diagnosing TB in endemic areas, such as South Africa, Asia, Brazil, Spain, and Eastern Europe (6-8). Based on literature a cut-off point of 40 U/L of ADA activity in a lymphocyte dominant pleural fluid is diagnostic for pleural TB. But the validity of the test is not generally accepted by consensus (9-11)

With the advancement in endoscopic techniques and video equipment, thoracoscopy has been suggested as a diagnostic and therapeutic modality in patients with pleural tuberculosis, and become more popular among the physicians. Thoracoscopic findings of these patients include caseous necrosis, miliary nodules, exudative pleural effusion, and pleural adhesion or fibrotic septa (12, 13).

Considering the important role of ADA in the diagnosis of pleural tuberculosis, evaluating the correlation between pleural ADA level and thoracoscopic findings of pleural tuberculosis seems necessary (14, 15).

This study aims to determine the correlation of the pleural fluid ADA activity and its diagnostic accuracy in histologically confirmed cases of pleural tuberculosis in order to reduce the use of thoracoscopy in patients with pleural effusion who represent a high ADA activity.

**Methods**

In this cross-sectional study, 113 patients who referred to cardiothoracic surgery department of Tabriz University of Medical Science with unexplained exudative pleural effusion throughout 18 months from
March 2016 were enrolled.

The study population was measured by GPOWER software with a confidence interval of 95% and a test power of 80%.

All patients had a pleural effusion with unknown etiology and candidate for thoracoscopy and biopsy for diagnosing their underlying disorder.

The exclusion criteria were patients with transudative pleural effusion, post-traumatic effusion, known pulmonary disorders, history of pulmonary or pleural malignancies and a history of radiotherapy on the thoracic cavity.

All patients underwent a thorascopic study. Direct evaluation of the pleural cavity with obtaining multiple biopsies from pleura and a fluid sample for ADA analysis was performed. All specimens were analyzed by a certain pathologist and laboratory. The ADA level of pleural fluid was measured in all patients. The ADA level of greater than or equal to 40 U/L considered as diagnostic for TB.

Based on pathologic findings, patients were divided into two groups of pleural effusion due to TB and pleural effusion due to non-TB causes.

Data were analyzed by SPSS® version 22 using chi² and logistic regression analysis to determine the relationship between the level of pleural fluid ADA and pleural TB.

Ethical Consideration

The study was approved by the ethics committee of Tabriz University of Medical Science under the approval number of 5/d/8716-94/5-6/3. All diagnostic and therapeutic interventions were performed regarding the routine management of patients; no additional intervention or cost was imposed on participants in this study. Patients’ data were recorded as encoded variables without mentioning the name of any participant. None of the patients' personal information was included in this research. Informed consent was obtained from each participant; nevertheless, patients were excluded from the study in cases which the patient was not willing to participate in the study.

Results

From the total of 113 patients enrolled in this study, 73 (58.4%) were male, and 40 (32.0%) were female. Among them 42 (33.6%) individuals were smokers. The mean age of the patients was 49.77 ±
18.71 years. The mean ADA level was measured 33.98 ± 33.63 within the study population.

According to the histopathologic reports of the biopsy specimens, the diagnosis of TB was confirmed in 40 patients. These patients were evaluated as the case group (known as group A), and the other 73 with a diagnosis of non-tuberculous pleural effusion were considered as the control group (known as group B).

Dyspnea, Cough, and pleuritic chest pain with a frequency of 66.37%, 48.67% and 40.7% respectively, were the most dominant symptoms of patients at the time of admission following by fever and weight loss with a frequency of 30.97%, and 28.31%, respectively.

Table-1 demonstrates demographic data of individuals separately for each study groups.

Regarding the thoracoscopic examination, pleural effusion, plural adhesion bond, miliary nodules, and caseous necrosis were found in 100%, 67.5%, 70%, and 60%, of patients in group A respectively (Table-2).

Among the control group (group B), pleural effusions, thickening of pleura and miliary nodules were the dominant manifestations with a frequency of 100%, 46.57%, and 30.13% respectively (Table-2).

The underlying cause of pleural effusion among the patients in control group was: metastasis (23.28%), mesothelioma (5.47%), inflammation (32.87%), fibrosis (36.98%), and fungal infection (1.36%) as shown in Table-2.

The mean ADA level in case group was 39.90 ± 22.13 IU/L. Meanwhile, the measured amounts of ADA in the control group were 30.74 ± 38.27 IU/L. The difference of ADA level between these two groups of patients showed a p-value of 0.167 hence, considered as statistically insignificant.

Table-3 demonstrates the frequency of individuals in each studied group with ADA level of greater than 40 (as a diagnostic cut-off for TB). Bar chart for these amounts also shown in figure-1.

