Original article

Serum transforming growth factor-beta1 in asthmatic children

**Background:** Transforming growth factor-beta1 is a multifunctional cytokine which has been linked to the pathogenesis of subepithelial fibrosis and airway wall remodeling in bronchial asthma.

**Objective:** To outline the changes in serum TGF-beta1 in children with bronchial asthma in relation to severity of asthma and different treatment modalities.

**Methods:** Twenty-three children with bronchial asthma recruited from the Pediatric Allergy and Immunology Clinic of Ain Shams University Children’s Hospital were enrolled in the study as well as 29 healthy controls. Asthmatic children were classified according to severity into two groups; the mild asthma group which included 12 children, 4 with mild intermittent and 8 with mild persistent asthma (none received steroid therapy), and the severe persistent asthma group which included 11 children (all were on steroid therapy). All patients were subjected to clinical evaluation and laboratory investigations including absolute eosinophilic count (AEC), total serum IgE% and biologically active serum TGF-beta1 by ELISA technique. All patients were studied during acute asthma exacerbations. Reevaluation during steady state asthma was carried out for 8 patients with mild persistent asthma and 9 with severe persistent asthma.

**Results:** During acute asthma exacerbations, the mean serum TGF-beta1 was significantly elevated in mild asthma (77.04 ± 57.04 ng/ml) compared to controls (21.81 ± 22.09 ng/ml). However for severe persistent asthma, the mean serum TGF-beta1 was significantly lower (4.23 ± 0.85 ng/ml) than in controls. Comparison of paired observations of serum TGF-beta1 revealed a significant drop, during steady state, in patients with mild asthma, whereas in severe asthma, a significant rise was observed. The levels of both asthma groups during steady state were comparable to the control values. A positive correlation, of borderline significance, between serum TGF-beta1 and total serum IgE% was observed among mild asthmatics during acute exacerbations (r = 0.55).

**Conclusion:** The behavior of serum TGF-beta1 in acute asthma exacerbations depends on asthma severity and is perhaps related to steroid inhalation therapy. The tendency towards normality of serum TGF-beta1 in steady state asthma is possibly a good prognostic sign.

**Key words:** TGF-beta1, bronchial asthma, children, remodeling.

**INTRODUCTION**

Chronic inflammation and airway remodeling are two key steps in asthma pathophysiology. Transforming growth factor-beta1 (TGF-beta1) is a multifunctional cytokine induced in pro- and anti-inflammatory pathways. It is produced by many types of cells that are activated in the asthmatic response. Recent studies highlighted this cytokine as an important negative regulator in an experimental model of asthma. In addition, TGF-beta1 is responsible for subepithelial fibrosis and airway smooth muscle cell (ASMC) hypertrophy, the principle features of airway wall remodeling in asthma.

Bronchial asthma requires early pharmacological treatment and long-term management. Anti-inflammatory agents, particularly inhaled corticosteroids, are currently the most effective long-term preventive medication. Moreover, early intervention with inhaled corticosteroids plays an important role in airway remodeling. Despite the fact that the role of TGF-beta1 in human asthma...
remains obscure, data derived from animal models encouraged further investigation of its suppressive mechanisms in order to develop novel therapies for asthma. This study was carried out to outline the changes in serum TGF-beta1 in children with bronchial asthma in relation to the exacerbations and severity of asthma.

**METHODS**

This work was conducted on 23 asthmatic children attending the Pediatric Allergy and Immunology Clinic, Children’s Hospital, Ain Shams University, in addition to 29 normal healthy children chosen as control group from the outpatient clinic.

**Patient group:**

Patients in this study were classified according to asthma severity into 2 subgroups:

**Mild asthma group:**

This group included 12 children; four with mild intermittent and 8 with mild persistent asthma, their ages ranged from 5 to 12 years with a mean value of 7.96 ± 2.63 years. They were 5 males and 7 females. They were studied during asthma exacerbations. Patients with mild intermittent asthma were on inhaled salbutamol 100 mcg/puff by MDI 3 to 4 times per day according to need as a quick relief medication for a duration of 4.63 ± 2.26 years with no long term controller therapy. During acute exacerbations, they received nebulized salbutamol solution in a dose of 0.01-0.02 ml/kg/dose every 6 hours. After initial response, therapy was continued on terbutaline oral preparation in a dose of 0.1-0.2 mg/kg/day every 8 hours. Oral short-acting theophylline (acephylline phenobarbitone) was given in a dose of 15-20 mg/kg/day every 8 hours. They also received oral antihistamine, promethazine in a dose of 0.5 mg/kg/day every 12 hours. Patients with mild persistent asthma were maintained on sustained-release oral theophylline tablets in a dose of 15-20 mg/kg/day every 12 hours and inhaled sodium cromoglycate (5 mg/puff by MDI) in a dose of 2 puffs/8 hours for a duration of 5.13 ± 1.98 years.

