Adenosine Deaminase Activity in COPD Patients and Healthy Subjects

Mohammad Taghi Goodarzi 1, Mohammad Abdi 2, Heidar Tavilani 3, Ebrahim Nadi 4, and Mojtaba Rashidi 3

1 Research Center for Molecular Medicine, Hamadan University of Medical Sciences, Hamadan, Iran
2 Department of Pathology and Medical Laboratory Sciences, Faculty of Para Medicine, Kurdistan University of Medical Sciences, Sannandaj, Iran
3 Department of Biochemistry and Nutrition, Faculty of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran
4 Research group of pulmonary disease and tuberculosis, Faculty of Medicine, Hamadan University of Medical Science, Hamadan Iran

Received: 30 April 2009; Received in revised form: 2 January 2010; Accepted: 16 February 2010

ABSTRACT

Chronic obstructive pulmonary disease (COPD) has been defined by the Global Initiative for Chronic Obstructive Lung Disease (GOLD), as a disease state characterized by airflow limitation which is not fully reversible. COPD consists of emphysema which is the destruction and inflammation of the lung alveoli. Adenosine deaminase (ADA, E.C.3.5.4.4) converts adenosine to inosine. There are two isoenzymes of ADA in serum; ADA1 and ADA2. It has been established that in COPD patients the adenosine levels increase, which can contribute to decrease of ADA activity. In this research we studied the ADA and its isoenzyme activity in COPD patients.

This descriptive analytical case-control study was performed on thirty patients who were hospitalized in the pulmonary wards with an acute exacerbation of COPD. ADA activity was determined in 30 COPD patients, 30 nonsmokers and 30 smokers controls. All subjects were male. We used colorimetric (Giusti) method for measuring of ADA activity. The data were analyzed using SPSS 13 software and Kruskall-Wallis and two-way ANOVA tests.

Total ADA activity in the COPD and smoker control groups was significantly lower than in non smoker group (18.99 ± 7, 19.03 ± 9.1 and 22.95 ± 6.7 U/L, respectively). There was a significant difference for ADA2 between the three groups. Whereas the ADA1 activity in the three groups had no significant difference.

Based on the obtained data, decrease of ADA activity may play an important role in the formation of pulmonary injury in COPD patients.

Key words: Adenosine deaminase (ADA); Adenosine deaminase Isoenzymes; Adenosine level; Serum; Chronic Obstructive Pulmonary Disease (COPD)
INTRODUCTION

Adenosine deaminase (ADA) catalyzes the conversion of adenosine to inosine in purine metabolism pathway. Since 1978, when ADA activity was found to be high in tuberculous pleural exudates, ADA has been used in the diagnosis of tuberculosis. The sensitivity and specificity of ADA were reported 99% and 93% respectively. High ADA levels can also be found in pleural effusions, secondary to other processes or lesions, especially pneumonia, empyema, lymphoma, neoplasia and systemic lupus erythematosus.

ADA consists of two major principals isoenzymes: ADA1 and ADA2. These principals have different optimal pH, Michaelis constants as well as relative substrate specificity frames. ADA1 has roughly a close connection with adenosine and 2'-deoxyadenosine with a 2'-deoxyadenosine deaminase/ADA activity with approximately a ratio of 0.75 which has been observed in a number of tissues.

Moreover, ADA2 has a greater connection with adenosine (2'-deoxyadenosine deaminase/ADA activity ratio approximately 0.25) and has been found in macrophages. Macrophages release ADA2 when stimulated by the presence of micro-organism in their interior. EHNA [Eryththro-9 (2-hydroxy-3- nonyl) adenine] can inhibit ADA1, but it has no effect on ADA2.

Chronic obstructive pulmonary disease (COPD) has been defined by the Global Initiative for Chronic Obstructive Lung Disease (GOLD), as a disease state characterized by airflow limitation that is not fully reversible.

COPD consists of emphysema which is the destruction and inflammation of the lung alveoli, chronic bronchitis that is a condition of chronic cough with phlegm, and small airway diseases with narrowing of the bronchioles. Chronic bronchitis is defined clinically as excessive cough and sputum production on most days for at least three months during at least two consecutive years. Emphysema is characterized by chronic dyspnea resulting from the destruction of lung tissue and the enlargement of air spaces. The aim of this study was to examine the changes in serum, total ADA levels and its isoenzymes (ADA1 and ADA2) in COPD patients and healthy subjects. In order to determine the possible contribution of these enzymes in COPD.

MATERIALS AND METHODS

From February 2007 until April 2008, data were collected from 30 male patients admitted to the Ekbabat Hospital, Hamadan-Iran, with an acute exacerbation of COPD. Patients were at least 40 years of age (mean±SD: 51.1±5.9 years) and had COPD as indicated by the criteria of the American Thoracic Society.

