Airway inflammatory biomarkers in different asthma phenotypes

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Background Asthma is a diverse disease with various phenotypes. Correlation of clinical asthma phenotypes with their underlying inflammatory biomarkers could help tailor asthma management and in turn improve the patient's outcome.

Aim of the study To validate the clinical classification of asthma phenotypes and to portray cough-predominant asthma phenotype and wheezy phenotype in accordance with their related inflammatory biomarkers.

Patients and methods This is a case–control study comprising 50 patients with cough-predominant asthma phenotype and 50 patients with wheezy asthma phenotype, together with 50 healthy controls. Serum interleukin-10 (IL-10), transforming growth factor-beta 1 (TGF-β1), and total serum immunoglobulin E (IgE) levels were assessed using immunoassay techniques.

Results The asthmatic children showed a significant increase of eosinophilic percentage, total serum IgE, and TGF-β1, when compared with the control group, whereas they showed a significant decrease of serum IL-10 when compared with the control group. As regards the clinical characteristics of both phenotypes, the prevalence of associated allergic rhinitis and atopic dermatitis in patients with cough-predominant asthma was significantly higher compared with the wheezy group. As regards laboratory biomarkers, total serum IgE was significantly elevated in cough-predominant asthma phenotype compared to wheezy phenotype. No significant differences were found between both phenotypes regarding serum TGF-β1 and IL-10.

Conclusion Cough-predominant asthma phenotype is characterized by prominent atopic features (allergic manifestations and elevated total IgE). However, cough-predominant asthma and wheezy asthma phenotypes were similar regarding serum TGF-β1 and IL-10.
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Introduction

Asthma is a heterogeneous disease consisting of different phenotypes with variable underlying mechanisms [1]. It is considered a collection of variable symptoms expressing different disease patterns. One of the ongoing challenges is exploring the underlying pathophysiological mechanisms that may be responsible for the varying responses to treatment [2].

Asthma was classified into different phenotypes based on various parameters such as precipitating factors, the course of the disease, or the prognosis. Clinical asthma phenotyping has been emerging. It focuses on asthma phenotypes that are associated with specific symptoms or clinical characteristics [3].

It has been noticed that asthmatic children vary widely in clinical presentation and time course of the disease [4]. Defining specific airway biomarkers that correlate with clinical symptoms and degree of airway obstruction is essential for effective future asthma treatments. Thus, characterization of asthma phenotypes based on the presenting symptoms in correlation with the underlying cytokine and genotypic pattern has been previously suggested [5,6].

Transforming growth factor-beta (TGF-β) is a main mediator involved in proinflammatory responses and fibrotic tissue remodeling in asthmatic patients [7]. TGF-β positively regulates T-regulatory cells, T-helper 17 (Th17), natural killer cells, and CD8+ T cells while inhibiting Th1 and Th2 differentiation [8]. Thus, TGF-β is implicated in the regulation of airway inflammatory responses, and repair of asthmatic tissues [9]. However, its role as a potential future therapeutic target including its importance in defining the emerging clinical asthma phenotypes and tailoring asthma management through TGF-β modulation is still controversial [7].

Interleukin (IL)-10 is a cytokine synthesized by various cell types, including B-cells, monocytes, dendritic cells, natural killer cells, and T cells. IL-10 has immunosuppressive functions and can decrease proinflammatory cytokine release [9].

This study aims to investigate the cytokine profile (TGF-β and IL-10) in two different clinical asthma phenotypes (cough-predominant asthma phenotype and wheezy phenotype).

Patients and methods

The study is a case–control study comprising 50 asthmatic patients presented with cough as the predominant symptom (cough-predominant asthma) and 50 asthmatic children presented with wheezes as the predominant symptom, together with 50 healthy controls of matched age and sex. After clinical examination and validation of asthma symptoms [10], the asthmatic children were classified according to their clinical phenotype. Written consents were obtained from all caregivers of patients and healthy controls and the protocol of this study was approved by the Institutional Research Board of our faculty.

Inclusion criteria

Children aged 6–18 years diagnosed as bronchial asthma with confirmation of diagnosis by [11]:

(1) The presence of typical asthma symptoms.
(2) Presence of variable expiratory airflow limitation as evidenced by increment in the forced expiratory value at 1 s more than 12% predicted after salbutamol (200 μg) inhalation.

Exclusion criteria

(1) Patients receiving immunotherapy.
(2) Patients with associated chronic illness.

