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Exhaled TGF- β 1 levels before and after an exercise challenge in asthmatic and healthy children, and during exacerbation

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ABSTRACT

Background: There is conflicting data regarding the role of transforming growth factor- β 1 (TGF- β 1) in the pathogenesis of airway hyper-reactivity and asthma exacerbation.

Objective: To investigate the role of exhaled-TGF- β 1 in exercise-induced bronchospasm (EIB) in asthmatic and nonasthmatic healthy children, and in asthma exacerbation and asthma control.

Methods: The exhaled-TGF- β 1 levels of 56 stable asthmatic children and 15 nonasthmatic healthy children were evaluated before and 30 min after an exercise challenge. The exhaled-TGF- β 1 levels of 20 additional children with asthma exacerbation were evaluated.

Results: While no significant difference in the exhaled-TGF- β 1 levels was found at the baseline, exhaled-TGF- β 1 levels after the exercise challenge were significantly higher in the non-EIB ($n=31$) asthmatics when compared to the asthmatic children with EIB ($n=25$) ($p=0.04$). Although there was a statistically significant increase in the concentration of the exhaled-TGF- β 1 after the exercise challenge in the non-EIB asthmatics ($p=0.008$), the concentration of the TGF- β 1 was not increased after the exercise challenge in EIB + asthmatics. The exhaled-TGF- β 1 was significantly correlated with the ACT score ($p=0.01$, $r=0.49$) and the baseline FEV₁ level ($p=0.02$, $r=0.35$). The exhaled-TGF- β 1 levels were significantly higher in the stable asthmatic children when compared to the nonasthmatic children ($p<0.0001$). There was no significant difference in exhaled-TGF- β 1 levels after the exercise challenge in the nonasthmatics. The exhaled-TGF- β 1 levels were significantly lower in those children with asthma exacerbation when compared to the stable asthmatic children ($p=0.0003$).

Conclusion: Our results suggest that TGF- β 1 may play a role in suppressing airway reactivity and its deficiency is associated with asthma exacerbation.

Abbreviations: ACT: Asthma control test; AHR: Airway hyper-reactivity; ASM: Airway smooth muscle; BAL: Bronchoalveolar lavage; ELISA: Enzyme-linked immunoassay; EIB: Exercise-induced bronchospasm; EBC: Exhaled breath condensate; FEV₁: Forced expiratory volume in the first second; FEV₁/FVC: Forced expiratory volume in the first second/forced vital capacity; FEF₂₅–75%: Forced expiratory flow, midexpiratory phase; GINA: Global Initiative for Asthma; (Ig)E: Immunoglobulin E; ISAAC: International Study of Asthma and Allergies in Childhood; IQR: Interquartile range; IL-10: Interleukin-10; TGF- β 1: Transforming growth factor- β 1; Treg cells: T regulatory cells

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Introduction

Exercise-induced bronchospasm (EIB), with a mean prevalence of 46% in children and adolescents with asthma, is a clinical reflection of bronchial hyper-reactivity deteriorating the quality of life (1,2). In a large cohort study with long-term follow-up, EIB under 5 years in childhood was found among the strongest predictors of persistent asthma in adulthood reflecting the chronicity of asthma (3). EIB, as the

earliest sign of asthma, often prevents asthmatics from living normal lives. A history of EIB and exercise-related respiratory symptoms occurs more commonly in patients with poorly controlled asthma (4,5).

The responsible mechanisms are not completely understood despite the clinical significance of EIB (6). It has been shown that EIB occurs in response to the loss of water from the lower airways, resulting in a hyperosmolar environment, which activates various

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cellular mechanisms to release mediators from the mast cells, eosinophils, epithelial cells and sensory nerves (7). In this regard, we showed that the level of exhaled RANTES (chemokine ligand 5; regulated upon activation, normal T-cell expressed and secreted) is increased after an exercise challenge in asthmatic children (8). In this context, limited number of studies showed that exhaled RANTES, cysteinyl leukotrienes and endothelin-1 concentrations were significantly increased in asthmatics with EIB after an exercise test and a decrease in EBC pH was associated with the development of EIB (9–12).