**Severe persistent asthma group:**

This group included 11 children studied during asthma exacerbation, 9 of whom were followed up during their symptom free state. Their ages ranged from 5 to 13 years with a mean value of 8.68 ± 2.28 years. They were 5 males and 6 females. All patients were on high-dose inhaled beclomethasone dipropionate (BDP) (100 mcg/dose), inhaled beta2 agonist and sustained-release oral theophylline in a dose of 15-20 mg/kg/day in two divided doses for a duration of 5.95±2.08 years. When needed, they received short-acting salbutamol inhaler in a dose of 3 to 4 puffs per day as quick relief medication. Three patients were on oral prednisone in a dose of 0.5-1 mg/kg/day. For acute asthma exacerbations, they intensified their inhaled corticosteroid and beta2 agonist therapy. Seven patients needed hospitalization and theophylline in a dose of 15-20 mg/kg/day given by infusion.

**Control group:**

Twenty-nine healthy children were included in this group. They were 14 males and 15 females. Their ages ranged from 5 to 13 years with a mean value of 8.66 ± 2.57 years. **Exclusion criteria for controls:** presence of history or clinical evidence of allergic problems, chronic disease or even family history of atopy.

**Children in this study were subjected to the following:**

1) **History taking:** laying stress on the duration of symptoms, frequency, severity, state of activity, precipitating factors, medications received during exacerbations and in symptom free state, the coexistence of other allergic problems and family history of atopy.

2) **Clinical examination** with special emphasis on weight centile, pulse, blood pressure, temperature, respiratory rate and chest examination.

3) **Investigations:**

   a- Plain X-ray, postero-anterior view during acute asthma exacerbation as needed to exclude pneumothorax, collapse or consolidation.

   b- Complete blood counts (CBC) for hemoglobin percent (Hb%), total leucocytic count (TLC) and absolute eosinophilic count (AEC) on Coulter Counter Model T660. Coulter Electronic, Inc., Hialeah, Florida, USA.

   c- Total serum IgE by ELISA technique (Genzyme Diagnostics, San Carlos, CA94070 USA). Owing to its variation with age, total serum IgE was taken as the percentage of the actual value of the subject in relation to the higher normal for age according to Kjellman et al.7.

   d- Biologically active serum transforming growth factor-beta1 (TGF-beta1) by ELISA using Predicta TGF-beta1 kit, Genzyme Corporation, One kendall Square Cambridge, MA02139, USA.

Eight patients with mild persistent asthma and 9 with severe persistent asthma were followed up and reevaluated for serum TGF-beta1 during steady state asthma.

**Statistical Methods:**

The results of this study were statistically analyzed through a standard computer program (Statview, version 5, USA). Descriptive statistical analysis was...
used to describe every parameter as mean, ± SD and range. Paired observations were compared using paired “t” test. Comparison among groups was done employing student “t” test for data showing normal distribution. For non-parametric data, comparisons were carried out using Wilcoxon signed rank test and Mann-Whitney test. Correlation of different parameters was performed using the Spearman’s correlation test. For all tests, p values less than 0.05 were considered statistically significant.

RESULTS

Patients and control groups were comparable regarding the age of the subjects (z = 0.5, p>0.05), furthermore, the 2 patient groups showed no differences in their mean ages (z = 0.7, p>0.05) or duration of asthma therapy (t = 1.15, p>0.05).

On comparing the mild and severe asthma groups regarding the AEC and IgE% , no significant differences were found (0.58±0.54 versus 0.89±0.74 x10³/mm³, p>0.05 and 310±467.3 versus 397±468.01%, p>0.05 respectively).

During acute asthma exacerbations, the mean serum TGF-beta1 was significantly elevated for mild asthma group in comparison to controls. However, the mean serum TGF-beta1 of severe persistent asthma group was significantly lower (4.23 ± 0.85 ng/ml) than that of controls. Table 1 and figure 1.

In patients with mild asthma, a significant drop in serum TGF-beta1 was noticed during steady state, while in patients with severe persistent asthma a significant rise occurred (table 1 and figure1). Furthermore, the steady state mean serum TGF-beta1 levels of both groups of asthmatic children were lower albeit insignificantly different from controls.

Comparison of patients with positive to those with negative steroid therapy showed the same results as the comparison of the 2 patient groups, this is because patients with severe asthma were all on steroid therapy and those with mild asthma did not receive any steroids.

