

# Assessment of severity of bronchial asthma by studying new markers: transforming growth factor- $\beta$ 1 and chitinase-3-like-1

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## Aim

The aim of this work was to look for non-invasive biomarkers that may enable us to assess asthma severity, as a surrogate for the invasive bronchial mucosa biopsy, by studying TGF- $\beta$ 1 and YKL-40.

## Objectives

TGF- $\beta$ 1 is used as biomarkers in the pathogenesis, prediction and follow up of asthma severity. YKL-40 has a role in airway inflammation; this relation suggests that YKL-40 and TGF- $\beta$ 1 can be used as biomarkers in the pathogenesis, prediction and follow-up of asthma severity.

## Background

Defective extracellular matrix (ECM) turnover characterizes airway remodeling. Transforming Growth Factor Beta-1 (TGF- $\beta$ 1) stimulate airway remodeling through activation of gene transcription via binding to specific subfamilies of cell transmembrane receptors.

## Methods

The work was done on a 60 subject aged between 20–40 years with equal sex. Classified into three groups; 20 patients with mild asthma, 20 patients with severe asthma and 20 normal subjects were taken as controls. For all subjects chest X-ray, pulmonary functions tests, allergy skin prick test, eosinophilic count, total IgE, YKL-40 and TGF- $\beta$ 1 in serum were performed.

## Results

The results showed that serum TGF- $\beta$ 1 and serum YKL-40 between the three groups were highly significantly different ( $P < 0.01$ ) between the three groups in asthmatic patients compared with control group.

## Conclusions

These variations were correlated positively with the severity of the disease indicating that their increased serum levels may be a biological characteristic of the disease exacerbation with a sentinel role in asthma.

## Keywords:

asthma severity, TGF- $\beta$ 1, YKL-40

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## Introduction

Transforming growth factor  $\beta$ 1 (TGF- $\beta$ 1) is thought to play a role in airway remodeling in asthmatic patients; however, controversy remains whether the concentration of TGF- $\beta$ 1 is correlated with the disease severity [1].

In airway remodeling, there is defective extracellular matrix turnover. There is increased expression of TGF- $\beta$ 1 molecule in mustard gas-harmed patients, as it plays an important role in stimulating extracellular matrix accumulation. So TGF- $\beta$ 1 may be involved in progression of airway remodeling of these patients [2].

YKL-40 is a measurable serum chitinase-like macromolecule and is synthesized in white cell

precursors at the myelocyte-metamyelocyte stages. It is localized inside the specific granules of neutrophils and discharged from totally activated cells as from neutrophils and macrophages that regulates the innate immune responses in inflammatory and tissue-transforming states [3].

It has been suggested by Saba *et al.* [4] that elevated serum level of YKL-40 could be a marker for asthma and its severity.

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We aim to look for 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 40 nonsmoking patients with asthma, diagnosed according to the Global Initiative for Asthma (GINA) guidelines (without additional diseases, who were aged between 15 and 40 years, with female : male ratio of 50 : 50), and 20 healthy controls, with equal sex ratio, nonsmokers, and of the same age group. They were all followed up between October 2014 and June 2015 in Internal Medicine Department and outpatient clinic at Al-Hussein University Hospital, Al-Azhar University. The smoking histories of patients were recorded. Our exclusion criteria were other infections within the last month, malignancy, chronic obstructive pulmonary disease, and other long-term diseases, and chest radiographs were evaluated to exclude other pathologies. The patients also underwent physical examinations. One or more positive results to allergens in skin prick test were accepted as atopy.

Participants were divided into three groups of individuals:

Group 1: 20 healthy nonsmoking individuals (female/male ratio of 10/10) as healthy control group.

Group 2: 20 patients (female/male ratio of 10/10) with mild intermittent asthma according to the GINA criteria.

Group 3: 20 patients (female/male ratio of 10/10) with severe but stable persistent asthma according to the GINA criteria.

The individuals participating in this study gave an informed verbal consent. Approval of the ethical committee of the Faculty of Medicine for Boys, Al-Azhar University, was also obtained.

The mild intermittent asthma group, which included 20 patients, received medication protocols according to GINA criteria. The patients received inhaled short-acting  $\beta_2$  agonists as needed. The 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 in last month according to GINA. During acute exacerbation period, the patients received inhaled short-acting  $\beta_2$  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 [5].

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

- (1) Chest radiography (computed tomography if needed) to exclude other pathology.
- (2) Pulmonary functions tests.
- (3) Allergy skin prick test.
- (4) Routine laboratory examination, including complete blood count, especially, eosinophilic count, liver enzymes, blood urea, and serum creatinine level.
- (5) IgE total.
- (6) YKL-40 in serum.
- (7) TGF- $\beta_1$  in serum.

