The diagnostic value of adenosine deaminase activity in pulmonary tuberculosis: Comparison between sputum and serum

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KEYWORDS
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Adenosine deaminase;
Pneumonia;
Lung cancer

Abstract  Background: Tuberculosis is a chronic specific bacterial infection caused by bacteria of the Mycobacterium tuberculosis. It remains one of the deadliest diseases in the world. It is the second leading infectious cause of death after HIV infection. Adenosine deaminase (ADA) activity is increased in various conditions such as liver disease, tuberculosis, typhoid, infective mononucleosis and certain malignancies, especially those of hematopoietic origin.

Objective: The aim of the work was to assess the diagnostic value of ADA in pulmonary tuberculosis by comparing levels in sputum and serum.

Subjects and methods: The present study included 15 patients with active pulmonary tuberculosis, 15 patients with pneumonia and 15 patients diagnosed as lung cancer. All patients were subjected to: Full history taking, complete clinical examination, laboratory investigations, and plain X-ray chest. Measurement of sputum and serum level of ADA of all patients using specific immunoassay method.

Results: There was a significant increase in sputum ADA in tuberculous group in relation to the other groups (mean sputum ADA was 159.76 ± 16.95, 84.34 ± 6.87 and 67.30 ± 7.47 U/L, respectively). The mean serum ADA was 31.99 ± 8.85, 24.15 ± 4.22 and 14.84 ± 2.43 U/L in tuberculous group, pneumonia group and bronchogenic carcinoma group, respectively. There was a statistically significant increase in serum ADA in the tuberculous group in relation to the other groups ($F_{10.65}$, $P = 0.001^*$). There was a statistically significant positive correlation between sputum and serum ADA ($r = 0.75$, $P = 0.0001$).

Conclusion: The results support the feasibility of using sputum ADA for diagnosis of pulmonary tuberculosis.
Introduction

Tuberculosis (TB) is a chronic specific bacterial infection caused by bacteria of the Mycobacterium tuberculosis [1]. TB remains one of the deadliest diseases in the world. It is the second leading infectious cause of death after Human Immunodeficiency Virus (HIV) infection [2]. It is an ancient disease with the evidence of the organism being present in skeletons over 4000 years ago [3]. The earliest records that are consistent with tuberculosis are the Egyptian wall paintings that described the typical hunchback deformities and correlate with the findings of spinal tuberculosis in mummies [4,5].

Adenosine deaminase (ADA) is an enzyme involved in purine catabolism, the enzyme catalyzes the hydrolytic and irreversible deamination of adenosine to inosine and deoxyadenosine to deoxyinosine [5].

ADA has been extensively used in the diagnosis of tuberculous pleural effusion, two isoenzymes, ADA1 and ADA2, have been described [6].

In humans two different isozymes are encoded by different genes, ADA1 is a single-chain Zn-binding protein and almost all activities are attributed to this protein. ADA2 is believed to be produced by monocytes and is found in negligible quantities. Mutations in the ADA1 gene, where expression is blocked, cause immunodeficiency, whereas mutations that cause overexpression cause hemolytic anemia [5].

Few studies have investigated the use of ADA activity in material other than pleural fluid. There are conflicting data regarding the use of ADA activity in bronchoalveolar lavage (BAL) as a diagnostic tool in pulmonary TB. Moreover, patients may have relative contraindications that make bronchoscopy difficult to carry out, whereas sputum is easily obtainable. However, sputum may be AFB-negative even in the presence of the disease [6].

Subjects and methods

The study included 45 patients divided into three groups:

Group I: 15 patients diagnosed as active pulmonary tuberculosis. All patients had symptoms and signs of active pulmonary tuberculosis, positive sputum smear for acid fast bacilli, with X-ray findings consistent with active pulmonary tuberculosis.

Group II: 15 patients diagnosed as pneumonia. All patients had symptoms and signs of pneumonia, with X-ray findings consistent with pneumonia and consolidation.

Group III: 15 patients diagnosed as bronchogenic carcinoma. All patients had symptoms and signs of bronchogenic carcinoma, with X-ray, CT and histopathological findings consistent with bronchogenic carcinoma.

After taking an informed consent, all subjects were subjected to detailed history taking including symptoms, complete clinical examination including general and local chest examinations, anthropometric measurements, routine laboratory investigations: including complete Blood count (CBC), liver and renal function tests, erythrocyte sedimentation rate (ESR) first and second hours and chest X-ray (standard postero-anterior (PA) chest radiographs).

Sputum smears for acid-fast bacilli: Early morning expectorated sputum specimens obtained after a deep, productive cough were collected on three days from each patient in a clean tightly closed plastic disposable container properly labeled and stained with Ziehl–Neelsen stain, quantitation scale for acid fast bacillus smears was done [7].