Since the hyperosmolar release of mediators from airway mast cells and epithelial cells is an important hypothesis in EIB pathogenesis, another important mediator in asthma pathogenesis, transforming growth factor- β 1 (TGF- β 1), may be worthy of research. TGF- β 1 is believed to be involved in the pathogenesis of asthma by promoting fibrosis and remodeling (13). In addition, TGF- β 1 has anti-inflammatory activity related to its induction of T regulatory (Treg) cells (14,15). However, there are conflicting data regarding the role of TGF- β 1 in the pathogenesis of airway hyper-reactivity (AHR) (16).

Another troublesome situation disrupting the quality of life of a great majority of children with asthma is the continuation of acute asthma exacerbations, despite optimal asthma management. It has been shown that 72%–97% of children with asthma experience an exacerbation; even mild forms of this disease may exhibit exacerbations (17).

Since the prevention of EIB and asthma exacerbation are important goals in asthma treatment, it is crucial to clarify their pathogenesis to assist in the diagnosis and treatment of asthma. Examining the TGF- β 1 levels (as a regulatory cell population cytokine) in the airways of asthmatic and nonasthmatic children before and after an exercise challenge, and during asthma exacerbation in asthmatics, and investigating its role in asthma control are inspiring topics for clarifying the pathogenesis of asthma in a clinical setting. To the best of our knowledge, no previous data has been reported on the role of exhaled TGF- β 1 levels in EIB and asthma exacerbation in children with asthma.

The primary aim of the present study was to determine the levels of exhaled TGF- β 1 following an exercise challenge in asthmatic and nonasthmatic healthy children, and during the exacerbation period in asthmatic children. The secondary outcome of our study was to determine the relationship between the exhaled TGF- β 1 level and the asthma control level and pulmonary function.

Methods

Subjects

Ninety one children were included in our study (Table 1). The exercise challenge part of our study included 56 children with stable asthma and 15 non-atopic, non-asthmatic healthy children. The second part of our study included 20 children with asthma exacerbation. Data were collected between 2011 and 2012.

Stable asthmatic children in the exercise challenge group

Fifty-six children with asthma, who presented at our Pediatric Allergy and Asthma Unit, were included. The subjects were diagnosed with asthma according to the criteria recommended by the Global Initiative for Asthma (GINA) (18). They were all in stable condition, and free from acute exacerbations and respiratory tract infections for the previous two months, with no systemic diseases other than asthma. None of the patients were being treated with leukotriene receptor antagonists. These children used β 2-agonists on demand, but refrained from taking this medication for at least 24 h before the test.

Nonasthmatic children in the exercise challenge group

Healthy children without asthma, allergic rhinitis, atopic dermatitis, atopy or any other systemic diseases, were included in our study (Table 2). All of the children were free from respiratory tract infections for the previous two months. These children were tested with allergy skin prick tests (SPT) to demonstrate that they were non-atopic, and we evaluated the presence of any asthma, allergic rhinitis or atopic dermatitis using an International Study of Asthma and Allergies in Childhood questionnaire (19).

Children with asthma exacerbation

Children who presented with moderate-severe asthma exacerbations at our Pediatric Allergy and Asthma Unit were the subjects of the exacerbation group (20). All of the children had previously demonstrated reversible airway obstruction, and had been diagnosed with asthma by a pediatric allergy specialist.

Table 1. Main characteristics of the study population.

	(n = 91)
Gender	
Male/Female	65/26
Age (years)*	11 $\pm$ 2.7
Atopy%	55

*Mean $\pm$ standard deviation.

Table 2. Main characteristics of the study population at the baseline.

	Healthy children (n = 15)	Stable asthmatics (n = 56)	Exacerbation group (n = 20)	p ^{&}	p [#]
Gender					
Male/Female	9/6	39/17	17/3	>0.05	>0.05
Age (years)*	10.6 ± 2	11.3 ± 2.2	10.2 ± 3.7	>0.05	>0.05
Duration of asthma (years)*	–	4.3 ± 3.5	4.2 ± 3.8	–	>0.05
Atopy %	0	63	72	<0.0001	>0.05
Regular use of ICS	–	56%	65%		
No regular treatment		44%	35%	–	>0.05
FEV ₁ (% pred) [†]	102.0 (99.0–110.0)	88.0 (81.0–97.0)	68.0 (60.0–89.0)	<0.0001	<0.0001
FEF _{25%–75%} (% pred) [†]	86 (82–92)	77.5 (65.3–96.0)	35.0 (29.0–74.0)	0.014	<0.0001
FEV ₁ /FVC (% pred) [†]	101.0 (99.0–105.0)	88.0 (83.0–94.8)	70.0 (64.0–83.0)	<0.0001	<0.0001
EBC TGF-β ₁ (pg/ml) [†]	22.9 (7.8–69.9)	121.9 (39.5–167.7)	30.3 (18.9–43.8)	<0.0001	0.0003
IgE (IU/L) [†]	19.0 (18.0–43.5)	172.0 (50.0–504.0)	568.0 (131.0–1050)	0.01	0.01
Eosinophil % [†]	1.4 (1.1–2.5)	3.8 (1.9–6.8)	5.5 (1.9–9.6)	0.01	>0.05