### Sample collection

In total, 5 ml of fasting (6–8 h) venous blood samples was collected from each patient participating in the study and divided into two parts. The first part (1.5 ml) was collected in EDTA-containing tube for complete blood count determination. The second part (3.5 ml) was allowed to clot for 30 min before centrifugation for 15 min at 3000g. The serum was removed and stored at less than or equal to 20°C for determination of IgE, YKL-40, and TGF- $\beta_1$ .

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

Serum IgE is measured by quantitative sandwich enzyme linked immunosorbent assay, and the kit was supplied by Abcam (Cambridge, UK) [6].

The determination of serum YKL-40 was performed using enzyme immunoassay method (Wang and Xing, 2014) [7], and the kit was supplied by MicroVue (Quidel Corporation, San Diego, California, USA).

Serum TGF- $\beta_1$  determination was performed using an enzyme immunoassay [8], 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 S.r.l., Rome, 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 performed. Small amounts of allergen are introduced into the epidermis and nonvascular superficial dermis, and they 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.

**Statistical analysis**

Statistical Package for Social Science (SPSS) version 17 was used (IBM Corporation, 1 New Orchard Road Armonk, New York 10504-1722, 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  $\chi^2$ -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 0.001.

**Results**

Our study included 60 participants, and they were classified into three groups: 20 (10 male/10 female)

normal individuals were taken as control, 20 (10 male/10 female) patients had mild asthma, and 20 (10 male/10 female) patients had severe asthma, with mean age of the control group being 24.800±2.505 years, mild group 24.250±5.129, and severe group 30.400±7.542 years.

There were highly significant differences in eosinophil count, FVC%, FEV<sub>1</sub>, FEV<sub>1</sub>/FVC%, PEF%, serum total IgE, serum YKL-40, and serum TGF-β1 between the three studied groups, with exception of FEV%, as a nonsignificant difference was observed between mild group and control group only (Table 1).

On comparing patients regarding skin prick test, there is a highly statistically significant difference (*P*<0.01) between the controls and the patients as shown in Table 2 and Fig. 1.

In our study, the cutoff point of TGF-β1 level between the patients and controls was more than 126.9 pg/ml, with sensitivity of 100%, specificity of 100%, and accuracy of 100%, as done by ROC curve and is shown in Table 3 and Fig. 2.

In our study, the cutoff point of YKL-40 level between the patients and controls was more than 71.3 ng/ml, with sensitivity of 100%, specificity of 100%, and accuracy of 100%, as done by ROC curve and is shown in Table 4 and Fig. 2.

**Table 1 Different laboratory data for all groups (mean±SD)**

Parameters	Groups			<i>P</i>	Tukey's test
	Control	Mild	Severe		
Eosinophils (%)	2.215±1.043	4.600±1.642	7.310±2.754	0.000	Ta: 0.001Tb: 0.000Tc: 0.0000
FVC%	99.950±6.962	97.200±6.221	68.550±13.153	0.000	Ta: 0.621Tb: 0.000Tc: 0:000
FEV <sub>1</sub>	99.150±12.115	68.950±3.677	33.600±7.358	0.000	Ta: 0.000Tb: 0.000Tc: 0:000
FEV <sub>1</sub> /FVC (%)	98.400±9.230	70.850±2.870	49.200±6.940	0.000	Ta: 0.000Tb: 0.000Tc: 0:000
PEF (%)	98.350±14.412	64.600±2.703	33.100±9.608	0.000	Ta: 0.000Tb: 0.000Tc: 0:000
Serum IgE (IU/ml)	52.650±62.086	111.330±92.495	400.950±327.971	0.000	Ta: 0.000Tb: 0.000Tc: 0:000
Serum YKL-40 (ng/ml)	55.770±9.675	157.220±24.458	230.110±27.697	0.000	Ta: 0.000Tb: 0.000Tc: 0:000
Serum TGF-β1 (pg/ml)	97.665±14.939	242.430±37.668	300.280±30.495	0.000	Ta: 0.000Tb: 0.000Tc: 0:000

FEV<sub>1</sub>, forced expiratory volume in first second; FVC, forced vital capacity; PEF, peak expiratory flow; Ig, immunoglobulin; Ta, Tukey's test comparing between control and mild group; Tb, comparing between control and severe; Tc, comparing between mild and severe; TGF-β1, transforming growth factor β1.

