Studying the correlation between transforming growth factor β1 and chitinase-3-like-1 in assessment of bronchial asthma severity

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Received 29 October 2017
Accepted 4 January 2018

Kasr Al Ainy Medical Journal
2018, 24:7–13

Background
Chronic inflammation and airway remodeling have important roles in asthma pathophysiology. Transforming growth factor β1 (TGF-β1) and YKL-40 play a very important role in the pathogenesis of asthma.
Aim
The aim of this work was to find noninvasive biomarkers that may enable us to assess asthma severity as a surrogate for invasive bronchial mucosa biopsy. Therefore, we studied the correlation between TGF-β1 and YKL-40 and asthma severity.
Patients and method
The work was done on 40 patients with asthma who were classified into two groups: 20 patients with mild asthma and 20 patients with severe but stable asthma. A third group of 20 normal participants was taken as control. Immunoglobulin E total, YKL-40, and TGF-β1 were determined in serum of all studied groups.
Results
The results showed highly significant increased serum TGF-β1 and serum YKL-40 in patients with asthma compared with control group, and they were positively correlated with disease severity.
Conclusion
We conclude that increased serum levels of TGF-β1 and YKL-40 may be a biological characteristic of asthma exacerbation.

Keywords:
asthma severity, correlation between asthma severity and transforming growth factor-β1, YKL-40

Kasr Al Ainy Med J 24:7–13

1687-4625

Introduction

Bronchial asthma is a chronic, complex, and heterogenic respiratory disease in which the mucosa of the airways becomes abnormal and inflamed. The etiology of the disease is unknown, but it may be accompanied with the increase of inflammatory serum markers [1].

YKL-40 is a measurable serum chitinase-like protein that downregulates or upregulates the innate immune responses in inflammatory and tissue-remodeling states [2,3]. Studies have found that increased serum level of YKL-40 could be a marker for asthma severity.

Transforming growth factor β1 (TGF-β1) is thought to play a role in airway remodeling in asthmatic patients; however, controversy remains whether the concentration of TGF-β1 is correlated with disease severity [4].

In airway remodeling, there is defective extracellular matrix (ECM) turnover. There is increased expression of TGF-β1 molecule in mustard gas-harmed patients, as it plays an important role in stimulating ECM accumulation. So TGF-β1 may be involved in progression of airway remodeling of these patients [5].

Aim

We aimed to find noninvasive biomarkers that may enable us to assess asthma severity, as a surrogate for the invasive bronchial mucosa biopsy.

Patients and methods

This case–control study included patients with asthma diagnosed according to the GINA guidelines [6]. A total of 40 nonsmoking patients with asthma (without additional diseases and aged between 15 and 40 years;

 DOI: 10.4103/kamj.kamj_35_17

female : male ratio was 50 : 50) and 20 healthy control participants who were also of equal sex and same age and did not smoke were followed up between October 2014 and June 2015 in Internal Medicine Department and Outpatient Clinic of Al-Hussein University Hospital and Al-Azhar University. The smoking histories of patients were recorded. Our exclusion criteria were other infections within the past month, malignancy, chronic obstructive pulmonary disease (COPD), and other chronic diseases. Chest radiographs were evaluated to exclude other pathologies. The participants also underwent physical examinations. One or more positive results to allergens in skin prick test were accepted as atopy.

Our participants were divided into the following three groups:

Group 1: 20 healthy nonsmoking participants (10 female and 10 male participants) as a control group.
Group 2: 20 patients with mild intermittent asthma according to GINA criteria (10 female and 10 male patients).
Group 3: 20 patients with severe but stable persistent asthma according to GINA criteria (10 female and 10 male patients).

The participants who participated in this study gave an informed verbal consent. Approval of the Ethical Committee of Faculty of Medicine for Boys, Al-Azhar University, was also obtained.

The 20 patients with mild intermittent asthma received medication protocols according to GINA criteria. The patients received inhaled short-acting β2 agonists as needed. Severe persistent asthma was defined as symptoms like dyspnea, cough, wheezing, shortness of breath, or chest tightness together with decrease in peak expiratory flow (PEF) and forced expiratory volume in first second (FEV_1) values according to GINA in the past month. During acute exacerbation period, the patients received inhaled short-acting β2 agonists 1–4 puffs/h [inhaled salbutamol 100 mcg or nebulized 0.15 mg/kg (2.5 mg)/20 min], systemic corticosteroids (0.5/2 mg/kg/day prednisolone), and/or oxygen therapy [7].