Also, sensitivity, specificity, positive predictive value and negative predictive value of ADA test were measured 35%, 86.30%, 58.33%, and 70.79% respectively (Table-4). The ROC curve of ADA test measures shown in Figure-2.

Discussion

Tuberculous pleurisy is the result of a delayed hypersensitivity reaction to Mycobacterium
tuberculosis bacilli (8).

Nowadays, obtaining thoracoscopic biopsies of pleura is the modality of choice for diagnosing tuberculous pleurisy between the majority of physicians. But, this procedure is invasive and has its own risks and dangers. So that, finding an alternative diagnostic method which is minimally invasive and safer with the same accuracy seems to be necessary (16, 17).

Adenosine deaminase (ADA) is known as a useful biomarker, especially for detecting tuberculosis in endemic areas (18-20). Initially, in the 1970s, it was introduced as a serologic biomarker for detecting lung cancer. After that, Perez et al. demonstrated the usefulness of ADA in diagnosing of tuberculous pleurisy (3, 21). An ADA level of greater than 40 IU/L in a lymphocyte dominant pleural fluid is generally acceptable for diagnosing pleural TB (9, 11). Also, several studies have been conducted in patients with non-tuberculous lymphocytic pleural effusion, among them, ADA levels exceeding 40 U/L detected in less than 3% of cases (11, 22, 23).

Technically, the predictive value of an indicator such as ADA does not only depend on its sensitivity and specificity, but also the incidence of the disease in the study region is also effective and in areas with high prevalence of disease, the positive predictive value increases. The high prevalence of pleural tuberculosis in patients with pleural effusion in our region suggests that the ADA level of the pleural fluid may have a higher predictive value based on the previous studies (8, 9, 11, 15, 24, 25).

In this study, 113 patients (73 males and 40 females) with unexplained pleural effusion were evaluated for probable pleural tuberculosis by using thoracoscopic examination and biopsy. Meanwhile, the ADA level was measured for all individuals regardless of pathologic findings. The diagnosis of pleural TB established only in 40 individuals according to the histopathologic reports. The ADA level among these tuberculous pleurisy patients was 39.90 ± 22.13 IU/L compared to a level of 30.74 ± 38.27 IU/L in non-tuberculous individuals, which was not statistically significant. Based on our results, the ADA test yield a sensitivity, specificity, positive predictive value and negative predictive value of 35%, 86.30%, 58.33%, and 70.79% respectively.

In a study performed by Van et al., the causes of pleural effusion were evaluated among 95 patients in Netherland. According to their results, they have found tuberculous pleurisy just in five patients,
among them the high ADA activity was only detected in four individuals. On the other hand, the underlying pathologies other than TB could raise the ADA activity based on their study. The authors conclude that the high ADA activity level in a country with low tuberculosis incidence is not accurate enough to establish the diagnosis of tuberculous pleurisy (26).

Tian et al. found a sensitivity and specificity of 84.4% and 91.8% for ADA in diagnosing tuberculous pleurisy by evaluating 190 patients with pleural effusion. The cause of pleural effusion was TB in 141 patients of their study population (27). The difference between the results of our study compared to the recently mentioned research is explainable by the high overall incidence of TB in the country in which Tian et al., performed their study.

Valdes et al., in their study revealed that measuring pleural ADA level is a useful parameter for the diagnosis of tuberculous pleurisy by evaluating 405 patients with pleural effusion. All 91 cases of pleural TB in their study showed an ADA level of greater than 47 IU/L, compared to the elevation just in 5% of non-tuberculous patients (14).

Zemlin et al. demonstrated that measuring the ADA₂ isoenzyme is more accurate and it is superior to ADA in diagnosing tuberculous pleurisy by performing a study on 951 pleural fluid samples, including 387 patients with TB. They suggested that measuring ADA₂ level is better to use as a routine test among patients with pleural effusion in endemic areas for TB (11).

The inconsistency between the results is due to the variable prevalence of TB and different sample sizes in which mentioned studies were performed.

**Conclusion**

By comparing our results with previous studies, it can be concluded that the sensitivity, specificity, and accuracy of this test are very low. Therefore, in patients with pleural effusion without an exact cause and in cases of suspicion of TB, despite the low ADA, direct pleural observation and obtaining multiple biopsies of pleura seems to be appropriate.

Also, in cases with high ADA level and lack of proper response to TB treatments, for further investigation and rule out the other diagnosis, thoracoscopy and pleural biopsy could be beneficial.
However, further studies with larger sample size are suggested.

