Bronchial Asthma and Serum Adenosine Deaminase Activity in Iraqi Patients

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Summary:

**Background:** Adenosine deaminase (ADA) catalyzes the irreversible deamination of adenosine in the purine degradation pathway, and it also inactivates a number of anti-viral and cancer therapeutic analogues. It is vital to the proper function of immune system. Adenosine, one of the main substrates for ADA, is known for its bronchoconstrictor and anti-arrhythmic actions. Some workers have reported an increase in ADA activity in the RBC of asthmatic patients.

**Objective:** The aim of this study was to measure the activity of ADA in serum of asthmatic Iraqi Patients of different ages & duration of their asthma. Moreover, the effects on this enzyme of the different drug treatment of bronchial asthma were studied.

**Subjects & methods:** Adenosine deaminase activity was measured in sera of 71 patients with bronchial asthma and compared to 62 controls. Serum ADA activity was assayed by the sensitive colorimetric methods of Galanti & Giusti.

**Results:** Serum ADA activity was significantly increased in asthmatic patients (p < 0.01) when compared to controls. ADA activity was independent of age or sex in both normal and asthmatics. It was also independent of the type of drug treatment. Purification of serum ADA in asthmatics by gel filtration chromatography revealed increased activity of isoenzymes 4 and 5.

**Conclusion:** Measurement of serum ADA and its isoenzymes may be used markers in diagnosis of asthma and is uninfluenced by the available drug treatment of asthma or its duration.

**Key Words:** Adenosine deaminase activity, isoenzymes, Bronchial asthma.

Introduction

Adenosine deaminase (ADA) catalyzes the irreversible deamination of adenosine in the purine degradation pathway, and it also inactivates a number of anti-viral and cancer therapeutic analogues (1, 2, 3). It is vital to the proper function of immune system, being essential for growth and proliferation of T-lymphocytes as well as maturation of monocytes to macrophages; it also protects lymphocytes from the toxic effect of high concentration of ADA substrate (4.5, 6). Adenosine, one of the main substrates for ADA, is known for its bronchoconstricting and anti-arrhythmic actions (7). Some workers have reported an increase in ADA activity in RBC of asthmatic patients (8). In order to elaborate on this, the present work was designed to measure ADA activity in serum of asthmatic Iraqi patients of different ages and duration of their asthma. Moreover, the effects of different drug treatment for bronchial asthma were studied.

**PATIENTS AND METHODS:**

A total of 133 subjects were included. They were 71 asthmatics, 38 males and 33 females, ranging in age from 4 to 75 years (mean age 36.3 y), and 62 controls (34 males, 28 females) ranging in age from 7 to 55 years (mean age of 38 y).

**Asthmatic patients were of 3 groups**

a: Untreated (5 patients)

b: Treated acutely with aminophylline (8 patients)

c: Treated chronically with single or multiple drugs for asthma as follows:

1 - salbutamol (16 patients)

2 - aminophylline + prednisolone or hydrocortison (14 patients)

3 - salbutamol + aminophylline (5 patients)

4 - salbutamol + prednisolone (8 patients)

5 - salbutamol + theophylline + prednisolone (15 patients)

The duration of asthma varied from 6 month to 27 years (41 patients less than 10 years duration, 21 patients between 10 to 20 years duration, and 9 patients more than (9 years duration).

**ADA Assay in Serum**

5 ml blood samples were withdrawn from each subject and were allowed to coagulate at room temperature before being centrifuged at 3000rpm for 10 minutes. The resulting sera were separated. Blood samples were collected, whenever possible, before treatment of acute attack of asthma with aminophylline, and after treatment of acute attacks.

Serum ADA activity was assayed by the sensitive colorimetric method of Galanti and Giusti (9), with adenosine being used as a...
substrate. The ammonia produced forms an intensely blue indophenol with sodium hypochlorite and phenol alkaline solution. Ammonia concentration is directly proportional to the absorbance of the indophenol at 630 nm.

One international unit (I.U.) of ADA activity is defined as the amount of enzyme that catalyzes the deamination of one micromole adenosine per minute at 37°C under standard assay conditions in 1 litre serum.

Purification of ADA in asthmatics’ serum

Separation and purification of serum ADA were done by gel filtration-chromatography (11), employing column (67x1.6cm) of sephacryl S 200 resin, using phosphate buffer 0.05 mM, pH 6.5. The crude enzyme was diluted by a minimum volume (1.5ml) to give a protein concentration of 32 mg/ml (12).

Statistics:
Comparisons were made by the students t-test for independent data unless otherwise indicated.

Results:

ADA activity in Asthma

The mean ADA in serum of asthmatic patients was 27.47 U/L, which was significantly higher than that of normal controls (18.034 U/L) (P < 0.001) (table 1). There was no significant difference in ADA activity between males and females in both asthmatics and normal controls (Fig. 1). Similarly, no significant difference was seen in enzyme activity between different age groups in normal and in asthmatics (Fig. 2).

Regarding disease duration, no significant change was seen except where duration exceeded 20 years in males (Fig.3).

Effect of drug treatment

Acute drug treatment with aminophylline IV induced a non-significant reduction in serum ADA activity (Fig.4)(p > 0.05). Similarly, no significant change in ADA activity occurred with different drug groups on chronic treatment for their asthma ADA Purification:

This yielded five isoenzymes; the peak for the third isoenzyme is less than that shown for normal; the fourth isoenzyme was twice the activity of normal serum, while the fifth isoenzyme in asthmatics had the highest activity of about 5 time that of normal serum (table 2).