For all participants participating in the study, the following investigations were performed:

(1) Chest radiograph, that is, chest radiography (computed tomography if needed) to exclude other pathology.
(2) Pulmonary functions tests.
(3) Allergy skin prick test.
(4) Routine laboratory tests: complete blood count, especially eosinophilic count, liver enzymes, blood urea, and serum creatinine level.
(5) Immunoglobulin (Ig)E total.
(6) YKL-40 in serum.
(7) TGF-β1 in serum.

Sample collection

Five milliliter of fasting (6–8 h) venous blood sample was collected from each patient participating in the study and divided into two parts: the first part (1.5 ml) was collected on EDTA-containing tube for CBC determination. The second part (3.5 ml) of the sample was allowed to clot for 30 min before centrifugation for 15 min at 3000*g*. The serum was removed and stored at up to 20°C for determination of IgE, YKL-40, and TGFβ1.

Determination of complete blood picture was performed on coulter counter T890 (Coulter Counter, Harpenden, UK).

Serum IgE is measured by quantitative sandwich ELISA, and the kit was supplied by Abcam (Cambridge, UK) [8].

The determination of serum YKL-40 was performed using enzyme immunoassay (EIA) method [9], and the kit was supplied by MicroVue (10165 McKellar Court; Quidel Corporation, San Diego, California, USA).

Serum TGF-β1 determination was performed using an enzyme immunoassay [10], and the kit was supplied by DRG Instruments GmbH (DRG International Inc., Marburg, Germany).

Pulmonary function testing (spirometry)

Baseline spirometric studies were carried out for cases and control groups (using MiniSpir; Mir Srl, Roma, Italy), including forced vital capacity (FVC), FEV_1, (FEV_1/FVC) ratio, and PEF, and the values were recorded.

Allergy skin prick test

The primary mode of skin testing for immediate IgE-mediated allergy was used. Small amounts of allergen are introduced into the epidermis and nonvascular superficial dermis and interact with specific IgE bound to cutaneous mast cells. Histamine and other mediators are released, leading to a visible 'wheal-and-flare' reaction peaking after ~15 min [11].

Statistical analysis

Statistical package for the social sciences (SPSS) version 17 was used (IBM Corporation, 1 New Orchard Road Armonk, New York, United States). Parametric data were expressed as mean±SD and nonparametric data were expressed as number and percentage of the total. The mean and SD were calculated. Comparing the mean ±SD of two groups was done using the unpaired *t*-test. Determining the extent that a single observed series of proportions differs from a theoretical or expected distribution was done using the χ^2-test. Receiver operator characteristic (ROC) curve was used, and sensitivity and specificity for various cutoff points were plotted. *P* was considered nonsignificant if more than 0.05, significant if less than 0.05, and highly significant if less than 0.01 and less than 0.001.

Results

Our study included 60 participants, and they were classified into three groups: 20 (10 male/10 female) normal participants were taken as controls, with a mean age of 24.800±2.505 years; 20 (10 male/10 female) patients with mild asthma, with a mean age of 24.250± 5.129 years; and 20 (10 male/10 female) patients with severe asthma, with a mean age of 30.400±7.542 years.

Table 1 shows that there were highly significant increases in serum YKL-40 and serum TGF-β1 levels in patient groups compared with control group and between mild and severe patient groups. Regarding serum total IgE, there no statistical significant difference between control and mild group, but there was a highly statistical significant difference between control and severe groups and between mild and severe groups.

Table 2 reveals that there was highly statistically significant difference regarding skin prick test between patients and control group.

Table 3 shows a significant positive correlation between both serum YKL-40 and serum TGF-β1 and serum TGF-β1, age, total IgE, eosinophil count, FVC, FEV_1, FEV_1/FVC, and PEF.