Measurement of serum and sputum ADA in all patients [8]: Early morning sputum samples and 5 cc venous blood samples were obtained from all patients.

Blood samples were centrifuged at 3500 rpm for 10 min to separate the sera. Sputum samples were homogenized with 70 milli-mol phosphate buffer (pH: 6.0) containing 0.5 mol NaCl (1 ml sputum + 5 ml buffer). They were centrifuged at 5000 rpm for 30 min and ADA activity of the supernatant was measured by Gusti method. Results were corrected by multiplying with the dilution coefficient.

We used Human Adenosine Deaminase ELISA kit with Catalog Number: MBS733123.

All reagents stored at 2-8 °C with valid period: six months for samples like: Cell culture fluid & body fluid & tissue homogenate Serum or blood plasma and for 96 tests.

Reagents handling: ADA reagent came in a liquid two reagent system ready to use for both manual and automated analyzer method. ADA controls and calibrator are in lyophilized form, and need to be reconstituted with 1.0 of DI water before use.

Assay procedure: ADA kit was used on automated clinical chemistry analyzers.

Calibration: 0.9% saline and ADA calibrator were needed for calibration. Results: The ADA results were printed out in U/L.

Results

The demographic data of the three studied groups as regards age and sex were shown in Table 1.

In tuberculous patients sputum ADA ranged between 125.4 and 180.3 U/L with a mean value of 159.76 ± 16.95 U/L, in pneumonia patients ranged between 72.5 and 95.3 U/L with a mean value of 84.34 ± 6.87 U/L and in bronchogenic carcinoma patients ranged between 51.8 and 83.2 U/L with a mean value of 67.30 ± 7.47 U/L. There was statistically significant difference between the three studied groups regarding sputum ADA (F = 25.65, P = 0.0001).

There was a significant increase in sputum ADA in group I than group II and group III and there was significant increase in sputum ADA in group II than group III (F = 25.65, P1 = 0.0001, P2 = 0.0001, P3 = 0.0001 respectively) Table 2.

Serum ADA in group I ranged between 17.7 and 54.1 U/L with a mean value of 31.99 ± 8.85 U/L, in group II ranged between 18.3 and 29.8 U/L with a mean value of 24.15 ± 4.22 U/L and in group III ranged between 11.3 and 180.3 U/L with a mean value of 159.76 ± 16.95 U/L. There was statistically significant difference between the three studied groups regarding sputum ADA (F = 10.65, P = 0.001).

There was a significant increase in serum ADA in group I than group II (P < 0.01), also there was a significant increase in serum ADA in group I than group III (P < 0.001), Group II serum ADA was significantly increased than group III (F = 10.65, P1 = 0.0022, P2 = 0.0001, P3 = 0.0001, respectively) Table 2.

ADA sputum/serum ratio, in group I ranged between 3.30 and 9.25 with mean value of 5.31 ± 1.47, in group II ranged between 2.99 and 4.84 with mean value of 3.59 ± 0.67 and
in group III ranged between 3.82 and 6.09 with mean value of 4.57 ± 0.64.

There was no statistically significant difference between the three studied groups regarding ratio ADA sputum/serum (P = 0.0685, 0.208 and 0.11 respectively) Table 2.

The ROC curve was done to determine the cut off value for both serum and sputum ADA to predict the presence or absence of tuberculosis, the sputum ADA was more sensitive and detective than serum ADA because the area under the curve for sputum ADA is 1.00 and the cut off value of sputum ADA was 93.4 U and the sensitivity of this marker was 100.0 and the specificity was 93.2% but in serum ADA the area under the curve was 0.89 and the cut off value of serum ADA was 17.9 U/L and the sensitivity of this marker was 93.3 and the specificity was 51.0% (Table 3, Fig. 1).

### Discussion

Mycobacterium tuberculosis infection is a main threat to mankind, with one third of the world population being infected. [9] Over nine million new cases of tuberculosis and two million deaths from this disease occur yearly worldwide. [10] The rising incidence of tuberculosis over the last decade has increased the need to define the host factors that control the resistance to tuberculosis. The chance of developing active disease after M. tuberculosis infection is about 10% in a lifetime in the non HIV infected host.

The major risk factor for developing active tuberculosis is immunodeficiency. Worldwide, malnutrition and starvation are major causes of immunosuppression and increased susceptibility to infectious diseases like tuberculosis [11,12]. Adenosine deaminase (ADA) is an enzyme involved in purine catabolism. The enzyme catalyzes the hydrolytic and irreversible deamination of adenosine to inosine and deoxyadenosine to deoxynosine. Two isoenzymes, ADA1 and ADA2 have been described [5,6].

