

## Serum Transforming Growth Factor – $\beta$ 1 (TGF- $\beta$ 1) in Asthmatics: Association between Disease Control, Severity and Duration

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### Abstract

**Rationale:** Asthma is a chronic inflammatory disorder of the airways in which many cells and cellular elements play a role. Assessment of airway remodelling by analysis of blood and sputum markers has been developed as non-invasive procedures to bypass tissue biopsy.

**Aim of the work:** evaluate the usefulness of estimation of serum TGF- $\beta$ 1, as non-invasive markers of airway remodelling in patients with bronchial asthma and correlation between its level and degree of asthma control, severity and duration.

**Patients and Methods:** the study included 68 asthmatics and 20 controls. They were classified according to level of control, severity and duration. Blood samples were taken to estimate the serum levels of TGF- $\beta$ 1.

**Results:** the serum level of TGF- $\beta$ 1 was significantly higher in asthmatics than controls and in patients with uncontrolled asthma compared to controlled ( $P < 0.001$ ). It was significantly higher in severe and moderate asthma than with mild asthma ( $P < 0.000$ ) and in patients with disease duration  $> 5$  years compared to disease duration  $< 5$  years ( $P < 0.001$ ). Significant positive correlation between TGF- $\beta$ 1 and FEV1 ( $P < 0.001$ ) were recorded.

**Conclusion:** Serum TGF- $\beta$ 1 could be considered as non-invasive markers of airway remodelling, severity of airflow limitation and as predictor of degree of asthma control.

**Keywords:** Serum TGF- $\beta$ 1; Growth factor; Asthma

### Introduction

Asthma and related atopic syndromes have emerged as major public health concerns, and studies from around the world suggest that the incidence and prevalence of asthma began to rise in the last two decades, and with no signs that these disturbing trends may be reversing. It is characterized by episodic dyspnoea, lung inflammation, and in some patients, progressive irreversible airway dysfunction [1,2].

Transforming growth factor - $\beta$  (TGF- $\beta$ ), a profibrotic cytokine, plays an important role in promoting the structural changes of airway remodelling. It affects the proliferation, differentiation and extracellular matrix (ECM) metabolism of airway structural cells. Furthermore, it decreases synthesis of enzymes that degrade the ECM, namely matrix metalloproteinase (MMPs) and increases the production of the tissue inhibitor of matrix metalloproteinase [3-5]. The TGF- $\beta$ , which is expressed in the airway in asthma, has the potential to induce peribronchial fibrosis through stimulation of fibroblasts to produce extracellular matrix proteins (collagen, fibronectin) [5]. This will lead to Fixed airflow obstruction which is regarded to be a late and irreversible manifestation of airway remodelling. For this reason, clinicians should adjust asthma therapy to prevent development or worsening of airway and tissue remodelling [3,6]. Assessment of airway remodelling by analysis of blood and sputum remodelling markers as TGF- $\beta$ 1 has been developed as minimally invasive way to bypass tissue biopsy [6].

### Aim of the Work

- 1- Estimate the usefulness of measuring serum TGF- $\beta$ 1 in asthmatic patients.
- 2- Evaluate the association of TGF- $\beta$ 1 with degree of asthma control, severity and disease duration.

### Patients and Methods

This study included 68 adult asthmatic patients attending Assiut University Hospital, Chest outpatient Clinic, their ages ranged from (18-64 years) and 20 apparently healthy subjects, age and sex matched as control group. Patients were diagnosed as bronchial asthma according to the Global Initiative for Asthma [7] criteria and also they were classified according to different levels of asthma control in the last three months [7] into: controlled asthma: 29 patients, and uncontrolled asthma: 39 patients. According to asthma severity the patients were divided into: mild: 14 patients, moderate: 25 patients and severe asthmatics: 29 patients, and according to disease duration into  $< 5$  years: 34 patients and  $> 5$  years: 34 patients.

The following were considered as exclusion criteria: Postoperative period, acute coronary artery disease, collagen vascular diseases, cancers, chronic renal or hepatic diseases, pregnancy and smoking (exclusion was done by history, clinical examination, as needed imaging techniques and laboratory exams). Patients who received any systemic corticosteroid therapy in the last three months were also excluded (other used drugs doesn't have effects on TGF level).