In our study, the cutoff point of TGF-β1 level between the patients and controls was more than 126.9 pg/ml, with sensitivity 100%, specificity 100%, and accuracy 100%, as done by ROC curve and shown in Table 4.

In our study, the cutoff point of YKL-40 level between the patients and controls was more than 71.3 ng/ml, with sensitivity 100%, specificity 100%, and accuracy 100%, as done by ROC curve and shown in Table 5.

In our study, the cutoff point of TGF-β1 level between the severe and mild groups was more than 260 pg/ml, with sensitivity 100%, specificity 55%, and accuracy 87.4%, as done by ROC curve and shown in Table 6.

In our study, the cutoff point of YKL-40 level between the severe and mild groups was more than 180 ng/ml, with sensitivity 100%, specificity 85%, and accuracy 97.5%, as done by ROC curve and shown in Table 7.

To our knowledge, regarding YKL-40 level in patients with asthma compared with controls, all results correlated with our results, and we did not find any opposing results.

Discussion

This study aimed to find the correlation between serum TGF-β1 and serum YKL-40 in patients with asthma of variable severity. Our study showed that there was highly significant increase in the serum TGF-β1 in both asthmatic groups compared with controls group, and this is attributed to airway wall thickening and

Table 1 Different laboratory data for all groups

Parameters	Groups (mean±SD)			*P*	Turkey's test
	Control	Mild	Severe		
Eosinophil's (%)	2.215±1.043	4.600±1.642	7.310±2.754	0.000	Ta: 0.001; Tb: 0.000; Tc: 0.0000
FVC (%)	99.950±6.962	97.200±6.221	68.550±13.153	0.000	Ta: 0.621; Tb: 0.000; Tc: 0:000
FEV_1	99.150±12.115	68.950±3.677	33.600±7.358	0.000	Ta: 0.000; Tb: 0.000; Tc: 0.000
FEV_1/FVC (%)	98.400±9.230	70.850±2.870	49.200±6.940	0.000	Ta: 0.000; Tb: 0.000; Tc: 0.000
PEF (%)	98.350±14.412	64.600±2.703	33.100±9.608	0.000	Ta: 0.000; Tb: 0.000; Tc: 0.000
Serum IgE (IU/ml)	52.650±62.086	111.330±92.495	400.950±327.971	0.000	Ta: 0.000; Tb: 0.000; Tc: 0.000
Serum YKL-40 (ng/ml)	55.770±9.675	157.220±24.458	230.110±27.697	0.000	Ta: 0.000; Tb: 0.000; Tc: 0.000
Serum TGF-β1 (pg/ml)	97.665±14.939	242.430±37.668	300.280±30.495	0.000	Ta: 0.000; Tb: 0.000; Tc: 0.000

FEV, forced expiratory volume in first second; FVC, forced vital capacity; Ig, immunoglobulin; Ta, Turkey's test comparing between control and mild group; Tb, comparison between control and moderate; Tc, comparison between mild and severe; TGF-β1, transforming growth factor β1.

Table 2 Skin prick test in the studied groups

Skin prick test	Groups [*n* (%)]			
	Controls	Mild	Severe	Total
Negative	20 (100.00)	11 (55.00)	7 (35.00)	38 (63.33)
Positive	0 (0.00)	9 (45.00)	13 (65.00)	22 (36.67)
Total	20 (100.00)	20 (100.00)	20 (100.00)	60 (100.00)
χ^2		25.436		
P-value		<0.001*		

*Meaning highly significant.

Table 3 Correlation between serum YKL-40, serum transforming growth factor-β1, and the parameters of the patients

	YKL-40		TGF-β1	
	r	*P*-value	*r*	*P*-value
TGF-β1	0.924	<0.001*		
Age	0.511	<0.001*	0.399	0.011*
Total IgE	0.792	<0.001*	0.756	<0.001*
HB	0.171	0.291	0.072	0.657
WBCs	0.028	0.863	0.020	0.904
Eosinophil count	0.888	<0.001*	0.926	<0.001*
PLT	0.075	0.644	0.121	0.458
FVC	–0.685	<0.001*	–0.546	<0.001*
FEV_1	–0.809	<0.001*	–0.646	<0.001*
FEV_1/FVC	–0.786	<0.001*	–0.627	<0.001*
PEF	–0.861	<0.001*	–0.717	<0.001*
ALT	–0.147	0.367	–0.242	0.133
AST	–0.118	0.467	–0.265	0.099
S. Cr	0.073	0.654	–0.026	0.871
Urea	0.379	0.016*	0.282	0.078

