

Serum TGF- β 1 in Atopic Asthma

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SUMMARY Asthma is a chronic inflammatory disease of the airway. Pathological repair of chronic inflammation leads to airway remodeling. Transforming growth factor- β (TGF- β), a profibrotic cytokine, plays an important role in promoting the structural changes of airway remodeling. TGF- β effects on the proliferation, differentiation and extracellular matrix (ECM) metabolism of airway structural cells. This study assessed serum TGF- β 1 in different severity of atopic asthma compared to non-atopic controls. Thirty-one atopic asthmatic patients and 34 non-atopic controls, aged 7-18 years, were recruited as to the asthma severity: steroid naïve mild asthma, moderate asthma, and asthma in remission. Serum TGF- β 1 was measured by enzyme-linked immunosorbent assay. There was a significant difference between serum TGF- β 1 in asthmatic patients and that in control patients (39.59 ng/ml vs. 0.26 ng/ml, $p < 0.001$). Serum TGF- β 1 was highest in steroid naïve mild asthma group when compared to the moderate asthma and asthma in remission groups (47.44 ng/ml vs. 38.64 ng/ml and 47.44 ng/ml vs. 35.94 ng/ml, $p = 0.013$ and 0.001 , respectively). There were no correlations among serum TGF- β 1 and pulmonary function test parameters, duration of asthma, and duration of inhaled corticosteroid treatment. These data support the role of TGF- β 1 in airway remodeling in asthma.

Asthma is a chronic inflammatory disease of the airways which contributes to airway hyperresponsiveness, airflow limitation, respiratory symptoms, and disease chronicity.¹ In addition to the presence of inflammatory cells in the airway, the airways of asthmatic patients exhibit varying levels of structural changes termed airway remodeling.²⁻⁵ The changes include epithelial cell mucus metaplasia, smooth muscle hypertrophy/hyperplasia, subepithelial fibrosis, myofibroblast hyperplasia and increased angiogenesis.⁶ Remodeling is considered to be responsible for the rapid decline in lung function over time in asthmatic patients.⁷

Transforming growth factor- β (TGF- β) plays an essential role in promoting the structural changes of airway remodeling. It is an extremely potent stimulus for the formation of the extracellular matrix

(ECM) by fibroblast such as collagen types I and III, fibronectin, vitronectin, tenascin, and proteoglycan. Furthermore, it decreases synthesis of enzymes that degrade the ECM, namely matrix metalloproteinase (MMPs) and increases the production of proteins that inhibit enzymes that degrade the ECM (tissue inhibitor of matrix metalloproteinase).⁵

There are three TGF- β isoforms identified in mammals, namely TGF- β 1, TGF- β 2 and TGF- β 3. In asthmatic airway, there were abundant TGF- β 1 expressions in epithelium⁸ and increased TGF- β 1 levels in the bronchoalveolar lavage fluids.⁹ Additionally,

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TGF- β 1 and TGF- β 2 are increased in BAL fluid following segmental allergen challenge.^{9,10} The TGF- β 1 immunostaining has been localized to the subepithelial airway.¹¹

Furthermore, it has been reported that elevated level of plasma TGF- β 1 are a predictor of lung fibrosis.¹² Serum TGF- β 1 has also been found to be associated with fibrosis in several diseases such as liver and cardiac fibrosis.^{13,14} To our knowledge, there have been only few reports regarding the role of serum TGF- β in atopic asthmatic patients.^{15,16} The current study was focused on the level of serum TGF- β 1 in different severity of asthma and attempted to elucidate the relationship between level of serum TGF- β 1 and duration of disease and pulmonary function parameters.

MATERIALS AND METHODS

Subjects

Thirty-one asthmatic patients aged 7-18 years who attended our asthma clinic regularly at least one year were recruited and assigned as to the severity according to GINA guideline: mild asthma with inhaled corticosteroids (ICS) naïve, moderate asthma and asthma in remission. They were also treated according to the guideline. Asthma in remission was defined as no asthmatic symptom, normal pulmonary function test (FEV₁% predicted > 80 and FEV₁/FVC > 80%) and not using inhaled corticosteroid for at least 12 months.¹⁷ All asthmatic patients had skin prick test positive to at least one common aeroallergen (Dust mite: *Dermatophagoides pteronyssinus*, *D. farinae*, Bermuda grass, cockroach, cat, and dog). All patients were clinically stable, were not suffering from respiratory tract infection, and had not experienced asthma exacerbation in the 4 weeks prior to blood sampling, and had no history of smoking. Thirty-four non-atopic controls aged 8-15 years were recruited from patients attending general pediatrics clinic on the basis of a negative history of allergy, asthma, atopic dermatitis, and infectious disease. We excluded the patients with chronic lung disease, cardiovascular disease, and congenital airway abnormalities. Written informed consent was obtained from parents or guardians before enrollment, and the study was approved by the Institutional Ethics Committee of Ramathibodi Hospital.

