ORIGINAL ARTICLE

Study of effect of inhaled versus oral corticosteroids on sputum granzyme B in patients with moderate persistent bronchial asthma

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Granzyme B;
Corticosteroids;
Bronchial asthma

Abstract  Background: Asthma is a major public health problem with a high economic burden. It involves several inflammatory cells and multiple mediators. Granzyme B is an inflammatory mediator expressed and secreted by both immune and non immune cells. Recently it was found to play a role in the pathogenesis of asthma.

The aim of this work: was to evaluate the effect of both inhaled and oral corticosteroids on sputum granzyme B in asthmatic patients with moderate severity.

Methods: The study included 25 patients with moderate persistent asthma plus 15 healthy subjects as a control group. Granzyme B was measured before treatment with corticosteroids then after inhalation therapy and oral therapy.

Results: It was found that expected pulmonary function parameters were significantly lower in asthmatic patients than in controls. Sputum granzyme B levels were significantly higher in asthmatic patients than in controls. Sputum granzyme B levels were significantly lower after treatment with inhaled corticosteroids than basal levels. Oral corticosteroids further significantly lowered granzyme B, but the lowering effect of inhaled steroids was significantly higher than that of oral drugs. There was no statistically significant correlation between granzyme B and PFTs in asthmatic patients.

Conclusion: Granzyme B levels are elevated in bronchial asthma. Granzyme B could play a role in the pathogenesis of bronchial asthma. Both inhaled and oral corticosteroids lowered granzyme B levels significantly. The lowering effect of inhaled corticosteroids on sputum granzyme B is more than that of the oral corticosteroids.

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Introduction

Granzymes are recently discovered inflammatory mediators claimed to play a role in asthma. Granzyme B is sorted to a granule compartment that can be mobilized in a way similar to histamine. Thus Granzyme B must be considered as a potential mediator in asthma [5]. Granzyme B is a multifunctional protease that may have important role in a number of inflammations. It can be expressed and secreted by both immune and non immune cells, so granzyme B may have a larger role in chronic inflammation, auto immune and degenerative diseases than previously believed [17]. Granzyme B may play important role in the pathogenesis of asthma, so reduction in GrB expression can inhibit the apoptosis of airway epithelial cells and tissue remodeling [18]. Induced sputum provides a non invasive tool for investigating mediators in various asthma subtypes. Sputum induction is performed by inhalation of hypertonic saline (NaCl 4.5%) or isotonic saline solution (NaCl 0.9%) 15 min after pre-medication with 400 microgram of inhaled salbutamol [6].

Subjects and methods

In this cross sectional prospective case-control study, 25 patients (14 females and 11 males) with moderate persistent asthma of those attending the Chest Department at Benha University Hospitals were included. The study was conducted between May 2012 and September 2013.15 healthy subjects were included as a control group.

Exclusion criteria

1. Patients received oral or inhaled corticosteroids in the preceding 2 weeks.
2. Patients on other immune suppressant or anti-inflammatory therapy.
3. Patients with renal or hepatic impairment.
4. Patients with systemic or cardiovascular diseases that may result in the elevation of Gr B.

Patients were submitted to the following

1. Full history taking.
2. Full clinical examination both general and local.
3. Body mass index (BMI) was calculated as the weight in kg divided by the height in square meter (kg/m²).
4. Radiological examination: Plain postero anterior chest X-ray was done to exclude any chest lesion if present.
5. Pulmonary function tests (spirometry) before and after bronchodilatation.

Table 1  Age distribution among patients and controls.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Age Range</th>
<th>Age Mean ± SD</th>
<th>T-test ( t )</th>
<th>P-value</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>19.000–61.000</td>
<td>46.960 ± 11.066</td>
<td>0.067</td>
<td>0.947</td>
<td>NS</td>
</tr>
<tr>
<td>Controls</td>
<td>28.000–66.000</td>
<td>47.200 ± 10.798</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

There was no statistically significant difference between patients and controls as regards their age.
### Table 3  Distribution of BMI among patients and controls.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Range</th>
<th>Mean ± SD</th>
<th>T-test</th>
<th>P-value</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>22.300–62.600</td>
<td>29.332 ± 7.941</td>
<td>0.925</td>
<td>0.361</td>
<td>NS</td>
</tr>
<tr>
<td>Controls</td>
<td>23.600–31.200</td>
<td>27.380 ± 2.297</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 4  Comparison of FVC% predicted between patients and controls.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Range</th>
<th>Mean ± SD</th>
<th>T-test</th>
<th>P-value</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>74.400–112.600</td>
<td>86.556 ± 10.644</td>
<td>0.048</td>
<td>0.962</td>
<td>NS</td>
</tr>
<tr>
<td>Controls</td>
<td>80.400–99.000</td>
<td>86.413 ± 5.224</td>
<td></td>
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</tr>
</tbody>
</table>