*Mean ± standard deviation.

[†]Median (interquartile range).[&]p values for comparison between healthy children and stable asthmatics.[#]p values for comparison between stable asthmatics and children with asthma exacerbation.

Those children who used systemic corticosteroids within the three weeks prior to the study, and those demonstrating signs of lower respiratory tract infections or systemic diseases other than asthma were excluded from the study.

Study design

This study was performed as a prospective trial. The personnel performing the enzyme linked immunosorbent assay (ELISA) tests and the exhaled breath condensate (EBC) analyses were unaware of the clinical statuses of the cases or the protocol of the study.

Study protocol in the exercise challenge group

Each child's family completed a questionnaire concerning the nature and duration of the child's asthma symptoms (Asthma Control Test (ACT) scores) (20), EIB and medication usage. Before the exercise test, the total eosinophil and immunoglobulin (Ig)E levels were assessed and forced expiratory volume in the first second (FEV₁) was performed in each asthmatic and nonasthmatic child.

All of the asthmatic and nonasthmatic children underwent physical examinations and EBC collection; then, they completed a standardized treadmill exercise test. The EBC was collected again 30 min after the exercise provocation.

Study protocol in the asthma exacerbation group

Upon enrollment, the children were grouped according to the GINA classification for exacerbations (20), and all of the patients were in the moderate or severe asthma exacerbation classes. In addition, the FEV₁ (VMAX22; SensorMedics Corporation, CA, USA) and oxygen saturation levels (Nellcor Oximax N-600 Pulse Oximeter; Discount Cardiology, Camarillo, CA, USA)

were determined. The EBC was collected, and the total eosinophil and IgE levels were assessed during asthma exacerbation.

Study measurements and procedures

Exercise challenge and spirometry

The exercise testing was done according to the American Thoracic Society (ATS) Task Force recommendations (21).

EBC collection

The EBCs (EcoScreen; Jaeger, Hoechberg, Germany) were collected before and 30 min after the exercise provocation in the asthmatic and nonasthmatic children, and during the clinical visit in the children with asthma exacerbation according to the ATS/European Respiratory Society Task Force recommendations (22). All of the subjects were instructed to breathe in a relaxed manner (tidal breathing) for 15 min. Approximately 1 ml of breath condensate was collected in a sterile Eppendorf tube, and immediately stored at –80 °C for later analysis.

EBC measurements

The exhaled TGF-β₁ concentrations were quantified using a specific ELISA kit (RayBiotech, Norcross, GA).

SPT

SPT for commonly encountered allergens (house dust mites, trees, weeds, grass, cats, *Alternaria* spp., *Cladosporium* spp., egg whites and cow's milk) were performed 1 week before exercise challenge day in each asthmatic and nonasthmatic child and were performed after the asthma exacerbation, within one month in the exacerbation group.

Table 3. Main characteristics of the stable asthmatics in the exercise challenge group.