ADA is an enzyme found in the majority of cells, but particularly in lymphocytes and monocytes. Its concentration is inversely related to the degree of differentiation [8].

High ADA activity has been used as a valuable marker for the diagnosis of tuberculous pleural effusion. As ADA is also high with other diseases, such as bronchogenic cancer, systemic lupus erythematosus, lymphoproliferative disorders, empyma, mesothelioma and rheumatoid arthritis, the sensitivity of the test is high and its specificity is low [13].

The present study was conducted on 45 patients, 15 patients with active pulmonary tuberculosis (group I), 15 patients with pneumonia (group II), and 15 patients diagnosed as lung cancer (group III).

In the current study there was a statistically significant difference between the three studied groups regarding sputum

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### Table 1 Comparison between the three studied groups regarding demographic data.

<table>
<thead>
<tr>
<th></th>
<th>Tuberculous group I</th>
<th>Pneumonia group II</th>
<th>Bronchogenic carcinoma group III</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age years</td>
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<td>37.80 ± 13.20</td>
<td>58.73 ± 7.33</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
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<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F = ANOVA test</td>
<td></td>
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</tr>
</tbody>
</table>

* P is significant at ≤0.05, P1 comparison between group I and group II, P2 comparison between group I and group III, P3 comparison between group II and group III.

### Table 2 Comparison between the three studied groups regarding sputum ADA, serum ADA and ADA sputum/serum ratio.

<table>
<thead>
<tr>
<th></th>
<th>Tuberculous (GI)</th>
<th>Pneumonia (GII)</th>
<th>Bronchogenic carcinoma (GIII)</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sputum ADA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>125.4–180.3 I</td>
<td>72.5–95.3</td>
<td>51.8–83.2</td>
<td>25.65</td>
<td>0.0001*</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>159.76</td>
<td>84.34</td>
<td>67.30</td>
<td>0.0001*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S.D.</td>
<td>16.95</td>
<td>6.87</td>
<td>9.47</td>
<td>0.0001*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum ADA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>17.7–54.1</td>
<td>18.3–29.8</td>
<td>11.3–19.2</td>
<td>10.65</td>
<td>0.0022*</td>
<td></td>
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<tr>
<td>Mean</td>
<td>31.99</td>
<td>24.15</td>
<td>14.84</td>
<td>0.001*</td>
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<tr>
<td>S.D.</td>
<td>8.85</td>
<td>4.22</td>
<td>2.43</td>
<td>0.0001*</td>
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<tr>
<td>Range</td>
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<td>3.82–6.09</td>
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<td>Mean</td>
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<td>3.59</td>
<td>4.59</td>
<td>0.208</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S.D.</td>
<td>1.47</td>
<td>0.67</td>
<td>0.64</td>
<td>0.11</td>
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</tbody>
</table>

* P is significant at ≤0.05, P1 comparison between group I and group II, P2 comparison between group I and group III, P3 comparison between group II and group III, F = ANOVA test.
The mean value of sputum ADA in tuberculous patients was 159.76 ± 16.95 U/L, the mean value of sputum ADA in pneumonia patients was 84.34 ± 6.87 U/L and the mean value of sputum ADA in bronchogenic carcinoma patients was 67.30 ± 7.47 U/L. There was a statistically significant difference between the three studied groups regarding sputum ADA ($F = 25.65, P = 0.0001$).

There was a significant increase in sputum ADA in group I than group II and group III and there was a significant increase in sputum ADA in group II than group III ($F = 25.65, P_1 = 0.0001, P_2 = 0.0001, P_3 = 0.0001$, respectively).

In accordance with our findings Dimakou et al. [14] tried to evaluate the diagnostic value of sputum ADA, ADA1 and ADA2 activity in pulmonary tuberculosis and found that sputum total ADA activity was significantly higher in TB than in lung cancer patients (median 18 U/L [range 3–70] vs. 6 U/L [2–16]; $P < 0.001$) respectively and sputum ADA2 activity was significantly higher in TB compared to lung cancer patients (9 U/L [0–65] vs. 5 U/L [0–12]; $P = 0.001$) respectively.

There was a statistically significant difference between the three studied groups regarding serum ADA. The mean value of serum ADA in tuberculous patients was 31.99 ± 8.85 U/L, the mean value of serum ADA in patients with pneumonia was 24.15 ± 4.22 U/L and the mean value of serum ADA in patient with bronchogenic carcinoma was 14.84 ± 2.43 U/L ($F = 10.65, P = 0.001$).

