

Clinical Significance of the Dynamic Changes in Serum Eotaxin, Interleukin 13 and Total IgE in Children with Bronchial Asthma

Xin Wang*, MD; Chunyan Ma, MD; Yajing Zhang, MD; Lihua Ning, MD; Hua Chen, MD; Fang Zhou, MD

Department of Pediatrics, the Third Affiliated Hospital of Inner Mongolia Medical University, China

Received: Nov 26, 2012; Accepted: Jul 24, 2013; First Online Available: Aug 13, 2013

Abstract

Objective: To determine the serum levels of eotaxin, IL-13 and total IgE (TIgE) in asthmatic children during the acute and clinical remission periods, as well as the changes in pulmonary function to determine their roles, relationships and clinical significance during asthma exacerbation.

Methods: A total of 30 asthmatic children and 22 healthy children were enrolled in the study. The serum eotaxin and IL-13 levels were detected using an enzyme-linked immunosorbent assay and the TIgE level was detected using a fluorescent enzyme-linked immunosorbent assay. The asthmatic children were subjected to pulmonary function tests.

Findings: The serum eotaxin, IL-13 and TIgE levels of the asthmatic children during the acute period significantly differed from those during clinical remission. The serum eotaxin, IL-13 and TIgE levels of the asthmatic children during both periods significantly differed from those of healthy children ($P < 0.001$). The serum eotaxin levels during the acute and clinical remission periods were positively correlated with serum IL-13 and with TIgE, and serum IL-13 was correlated with serum TIgE. The pulmonary function indices of asthmatic children during the acute period significantly differed from those during clinical remission ($P < 0.001$). The serum eotaxin and IL-13 levels in the asthmatic children were positively correlated with the forced expiratory volume in 1 second (FEV1) and the peak expiratory flow (PEF) during the acute and clinical remission periods ($P < 0.05$). However, the serum TIgE levels in asthmatic children were not significantly correlated with the FEV1 and PEF during both periods ($P > 0.05$).

Conclusion: Serum TIgE, IL-13 and eotaxin influence each other during exacerbation of bronchial asthma and influence the corresponding pathophysiologic changes. Serum IL-13 and eotaxin could be used as markers for evaluating the severity of bronchial asthma.

Iranian Journal of Pediatrics, Volume 23 (Number 5), October 2013, Pages: 525-530

Key Words: Asthma; Eotaxin; interleukin 13; Total IgE; Pulmonary Function; Children

Introduction

Bronchial asthma is the most common chronic inflammatory disease of the airway in children. It is a group of clinical syndromes caused by the synergism of immune, genetic and environmental factors. Its pathogenesis is complex. An imbalance

in Th1/Th2 cells and disorders of the cytokine network are generally considered the etiologies of asthma^[1-3]. Eosinophils (EOS) are the major effector cells that cause tissue injury and pulmonary dysfunction in the inflammatory response of asthma. EOS infiltration is the basic pathologic feature of asthma. Both IgE-mediated

* Corresponding Author:

Address: Department of Pediatrics, the Third Affiliated Hospital of Inner Mongolia Medical University, Baotou 01410, China

E-mail: wxzfen@yeah.net

© 2013 by Pediatrics Center of Excellence, Children's Medical Center, Tehran University of Medical Sciences, All rights reserved.

rapid-onset asthma and inflammation-induced late-onset asthma cause both bronchospasm and airflow obstruction, which are the main pathophysiologic changes in asthma.

Recent studies on the relationship between asthma and serum eotaxin, Interleukin (IL)-13 and IgE indicate that several factors influence the pathogenesis of asthma, although the exact mechanism and relationships are still unclear^[4-6]. The current research aims to determine the serum levels of eotaxin, IL-13 and total IgE (TlgE) in asthmatic children during different periods, as well as the corresponding changes in pulmonary function, to determine their significance in the exacerbation of asthma, their relationships and their clinical significance.

Subjects and Methods

Subjects

A total of 30 asthmatic children during the acute period were selected from the outpatients hospitalized from August 2010 to August 2011. The subjects consisted of 14 males and 16 females, with a mean age of 8.53 ± 2.61 years. The subjects were diagnosed in accordance with the criteria established by the Respiratory Study Group of the Paediatrics Branch of the Chinese Medical Association in 2008^[7]. The acute period is characterised by sudden wheezing, coughing, polypnea and respiratory distress, or a sharp deterioration of the initial symptoms. The severity was scaled according to the clinical manifestations and pulmonary function, with 14 cases classified as mild persistent and 16 classified as moderate persistent. The children were followed up

regularly. Clinical remission was defined as improvement of symptoms and pulmonary function, their acute levels and maintained for more than 3 months. The acute period ranged from 1 day to 5 days. The asthma group consisted of children 5 years to 12 years old, without histories of parasitic infections or a history of other infectious or autoimmune diseases. The control group consisted of 22 healthy children (14 males and 8 females, with a mean age of 8.59 ± 2.24 years, without personal or family histories of allergic diseases or histories of parasitic infections, with no wheezing, coughing, polypnea and respiratory distress. This study was conducted in accordance with the declaration of Helsinki. This study was conducted with approval from the Ethics Committee of the Third Affiliated Hospital of Inner Mongolia Medical College. Written informed consent was obtained from the parents of the all participants.