ALT, alanine transaminase; AST, aspartate transaminase; FEV, forced expiratory volume in first second; FVC, forced vital capacity; HB, hemoglobin; Ig, immunoglobulin; PEF, peak expiratory flow; PLT, platelet; S. Cr, serum creatinine; TGF-β1, transforming growth factor β1; WBC, white blood cells. *Meaning highly significant.

Table 4 Receiver operator characteristic curve between patients and controls regarding transforming growth factor-β1

Cutoff	Sensitivity	Specificity	PPV	NPV	Accuracy
>126.9	100.0	100.0	100.0	100.0	100.0

PPV, positive predictive value; NPV, net present value.

Table 5 Receiver operator characteristic curve between patients and controls regarding YKL-40

Cutoff	Sensitivity	Specificity	PPV	NPV	Accuracy
>71.3	100.0	100.0	100.0	100.0	100.0

PPV, positive predictive value; NPV, net present value.

Table 6 Receiver operator characteristic curve between severe and mild asthma groups regarding transforming growth factor-β1

Cutoff	Sensitivity	Specificity	PPV	NPV	Accuracy
>260	100.0	55.0	69.0	100.0	87.4

PPV, positive predictive value; NPV, net present value.

Table 7 Receiver operator characteristic curve between severe and mild asthma groups regarding YKL-40

Cutoff	Sensitivity	Specificity	PPV	NPV	Accuracy
>180	100.0	85.0	87.0	100.0	97.5

PPV, positive predictive value; NPV, net present value.

airflow limitation. Serum YKL-40 also showed a highly significant increase in asthmatic groups compared with control group, so we can use both as a biomarker of inflammation and asthma severity.

Moreover, we found a significant positive correlation between mean serum level of both TGF-β1 and YKL-40 and mean serum total IgE, eosinophil count, FVC, FEV_1, FEV_1/FVC, PEF, and age. In fact, there is no evidence that TGF-β1 production or metabolism might be different relative to age, and this may be a limitation of our study.

In this study, we found a significantly higher serum level of TGF-β1 in patients with severe asthma compared with those with mild asthma. This is owing to the presence of different types of structural or inflammatory cells that secrete high levels of TGF-β1 [12]. This higher expression of TGF-β1 correlated with basement membrane thickness as evidenced by airflow limitation by pulmonary function test. The higher level of serum TGF-β1 level was significant correlated with asthma severity, so it is considered as a noninvasive biomarker of airway remodeling and asthma severity [12].

This is in agreement with Hassan *et al.* [13] who found increased serum levels of TGF-β1 in patients with asthma, and it was associated with decreased asthma control, increased disease duration, and increased asthma severity.

Moreover, another study showed that elevated TGF-β1 in induced sputum was associated with airflow limitation, and this correlated with functional indices, such a severity of the disease and airflow obstruction. Airway wall thickness correlates positively with tissue inhibitor of

metalloproteinases-1 and also with eosinophilic cationic protein levels in bronchoalveolar lavage [14].

Our results agreed with Joseph *et al.* [15] and Ozyilmaz *et al.* [16] as they found significantly higher TGF-β1 levels in patients with asthma compared with controls.

On the contrary, El-Sayed *et al.* [17] found significant increase in the serum TGF-β1 in mild bronchial asthma group and significant decrease in the serum TGF-β1 in the severe asthma group compared with controls participants. These results can be explained by the spontaneous release of significantly higher levels of TGF-β1 from neutrophils of patients with asthma than those from normal participants [18]. The other explanation is that the increase in serum TGF-β1 could be secondary to its increase in the respiratory tract during acute asthma [19]. The behavior of serum TGF-β1 in acute asthma exacerbation is dependent on severity of asthma: it was significantly higher in mild asthma, whereas in severe asthma, it was low, perhaps, related to an inherent defect in TGF-β1 secretion, exhaustion of TGF-β1 secreting cells, or to steroid inhalation therapy [17].

