Evaluation of Serum Adenosine Deaminase in Cystic Fibrosis Patients in an Iranian Referral Hospital

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Abstract

Background: Adenosine, a signaling nucleoside, is controlled in part by the enzyme adenosine deaminase (ADA). There are rare reports on the role of adenosine levels and ADA in cystic fibrosis (CF) patients.

Objectives: The aim of this study was to assess serum ADA in CF patients in order to find whether the severity of lung disease in CF is related to significant changes of ADA or not.

Patients and Methods: Venous blood serum ADA was measured in CF patients (3-15 years) and 49 healthy children (3-15 years) referred to Children’s Medical Center. Classification of respiratory and gastrointestinal disease severity in CF patients as well as Body Mass Index (BMI) was performed. The results were compared with values obtained from healthy children matched for age and gender.

Results: This study included 49 children of both genders (20 females and 29 males) with CF (mean age: 6.36 ± 2.22 years). Mean serum ADA in CF patients group and control group was 9.38 ± 2.72 and 16.04 ± 1.27, respectively (P value = 0.001). Mean serum ADA in CF patients with normal BMI was higher than in patients with low BMI (P value = 0.002).

Conclusions: In this study the lower serum level of ADA was seen in CF patients compared to control group. The clinical symptoms, especially respiratory symptoms, in CF patients might be associated with reduction of serum ADA and rising serum adenosine; therefore, further studies on the use of ADA enzyme therapy in CF patients are highly recommended.

Keywords: Adenosine Deaminase, Cystic Fibrosis, Children

1. Background

Cystic Fibrosis (CF) is an autosomal recessive genetic disorder caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. Adenosine, a signaling nucleoside, has both tissue-protective and tissue-destructive effects and its level in tissues is controlled in part by the enzyme adenosine deaminase (ADA) (1).

ADA is an enzyme of the purine metabolism. There are two isoenzymes of ADA in humans mainly in two forms of adenosine deaminase 1 (ADA1) and adenosine deaminase 2 (ADA2) (2). Adenosine has 3 receptors including A1, A2B, and A3. Adenosine produces cytokine through A2B receptor and increases production of mucus and eosinophil cells in respiratory system through A3 receptor (3, 4). Adenosine level rises in cellular damage, cellular stress, hypoxia and reduced ADA (5). Deficiency of ADA conduces to pulmonary inflammation in SCID (6, 7). In addition, the elevated level of ADA was demonstrated in several infections such as tuberculosis (8, 9) and upper respiratory tract infection (10), while in chronic obstructive pulmonary disease (COPD) and asthma its level was decreased (11, 12). It has been suggested that adenosine signaling might play a role in the pathogenesis of fibrosis in many disorders such as hepatic fibrosis, cirrhosis and chronic renal scarring in patients with glomerulonephritis (1). Tissue damage is observed in CF caused by activation of macrophages and T-lymphocytes that release myeloperoxidase (MPO) and ADA (13).

According to a previous experimental study, adenosine was accumulated in multiple tissues including the lung of ADA-deficient mice which was supposed to contribute to the development of pulmonary inflammation (1).

2. Objectives

There are rare reports on the role of adenosine level and ADA in CF patients. The aim of this study was to assess serum ADA in CF patients in order to find whether
the severity of lung disease in CF is related to significant changes of ADA or not.

3. Patients and Methods

Between October 2013 and February 2014, 49 patients with CF and 49 healthy controls were recruited at gastroenterology clinic of Children’s Medical Center in Tehran, Iran. Case and Control individuals were 3 to 15 years old. The Control group had no previous history of functional abdominal pain and had no symptoms during the study. Written informed consent was obtained from participant’s parents or legal guardian. Requirements for a CF diagnosis included either positive genetic testing or positive sweat chloride test findings and at least one clinical manifestation including typical chronic obstructive pulmonary disease (COPD), documented exocrine pancreatic insufficiency, and positive family history (usually an affected sibling) (14).

The sweat test was performed according to Clinical and Laboratory Standards Institute guidelines. A sweat chloride level $\geq 60$ mmol/L was interpreted as within the CF range, 30–60 mmol/L as equivocal, and $\leq 29$ mmol as normal (15). Classification of respiratory disease severity was based on the number of hospital admissions during the year, radiographic findings and the presence of exertional dyspnea. Classification of gastrointestinal disease severity was based on the sufficiency/insufficiency of pancreas. BMI was obtained according to children’s sex and age.

1 cc venous blood sample was taken and serum ADA measured using ADA kit (Diazyme Laboratories Company, California, USA). The results were expressed in unit per liter (U/L).

Data are presented as the mean $\pm$ standard deviation (SD) (normally distributed variables), Group comparisons were performed using the Student’s t test or ANOVA test. Statistical significance was defined as $P < 0.05$. All analyses were performed with IBM SPSS Statistics.

4. Results

The study included 49 children (mean age: 6.36 $\pm$ 2.22 years) of both genders (20 females and 29 males) with CF. The characteristics of CF patients are shown in Table 1. Among control individuals (mean age 6.14 $\pm$ 2.26.24 years) 24 were females and 25 males.

Mean serum ADA in CF patients group and control group was 9.38 $\pm$ 2.72 and 16.04 $\pm$ 1.27, respectively ($P$ value = 0.001). Mean serum ADA in CF patients with mild respiratory involvement was 10.8 $\pm$ 2.45, whereas it was with 7.14 $\pm$ 1.19 higher than in CF patients with severe respiratory ($P$ value = 0.001). Mean serum ADA in CF patients with mild and severe gastrointestinal involvement was 8.87 $\pm$ 2.38 and 9.58 $\pm$ 2.85, respectively ($P$ value = 0.41). Mean serum ADA in CF patients with normal BMI was 10.3 $\pm$ 2.7 that was higher than in patients with low BMI (7.9 $\pm$ 2.08) ($P$ value = 0.002). Mean serum ADA in CF patients with consanguinity of parents was lower than in those without consanguinity ($P$ value = 0.01) (Table 2).