	Healthy children (n = 15)	EIB children (n = 25)	Non-EIB children (n = 31)	<i>p</i> ^{&}
Gender				
Male/Female	9/6	21/4	18/13	0.03
Age (years)*	10.6 ± 2	11.5 ± 2.1	11.2 ± 2.1	>0.05
Duration of asthma (years)*	–	4.3 ± 3.5	4.2 ± 3.8	>0.05
Atopy%	0	78	51	0.04
FEV ₁ (% pred) [†]	102.0 (99.0–110.0)	85.0 (76.0–92.0)	91.0 (85.0–99.0)	0.03
FEF _{25%–75%} (% pred) [†]	86 (82–92)	68.0 (51.0–83.0)	86.0 (70.0–100.0)	0.01
FEV ₁ /FVC (% pred) [†]	101.0 (99.0–105.0)	86.0 (79.0–89.0)	92.0 (85.0–97.0)	0.002
Maximum FEV ₁ decrease (%) [†]	–4 ((–5)–(–2))	–22 ((–32)–(–14))	–3 (–6–0)	<0.0001
Pre-exercise EBC TGF-β1 (pg/ml) [†]	22.9 (7.8–69.9)	124.1 (20.7–149.1)	117.9 (36.7–181.3)	0.93
Post-exercise EBC TGF-β1 (pg/ml) [†]	24.1 (7.8–41.5)	106.4 (91.4–126.4)	125.4 (104.2–186.7)	0.04
IgE (IU/L) [†]	19.0 (18.0–43.5)	270.0 (156.0–768.0)	68.2 (25.2–363)	0.008
Eosinophil % [†]	1.4 (1.1–2.5)	6.7 (5.4–8.5)	2.6 (1.2–4.3)	<0.0001

*Mean ± standard deviation.

[†]Median (interquartile range).[&]*p* values for comparison between EIB children and non-EIB children.

Ethics

All of the study procedures were done in accordance with a protocol previously approved by the Ethics Committee of Gaziantep University. All of the parents provided written informed consent for the study procedures, and the children gave their verbal assent.

Statistical analyses

Data analysis was performed with nonparametric tests using the SPSS V11.5 statistical software package. *p* values of <0.05 was considered to be significant.

The sample size was estimated using a power calculation based on a 50% difference in the TGF-β1 measurements. It was estimated that at least 12 patients would be required to detect a significant difference at the 80% power level, and an alpha error of 5%.

Results

EBC TGF-β1 levels before and after the exercise challenge in the stable asthmatics

Fifty-six consecutive asthmatic children (39 males, 17 females) aged 6–18 years old (mean 11.3 ± 2.2) were tested, and their characteristics are shown in Table 2. Twenty-six of the children with mild-to-moderate persistent asthma had been on maintenance therapy with low-to-medium constant doses of inhaled corticosteroids for at least two months. Thirty of the children were steroid-free. None of the patients was being treated with leukotriene receptor antagonists.

Twenty-five of the 56 children with stable asthma had a decrease of 10% or greater in the FEV₁ after the exercise (EIB + asthmatics), while 31 had a <10% decrease (non-EIB asthmatics). According to the different cutoff values of 15%, 12% and 10% FEV₁

decrease, 34%, 43% and 45% of the stable asthmatics had positive EIB, respectively.

At the baseline, no significant difference in the EBC TGF-β1 levels was found between the EIB + asthmatics and the non-EIB asthmatic group (124.1 pg/ml [IQR: 20.7–149.1] vs. 117.9 pg/ml [IQR: 36.7–181.3], respectively; *p* = 0.93) (Table 3, Figure 1). After the exercise challenge, the EBC TGF-β1 levels were significantly higher in non-EIB asthmatic group when compared to the EIB + asthmatics (125.4 pg/ml [IQR: 104.2–186.65] vs. 106.4 pg/ml [IQR: 91.4–126.4], respectively; *p* = 0.04) (Table 3, Figure 1).

There was a statistically significant increase in the concentration of the TGF-β1 after the exercise challenge in the non-EIB asthmatic group (*p* = 0.008; Wilcoxon signed rank test) (Figure 2). However, an increase in the concentration of the TGF-β1 was not seen after the exercise challenge in the asthmatic children with EIB (*p* = 0.56; Wilcoxon signed rank test) (Figure 2).

EBC TGF-β1 levels before and after the exercise challenge in the non-asthmatic children

Fifteen non-asthmatic healthy children (10 males, 5 females), aged 6–18 years old (mean 10.2 ± 3.7), underwent the exercise challenge, and the characteristics of these children are shown in Tables 2 and 3. All of them had a <10% decrease in the FEV₁ after the exercise.