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**Received:** February 20, 2015; **Accepted:** July 25, 2015; **Published:** July 27, 2015

**Citation:** Hassan NAE, Mohamed-Hussein AAR, Mohammed EF, Mohamed OAE, Mohamed HO, et al. (2015) Serum Transforming Growth Factor - $\beta$ 1 (TGF- $\beta$ 1) in Asthmatics: Association between Disease Control, Severity and Duration. *Biochem Anal Biochem* 4: 200. doi:10.4172/2161-1009.1000200

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### All patients and controls included in this study were subjected to the following

- Careful history taking and clinical examination,
- Chest X-Ray and pulmonary function tests {as Forced vital capacity (FVC), Forced expiratory volume in the first second ( $FEV_1$ ) and  $FEV_1/FVC$  ratio} were done utilizing Sensor Medics Corporation Spirometer (Model CA92687, SN 54065, Osaka, Japan).
- Blood samples were taken and serum was separated for all patients and controls and subjected to the following investigations: Complete blood picture on micros 60, Serum glucose level, liver function tests, renal function tests on Hitachi 911- Boehringer Mannheim.
- Serum level of active TGF- $\beta_1$  was determined by sandwich enzyme-linked immunosorbent assay (ELISA) by using DRG TGF- $\beta_1$  ELISA kit catalog no. EIA 1864, purchased from DRG diagnostic, Germany.

The study was approved by Faculty of Medicine, Assiut University Ethical Committee and all participants gave informed signed consent.

### Statistical analysis

Data entry and analysis were done by using SPSS software v.17 (Chicago, IL, USA). Continuous values were described by mean and standard deviation. Univariate analysis for determining the difference of lab variables between studied groups was performed using student's T test for continuous variables. The Analysis of Variance (ANOVA) was used in determining the difference of lab variables in cases where there are more than two groups. Correlations among the studied variables were tested by spearman's correlation coefficient. Bonferroni correction was performed for significant results and the difference was statistically significant if P value was less than 0.05.

### Results

This study included 68 adult asthmatic patients attending Assiut University Hospital, Chest outpatient Clinic, their ages ranged from 18-64 years (Mean  $\pm$  SD 43.6  $\pm$  13.1 years, Male/female 1:1 ) and 20 apparently healthy subjects (Mean  $\pm$  SD 41.3  $\pm$  11.9 years) as control group (Male/female 1:1).

Figure 1 shows that serum level of TGF- $\beta_1$  in asthmatics (26.69  $\pm$  3.35 pg/ml) was significantly higher than in controls (14.45  $\pm$  3.36 pg/ml),  $P < 0.001$ . The serum level of TGF- $\beta_1$  was significantly higher in uncontrolled patients (28.29  $\pm$  2.69 pg/ml) vs. controlled (24.53  $\pm$  2.94 pg/ml,  $P < 0.01$ ) and in patients with disease duration  $> 5$  years (28.29  $\pm$  2.87 pg/ml) compared to those with disease duration  $< 5$  years (25.09  $\pm$  3.04 pg/ml,  $P < 0.01$ ) (Figures 2 and 3).

As regards disease severity patients with severe asthma recorded significantly higher serum level of TGF- $\beta_1$  (28.21  $\pm$  3.02 pg/ml) compared to moderate (27.04  $\pm$  2.43 pg/ml) and mild asthma (22.93  $\pm$  2.59 pg/ml,  $P < 0.001$  each). There was significant negative correlation between  $FEV_1$  in the studied group and the serum level of TGF- $\beta_1$  ( $r = -0.692$ ,  $P < 0.0001$ ) (Figure 4). Negative correlation was also found between  $FEV_1/FVC$  and serum level of TGF- $\beta_1$  ( $r = -0.597$ ,  $P < 0.001$ ).

### Discussion

Asthma is a chronic inflammatory disorder of the airways. The chronic inflammation is associated with airway hyper responsiveness that leads to recurrent episodes of wheezing, dyspnoea, breathlessness, chest tightness and coughing, particularly at night or in the early

morning [8,9]. These episodes are usually associated with widespread, but variable, airflow obstruction within the lung that is often reversible either spontaneously or with treatment [2,7,10,11].