A positive correlation, of borderline significance, between serum TGF-beta1 and total serum IgE% was observed among mild asthmatics during acute exacerbations (r = 0.55). Figure 2.

While a non-significant negative correlation was found between TGF-beta1 and hemoglobin among patients of group I.

![Figure 1: Comparison of serum TGF-beta1 during and after acute asthma exacerbations among the studied groups.](image)

![Figure 2: Correlation between serum total IgE and serum TGF-beta1 during acute asthma exacerbations among patients with mild asthma.](image)

Table 1: Comparison of serum TGF-beta1 during and after acute asthma exacerbations among the studied groups.

<table>
<thead>
<tr>
<th>Serum TGF-beta1 (ng/ml)</th>
<th>All patients</th>
<th>Mild</th>
<th>Severe</th>
<th>Control</th>
<th>p1</th>
<th>p2</th>
<th>p3</th>
<th>p4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute exacerbation</td>
<td>42.22±54.9</td>
<td>77.04±57.07</td>
<td>4.23±0.85</td>
<td>21.81±22.09</td>
<td>&gt;0.05</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Steady-state</td>
<td>9.21±9.81</td>
<td>7.31±4.94</td>
<td>10.89±12.82</td>
<td>21.81±22.09</td>
<td>&gt;0.05</td>
<td>&lt;0.01</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

p1, all patients vs. control; p2, mild vs. control; p3, severe vs. control; p4, mild vs. severe.
DISCUSSION

In the present work, a significant rise in the mean serum TGF-beta1 was found in mild intermittent and mild persistent asthma during exacerbations as compared to steady state (p<0.05), and to the control group (p<0.01). Two explanations of this finding are suggested; the first is based on the report of Chu et al.\(^8\) who found that peripheral blood neutrophils from asthmatic subjects spontaneously released significantly higher levels of TGF-beta than those from normal subjects. The second explanation is that the rise in serum TGF-beta1 could be secondary to its rise in the respiratory tract during acute asthma as proved by Redington et al.\(^9\).

TGF-beta1 is upregulated in response to tissue injury and is produced by many cells such as activated alveolar macrophages, endothelial cells, T and B lymphocytes\(^10\). Eosinophils are thought to be the main source of TGF-beta1 mRNA in bronchial biopsies from acute severe asthma patients\(^11\). TGF-beta1 in turn, has been ranked as a potent mast cell chemoattractant\(^12\). In addition, it has a similar effect on neutrophils, T lymphocytes, and unactivated monocytes\(^13\). All these effects, superadded to its capability of inducing gene transcription of IL1, TNF and IL6, account for its proinflammatory activity\(^14\). However, its exact role in asthma remains obscure\(^3\). Kim and Lee\(^15\) interestingly reported that TGF-beta1 has an important role in the elicitation of IgE dependent anaphylaxis. It has been shown that treatment of passive cutaneous anaphylaxis (PCA) sites with neutralizing TGF-beta1 antibodies significantly blocked PCA reaction in these sites. On the other hand, TGF-beta has been demonstrated to have immunosuppressive and anti-inflammatory effects as well. These contradictory effects make it difficult to envisage its overall contribution to asthma.

Contrary to the situation in mild asthma, acute exacerbations of severe persistent asthma were associated with significantly lower TGF-beta1 compared to steady state (p<0.05) and to controls (p<0.01). Hansen et al.\(^16\) were able to abolish airway reactivity in mice through genetic engineering of Th2 cells to express latent TGF-beta1. They demonstrated that TGF-beta1 secreting T-cells play an important regulatory role in asthma. It is speculated that patients with severe asthma probably have a defect in TGF-beta1 responses, perhaps a genetic one, which results in an uncontrolled Th2-induced airway inflammation that manifests clinically as severe asthma.

Another possible explanation is based on the fact that all patients in this group were under long-term therapy with inhaled beclomethasone dipropionate (BDP) and 3 of them were additionally receiving oral steroids. Furthermore, intensification of the doses of inhalation therapy is adopted by many of them with the onset of the acute attacks. There is accumulating evidence, from pharmacokinetic studies, suggesting that absorption across the lung vascular bed is an important determinant of systemic bioactivity and adverse effects, this being particularly the case with inhaled corticosteroids, where there is extensive first-pass metabolism in the liver but not in the lung\(^17\). The presence of a putative glucocorticoid responsive element (GRE) in the promoter region of the human TGF-beta1 gene has been identified\(^18\). Glucocorticoids induce the production of TGF-beta by various cell types including T-cells. The biological significance of this finding is not clear, but might imply that glucocorticoids by inducing TGF-beta, enhance an autocrine control of resting and active T-cells that restrict systemic inflammation\(^19\). The relationship between serum cortisone and TGF-beta1 was further clarified by Otsuka et al.\(^20\), who found a transient increase in TGF-beta1 mRNA expression with raised corticosterone levels resulting from long-term adrenocorticotropic hormone (ACTH) therapy. Surprisingly, it has been documented that children receiving inhaled BDP had lower cortisol secretion during the night than those who were not taking BDP, with a delayed rise from the nocturnal nadir and low early morning levels. Also, inhaled BDP produces a dose-dependent adrenal suppression\(^21\). Thus decreased endogenous cortisol might be the reason behind the absence of a rise in serum TGF-beta1 during acute exacerbations in severe asthmatics receiving inhaled steroids.