The concentration of the exhaled TGF-β1 before the exercise challenge in the non-asthmatic children was significantly lower than that in the stable asthmatics (22.9 pg/ml [IQR: 7.8–69.9] vs. 121.9 pg/ml [IQR: 39.5–167.7], respectively; *p* < 0.0001) (Table 2, Figure 3). After the exercise challenge, the concentration of TGF-β1 in the non-asthmatic children was significantly lower than that in the stable asthmatics (24.1 pg/ml [IQR: 7.8–41.5] vs. 118.6 pg/ml [IQR: 99.6–176.7], respectively; *p* < 0.0001). Overall, there was no

statistically significant difference in the TGF- β 1 concentration after the exercise challenge, when compared to the pre-exercise levels in the non-asthmatic children ($p = 0.6$; Wilcoxon signed rank test) (Figure 2).

EBC TGF- β 1 levels and atopy in stable asthmatics

There was no significant difference in the exhaled TGF- β 1 concentrations between the atopic and non-atopic asthmatics (79.7 pg/ml [IQR: 21.2–152.3] vs. 118.8 pg/ml [IQR: 45.2–191.5], respectively; $p = 0.18$).

Factors affecting exhaled TGF- β 1 levels in the stable asthmatics

The pre-exercise TGF- β 1 levels were correlated with the ACT scores and the baseline FEV₁ in the stable

asthmatic children ($p = 0.01$, $r = 0.49$ and $p = 0.02$, $r = 0.35$, respectively). The post-exercise TGF- β 1 levels were negatively correlated with the eosinophil percentage ($p = 0.005$, $r = -0.42$) and the maximal post-exercise decrease in the FEV₁ ($p = 0.047$, $r = -0.31$) in the stable asthmatic children.

When we assessed the influence of the inhaled corticosteroid treatment on the exhaled TGF- β 1, we could not show any relationship between the treatment and exhaled TGF- β 1 levels before or after the exercise challenge in stable asthmatics (data not shown).

EBC TGF- β 1 levels in the asthmatic children with exacerbation

Twenty children (17 males, 3 females) aged 6–18 years old (mean 10.2 ± 3.7) who presented with moderate-severe asthma exacerbations at our Pediatric Allergy and Asthma Unit, were the subjects of the exacerbation group (20). The concentration of the exhaled TGF- β 1 in the asthmatics with moderate-severe acute exacerbation was significantly lower than in the stable asthmatics (30.3 pg/ml [IQR: 18.9–43.8] vs. 121.9 pg/ml [IQR: 39.5–167.7], respectively; $p = 0.0003$) (Table 2, Figure 3). Unlikely, there was no significant difference in exhaled TGF- β 1 levels between exacerbation group and non-asthmatic children (30.3 pg/ml [IQR: 18.9–43.8] vs. 22.9 pg/ml [IQR: 7.8–69.9], respectively; $p = 0.68$).

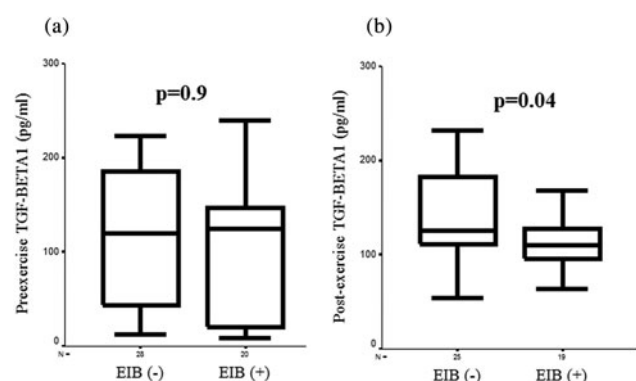


Figure 1. Exhaled TGF- β 1 measurements (a) before and (b) after the exercise challenge in asthmatic children with EIB and without EIB. Median and interquartile range values are shown.