### Table 5  Comparison of FEV1% predicted between patients and controls.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Range</th>
<th>Mean ± SD</th>
<th>T-test</th>
<th>P-value</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>60.000–79.300</td>
<td>65.700 ± 5.925</td>
<td>11.231</td>
<td>&lt;0.001*</td>
<td>S</td>
</tr>
<tr>
<td>Controls</td>
<td>81.400–104.100</td>
<td>89.827 ± 7.567</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 6  Comparison of FEV1/FVC% ratio between patients and control.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Range</th>
<th>Mean ± SD</th>
<th>T-test</th>
<th>P-value</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>50.660–69.630</td>
<td>62.247 ± 6.826</td>
<td>11.813</td>
<td>&lt;0.001*</td>
<td>S</td>
</tr>
<tr>
<td>Controls</td>
<td>79.450–95.860</td>
<td>85.979 ± 4.777</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

### Table 7  Comparison of FEF 25–75% between patients and controls.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Range</th>
<th>Mean ± SD</th>
<th>T-test</th>
<th>P-value</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>31.200–56.800</td>
<td>41.440 ± 7.415</td>
<td>13.727</td>
<td>&lt;0.001*</td>
<td>S</td>
</tr>
<tr>
<td>Controls</td>
<td>66.000–110.400</td>
<td>85.787 ± 13.090</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 8  Comparison of granzyme B levels in pg/ml between asthmatic patients and controls.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Range</th>
<th>Mean ± SD</th>
<th>T-test</th>
<th>P-value</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>162.700–809.800</td>
<td>392.076 ± 125.690</td>
<td>5.580</td>
<td>&lt;0.001*</td>
<td>S</td>
</tr>
<tr>
<td>Controls</td>
<td>104.400–292.300</td>
<td>201.560 ± 50.815</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 9  Comparison of granzyme B levels in pg/ml between males and females before and after inhaled corticosteroids (ICS) and after oral corticosteroids (OCS).

<table>
<thead>
<tr>
<th>Granzyme B Level in sputum</th>
<th>Female</th>
<th>Male</th>
<th>T-test</th>
<th>P-value</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before ICS</td>
<td>428.036 ± 134.485</td>
<td>346.309 ± 101.433</td>
<td>1.673</td>
<td>0.108</td>
<td>NS</td>
</tr>
<tr>
<td>After ICS</td>
<td>275.350 ± 71.213</td>
<td>249.291 ± 85.638</td>
<td>0.831</td>
<td>0.414</td>
<td>NS</td>
</tr>
<tr>
<td>After OCS</td>
<td>182.957 ± 73.977</td>
<td>173.409 ± 57.575</td>
<td>0.352</td>
<td>0.728</td>
<td>NS</td>
</tr>
</tbody>
</table>
Table 7 shows a significantly higher FEF 25–75% in the control group than the asthmatic group.

Table 8 shows a significantly higher granzyme B levels in asthmatic patients than controls.

Table 9 shows a non-statistically significant difference in granzyme B levels between males and females before and after treatment with corticosteroids.

Table 10 shows that granzyme B levels were significantly lower after treatment with inhaled corticosteroids. Oral steroids further significantly lowered granzyme B levels, but the lowering effect of inhaled steroids was significantly higher than that of oral drugs.

Discussion

The present study aimed to measure the sputum level of granzyme B of asthmatic patients with inhaled and oral corticosteroids treatment compared to those of healthy controls. There was no statistically significant difference between patients and controls as regards age (Table 1) and sex (Table 2). Male sex is a risk factor for asthma in children. Prior to the age of fourteen, the prevalence of asthma is nearly twice as great in boys as in girls. As children get older, the difference between the sexes narrows, and by adulthood, the prevalence of asthma is greater in women than in men ([10]). Absence of a significant difference between males and females in our asthmatic patients is related to the small number of the sample and the age range (as sex difference is more evident with extreme of age). The reasons for sex-related difference in asthma prevalence are not clear. However, lung size is smaller in males than in females at birth but larger in adulthood [12]. There was no statistically significant difference between patients and controls as regards BMI. Although higher incidence of asthma was seen in obese patients (especially severe forms) [8], the absence of a significant difference between our patients and controls is almost related to the smaller number of the sample studied. Higher incidence of severe form of asthma in obese patients is related to certain mediators such as leptins that affect airway function and increase the likelihood of asthma development [3].