The absence of respiratory inflammation in normal, non-atopic, non-asthmatic subjects is maintained by influences which promote the development of non-responsiveness toward inhaled aerosols. The cytokine milieu of the healthy respiratory tract contributes to this immune non-responsiveness through the constitutive expression of the anti-inflammatory cytokine IL-10\(^22\), and TGF-beta1\(^23\).

It was found, in the present study, that the steady state in patients with mild persistent asthma was accompanied by a significant drop in the levels of TGF-beta1 so that it became insignificantly different from that of the control group, achieving a cytokine milieu comparable to that found in normal non-atopic subjects. This drop in the levels of serum TGF-beta1 is probably a consequence of the subsidence of the allergic inflammatory response
that triggered its release together with other cytokines. On the other hand, patients with severe persistent asthma experienced a mild but significant rise in serum TGF-beta1 as they entered the steady state. It seems that TGF-beta1 secretion has been restrained during exacerbation by an inhibitory influence, perhaps that of inhaled steroid therapy. This influence is relieved during steady state so that the levels of TGF-beta1 in serum achieved normality.

Many reports emphasize the role of TGF-beta1 in tissue remodeling and repair. However, overproduction and activation have been linked to the fibrotic complications associated with chronic inflammatory conditions such as idiopathic pulmonary fibrosis and pulmonary tuberculosis. Fortunately, high levels of TGF-beta1 of mild asthma patients are not maintained through steady state and the levels in severe asthma patients do not exceed the normal limit, otherwise, the course of illness in these patients would have changed into one of chronic obstructive airway disease.

Interestingly, mild intermittent and mild persistent asthma patients with positive family history of asthma had significantly lower levels of serum TGF-beta1 during steady state compared to those with negative family history of the same group (p<0.05). Insignificantly higher levels were also encountered during acute exacerbations (p>0.05). Hobbs et al.24 demonstrated the presence of polymorphism in the promoter region of the IL-10 gene and four in the TGF-beta promoters (3 in TGF-beta1 and 1 in TGF-beta2) in DNA taken from families with asthmatic proband. These families have been identified by the presence of a pregnant mother with asthma, and the proband is the child who has been followed up since birth. It is speculated that asthmatic patients with positive family history of asthma have some form of TGF-beta1 polymorphism that is evidenced by a lower serum TGF-beta1. This polymorphism in TGF-beta1 gene might also explain the low but statistically insignificant differences in serum TGF-beta1 in the studied asthma groups during steady state when compared to controls (9.21±9.81 vs. 21.81±22.09 ng/ml respectively).

A significant negative correlation of serum TGF-beta1 with Hb% during steady state (r = -0.64, p>0.05) in mild asthmatics was found in the present study. It has been previously shown that in vivo administration of TGF-beta1 either systemically or locoregionally resulted in suppression of hematopoiesis. Furthermore, depending on the target cell, TGF-beta1 can enhance or inhibit proliferation. Early erythroid and myeloid progenitors are markedly inhibited in the presence of TGF-beta1 whereas more differentiated myeloid progenitors forming granulocytes, macrophages, granulocyte-macrophage colonies and erythroid progenitors are not inhibited by TGF-beta1.25. Zermati et al.26 studied the mechanisms by which TGF-beta1 inhibits erythropoiesis using in vitro serum-free system of human red blood cell production. They found that TGF-beta 1 is a paradoxical inhibitor of erythropoiesis that acts by blocking proliferation and accelerating differentiation of erythroid progenitors.

It is concluded that the relationship of TGF-beta1 to asthma is not fully understood. The behavior of serum TGF-beta1 in acute asthma exacerbation is dependent on the severity of asthma: it was significantly higher in mild asthma, while in severe asthma it was low, perhaps, related to an inherent defect in TGF-beta1 secretion or to steroid inhalation therapy. The tendency towards normality during steady state might signify a good prognosis in terms of the remote outcome. However, further longitudinal studies are needed to confirm this and to throw more light on the role of TGF-beta1 in asthma.

REFERENCES