List Of Abbreviations
ADA: Adenosine deaminase
CT: Computerized Tomography
TB: Tuberculosis
SPSS: Statistical Package for the Social Science (Software)
ROC-Curve: Receiver Operating Characteristic Curve

Declarations

Ethics approval and consent to participate
The study was approved by the ethics committee of Tabriz University of Medical Science under the registration number of 5/d/8716-94/5-6/3.

Consent for Publication
A written informed consent was obtained from each participant. Patients were excluded from the study in cases which they were not willing to participate in the study.

Availability of Data and Materials
The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests
The authors declare that they have no competing interests

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Authors' contributions
F.R., M.S., S.B.R. and S.P. conceived of the presented idea. F.R. and M.S. diagnosed the disease and selected the patients. F.R., M.S. and S.B.R. provided the management methods, F.R. and S.P. collected the data sets, S.P. acquired additional data from the database. F.R. and S.P. wrote the
manuscript. S.P. analyzed the data, M.S. and S.B.R. made a revision on the manuscript. M.S. supervised the whole project.

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Tables

Table-1. Demographic data of studied individuals

| P-value | Total | In Group B | In Group A | Number of Patients |
|---------|-------|------------|------------|--------------------|
|         |       | 113        | 73         | 40                 |
| 0.006   | 49.77 ± 18.71 | 53.31 ± 18.61 | 43.32 ± 17.31 | Age (years) |
| 0.453   | 73    | 49         | 24         | Male               |
| 0.957   | 42    | 28         | 14         | Female             |
| 0.045   | 55 (48.6%) | 25 (34.2%) | 30 (75%)  | Yes                |
| 0.000   | 46 (40.7%) | 10 (13.7%) | 36 (90%)  | No                 |
| 0.19    | 75 (66.3%) | 36 (49.3%) | 39 (97.5%) | Cough              |
| 0.000   | 32 (28.3%) | 25 (34.2%) | 7 (17.5%)  | Pleuritic Chest Pain |
| 0.036   | 35 (30.9%) | 15 (20.5%) | 20 (50%)  | Dyspnea            |
|         | 32 (28.3%) | 25 (34.2%) | 7 (17.5%)  | Signs and Symptoms |
|         | 0.000   | 32 (28.3%) | 25 (34.2%) | Weight Loss        |
| 0.036   | 35 (30.9%) | 15 (20.5%) | 20 (50%)  | Fever              |
### Table-2. Surgical and paraclinical findings of patients

| CT-Scan Findings                  | Total | In Group B | In Group A | Number of Patients |
|-----------------------------------|-------|------------|------------|-------------------|
| Pleural Effusion                  | 113 (100%) | 73 (100%) | 40 (100%) | 39 (34.5%) |
| Pleural Effusion + Pleural Thickening | 113 (100%) | 73 (100%) | 40 (100%) | 40 (42.5%) |
| Thoracoscopic Findings            | 113 (100%) | 73 (100%) | 40 (100%) | 17 (42.5%) |
| Solitary Nodule                  | 1 (1.3%) | 0 (0.0%) | 1 (2.5%) | 22 (30.1%) |
| Miliary Nodules                  | 50 (44.2%) | 22 (30.1%) | 28 (70%) | 28 (70%) |
| Caseous Necrosis                 | 24 (21.2%) | 0 (0.0%) | 24 (60%) | 24 (60%) |
| Adhesion Bonds                   | 27 (23.9%) | 0 (0.0%) | 27 (67.5%) | 17 (42.5%) |
| Pleural Thickening               | 46 (40.7%) | 34 (46.5%) | 12 (30%) | 12 (30%) |
| Edema                            | 7 (6.2%) | 0 (0.0%) | 7 (17.5%) | 7 (17.5%) |
| Tuberculosis                      | 40 (35.4%) | 0 (0.0%) | 40 (100%) | 2 (5.4%) |

### Table-3. Measured ADA level of the patients

| ADA Level | P-value |
|-----------|---------|
| < 40      | 0.993   |
| ≥ 40      | 0.993   |

### Table-4. Characteristics of the ADA test and its diagnostic power for diagnosing TB (CI: Confidence Interval, TB: Tuberculosis)

|                         | Value   | 95% CI                |
|-------------------------|---------|-----------------------|
| Sensitivity             | 35.00%  | 20.63% to 51.68%      |
| Specificity             | 86.30%  | 76.25% to 93.23%      |
| Positive Predictive Value | 58.33% | 40.67% to 74.409%     |
| Negative Predictive Value | 73.00% | 65.47% to 75.59%      |

**Figures**
Figure 1

Frequency of patients with ADA activity level of greater than 40 in each TB-positive and TB-negative groups (ADA: Adenosine deaminase, TB: Tuberculosis)
Figure 2

ROC curve for sensitivity and specificity of ADA biomarker (ADA: Adenosine deaminase)

Supplementary Files

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