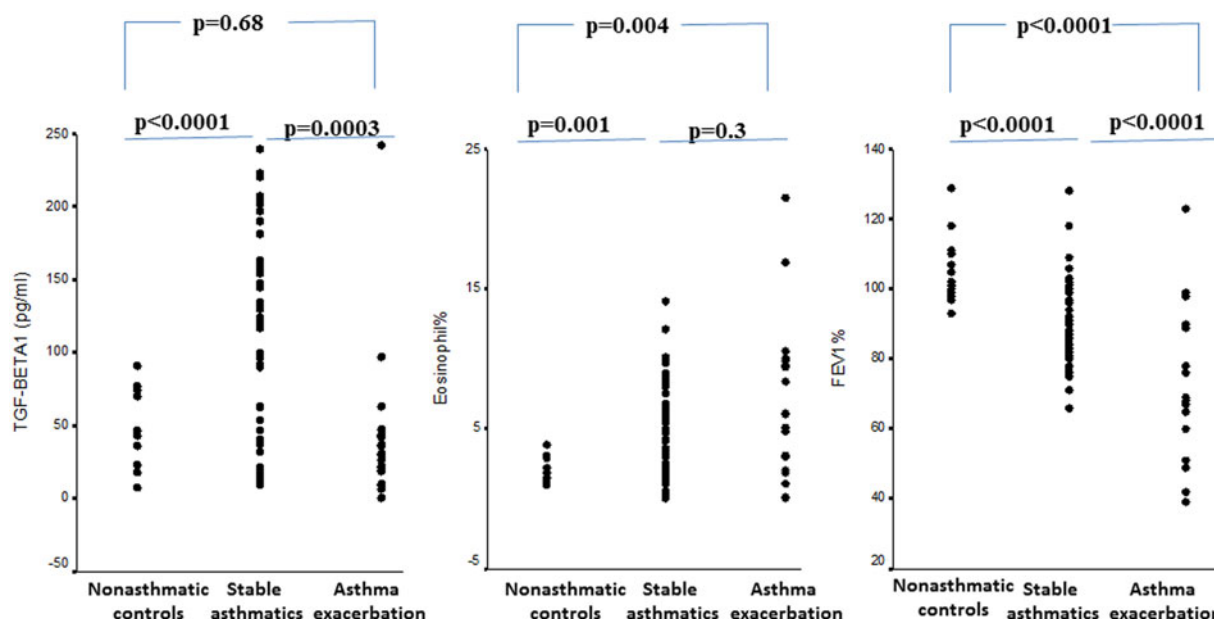


Figure 2. Exhaled TGF- β 1 measurements before and after the exercise challenge in asthmatic children (a) without EIB, (b) with EIB and (c) in healthy children. Median and interquartile range values are shown.