The pathological repair of the chronic inflammation in asthma leads to airway remodelling. Transforming growth factor- $\beta$  (TGF- $\beta$ ), a profibrotic cytokine, plays an important role in promoting the structural changes of airway remodelling through stimulation of proliferation, differentiation and extracellular matrix (ECM) metabolism of airway structural cells [3,4]

In this study it was found that the serum level of TGF- $\beta_1$  was significantly higher in asthmatics than in control group. Similar results were recorded by Joseph et al. [10] and Ozyilmaz et al [11], who found that TGF- $\beta_1$  was significantly higher in asthmatic patients compared with controls [10,11]. Also Manuyakorn et al. [3] demonstrated that atopic asthmatic patients had significantly higher levels of serum TGF- $\beta_1$  compared to controls. Furthermore, TGF- $\beta_1$  was released in higher amounts in allergen-challenged sites in the BAL fluid of asthmatic patients than in controls [3]. The TGF- $\beta$ , which is expressed in the airway in asthma, has the potential to induce peribronchial fibrosis through stimulation of fibroblasts to produce extracellular matrix proteins (collagen, fibronectin) [5].

Yang et al. [12] found that anti-TGF- $\beta_1$  antibody was effective in inhibiting pulmonary fibrosis and significantly reduced collagen deposition, smooth muscle cell proliferation, and goblet cell mucus production in an asthma model [12].

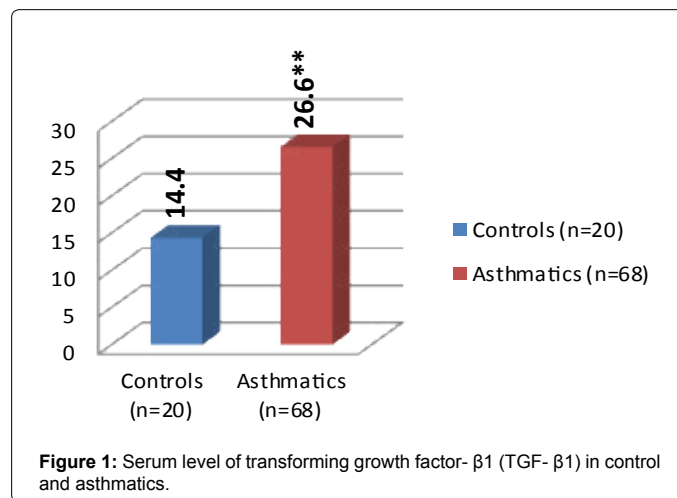


Figure 1: Serum level of transforming growth factor-  $\beta_1$  (TGF-  $\beta_1$ ) in control and asthmatics.

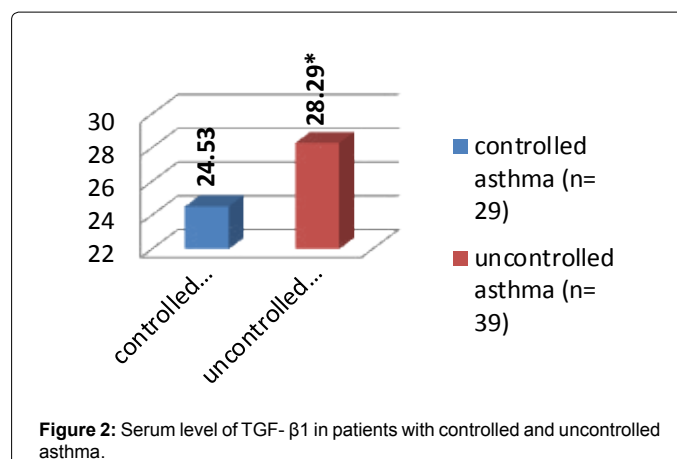


Figure 2: Serum level of TGF-  $\beta_1$  in patients with controlled and uncontrolled asthma.

