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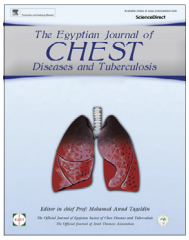

ORIGINAL ARTICLE

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

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KEYWORDS

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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 Granzyme B 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/m^2).
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.

Ventilatory function tests were performed using a Sensor-medics V max series, 2130 spirometer, V6200 Autobox, 6200DL.

Measurement of granzyme B level in sputum

Basal levels of GrB were measured at the start then patients were given high dose of inhaled steroid in the form of Budesonide 800 μg (Miflonide, caps, 400 mg, Novartis) twice daily for 2 weeks then low dose of oral steroid in the form of prednisolone 10 mg (2 tablets/day) for another 2 weeks.

Granzyme B was measured again after 2 weeks of inhalation therapy and at the end of oral therapy. Granzyme B was measured by an ELISA assay using a Human Granzyme B ELISA Kit by Wuhan EIAab Science Co., Ltd. (www.eiaab.com). Levels of granzyme B were estimated in pg/ml.

Sputum processing

Sputum was processed within 2 h of collection. Sputum plugs were harvested and processed with 4× weight/volume of 0.1% dithiothreitol in phosphate buffered saline (PBS).

Samples were filtered through 48 mm mesh and centrifuged at 1200 rpm for 5 min. to remove cells. Supernatants were stored at −80 °C until ELISA test [13].

Results

Table 2 shows a non statistically significant difference between patients and controls as regards sex distribution.

Table 3 shows a non statistically significant difference between patients and controls as regards BMI.

Table 4 shows a non statistically significant difference in FVC% between patients and controls.

Table 5 shows that FEV1% of the control group is significantly higher than the asthmatic group.

Table 6 shows that FEV1/FVC% is significantly higher in the control group than the asthmatic group.

Table 2 Sex distribution among patients and controls.

Sex		Patients	Controls	Total
Female	N	14	6	20
	%	56.00	40.00	50.00
Male	N	11	9	20
	%	44.00	60.00	50.00
Total	N	25	15	40
	%	100.00	100.00	100.00
Chi-square	X^2	0.965		
	P-value	0.326		

Table 1 Age distribution among patients and controls.

Groups	Age		*T*-test		
	Range	Mean ± SD	*t*	*P*-value	Sig.
Patients	19.000–61.000	46.960 ± 11.066	0.067	0.947	NS
Controls	28.000–66.000	47.200 ± 10.798			

There was no statistically significant difference between patients and controls as regards their age.

Table 3 Distribution of BMI among patients and controls.

Groups	BMI		*T*-test		
	Range	Mean ± SD	*t*	*P*-value	Sig.
Patients	22.300–62.600	29.332 ± 7.941	0.925	0.361	NS
Controls	23.600–31.200	27.380 ± 2.297			

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

Groups	FVC%		*T*-test		
	Range	Mean ± SD	*t*	*P*-value	Sig.
Patients	74.400–112.600	86.556 ± 10.644	0.048	0.962	NS
Controls	80.400–99.000	86.413 ± 5.224			

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

Groups	FEV1%		*T*-test		
	Range	Mean ± SD	*t*	*P*-value	Sig.
Patients	60.000–79.300	65.700 ± 5.925	11.231	$<0.001^{*}$	S
Controls	81.400–104.100	89.827 ± 7.567			

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

Groups	FEV1/FVC%		*T*-test		
	Range	Mean ± SD	*t*	*P*-value	Sig.
Patients	50.660–69.630	62.247 ± 6.826	11.813	$<0.001^{*}$	S
Controls	79.450–95.860	85.979 ± 4.777			

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

Groups	FEF 25–75%		*T*-test		
	Range	Mean ± SD	*t*	*P*-value	Sig.
Patients	31.200–56.800	41.440 ± 7.415	13.727	$<0.001^{*}$	S
Controls	66.000–110.400	85.787 ± 13.090			

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

Groups	Granzyme B Level in sputum (before):		*T*-test		
	Range	Mean ± SD	*t*	*P*-value	Sig.
Patients	162.700–809.800	392.076 ± 125.690	5.580	$<0.001^{*}$	S
Controls	104.400–292.300	201.560 ± 50.815			

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).

Granzyme B Level in sputum	Female	Male	*T*-test		
	Mean ± SD	Mean ± SD	*t*	*P*-value	Sig.
Before ICS	428.036 ± 134.485	346.309 ± 101.433	1.673	0.108	NS
After ICS	275.350 ± 71.213	249.291 ± 85.638	0.831	0.414	NS
After OCS	182.957 ± 73.977	173.409 ± 57.575	0.352	0.728	NS

Table 10 Comparison of granzyme B levels in asthmatic patients in pg/ml before and after inhaled corticosteroids (ICS): and oral corticosteroids (OCS).

Descriptive Statistics	Granzyme B Level in sputum			Difference		Paired *t*-test	
	Range	Mean ± SD		Mean	SD	*t*	P-value
Before ICS	162.700–809.800	392.076 ± 125.690	*t*1	128.192	136.212	4.706	< 0.001*
After ICS	117.700–440.500	263.884 ± 77.311	*t*2	213.320	147.621	7.225	< 0.001*
After OCS	50.200–312.200	178.756 ± 66.098	*t*3	85.128	87.355	4.873	< 0.001*

t1: change between before and after ICS. **t2**: change between before and after OCS. **t3**: change between after ICS and after OCS.* significant.

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 asthmatic 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 eosinophilic 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 asthmatic 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)

Table 11 Correlation between granzyme B and each of age and BMI, in asthmatic patients and the control group.

Basal granzyme B Level in sputum			
	R	*P*-value	Sig.
Age	−0.237	0.254	NS
BMI	−0.047	0.824	NS

There was no statistically significant correlation between granzyme B and both of age and BMI, in asthmatic patients and controls.

Table 12 Correlation between granzyme B and PFTs in asthmatic patients.

Basal granzyme B Level in sputum			
	R	*P*-value	Sig.
FVC%	−0.103	0.624	NS
FEV1/FVC	0.142	0.497	NS
FEV1%	−0.096	0.649	NS
change in FEV1%	0.099	0.638	NS
FEF25–75%	0.216	0.300	NS

There was no statistically significant correlation between granzyme B and PFTs in asthmatic patients.

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.

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