Specimens and preservation

Fasting venous blood samples (3 ml) were collected from the asthma group during the acute period and clinical remission, as well as from the healthy controls. The samples were allowed to clot and were centrifuged at 3000 r/min for 10 min. Serum was collected from the blood samples and placed in Eppendorf tubes. The sera were temporarily stored at -20°C and transferred into a -70°C ultralow-temperature refrigerator after 2 months.

Enzyme-linked immunosorbent assay

Eotaxin and IL-13 were determined using kits provided by Shanghai Rui Cong Laboratory Equipment Co., Ltd. and produced by the ADL Company, USA. The measurement range for IL-13 was from 0 pg/ml to 800 pg/ml with a sensitivity

Table 1: Comparison of the pulmonary function in children with asthma between the acute-outbreak periods (n=30) and the clinical remission (n=30)

Groups	FEV1 (L/min)	PEF (%)	FEV1/FVC	V50%	V50 (L/min)	V25%	V25 (L/min)
Acute-outbreak period	1.3 (0.5)	69.7 (21.7)	71.5 (11.5)	45.4 (22.4)	1.3 (0.7)	37.5 (22.2)	0.6 (0.4)
Clinical remission period	2.0 (0.5)	98.5 (15.8)	84.2 (8.5)	77.6 (22.2)	2.3 (0.8)	67.3 (20.3)	1.1 (0.4)
t	-4.844	-5.879	-4.858	-5.591	-4.683	-5.421	-4.045
P. value	≤ 0.001	≤ 0.001	≤ 0.001	≤ 0.001	≤ 0.001	≤ 0.001	≤ 0.001

FEV1: Forced expiratory volume in 1 second; PEF: Peak expiratory flow; FVC: Forced vital capacity; V50% & 25%: 50% and 25% of vital capacity

Table 2: Comparison of serum eotaxin, interleukin (IL)-13 and total IgE (TIgE) levels between the children with asthma and the healthy control group

Groups	Cases	Eotaxin (ng/L) Mean (SD)	IL-13 (ng/L) Mean (SD)	TIgE (ng/L) Mean (SD)
Acute-outbreak period	30	53.13 (24.03) ^{ab}	184.61 (114.14) ^{ab}	924.17 (599.75) ^{ab}
Clinical remission period	30	20.14 (11.19) ^a	58.03 (26.17) ^a	241.75 (109.82) ^a
Healthy control group	22	4.75 (3.98)	23.94 (11.84)	42.64 (32.54)

Compared with the healthy control group, ^a $P < 0.001$; compared with the clinical remission period, ^b $P < 0.001$

of 1.0 pg/ml, whereas those for eotaxin was from 0 pg/ml to 240 pg/ml with a sensitivity of 0.1 ps/ml. A fluorescent enzyme-linked immunosorbent assay was used to determine TIgE. The kits were provided by Sweden Pharmacia Company. The Pharmacia Uin CAP allergen detection system was used to detect TIgE. The specimens were tested using kits from the same batch.

Pulmonary function test

The children in the asthma group underwent pulmonary function tests in the acute-outbreak and clinical remission periods with an Italian COSMED PFT4 pulmonary function tester. The indicators were measured simultaneously and the parameters included forced vital capacity (FVC), forced expiratory volume in 1 second (FEV1) and peak expiratory flow (PEF). The PEFs were set to 75%, 50% and 25% of vital capacity (V75, V50 and V25). The subjects were measured repeatedly at least twice after practice runs until the steps were mastered. The predicted values were adjusted for individual age, height, gender and body mass and the best values during the repeated measurements were used for analysis.

Statistical analysis

The measurement data are presented as mean \pm standard deviation ($\bar{x} \pm s$). SPSS (version 13.0)

statistical software was used for statistical analysis. A t-test was used to compare two-sample means. An ANOVA was used to compare three-sample means. A chi square test was used to compare gender between groups. The linear correlation was used to analyse the relationship between the two groups. Differences with $P < 0.05$ were considered significant.

Findings

Comparison of pulmonary function: There were significant statistical differences in the various indexes of the pulmonary function in asthmatic children during the acute and clinical remission periods ($P < 0.001$; Table 1).

Serum levels of eotaxin, IL-13 and TIgE: The serum eotaxin, IL-13 and TIgE levels in the asthma group during the acute period significantly differed from those during clinical remission ($P < 0.001$). The serum eotaxin, IL-13 and TIgE levels in the asthma group during both periods significantly differed from those of the healthy controls ($P < 0.001$; Table 2).