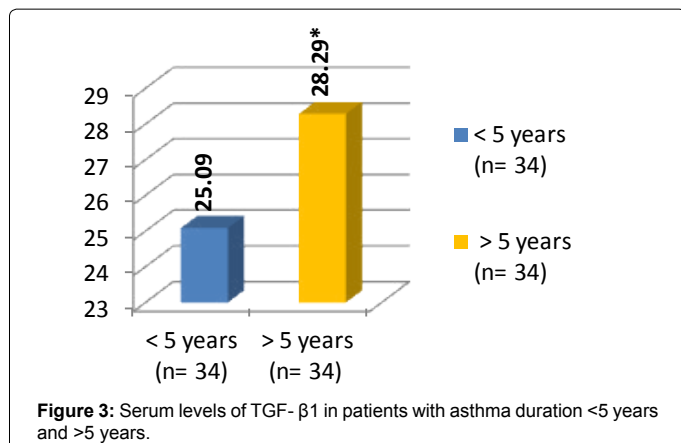


Figure 3: Serum levels of TGF-  $\beta$ 1 in patients with asthma duration <5 years and >5 years.

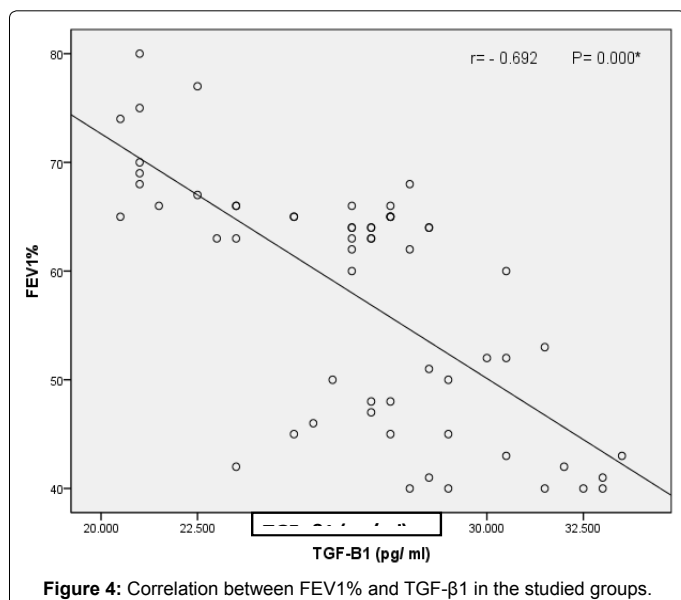


Figure 4: Correlation between FEV1% and TGF- $\beta$ 1 in the studied groups.

The results of this study revealed that the serum level of TGF- $\beta$ 1 was significantly higher in patients with uncontrolled asthma than in patients with controlled asthma. Similar results were recorded by many authors. Balzar et al. [13] reported that the serum level of TGF- $\beta$ 1 was significantly higher in the uncontrolled patients than in the controlled patients [13]. Also, Ozyilmaz et al. [11] reported that there was positive correlation between uncontrolled asthma, pulmonary function tests and plasma TGF- $\beta$ 1 levels. He suggested that plasma TGF- $\beta$ 1 level may be a systemic marker of asthma control [11]. It was added that asthmatic patients with decreased expression of TGF- $\beta$ 1 had better asthma control as well as longer disease free period than other ones with increased expression of TGF- $\beta$ 1 [14].

Concerning the effects of asthma severity on TGF- $\beta$ 1 levels, this study showed that serum level was significantly higher in all cases with severe or moderate disease than in cases with mild disease. Many authors agreed that increased TGF- $\beta$ 1 levels in the airways of asthmatic patients correlate with the severity of asthma and with the thickness of sub-epithelial basement membrane [15-17].

To study the effects of asthma duration on TGF- $\beta$ 1 levels this

study revealed that serum level of TGF- $\beta$ 1 was significantly higher in cases with disease duration more than 5 years than in cases with disease duration less than 5 years. Chung and Kimb [18] found that plasma level of TGF- $\beta$ 1 in children with persistent asthma was significantly higher than in the children who were diagnosed with asthma for the first time and controls. The secretion of TGF- $\beta$ 1 after an allergic disorder takes part in fibrosis and the irreversible changes associated with airway remodelling in chronic asthma [18]. Furthermore, it has been reported that elevated level of plasma TGF- $\beta$ 1 was a predictor of lung fibrosis [3].

## Conclusions

Serum levels of TGF- $\beta$ 1 are increased in asthmatic patients and are associated with increased asthma severity, decreased asthma control and prolonged disease duration and negatively correlate with pulmonary function tests. Serum TGF- $\beta$ 1 could be considered as non-invasive marker of airway remodelling. The serum sample is easily handled and not subjected to technical error as sputum or bronchoalveolar lavage fluid.

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