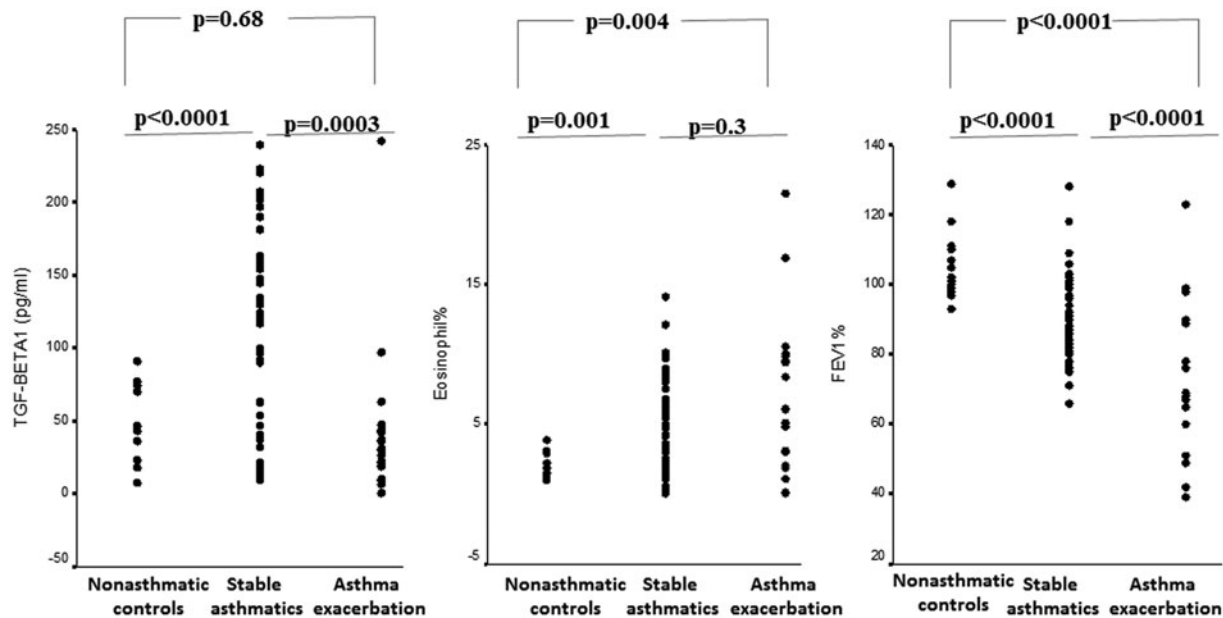


Figure 3. (a) Exhaled TGF- β 1 measurements before the exercise challenge, (b) Eosinophil and (c) FEV1 measurements in healthy children and stable asthmatic children, and during exacerbation in children with asthma exacerbation. Mann Whitney U test was used.

Discussion

By showing that the exhaled TGF- β 1 was significantly increased after exercise provocation in only the non-EIB asthmatics, our study demonstrated that TGF- β 1 might play a role in the suppression of EIB in asthmatic children, which is a physiological example of AHR. In this regard, it has been shown in one animal study that the blockage of TGF- β 1 may result in AHR (16). Alcorn et al. found that while the anti-TGF- β 1 antibody may provide desired anti-fibrotic effects in a mouse model of allergic inflammation, it actually exacerbates the AHR associated with the allergic response. One possible explanation is that the suppressive effect of the TGF- β 1 on AHR may be related to the mechanical and/or immunological pathways, or that these two may collectively inhibit the AHR (23,24).

With regard to the immunological aspect of this scenario, the TGF- β 1 suppresses immune responses through the promotion of Treg production and the inhibition of inflammatory cell function (25,26). Previous studies have shown that Treg cells inhibit the development and progression of allergic diseases including asthma, prevent development of airway mucus hyper-secretion, smooth muscle hypertrophy and collagen synthesis (27).

Antigens may induce Treg to produce TGF- β 1, which successively mediates immunosuppression (28). Via the regulation of the homeostasis of lymphocytes and Tregs, inflammation can be restricted. For

example, it has been shown that TGF- β 1 prevented house dust mite-induced allergic reactions in murine lungs by producing Treg cells (29). Moreover, TGF- β 1 applied into the trachea suppresses allergen-induced inflammation, suggesting its potential therapeutic role (30).

The retroviral transduction of unselected CD4+ T cells with latent TGF- β 1 has been shown to improve AHR and airway inflammation upon adoptive transfer (31). Moreover, intra-tracheally administered TGF- β 1 was associated with a decrease in AHR in another study (32). Similarly, we showed that the levels of exhaled TGF- β 1, a well-known Treg cytokine, could increase only in asthmatic children without EIB following an exercise challenge. This increase in exhaled TGF- β 1 might help in the toleration of exercise. We also found that the FEV₁ decrease following the exercise challenge was significantly lower in the asthmatic children with higher post-exercise TGF- β 1 levels, when compared to the asthmatics with lower post-exercise TGF- β 1 levels. Moreover, since our study has demonstrated that the exhaled TGF- β 1 level was correlated with the ACT score and FEV₁ percentage, TGF- β 1 may play a role in good asthma control and better outcome of the pulmonary function tests.

In other mouse models, the impairment of TGF- β 1 signaling in T cells led to an exacerbation of the asthmatic phenotype (33,34). It has also been shown that the serum TGF- β 1 levels were lower in children with asthma exacerbation, when compared to stable asthmatics (35). In agreement with these studies, we have

shown that the exhaled TGF- β 1 concentrations were significantly lower in the children with moderate-severe exacerbation of asthma, when compared to the stable asthmatics, suggesting that TGF- β 1 may have a role in preventing asthma exacerbation in asthmatics. However, when it decreases, does not increase or is suppressed as a result of an enormous increase in Th2 allergic inflammation, the exacerbation of asthma may occur. In asthma exacerbation, the lower level of TGF- β 1 could not prevent asthma exacerbation, and could not protect against the progression of inflammation.

On the other hand, high levels of TGF- β 1 have been found in the BAL fluid of asthma patients, when compared to healthy controls (36) and TGF- β 1 expression was also significantly higher in the bronchial biopsies of the patients with mild-to-severe asthma than in the controls (37). Accordingly, our study showed that stable asthmatic children have higher exhaled TGF- β 1 levels than non-asthmatic children. This increase in the TGF- β 1 levels in asthmatics may be a counter-regulatory protective reflex mechanism to suppress allergic inflammation in asthma. Accordingly, there was no significant difference in exhaled TGF- β 1 levels between asthmatic children with moderate-severe exacerbation and non-asthmatic children in our study.