**Table 2 Skin prick test between 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)
$\chi^2$	25.436			
<i>P</i> -value	<0.001*			

\*Highly significant.

In our study, the cutoff point of TGF- $\beta$ 1 level between the severe and mild groups was more than 260 pg/ml, with sensitivity of 100%, specificity of 55%, and accuracy 87.4%, as done by ROC curve and is shown in Table 4.

In our study, the cutoff point of YKL-40 level between the severe and mild groups was more than 180 ng/ml, with sensitivity of 100%, specificity of 85%, and accuracy 97.5%, as done by ROC curve and is shown in Table 4.

## Discussion

The aim of this work was to assess bronchial asthma severity by studying new biomarkers such as TGF- $\beta$ 1 and YKL-40 in patients with asthma of variable severity in comparison with normal population.

This study showed a highly significant increase in the serum TGF- $\beta$ 1 in both asthmatic groups (242.430  $\pm$  37.668 pg/ml in patients with mild asthma and 300.280  $\pm$  30.495 pg/ml in patients with severe asthma) compared with the controls (97.665  $\pm$  14.939 pg/ml) ( $P < 0.001$ ).

Also we found a highly significant correlation ( $P < 0.001$ ) between the mean serum level of TGF- $\beta$ 1 and the level of YKL-40, total IgE, eosinophil, FVC, FEV<sub>1</sub>, FEV<sub>1</sub>/FVC, and PEF and significant correlation with age.

Regarding disease severity, patients with severe asthma recorded highly significant increase in mean serum level of TGF- $\beta$ 1 (300.280  $\pm$  30.495 pg/ml) compared

with those with mild asthma (242.430  $\pm$  37.668 pg/ml,  $P < 0.001$  each). The elevated TGF- $\beta$ 1 level was significantly associated with severity of asthma; therefore, it could be used as a predictor of asthma severity and could be considered as noninvasive marker of airway remodeling, as it is increased in case of severe asthma compared with mild form of the disease.

This is in agreement with Hassan *et al.* [9], who found increased serum levels of TGF- $\beta$ 1 in patients with asthma, and it negatively correlates with pulmonary function tests. It was associated with prolonged disease duration, increased asthma severity, and decreased asthma control.

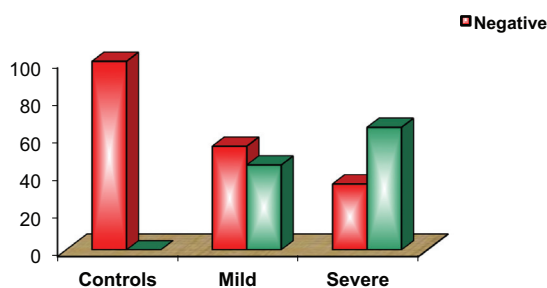
Also Manuyakorn *et al.* [10] found that significantly higher levels of serum TGF- $\beta$ 1 in patients with atopic asthma compared with nonatopic controls. This is because of the role of TGF- $\beta$ 1 in airway remodeling in asthma.

Joseph *et al.* [11] and Ozyilmaz *et al.* [12] found that TGF- $\beta$ 1 was significantly higher in patients with asthma as compared with controls. These results were in agreement with our results.

On the contrary, Zeinab *et al.* [13] found a significant increase in the serum TGF- $\beta$ 1 level in mild bronchial asthma group and significant decrease in the serum TGF- $\beta$ 1 level in the severe asthma compared with controls. These results can be explained by the spontaneous release of significantly higher levels of TGF- $\beta$ 1 from neutrophils of patients with asthma than those from normal individuals [14]. The other explanation is that the increase in serum TGF- $\beta$ 1 level could be secondary to its increase in the respiratory tract during acute asthma [15]. The behavior of serum TGF- $\beta$ 1 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- $\beta$ 1 secretion, exhaustion of TGF- $\beta$ 1 secreting cells, or to steroid inhalation therapy [13].