Other mechanisms by which obesity could potentially influence asthma are thought to be mechanical factors leading to a decreased functional residual capacity and tidal volume with less smooth muscle stretch or through inflammatory or immune modification, increase in estrogen, or precipitating gastro-esophageal reflux [15].

Majority of asthmatic patients in the study were non-smokers only one patient was ex-smoker. Tobacco smoking is associated with accelerated decline of lung function in people with asthma and increase asthma severity, and may render patients less responsive to treatment with inhaled glucocorticoids, and reduces the likelihood of asthma being controlled [2].

In this study, pre and post bronchodilator spirometry were done among 25 cases to confirm the diagnosis of asthma. Statistical comparisons of pulmonary function tests were done among all studied groups. FEV1% predicted, FEV1/FVC% and FEF25–75% were significantly lower in asthmatic patients than controls while FVC% was not statistically different (Tables 5–7) [1].

The results of this study showed a significantly higher levels of Gr B in asthmatic patients (392.076 ± 125.690 pg/ml) than controls (201.560 ± 50.815 pg/ml) (Table 8). This result is in agreement with Athanasia et al. [1] who found that Gr B levels were significantly increased in moderate persistent asthma compared to mild asthmatics. The study was done on 10 patients with moderate persistent asthma compared to 10 patients with mild asthma to investigate granzyme B levels in induced sputum; it was 325.6 ± 44 versus 253 ± 24 pg/ml.

Also Sandra et al. [14] found that granzyme B levels were increased in patients with non-cosinophilic asthma (NEA) (n = 6) and in patients with mixed EA/NEA (n = 8) versus controls (n = 9) but granzyme B levels in EA (n = 7) were not significantly different from controls.

Results of Sandra et al. [14] indicate that granzyme B is more important in non-allergic asthma, however Khoa et al. [11] found increased granzyme B and perforin expression in allergic asthma CD4 + I NKT cells compared to healthy controls and allergic asthma. Sandra et al. [14] showed increased Gr B levels in BAL from a topic asthmatic following segmental allergen provocation and concluded that it is possible to play a role in the pathogenesis of the disease as they found increased expression in T-lymphocytes, a result similar to that of Khoa et al. [11]. Also, Tschopp et al. [16] showed induced production of Gr B, IL 13 and Leukotriene C4 by blood basophils following allergen sensitization in asthmatic patients. In the present study Gr B levels were significantly lower following inhaled steroids (263.884 ± 77.311)
than basal levels (392.076 ± 125.690) and further significant reduction was seen after oral steroids (178.756 ± 66.098). Table 10. Our result is in agreement with Chiara et al. [4] who found that methylprednisolone-treated normal NK cells display reduced levels of intracytoplasmic granzyme B that was done on 10 patients receiving methylprednisolone. In the present study there was no statistically significant correlation found between granzyme B and pulmonary function tests (FEV1%, FVC%, FEV1/FVC, FEF25–75%) in asthmatic patients. (Table 12). This result is in agreement with Hodge et al. [9] who found that there was no correlation between sputum granzyme B levels and airflow obstruction. Also Khoa et al. [11], did not find any significant correlation between granzyme B levels and forced expiratory volume in 1 s (FEV1).

The study showed that there was no statistically significant correlation between granzyme B and both age and BMI in asthmatic patients and controls. This result is in agreement with Hodge et al. [11] who found that there was no correlation between granzyme B levels and clinical parameters of asthma such as patient age, disease duration and age of onset. However, Hala et al. [7] found that granzyme B levels positively correlate with measures of obesity and insulin resistance and other metabolic parameters which support our result (see Table 11).

### Conflict of interest

None declared.

### References


[14] H. Sandra, R. Sarah, L.S. Jodie, G. Calida, H. Kirsty, Y. Lan, T – cell granzyme B is increased in induced sputum both from patients with COPD and non eosinophilic asthma, Asian pac. so. of Resp. 16 (2011) 37-86.