Asthmatic patients were measured for pulmonary function tests by a Jaeger MasterScope® (VIASYS Healthcare GmbH, Hoechberg, Germany), according to American Thoracic Society guidelines.¹⁸

Measurement of active human transforming growth factor- β 1

Blood samples from asthmatic patients and control patients were taken to the serum separator tubes and stored for 30 minutes at 26°C to clot. Then, to obtain complete release of TGF- β 1, the samples were incubated overnight at 4°C before centrifugation (1,000 x g/10 minutes). The serum was immediately harvested for storage at -80°C until analysis. Serum levels of TGF- β 1 were measured with enzyme-linked immunosorbent assay (ELISA) using a commercially available kit (Quantikine; R & D Systems Inc., Minneapolis, MN, USA). The TGF- β 1 assay was performed according to methods outlined in the package insert. Before the assay, latent TGF- β 1 was activated to immunoreactive TGF- β 1, detectable by the Quantikine TGF- β 1 immunoassay. Aliquots of standard or activated samples were added to each well of an assay plate and the plate was incubated at 26°C for 3 hours. After aspirating/washing three times, 200 μ l of conjugate was added to each well and the plate was incubated again at 26°C for 1.5 hours. Again, after aspiration and washing, 200 μ l of substrate solution was added to each well and the plate was incubated at 26°C for 20 minutes. Finally, 50 μ l of stop solution were added to each well. The optical density in each well was measured within 30 minutes by using a microplate reader set to absorbance 450 nm. During measurement, serum samples were analyzed in randomly ordered duplicates to reduce systematic and interassay errors. All assays were performed and interpreted by individuals who were blinded to the case-control status of samples. The minimum detectable level of TGF- β 1 is 7 pg/ml.

Statistical analysis

Data were analyzed using SPSS version 15 computer software (SPSS Inc. Chicago, IL). Comparisons of data among groups were analyzed by using Chi-square test, Student's *t*-test, or one-way ANOVA with multiple comparisons. Pearson's cor-

relation coefficient was calculated to study the correlation between levels of serum TGF- β 1, pulmonary function test measurements, duration of asthma and duration of ICS treatment.

RESULTS

A total of 31 asthmatic children (7 mild intermittent, 12 moderate persistent, and 12 asthma in remission) and 34 non-atopic healthy control patients participated in the study. The demographic data of patients enrolled in this study are summarized in Table 1. All asthmatic groups were equivalent in term of age and duration of asthma. Both mild intermittent and asthma in the remission groups had the same level of FEV₁% predicted. However, there was a significant difference in duration of ICS treatment in moderate persistent asthma and asthma in remission groups (6.6 ± 3.2 vs. 3.2 ± 2.6 years, $p < 0.001$). There were no significant differences in age and sex between asthmatic and control groups.

Levels of serum TGF- β 1 in asthmatic patients and control patients

There was a significant difference between serum TGF- β 1 in asthmatic patients and that in control patients (39.59 ng/ml vs. 0.26 ng/ml, $p < 0.001$). In asthmatic group, steroid naïve mild asthma group

had the significantly highest serum TGF- β 1 compared to moderate persistent asthma and asthma in remission groups (47.44 ng/ml vs. 38.64 ng/ml and 47.44 ng/ml vs. 35.94 ng/ml, $p = 0.013$ and 0.001 , respectively). However, there was no significant difference in serum TGF- β 1 between moderate persistent asthma and asthma in remission groups.

Correlation between TGF- β 1 and duration asthma, duration of ICS treatment, and pulmonary function test parameters

There were no significant correlations between duration of asthma, duration of ICS treatment, FEV₁% predicted, FEV₁/FVC, and serum TGF- β 1 as shown in Table 2.