Although Yang *et al.* [1] suggested that TGF- $\beta$ 1 plays a key role in tissue remodeling, and because of its immunoregulatory role, a new therapeutic intervention

Figure 1



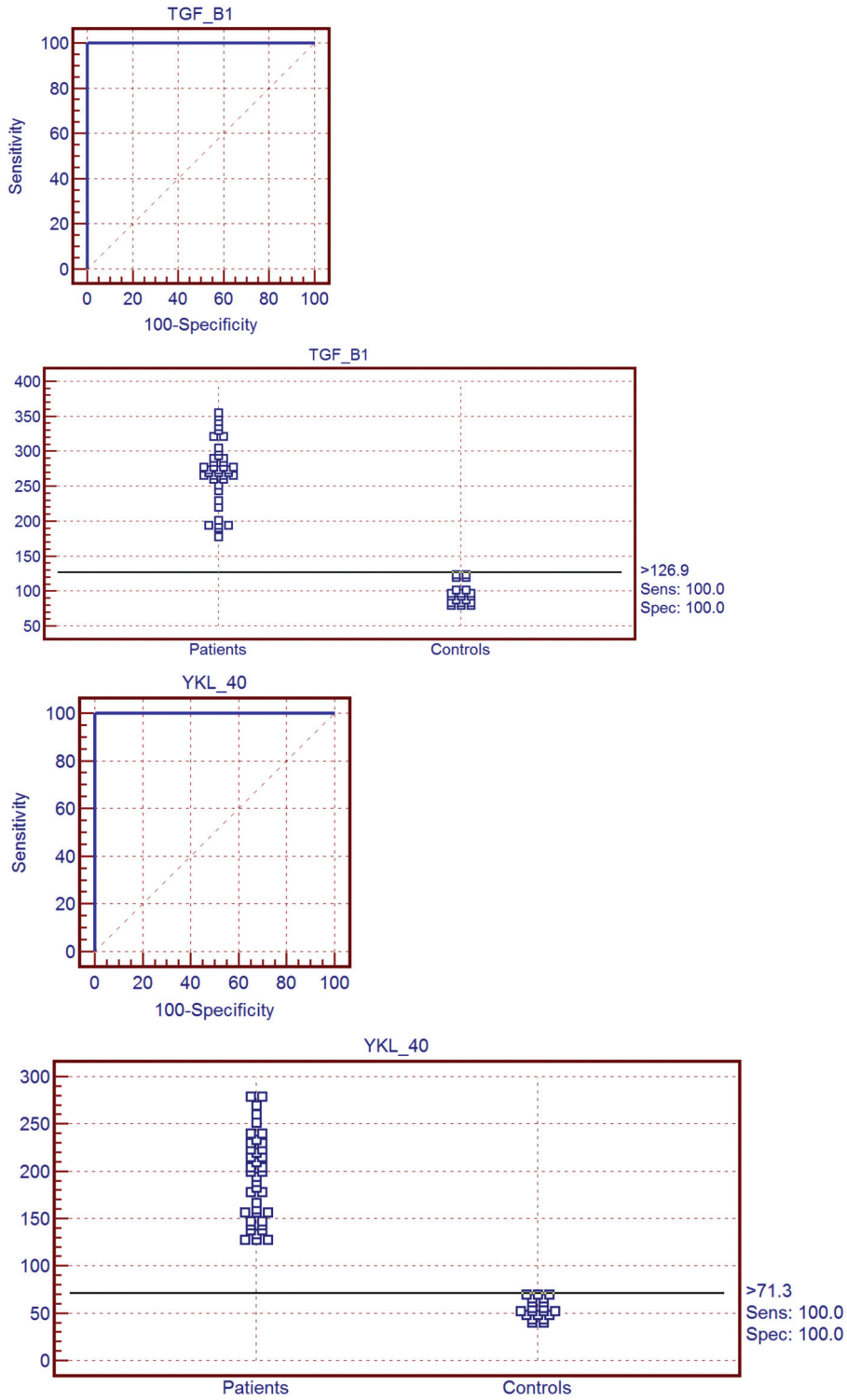
Skin prick test between the studied groups

Table 3 Receiver operating characteristic curve between patients and controls regarding serum transforming growth factor  $\beta$ 1 and serum YKL-40

Parameters	Cutoff	Sensitivity	Specificity	PPV	NPV	Accuracy
TGF- $\beta$ 1	>126.9	100.0	100.0	100.0	100.0	100.0
YKL-40	>71.3	100.0	100.0	100.0	100.0	100.0

NPV, negative predictive value; PPV, positive predictive value; TGF- $\beta$ 1, transforming growth factor  $\beta$ 1.

Figure 2



Receiver operating characteristic curves between patients and controls regarding serum TGF-β1 and serum YKL-40. TGF-β1, transforming growth factor β1

**Table 4 Receiver operating characteristic curves between patients with severe asthma and patients with mild asthma regarding serum transforming growth factor β1 and serum YKL-40 levels**

Parameters	Cutoff	Sensitivity	Specificity	PPV	NPV	Accuracy
TGF-β1	>260	100.0	55.0	69.0	100.0	87.4
YKL-40	>180	100.0	85.0	87.0	100.0	97.5

NPV, negative predictive value; PPV, positive predictive value; TGF-β1, transforming growth factor β1.



for asthma should be considered. Also they found that anti-TGF- $\beta$ 1 antibody significantly decreased mucus production, collagen deposition, and smooth muscle cell proliferation in an asthma model.