There was a significant increase in serum ADA in group I than group II and group III and group II show a significant increase than group III ($F = 10.65, P_1 = 0.0022, P_2 = 0.0001, P_3 = 0.0001$, respectively).

Afrasiabian et al. [15] found that the average (SD) of serum ADA in TB and non-TB patients were 20.88 (± 5.97) and 10.69 (± 2.98) U/L, respectively ($P$ value < 0.05). The best cut-off point was 14 U/L. The calculated area under the receiver operating characteristic (ROC) curve was 0.955 (95% CI, 0.914–0.995); sensitivity was 92.7% (95% CI, 84.7–100) and specificity was 88.1% (95% CI, 78.3–97.8) ($P < 0.001$).

There was a statistically significant positive correlation between sputum and serum ADA ($r = 0.75, P = 0.0001$).

In the present study, there was no statistical significant difference between the three studied groups regarding ratio ADA sputum/serum ($P = 0.0685, 0.208$ and $0.11$, respectively).

Similarly Dilmac et al. [16] tried to assess the diagnostic value of adenosine deaminase activity in sputum of patients with pulmonary tuberculosis, their aim was to determine and compare sputum ADA activity in pulmonary tuberculosis,

<table>
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<th>Test result variable(s)</th>
<th>Positive if greater than or equal to</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sputum ADA</td>
<td>93.4000</td>
<td>1.000</td>
<td>0.932</td>
<td>95.0</td>
<td>90.0</td>
</tr>
<tr>
<td>Serum ADA</td>
<td>17.9000</td>
<td>0.933</td>
<td>0.510</td>
<td>62.5</td>
<td>88.0</td>
</tr>
</tbody>
</table>

* Positive predictive value (PPV) is the% probability that an individual has the disease if he has a positive test. %PPV = TP/(TP + FP) × 100. Its value lies in that it combines the disease prevalence with test sensitivity and specificity. The efficiency of the laboratory test lies in that A perfect laboratory test would have 100% sensitivity–specificity and predictive values of positivity and negativity.

Figure 1 ROC curve assessing the ability of sputum and serum ADA to discriminate patients with tuberculous disease. The area under the ROC curve was 1.00 and 0.89 (95% CI, 0.00–1.00; and 0.791 and 0.989, $P$ was 0.0001, 0.0001) respectively for both sputum and serum ADA.
lung cancer and chronic obstructive pulmonary disease (COPD) patients in order to assess its diagnostic value.

Dilmacı et al. [16] found that sputum ADA activities in tuberculous group were significantly higher than the other two groups (P < 0.01 for lung cancer group, P < 0.05 for COPD) and this coincide with our findings. Also there was a significant increase in serum ADA activities in tuberculous patients than bronchogenic carcinoma and chronic obstructive pulmonary disease patients (P < 0.05) and this also coincide with the present study.

In contrast to our result, Boonsarnsuk et al. [17] found that BALF ADA had limited value in differentiating pulmonary TB from some other pulmonary diseases. To differentiate TB from solid tumor without endobronchial obstruction, a combination of BALF ADA and TB PCR had marked additive diagnostic value.

In the current study the cut off value of sputum ADA was 93.4 IU/L, if the value was more than this cut off value the disease was present by 100.0%, while if the value was less than 93.4 IU/L, the disease was absent by about 93.2%, i.e. the sensitivity of this marker was 100.0%, specificity was 93.2%, positive predictive value was 95% and negative predictive value was 90%.

On the other hand the cut off value of serum ADA was 17.9 U/L, if the value was more than this cut off value the disease was present by 93.3%, while if the value was less than 17.9 U/L, the disease was absent by about 51.0%, the sensitivity of this marker was 93.3%, specificity was 51.0%, positive predictive value was 62% and negative predictive value was 88%.

Dimakou et al. [14] used a cut-off level of 16 U/L and 5 U/L respectively for sputum total ADA and ADA2, sensitivity and specificity were 55.6% and 100% for total ADA and 81.5% and 63.2% for ADA2.

Conclusion

Sputum ADA was significantly increased in patients with pulmonary tuberculosis than with pneumonia and lung cancer patients.

The sputum ADA was more sensitive than serum ADA in the diagnosis of pulmonary tuberculosis and there was a statistically significant positive correlation between sputum and serum ADA.

Recommendation

Further studies are needed to measure ADA subtypes ADA1 and ADA2 separately to assess the diagnostic value of each subtype in serum and sputum of tuberculous patients.

Further studies are needed to measure the level of sputum and serum ADA before and after treatment of pulmonary Tuberculosis to assess the effect of antituberculous drugs on the level of ADA in sputum and serum.