DISCUSSION

Asthma is a chronic inflammatory disease of the airways characterized by Th2 type inflammation. Pathological repair of chronic inflammation may lead to structural alteration in the airways that are called airway remodeling. TGF- β belongs to a family of growth factors that has an important role in wound healing and fibrogenesis. It promotes differentiation of fibroblasts into myofibroblasts, which are major producers of ECM.¹⁹

Table 1 Patient characteristics

Parameters	Asthmatic patients			Control patients
	Mild asthma steroid naïve	Moderate asthma	Asthma in remission	
No. of subjects	7	12	12	34
Age (years) mean \pm SD	12.3 \pm 3.6	12.4 \pm 3.2	13.4 \pm 2.9	12.35 \pm 1.34
Sex (M/F)	5/2	4/8	8/4	17/17
FEV ₁ % predicted* mean \pm SD	90.9 \pm 9.3	68.7 \pm 5.6	91.3 \pm 9.1	NA
Positive skin test to				NA
Dust mites	7	12	12	
Cockroach	1	3	5	
Cat	2	5	2	
Dog	0	1	1	
Duration of asthma (years) mean \pm SD	7.1 \pm 4.8	10.2 \pm 2.7	9.0 \pm 2.8	NA
Duration of inhaled corticosteroid (year)* mean \pm SD	NA	6.6 \pm 3.2	3.2 \pm 2.6	NA

* $p < 0.001$

TGF- β is increased and associated with submucosal and inflammatory cells, including fibroblasts, smooth muscle cells, eosinophils, macrophages and the connective tissue of the airway, with variable expression in the epithelial cells.²⁰ Expression of TGF- β has been shown to correlate with fibroblast numbers, subepithelial thickening and severity of asthma.^{6,20} An elevated level of TGF- β 1 has been reported in the bronchoalveolar lavage (BAL) fluid of asthmatics.⁹ These findings have also been confirmed by measurements of TGF- β 1 in BAL fluids after segmental allergen challenge in asthmatic subjects.¹⁰ In addition, the secretion of TGF- β 1 after an allergic disorder takes part in fibrosis and the irreversible changes associated with airway remodeling in chronic asthma.²¹

The present study has shown that TGF- β 1 participated in the pathogenesis of asthma. This was documented by increased TGF- β 1 serum levels in atopic asthmatic children compared with levels in healthy non-atopic children. In contrast to the previous study, Lommatzsch *et al.*¹⁶ have found that levels of serum TGF- β 1 in atopic asthmatic adults are not different from control subjects. However, there was a significantly shorter duration of asthmatic symptom in their subjects compared to our subjects (107.83 ± 41.09 vs. 9.0 ± 8.0 months). This may emphasize the role of TGF- β 1 in the chronic inflammatory process involved in asthma pathogenesis.

Moreover, the present study has demonstrated that TGF- β 1 serum levels were highest in mild asthmatic children who were steroid naïve. This may explain by the effect of corticosteroids which are the main therapy for the majority of mild

to severe, persistent asthmatic patients. Corticosteroids exert an anti-inflammatory effect, but can also have an anti-fibrotic action by decreasing collagen synthesis.²² Recent studies in mouse model have demonstrated that corticosteroid significantly decrease TGF- β expression and peribronchial fibrosis in the airways.^{23,24} Additionally, it had been demonstrated in the human fetal lung that corticosteroid treatment inhibits the production of TGF- β 1 and TGF- β 2.²⁵ Karagiannidis *et al.*²⁶ also found that TGF- β 1 serum levels in asthmatic patients without corticosteroids were higher than the levels in patients with both systemic and ICS.

Furthermore, high level of serum TGF- β 1 in mild asthma group supports the previous study which found that airway basement membrane thickening occurred in mild asthma.²⁷ Due to the limitation of our study, we did not have the information on the type of inflammatory cells which our asthma patients were encountering. If most of our moderate patients had no eosinophilic or neutrophilic inflammation, they might have more tissue destruction and airway remodeling.²⁸ This might be the reason why we could not find the difference in TGF- β 1 serum levels between moderate asthma and asthma in remission patients.

Taken together, we have demonstrated that atopic asthmatic patients had significantly higher levels of serum TGF- β 1 compared to non-atopic control patients. This will emphasize the role of TGF- β 1 in airway remodeling in asthma.

ACKNOWLEDGEMENTS

This study was funded by Faculty of Medi-

Table 2 Correlations between TGF- β 1 and duration of asthma, duration of inhaled corticosteroid treatment, and pulmonary function test parameters

Parameters	Pearson correlation	p value
Duration of asthma	0.29	0.12
Duration of inhaled corticosteroid treatment	-0.02	0.9
FEV ₁ % predicted	0.22	0.23
FEV ₁ /FVC	-0.10	0.59

cine Ramathibodi Hospital, Mahidol University. The authors would like to thank you Professor Amnuay Thithapandha for editing our manuscript.

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