The following were done for patients and controls:

(1) Thorough history taking and clinical examination.
(2) Peripheral eosinophilic percentage and total serum immunoglobulin E (IgE).
(3) Serum TGF-β1 and IL-10 levels.

Laboratory tests

Blood samples were obtained from each patient and healthy controls. Blood was collected in two aliquots. One aliquot was used for eosinophilic count. The other was allowed to clot for 1 h at room temperature, centrifuged for 10 min and serum was extracted. In each sample IL-10, TGF-β1, and total serum IgE levels were measured using immunoassay techniques. IL-10 and TGF-β1 were measured by using a special kit (Boster's Human IL-10 ELISA Kit and Boster's Human TGF-β1 ELISA Kit). Serum IgE levels were measured by using IgE ELISA Kits (Chemux Bioscience Inc., USA) from Aptech Services.

Statistical analysis

Data were analyzed with statistical package for the social sciences (SPSS), version 22 (SPSS 22 for Windows Inc., Chicago, Illinois). The normality of data was first tested with one-sample Kolmogorov–Smirnov test. Quantitative data were described using mean and SD. Qualitative data were described using number and percent. Association between categorical variables was tested using χ^2 test. One-way analysis of variance was used to compare the means of three groups. Statistical significance was defined as P value less than 0.05.

Results

This study was a case–control study comprising 100 asthmatic children and 50 healthy controls. The asthmatics were later classified into two clinical phenotypes; 50 children with cough-predominant asthma phenotype and 50 children with wheezy phenotype. The demographic and clinical data of the studied groups are represented in Table 1. No statistically significant differences were found between groups as regards their age and sex. However, the prevalence of associated allergic rhinitis (AR) and atopic dermatitis (AD) in the group of patients with cough-predominant asthma was significantly higher compared with the wheezy group ($P<0.05$) (Table 1).

Both cough-predominant asthma phenotype and wheezy phenotype showed a significant increase of

Table 1 Demographic and clinical data of the studied groups

	Wheezy phenotype (N=50)	Cough-predominant asthma phenotype (N=50)	Healthy controls (N=50)	P value
Age (years)	9.2±1.3	8.5±2.1	8.9±2.7	0.2
Sex				
Male	30 (60)	28 (56)	33 (66)	0.6
Female	20 (40)	22 (44)	17 (34)	
Allergic rhinitis				
Positive	20 (40)	40 (80)	–	0.0004*
Negative	30 (60)	10 (20)	–	
Atopic dermatitis				
Positive	10 (20)	25 (50)	–	0.002*
Negative	40 (80)	25 (50)	–	

Data are expressed as mean±SD or n (%). P value is assessed via one-way analysis of variance and χ^2 test. *P value is considered significant if less than 0.05.

eosinophilic percentage, total serum IgE, and TGF-β1, when compared with the control group, whereas, they showed a significant decrease of serum IL-10 when compared with the control group (Table 2).

As regards comparison between both clinical phenotypes, total serum IgE was significantly elevated in the cough-predominant asthma group compared with the wheezy group. However, no statistically significant differences were found between both groups regarding serum TGF-β1 and IL-10 (Table 2).

Discussion

The concept of asthma heterogeneity has been established recently. Asthma phenotyping was initially based on the clinical features, but they later evolved into linking the biology to phenotype, which could lead to individualized asthma management [12]. Thus, the aim of the present study was to correlate the patient's clinical features to his/her inflammatory biomarker.

As regards the clinical characteristics of both clinical phenotypes in the present study, the prevalence of AR and AD among patients with cough-predominant asthma was significantly higher compared with those with wheezy phenotype, whereas, in a

Table 2 Airway inflammatory biomarkers of the studied groups

	Wheezy phenotype (N=50)	Cough-predominant asthma phenotype (N=50)	Healthy controls (N=50)	P value
Eosinophilic percentage	6.3±1.5	6.7±2.0	0.97 ±0.35	0.000* P1=0.3 P2=0.000* P3=0.000*
Total serum IgE (IU/ml)	324.8±150	405.5±180	72.9±26	0.000* P1=0.009* P2=0.000* P3=0.000*
Serum IL-10 (pg/ml)	3.7±1.5	2.8±1.2	7.03 ±3.2	0.000* P1=0.09 P2=0.000* P3=0.000*
Serum TGF-β (pg/ml)	300±120	280±100	31±13.1	0.000* P1=0.5 P2=0.000* P3=0.000*