Blood samples from 60 male healthy people (mean±SD: 51.01±6.2 years) were used as control. For investigation the possible effects of smoking on ADA activity we used two control groups, 30 smokers (CS) and 30 nonsmokers (CNS). The controls had no specific pulmonary disease. World Health Organization (WHO) definition for smoker was used for a person who smokes more than 100 cigarettes in his lifetime. There was no statistically significant difference in age of these three studied groups. This descriptive analytical case-control study was approved by the Ethics Committee of Hamadan University of Medical Sciences. To prepare serum, blood samples were centrifuged for 10 min at 3000 rpm, and the supernatants were stored at -70°C pending assay.

Adenosine was obtained from Sigma-Aldrich (Saint Louis, Missouri 63103, USA). Sodium di-hydrogen phosphate \(\text{Na}_2\text{HPO}_4\cdot\text{H}_2\text{O}\), di-sodium hydrogen phosphate \(\text{Na}_2\text{HPO}_4\cdot12\text{H}_2\text{O}\), Ammonium sulfate \((\text{NH}_4)_2\text{SO}_4\), Phenol \(\text{C}_6\text{H}_5\text{OH}\), Sodium nitroprusside \(\text{Na}_2(\text{Fe(CN)}_5\text{NO})\), soda \(\text{NaOH}\) and EHNA were obtained from (Merck Chemicals Ltd, Germany).

ADA total, ADA1 and ADA2 activities were determined calorimetrically by Giusti method. ADA isoenzymes being distinguished by their different inhibition by EHNA. Briefly, duplicate 25 µL samples were incubated for 60 min at 37°C with 500 µL of 21 mM adenosine (one sample) and 4 mM EHNA (the other) in 50 mM phosphate buffer, and the released ammonia was determined by its reaction with 1.5mL phenol nitroprusside 30 min (106 mM phenol nitroprusside) in the presence of 1.5 mM sodium nitroprusside) in the presence of 1.5 mM of sodium hypochlorite (11 mM NaOCl plus 125 mM NaOH), using a spectrophotometer absorption was read at 630 nm. To control the presence of ammonium before addition of exogenous adenosine, untreated samples were run in parallel. Estimated ADA1 activity was calculated by subtracting the ADA2 and ADAt activities using the inhibition of ADA1 by EHNA.

The results were expressed in U·L$^{-1}$. 

8/ IRANIAN JOURNAL OF ALLERGY, ASTHMA AND IMMUNOLOGY Vol. 9, No. 1, March 2010
Table 1. Total ADA, ADA1 and ADA2 activities (X±SD) in serum of COPD patients and control groups.

<table>
<thead>
<tr>
<th>The studied groups</th>
<th>ADA (U/L)</th>
<th>ADA1 (U/L)</th>
<th>ADA2 (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>COPD</td>
<td>18.99 ± 7.0*</td>
<td>5.77 ± 3.5*</td>
<td>14.00 ± 6.5*</td>
</tr>
<tr>
<td>Smoker control</td>
<td>19.03 ± 9.1*</td>
<td>6.20 ± 2.9*</td>
<td>14.25 ± 9.2*</td>
</tr>
<tr>
<td>Non-smoker control</td>
<td>22.95 ± 6.7*</td>
<td>7.65 ± 3.9*</td>
<td>17.57 ± 6.5*</td>
</tr>
</tbody>
</table>

*: P<0.05

Statistical analysis was carried out using the Kruskall-Wallis and two-way ANOVA tests. A p-value < 0.05 was considered statistically significant. The correlation between three variables was plotted on an Error Bar plot and Spearman rank coefficients were used to express the relationships.

The significance of the correlation was denoted by a p-value < 0.05. All statistical analyses were done using software SPSS version 13 (SPSS Inc., Chicago).

RESULTS

Table 1 shows the means and standard deviations of ADA, ADA1 and ADA2 activities in serum for each group of subjects. All patients were men, between 40 to 60 years old.

![Figure 1](image1.png)

**Figure 1.** Difference between three groups for ADAt activity

*COPD: Chronic obstructive pulmonary disease
CNS: Control nonsmoker
CS: Control smoker*

Attention to the error plot (Figures 1 and 2), the mean differences between the patient group (COPD) with non smokers control group (CNS) and smokers control group (CS) were statistically significant for ADAt (p<0.05) and COPD with CNS for ADA2, whereas there was not any significant differences between three groups for ADA1.

**DISCUSSION**

The above results confirm that in COPD patients ADA activity decreased and the low total ADA activity in serum of COPD patients are largely due to low ADA2 activity. Although many reports demonstrated the changes in ADA activity in a whole variety of diseases including tuberculosis, there are few studies in COPD. However there are reports indicating higher...
serum activity of ADA in different disease compared to healthy status.\textsuperscript{5,6} We found low activity of ADA in COPD patients. Adenosine concentration and adenosine receptor levels are elevated in the lung of patients with asthma and COPD.\textsuperscript{15} The high formation of adenosine in the lung cause profibrotic pathways and therefore may result in pulmonary fibrosis development and/or maintenance. Low activity of ADA in serum of patients with COPD may contribute to high level of adenosine.