Regarding YKL-40, we found that the serum YKL-40 level was significantly higher in asthmatic groups

(157.220 $\pm$ 24.458 ng/ml in patients with mild asthma, and 230.110 $\pm$ 27.697 ng/ml in patients with severe asthma) compared with the controls (55.770 $\pm$ 9.675 ng/ml) ( $P$ <0.001), and this elevation reflects airway obstruction in asthma.

Also we found a significant correlation ( $P$ <0.001) between the mean serum level of YKL-40 and the level of TGF- $\beta$ 1, age, total IgE, eosinophils, FVC, FEV<sub>1</sub>, FEV<sub>1</sub>/FVC, and PEF.

Serum YKL-40 can be used to predict asthma severity and also can be used as a biomarker for inflammation as confirmed by our results, as serum YKL-40 level is increased in parallel to asthma severity.

This comes in agreement with Chupp *et al.* [16] who showed that serum levels of YKL-40 were increased in patients with asthma compared with healthy participants. Similar to our finding, Kuepper *et al.* [17] found significantly higher levels of YKL-40 in serum of patients with asthma after challenging them with various allergens.

Also Ober *et al.* [18] found that YKL-40 levels were significantly higher in the serum of asthmatic patients compared with nonasthmatic individuals. These levels were significantly positively correlated with asthma severity, the degree of airway obstruction, and Pediatric Appendicitis Score.

The reason behind the significant increase of YKL-40 in patients with severe asthma could be related to its pathophysiological role in asthma, and this has been supported by Kuepper *et al.* [17]. Shuhui *et al.* [19] revealed that YKL-40 can attenuate airway inflammation and hyper-responsiveness, so it protects the lungs. The persistent elevation of serum YKL-40 level could be used as a significant marker of antigen-driven inflammation and remodeling in the asthmatic airway.

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

In our study, we found a highly significant correlation ( $P$ <0.01) between the level of YKL-40 with asthma severity and the level of total IgE.

Our results are consistent with Tang *et al.* [20] who found that total serum IgE levels in patients with asthma compared with control participants, especially those with acute exacerbation and significantly, correlated with the serum YKL-40 level.

Also Sohn *et al.* [21] found a positive correlation between serum total IgE and the serum YKL-40 level in atopic patients, and they are related to genotype.

On the contrary, Duru *et al.* [22] found no correlation between the serum YKL-40 levels with asthma severity and total serum IgE levels in Turkish patients, and this might be because of genotypic structure of these patients.

Regarding serum total IgE level, in our study, there was no statistical significant difference ( $P$ >0.05) between control and mild groups, but there was highly statistical significant difference ( $P$ <0.01) between the control and severe groups and between mild and severe groups.

Our results are consistent with Rotsides *et al.* [23] who found a high positive association between increased IgE level and asthma intensity. Also, they suggested that serum total IgE level can be used as a strong predictor of allergy in children with asthma.

In our study, the cutoff point of TGF- $\beta$ 1 level between the patients and controls was more than 126.9 pg/ml, with sensitivity of 100%, specificity of 100%, and accuracy 100%, as done by ROC curve.

In our study, the cutoff point of mean serum YKL-40 level between the patients and controls was more than 71.3 ng/ml, with sensitivity of 100%, specificity of 100%, and accuracy of 100%, as done by ROC curve. Also we found that the cutoff point of mean serum TGF- $\beta$ 1 level between the severe and mild groups was more than 260 pg/ml, with sensitivity of 100%, specificity of 55%, and accuracy of 87.4%, as done by ROC curve.

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

## Conclusion

Serum TGF- $\beta$ 1 and serum YKL-40 levels could be used as a predictor of asthma severity and could be considered as noninvasive marker of airway remodeling, as it is increased in severe asthma compared with mild form of the disease.

## Limitations

Our study had some limitations. First, we did not correlate the serum levels of TGF- $\beta$ 1 and YKL-40 with their bronchoalveolar lavage levels as well as thickness of the subepithelial basement membrane in biopsy specimens of the lung. Second, further studies to investigate genetic polymorphism of YKL-40 and TGF- $\beta$ 1 are suggested to be performed. Third, it was difficult to find patients having asthma without any additional diseases. Finally, further multicenter studies with a larger number of patients are needed.

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## Conflicts of interest

There are no conflict of interests.

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