Besides the negative feedback role to control airway inflammation, TGF- β 1 is involved in the repair of injured asthmatic airways and induction of fibrosis (38), together which play an important role in wound healing. Nevertheless, this process of wound healing in injured asthmatic airways may play a role in remodeling of airways. It has been shown that TGF- β 1, as a well-known mediator of airway remodeling, increases the proliferation of airway smooth muscle (ASM) (39). However, the effects of TGF- β 1 on cell proliferation are complex, and depend on the local environment. TGF- β 1 inhibits ASM cell proliferation in sub-confluent culture, but promotes proliferation when the cells are confluent (40). Furthermore, TGF- β 1 induces ASM cell proliferation at low doses, but inhibits proliferation at higher doses (39,41,42). This observation is very important in the evaluation of the complicated role of TGF- β 1 in the pathogenesis of asthma.

In this context, Ojiaku et al. (43) reviewed the literature and emphasized that although current therapies targeting TGF- β 1 signaling show encouraging applications in chemotherapy, fibrosis, and tumor vaccines in patients with cancer or idiopathic pulmonary fibrosis, adverse consequences of reducing TGF- β 1 expression in asthmatic airways should be considered

carefully. Researches supporting this idea demonstrated that TGF- β 1 reduction increased eosinophilic inflammation (34), and TGF- β 1 decreases AHR and fibrosis in the airway (16) in experimental asthma models. Furthermore, it has been shown that TGF- β 1 expression is inversely correlated with collagen III deposition and total basement membrane thickness in the asthmatic airway (44).

Since there are studies demonstrating significant correlation in the mediators between EBC and BAL, the levels of TGF- β 1 levels in samples obtained in EBC could reflect airway inflammation similar to samples obtained in BAL fluid for the analysis of the activity of fibrosis and inflammation (45,46). Showing the success of EBC reflecting airway inflammation, it was demonstrated that while EBC periostin levels correlated with tissue periostin expression in asthma, no significant correlations between tissue periostin expression and BAL, induced sputum, or serum periostin levels were found (47).

Although there are studies reporting the prevalence of EIB to be higher in girls than in boys (48,49), our study showed that male prevalence was higher than females in EIB + asthmatics. Also, a study reporting no gender difference for EIB positivity was published (50).

There are some limitations of our study. Since our study is a cross-sectional study, we could not compare exhaled TGF- β 1 levels between asthma exacerbation period and stable controlled asthma period in the same individual child. Further studies are needed with longitudinal study design. In addition, EBC TGF- β 1 level could be influenced from differences in dilution. To standardize the level of a biomarker assessed in EBC, dilution factor must be considered. Further studies using of different dilution factors (e.g. urea, conductivity or total cations) can be proposed for better standardization of EBC TGF- β 1 levels using TGF- β 1-to-urea ratios to control for dilution (51,52). Another limitation of our study may be related with the difference in the number of subjects in each group, which may have contributed to the results.

Conclusion

In conclusion, although the concentration of TGF- β 1 is increased in children with asthma, (when compared to children without asthma) as a possible control mechanism to prevent the negative effects of Th2 inflammation, interestingly its level does not increase, or even, decreases during asthma exacerbation. Treg cells may prevent asthma exacerbation and EIB

through TGF- β 1 pathway, which could lead to a good asthma control in asthmatics, suggesting a potential therapeutic role of TGF- β 1 in the prevention of both asthma exacerbation and EIB. However, potential remodeling effects of TGF- β 1, in addition to anti-inflammatory role, in airways may cause negative consequences in asthma. The cytokine milieu may determine whether TGF- β 1 will act in a regulatory or tissue remodeling manner, therefore TGF- β 1 may have different effects in different asthma phenotypes, and different asthma clinical status with different cytokine milieu. To succeed the personalized approaches to asthma therapy with regards to TGF- β 1, more human studies are needed to find the predictive value of TGF- β 1 as a biomarker to characterize asthma phenotypes. We believe our study opens a gate for future studies.

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Declaration of interest

None of the authors has a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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