5. Discussion

Several studies have demonstrated a correlation between elevations in lung adenosine levels and development of pulmonary fibrosis (1, 5, 16). The mechanisms by which adenosine elevation leads to pulmonary fibrosis are not clear; however, it might activate the profibrotic pathways in the lung. ADA-deficient mice accumulate adenosine in multiple tissues, including the lung, where adenosine contributes to the development of pulmonary inflammation (1). It has been reported that TGF-$\beta$1 is elevated in the lungs of partially ADA-deficient mice, suggesting that this molecule may increase the abundance of myofibroblasts and collagen deposition (1).

Although the direct stimulation of TGF-$\beta$1 by adenosine has not been shown, the use of exogenous ADA en-

| Table 1. CF Patients’ Characteristics $^a$ | Variables | Values |
|------------------------------------------|-----------|--------|
| Gender                                   |           |        |
| Male                                     | 20 (41)   |        |
| Female                                   | 29 (59)   |        |
| Consanguinity of parents                 |           |        |
| Near consanguinity                       | 18 (38)   |        |
| h distant consanguinity                  | 5 (10)    |        |
| No consanguinity                         | 6 (12)    |        |
| Body Mass Index (BMI)                    |           |        |
| Normal                                   | 29 (59)   |        |
| Abnormal                                 | 20 (41)   |        |
| Severity of gastrointestinal disease     |           |        |
| Mild                                     | 14 (29)   |        |
| Severe                                   | 35 (71)   |        |
| Severity of respiratory disease          |           |        |
| Mild                                     | 21 (43)   |        |
| Severe                                   | 28 (57)   |        |

$a$ Data are presented as No. (%).
Table 2. The Level of Serum ADA in CF Patients and Control Group

| Variables                          | Mean ± SD  | P Value |
|------------------------------------|------------|---------|
| **Severity of respiratory disease**|            |         |
| CF patients                        |            | 0.001   |
| Severe                             | 10.8 ± 2.45|         |
| Mild                               | 7.34 ± 1.9 |         |
| Control                            | 16.04 ± 3.9|         |
| **Severity of gastrointestinal disease**|          | 0.41    |
| CF patients                        |            |         |
| Severe                             | 9.58 ± 2.85|         |
| Mild                               | 8.87 ± 2.38|         |
| Control                            | 16.04 ± 1.2|         |
| **BMI, Kg/m²**                     |            |         |
| CF patients                        |            | 0.002   |
| Low (abnormal)                     | 7.9 ± 2.08 |         |
| Normal                             | 10.3 ± 2.7 |         |
| Control                            | 0.4         |         |
| Low (abnormal)                     | 16.2 ± 1.3  |         |
| Normal                             | 15.9 ± 1.2  |         |
| **Consanguinity of parents**       |            | 0.01    |
| CF patients                        |            |         |
| Near consanguinity                 | 8.6 ± 2.09 |         |
| Distant consanguinity              | 10.8 ± 4.2 |         |
| No consanguinity                   | 11.5 ± 2.3 |         |
| Control                            | 0.6         |         |
| Near consanguinity                 | 15.7 ± 1.1 |         |
| Distant consanguinity              | 16.1 ± 1.5 |         |
| No consanguinity                   | 16.1 ± 1.2 |         |

zyme therapy in completely ADA-deficient mice led to lower adenosine levels in the lung and resolved the pulmonary fibrosis (16).

In this study, the mean serum ADA decreased in CF patients compared to control group. There are rare data on the level of serum ADA in CF patients. In the study of Perris et al. in Argentina, the increase in the activity of ADA was observed in patients with CF (17).

Interestingly, in our study the ADA level was lower in CF children with parental consanguinity compared to children without parental consanguinity (P value = 0.01), while the difference was not significant in control children with and without parental consanguinity. In addition, the lower level of ADA was found in CF patients with low BMI compared to CF patients with normal BMI (P value = 0.002).

In Goodarzi et al. study, the serum level of ADA was decreased in COPD patients (11). Vass et al. demonstrated a protective role of ADA and a pro-inflammatory function for adenosine in asthmatic patients (12). In addition, Hirsh et al. suggested that airway inflammation is due to increased level of adenosine in airway (18).

It has been reported that 8-cyclopentyl-1,3-dipropylxanthine (CPX) and 1,3-diallyl-8-cyclohexylxanthine (DAX), the adenosine antagonists, could improve the symptoms in CF patients through reduction of adenosine (19). According to previous reports (20, 21), the increased level of serum adenosine and disorder of alveogenesis was seen in ADA-deficient mice and its treatment with polyethylene glycol-modified ADA (PEG-ADA) improved the alveolar growth and clinical...
symptoms.

Advances in understanding the role of adenosine in respiratory diseases should lead to effective treatment options. Pharmacological manipulation of adenosine signaling pathway is of great interest and exploitation of new therapeutic target with agonist and antagonist activities against known adenosine receptors would be so useful for the treatment of different conditions of the respiratory system and cystic fibrosis (22).

In addition, the uses of ADA enzyme therapy are able to prevent the abnormal alveolar development by prevention of adenosine and to a lesser extent deoxyadenosine accumulation in the lung (20).

In conclusion, the clinical symptoms in CF Patients especially respiratory symptoms might be associated with reduction of serum ADA and rising serum adenosine; particularly respiratory symptoms might be associated with antagonists and agonists of adenosine receptors are highly recommended.

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