However, [4] suggested that TGF-β1 plays a key role in tissue remodeling, and owing to its immunoregulatory role, a new therapeutic intervention for asthma should be considered. Moreover, they found that anti-TGF-β1 antibody was significantly associated with decreased mucus production, collagen deposition, and smooth muscle cell proliferation in an asthma model.

This study showed that mean serum YKL-40 level was significantly higher in both asthmatic groups compared with control group. Moreover, we found a significant positive correlation between mean serum YKL-40 and mean serum TGF-β1, age, serum total IgE, eosinophil count, FVC, FEV_1, FEV_1/FVC, and PEF.

This study showed significantly higher serum level of YKL-40 in patients with severe asthma compared with mild asthma. Therefore, it can be used as a biomarker for inflammation and to predict asthma severity.

This comes in agreement with Chupp *et al.* [20] who measured serum YKL-40 levels in three groups of patients with asthma and found that serum levels of YKL-40 were increased in patients with asthma compared with healthy persons. Ober and Chupp [2] found that YKL-40 levels were significantly higher in the serum of patients with severe asthma compared with patients with mild to moderate asthma. These levels were significantly positively correlated with asthma severity, the degree of airway obstruction, and pediatric asthma score (PAS) score.

The reason behind the significant increase of YKL-40 in severe asthmatics could be related to its pathophysiological role in asthma, as it is Th2-cytokine interleukin-13 dependent, and this has been supported by Kuepper *et al.* [21] and Shuhui *et al.* [22]. They revealed that YKL-40 might have a protective role in the lungs through attenuating airway inflammation and airway hyperresponsiveness. Persistently elevated serum YKL-40 level was a significant marker of antigen-driven inflammation and remodeling in the asthmatic airway.

In our study, we found highly significant correlation between the level of YKL-40 with asthma severity and the level of total IgE.

Our results are consistent with Tang *et al.* [23] who found that total serum IgE levels of patients with asthma in the acute exacerbation period were found higher than the well-controlled asthma and the control groups and significantly correlated with the serum YKL-40 level.

Moreover, Sohn *et al.* [24] found a positive correlation between serum total IgE and the serum YKL-40 levels, and they were demonstrated to be higher in atopic patients and are related to genotype.

Owing to genotypic structure of Turkish patient population, Duru *et al.* [7] found no correlation between serum YKL-40 levels and asthma severity and total serum IgE levels in these patients.

Regarding total IgE, in our study, there was no statistical significant difference between control and mild group, but there was a highly significant difference between the control and severe groups and between mild and severe groups. Our results are consistent with Rotsides *et al.* [25] who found a high positive correlation between increased IgE and asthma intensity. Moreover, serum total IgE level is a strong

predictor of allergy in asthmatic children. Satwani *et al.* [26] and Mojtaba *et al.* [27], found that IgE may be an accurate predictor of asthma diagnosis, as its serum levels were increased in patients with asthma compared with healthy participants. A negative association was found between serum IgE and FEV_1, FVC, and FEV_1/FVC.

Our results agreed with Anupama *et al.* [28] who was found that serum IgE levels reflect asthma severity, as degrees of inflammation and the subsequent severity of airway obstruction in bronchial asthma are proportional to the serum IgE levels.

Our study showed a significant increase in the blood eosinophil count in patients with asthma. Eosinophilic asthma has been used to characterize asthma phenotype, as this phenotype can be identified by peripheral eosinophil count [29].

Our result was in agreement with Ulrik [30] who found that in asthmatics with peripheral eosinophilia, there is a correlation with severity of asthma symptoms and an inverse correlation in pulmonary function.

Our results agreed with a study performed in 2010 that showed significant relation between platelet and eosinophil activation in airways of patients with asthma. These data showed the important role of platelets in airway eosinophilia [31].

Conclusion

We conclude that increased serum levels of these markers (TGF-β1 and YKL-40) have a direct correlation asthma exacerbations as well as severe asthma and reverse correlation with lung function.

Acknowledgements

The authors thank members of our laboratory in Al-Hussein University Hospital and also for members of laboratory in National Research Center for aid in preparation of this manuscript.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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