DISCUSSION

It could be seen that ADA activity in plasma is higher in patients with bronchial asthma than in normal controls, and that this increase is independent of age, sex or disease duration. Other workers reported increased ADA activity in RBC of asthmatics but our work is the first to show this increase in the serum of asthmatics; this higher ADA activity may be related to the higher adenosine level reported in asthmatics (13). It might be that adenosine inhibits activity of suppressor T-lymphocytes which would perpetuate the immune reaction in asthma, and in that sense, the increase in ADA activity is protective by reducing adenosine accumulation in asthmatics. Although excess adenosine causes bronchoconstriction, its physiological level inhibits IgE-induced release of histamine from basophils or mast cells, probably by being utilized to increase cAMP level in these cells (14). It should be noted that conversion of ATP into cAMP would result in reduced adenosine formation which might result in a secondary decrease in ADA activity. The different drugs used by bronchial asthma patients had no significant effect on ADA activity in patients' sera. Salbutamol increases cAMP level by stimulation of B2-adrenergic receptors in bronchial smooth muscle (15), while theophyllines including aminophylline inhibits phosphodiesterase enzyme that catalyzes cAMP catabolism. In addition, theophyllines act as competitive antagonists of adenosine at adenosine receptors (16), and inhibits free superoxide radicals release from eosinophils (17). Hydrocortisone stabilizes the membranes of different reactive cells, and may enhance the action of bronchodilators like salbutamol or theophylline in asthmatic However high concentration of these drugs in vitro directly inhibited ADA enzyme activity. Furthermore, it was shown that this inhibition is competitive in case of salbutamol and hydrocortisone and of mixed type with aminophylline (unpublished data) (18).

The purification of ADA in asthmatics' serum revealed 5 isoenzyme peaks. This is done for the first time in asthma, and comparing our isoenzymes with their normal profile in normal serum, as done by Al -Nify (19), showed that isoenzymes 4 and especially 5 are much higher than in normal subjects and they might be of diagnostic value or as markers in asthma. This increase may be secondary to the increase in adenosine in asthmatics and possibly also due to leak of these isoenzyme from inflammatory cells accumulating in bronchi in asthmatics.

In conclusion, it seems that the increased ADA in asthmatic patients is independent of age, sex, duration of disease, or type of drug treatment. Purification of ADA revealed increased activity of isozymes 4 and 5, which could serve as a useful diagnostic marker in early asthma and to differentiated it from disorders of psychogenic, respiratory, or cardiac origin.
Figure (1): Serum ADA activity of asthmatic patients and normal healthy controls in accordance with sex (mean ± SD).

Figure (2): Serum ADA activity of asthmatic patients and normal healthy controls in accordance with age (mean ± SD).

Figure (3): Serum ADA activity in accordance with duration of chronic treatment and sex (mean ± S.D).

Figure (4): Serum ADA of asthmatic patients before and after acute treatment with aminophylline i.v. (mean ± S.D).
Bronchial asthma & serum adenosine deaminase activity in Iraqi patients

References:


Table 2: Purification of asthmatic serum ADA by gel filtration chromatography using Sephacryl S-200

Table 1: Serum ADA activity (I.U./L) in asthmatic patients and control group.

<table>
<thead>
<tr>
<th>Number of blood samples</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>20</th>
<th>25</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activity (I.U./L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

Figure (4): Serum ADA activity of asthmatic patients using different combinations of chronic treatment (mean ± S.D.).

Table 2:

<table>
<thead>
<tr>
<th>Volume of fraction (ml)</th>
<th>Concentration of protein (mg/ml)</th>
<th>Total amount of protein (mg)</th>
<th>Activity (units/ml)</th>
<th>Specific activity (units/mg protein)</th>
<th>Total activity (unit)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude enzyme</td>
<td>1.5</td>
<td>32.000</td>
<td>48.000</td>
<td>0.016</td>
<td>5 x 10³</td>
</tr>
<tr>
<td>Isoenzyme 1</td>
<td>2</td>
<td>0.074</td>
<td>0.148</td>
<td>9.770 x 10⁴</td>
<td>0.013</td>
</tr>
<tr>
<td>Isoenzyme 2</td>
<td>2</td>
<td>0.010</td>
<td>0.200</td>
<td>1.465 x 10⁴</td>
<td>0.015</td>
</tr>
<tr>
<td>Isoenzyme 3</td>
<td>2</td>
<td>0.172</td>
<td>0.344</td>
<td>2.920 x 10⁴</td>
<td>0.017</td>
</tr>
<tr>
<td>Isoenzyme 4</td>
<td>2</td>
<td>0.148</td>
<td>0.296</td>
<td>2.441 x 10⁴</td>
<td>0.016</td>
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<tr>
<td>Isoenzyme 5</td>
<td>2</td>
<td>0.055</td>
<td>0.070</td>
<td>0.859 x 10⁴</td>
<td>0.167</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Controls (A)</th>
<th>Asthmatics (B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample size</td>
<td></td>
</tr>
<tr>
<td>n = 62</td>
<td>n2 = 71</td>
</tr>
<tr>
<td>Mean</td>
<td>x1 = 18.034</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>s1 = 3.734</td>
</tr>
<tr>
<td>Standard error</td>
<td>s x1 = 0.474</td>
</tr>
<tr>
<td>Range</td>
<td>11.300-25.625</td>
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\( t \)-test between A/B = P < 0.001