ADA is a polymorphic enzyme that is involved in purine metabolism. ADA catalyzes deamination of adenosine and deoxyadenosine to produce inosine and deoxyinosine, respectively.\textsuperscript{16} The cause of increased adenosine levels in chronic pulmonary disease is still unknown.

Role of adenosine in the regulation of chronic lung disease has been established. A high total ADA activity in tuberculosis pleural effusion has been reported.\textsuperscript{6,17} Adenosine is a potent and an ubiquitous signaling molecule. In times of damage and cellular stress it accumulates. In cases of inflammation it is formed by ATP catabolic breakdown. It also functions in order to regulate the inflammatory reaction and response by the aid of putative adenosine receptors.\textsuperscript{17} Consistent with this, asthmatic patients have increased adenosine levels in their BAL fluid and exhaled breath condensates and the degree of adenosine elevations correlate with disease severity.\textsuperscript{18} The findings of the current study suggest that adenosine may contribute to pathogenesis of chronically inflamed asthmatic lungs.\textsuperscript{19} We supposed that low activity of ADA in serum of patients with COPD may contribute to high level of adenosine.

Fozard et al demonstrated elevated level of adenosine in a mouse model of chronic pulmonary inflammation.\textsuperscript{20} Elevation in serum adenosine level that was found in this study may reflect high level in lung of COPD patients.

Seven transmembrane proteins which couple to generation of heterotrimeric, distinct subtypes of P1 purinergic (A1, A2A, A2B, and A3 adenosine receptors) are used by adenosine.\textsuperscript{21} Hays W. J. Young et al demonstrated that A3R levels are elevated in the lungs of ADA\textsuperscript{−/−} mice that exhibit adenosine-mediated lung disease. Treatment of ADA\textsuperscript{−/−} mice with a selective A3R antagonist or genetically without this receptor prevented the production of inflammation and mucus. This suggests that A3R signals has a major role in the regulation of these aspects of chronic lung disease.\textsuperscript{22} Janci et al revealed that controlling adenosine levels with the use of exogenous ADA treatments may provide a significant approach to seize the progression or alter the features of pulmonary fibrosis not only in IPF but also in severe asthma as well as COPD.\textsuperscript{23}

Our finding represent decrease of ADA total activity in COPD patients compared with healthy subjects and this decrease compound with increase of adenosine levels may cause change in lung tissue and presumably play an important role in creation of COPD. Results show that smoking cause a decrease in enzyme activity. It seems that determination of ADA activity with adenosine level can play an important role in COPD treatment. Future studies in this base could be helpful.

In human, the isoenzyme ADA1 is present in many tissues, which are equipped with an efficient mechanism to capture and internalize 2\textsuperscript{'-}deoxyadenosine.\textsuperscript{7} The malfunction of the immune response in subjects which congenitally lack ADA1 because of inadequate homeostasis of 2\textsuperscript{'-}deoxyadenosine in their immune cells, reveal the importance of ADA1 in cells.\textsuperscript{7} Our study, represent the decrease of ADA1 activity in COPD patients compared to healthy smokers and nonsmokers groups. However, there was not any significant difference for ADA1 activity between three groups.

We determined and compared ADA2 activity in patient and control groups. The isoenzyme ADA2 coexists with ADA1 in monocytes-macrophages. ADA2 and ADA1 are coded by different gene loci.\textsuperscript{7} ADA2 is the predominant isoenzyme in the sera in all infectious diseases. Hence why ADA2 is the predominant serum enzyme is not yet determined.\textsuperscript{24} Our study represent decrease of ADA2 activity in COPD group compared with two control groups. Obtained results represent the role of smoking in decrease of ADA2 activity in CS group. This decrease of activity may have an effective role in monocytes-macrophages system and involved in pathogenesis of COPD. High total activity of ADA that is reported in many pathological states is due to high activity of ADA2 isoenzyme.\textsuperscript{5} The monocytes (and macrophages) are shown as the main source of ADA2, therefore the ADA2 level of monocytes in COPD patients can be the reason for the cause of decreased ADA activity in COPD. Our finding show decreased ADA activity in COPD may be largely due to decreased activity of the ADA isoenzyme ADA2, together with the fact that the
only group of cells in which ADA2 has been found are monocytes/macrophages, which suggest that decreased ADA activity in COPD may be due to decrease in monocytes/macrophages activity.

In conclusion, we believe that low ADA activity in serum of COPD patients is mainly as the result of a decrease in ADA2 activity. In this study, ADAt activity was a more efficient marker of COPD than the total ADA2 activity. ADA1 activity shows no change in COPD.

Decrease of ADA activity may play an important role in the formation and treatment of pulmonary injury in COPD patients. Future research should investigate the origin of low ADA2 and total ADA activity in COPD patients.

REFERENCES