IgE, immunoglobulin E; IL, interleukin; pg, picogram; IU, international unit; TGF-β, transforming growth factor-beta. P1: wheezy group versus cough-predominant asthma group. P2: wheezy group versus control group. P3: cough-predominant asthma group versus the control group. Data are expressed as mean±SD. P value is assessed via one-way analysis of variance. *P value is considered significant if less than 0.05.

previous study, perennial/or seasonal AR was found to be more prevalent in classic asthma patients compared with cough-variant asthma (CVA) patients [13]. The different prevalence in our study may be partly explained by the degree of atopic status that was assessed with total IgE levels. Total IgE levels in studied patients were found to be significantly higher in cough-predominant asthma compared with the wheezy phenotype. Further, a strong relationship between IgE and AD was previously reported but the functional pathomechanism of IgE in patients with AD is still unclear [14].

TGF-β has a fundamental role in asthma. It orchestrates the formulation and maintenance of an inflammatory response. It also has a fibrogenic and immunomodulatory function, thus playing a main role in airway remodeling [15].

In the present study, TGF-β1, total serum IgE, and the eosinophilic percentage were significantly higher in asthmatic children compared with the control group. In agreement with our findings, previous studies reported elevated levels of TGF-β1 in the serum and bronchoalveolar lavage fluid of asthmatics [16,17]. Moreover, a recent study reported a significant increase of TGF-β1 in bronchoalveolar lavage and serum in moderate and severe asthma compared with controls [18]. Further, the expression of TGF-β1 in severe asthma and its association with airway remodeling has been previously reported [19]. This emphasizes the role of TGF-β1 in the chronic inflammatory process involved in asthma pathogenesis, and proclaims the role of TGF-β1 not only as a biomarker but as a potential therapeutic target in asthma.

IL-10 has a significant function in asthma pathogenesis. It is an anti-inflammatory factor that has been suggested to control the allergic inflammation by inhibiting the production of proinflammatory cytokines [20]. The current study showed significant lower levels of serum IL-10 in asthmatic cases compared with controls. This goes hand in hand with a previous study that reported lower serum levels of IL-10 and significantly fewer IL-10 producing cells in asthma patients compared with controls [21].

In the present study, comparing both clinical phenotypes regarding their airway inflammatory biomarkers showed no statistically significant differences in serum levels TGF-β1, IL-10, and

eosinophilic percentage. However, serum total IgE was found to be significantly elevated in cough-predominant asthma group compared with the wheezy group. This was in agreement with Wang *et al.* [22], who found significant increase of total serum IgE levels in patients with CVA either in the acute or convalescence stage compared with controls. On the contrary, another study has found total serum IgE to be significantly increased in CA compared with patients with CVA [23].

In conclusion, cough-predominant asthma phenotype was found to have prominent atopic features compared with the wheezy phenotype. Yet, serum levels of TGF-β1 and IL-10, together with the eosinophilic percentage, were not significantly different in both phenotypes.

To the best of our knowledge, this is the first study to explore the clinical and laboratory characteristics (TGF-β1, IL-10, total serum IgE, eosinophilic percentage) of two different asthma phenotypes (cough-predominant asthma and wheezy asthma phenotypes) in children. However, an important limitation of this study is the small sample size. Therefore, future studies that include a larger number of patients are required to validate the results of the present study including systemic and local airway inflammatory biomarkers.

Conflicts of interest
There are no conflicts of interest.

References
1 Chung KF. Precision medicine in asthma: linking phenotypes to targeted treatments. *Curr Opin Pulm Med* 2018; **24**:4–10.

2 Deliu M, Belgrave D, Sperrin M, Buchan I, Custovic A. Asthma phenotypes in childhood. *Expert Rev Clin Immunol* 2017; **13**:705–713.

3 Hekking PP, Bel EH. Developing and emerging clinical asthma phenotypes. *J Allergy Clin Immunol Pract* 2014; **2**:671–680.

4 Spycher BD, Silverman M, Pescatore AM, Beardsmore CS, Kuehni CE. Comparison of phenotypes of childhood wheeze and cough in 2 independent cohorts. *J Allergy Clin Immunol* 2013; **132**:1058–1067.

5 Zedan M, Attia G, Zedan MM, Osman A, Abo-Elkheir N, Maysara N, et al. Clinical asthma phenotypes and therapeutic responses. *ISRN Pediatr* 2013; **2013**:824781.

6 Zedan M, Bakr A, Shouman B, Zaghloul H, Al-Haggar M, Zedan MM, et al. Single nucleotide polymorphism of IL4C-590T and IL4RA 175V and immunological parameters in Egyptian asthmatics with different clinical phenotypes. *J Allergy Ther* 2014; **5**:189.

7 Al-Alawi M, Hassan T, Chotirmall SH. Transforming growth factor β and severe asthma: a perfect storm. *Respir Med* 2014; **108**:1409–1423.

8 Saito A, Horie M, Nagase T. TGF-β signaling in lung health and disease. *Int J Mol Sci* 2018; **19**:E2460.

9 Martín-Orozco E, Norte-Muñoz M, Martínez-García J. Regulatory T Cells in allergy and asthma. *Front Pediatr* 2017; **5**:117.

10 Zedan M, Gamil N, El-Chennawi F, Maysara N, Abdel Hafeez H, Nasef N, et al. Evaluation of different asthma phenotype responses to montelukast versus fluticasone treatment. *Pediatr Asthma Allergy Immunol* 2009; **22**:63–68.

11 GINA Report (2018). *Global strategy for asthma management and prevention. Report 2018*. Available at: https://ginasthma.org/wp-content/uploads/2018/04/wms-GINA-2018-report-V1.3-002.pdf. [Accessed 9 March 2019].

12 Wenzel SE. Asthma phenotypes: the evolution from clinical to molecular approaches. *Nat Med* 2012; **18**:716–725.

13 Tajiri T, Niimi A, Matsumoto H, Ito I, Oguma T, Otsuka K, et al. Prevalence and clinical relevance of allergic rhinitis in patients with classic asthma and cough variant asthma. *Respiration* 2014; **87**:211–218.

14 Werfel T, Allam JP, Biedermann T, Eyerich K, Gilles S, Guttman-Yassky E, et al. Cellular and molecular immunologic mechanisms in patients with atopic dermatitis. *J Allergy Clin Immunol* 2016; **138**:336–349.

15 Tirado-Rodriguez B, Ortega E, Segura-Medina P, Huerta-Yepez S. TGF-β: an important mediator of allergic disease and a molecule with dual activity in cancer development. *J Immunol Res* 2014; **2014**:318481.

16 Manuyakorn W, Kamchaisatian W, Atamasirikul K, Sasisakulporn C, Direkwattanachai C, Benjaponpitak S. Serum TGF-beta1 in atopic asthma. *Asian Pac J Allergy Immunol* 2008; **26**:185–189.

17 Redington AE, Madden J, Frew AJ, Djukanovic R, Roche WR, Holgate ST, et al. Transforming growth factor-β 1 in asthma: measurement in bronchoalveolar lavage fluid. *Am J Respir Crit Care Med* 1997; **156**:642–647.

18 Frahat A, Mansour Y, Eldib A, Alsed D. Diagnostic value of 8-isoprostane and transforming growth factor-β in bronchial asthma patients. *Egypt J Bronchol* 2018; **12**:295.

19 Chakir J, Shannon J, Molet S, Fukakusa M, Elias J, Laviolette M, et al. Airway remodeling-associated mediators in moderate to severe asthma: effect of steroids on TGF-β, IL-11, IL-17, and type I and type III collagen expression. *J Allergy Clin Immunol* 2003; **111**:1293–1298.

20 Huang ZY, Cheng BJ, Wan Y, Zhou C. Meta-analysis of the IL-10 promoter polymorphisms and pediatric asthma susceptibility. *Genet Mol Res* 2016; **15**:2.

21 Tomiita M, Campos-Alberto E, Shima M, Namiki M, Sugimoto K, Kojima H, et al. Interleukin-10 and interleukin-5 balance in patients with active asthma, those in remission, and healthy controls. *Asia Pac Allergy* 2015; **5**:210–215.

22 Wang MZ, He QN, Yuan HX, Liu XL. Roles of IL-4, IL-5 and IgE in childhood cough variant asthma. *Zhongguo Dang Dai Er Ke Za Zhi* 2006; **8**:382–384.

23 Takemura M, Niimi A, Matsumoto H, Ueda T, Yamaguchi M, Matsuoka H, et al. Atopic features of cough variant asthma and classic asthma with wheezing. *Clin Exp Allergy* 2007; **37**:1833–1839.