Relationship of serum eotaxin and IL-13 levels with FEV1 and PEF: The serum eotaxin and IL-13 levels in the asthma group were positively

Table 3: Relationships between the serum eotaxin, Interleukin 13 and total IgE levels in the acute-outbreak periods and the clinical remission periods in children with asthma and FEV1, PEF

Items	Acute-outbreak period				Clinical remission period			
	FEV1		PEF		FEV1		PEF	
	<i>r</i>	<i>P. value</i>	<i>r</i>	<i>P. value</i>	<i>r</i>	<i>P. value</i>	<i>r</i>	<i>P. value</i>
Eotaxin	-0.616	0.000	-0.489	0.006	-0.575	0.001	-0.277	0.138
Interlukine-13	-0.792	0.000	-0.707	0.000	-0.431	0.017	-0.403	0.027
Total IgE	-0.358	0.052	-0.247	0.189	-0.167	0.377	-0.212	0.260

FEV1: Forced expiratory volume in 1 second; PEF: Peak expiratory flow

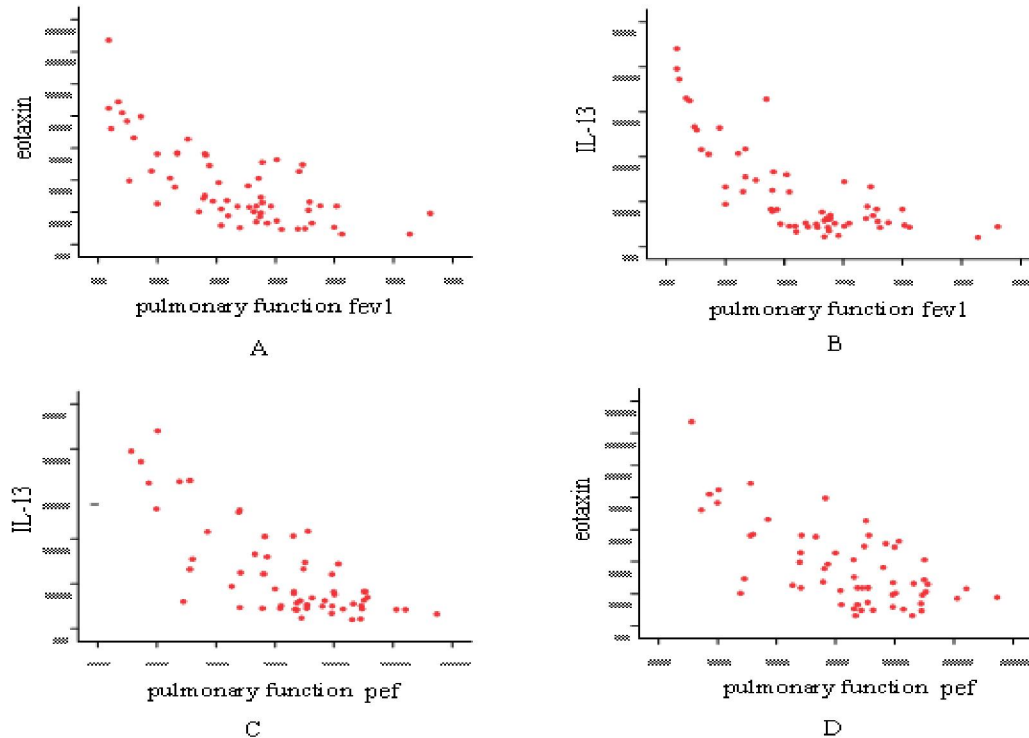


Fig. 1: **A.** The relationship of eotaxin and FEV1; **B.** The relationship of IL-13 and FEV1; **C.** Relationship of serum IL-13 with PEF; **D.** Relationship of serum eotaxin with PEF

FEV1: Forced expiratory volume in 1 second; PEF: Peak expiratory flow; IL-13: interleukin 13

correlated with FEV1 and PEF during both periods ($P < 0.05$; Table 3, Fig 1). Relationship of serum TIgE levels with FEV1 and PEF: TIgE levels were not significantly correlated with FEV1 and PEF during both periods ($P > 0.05$; Table 3, Fig. 2).

Relationships among serum levels of eotaxin, IL-13 and TIgE: During the acute period, the serum eotaxin in the asthma group was positively correlated with serum IL-13 ($r = 0.695$, $P \leq 0.0001$),

as well as with serum TIgE ($r = 0.382$, $P = 0.04$), and serum IL-13 was positively correlated with serum TIgE ($r = 0.403$, $P = 0.03$). During clinical remission, the serum eotaxin of the asthma group was positively correlated with serum IL-13 ($r = 0.498$, $P \leq 0.0001$), as well as with serum TIgE ($r = 0.437$, $P = 0.02$), and IL-13 was positively correlated with TIgE ($r = 0.369$, $P = 0.045$; Fig. 3).

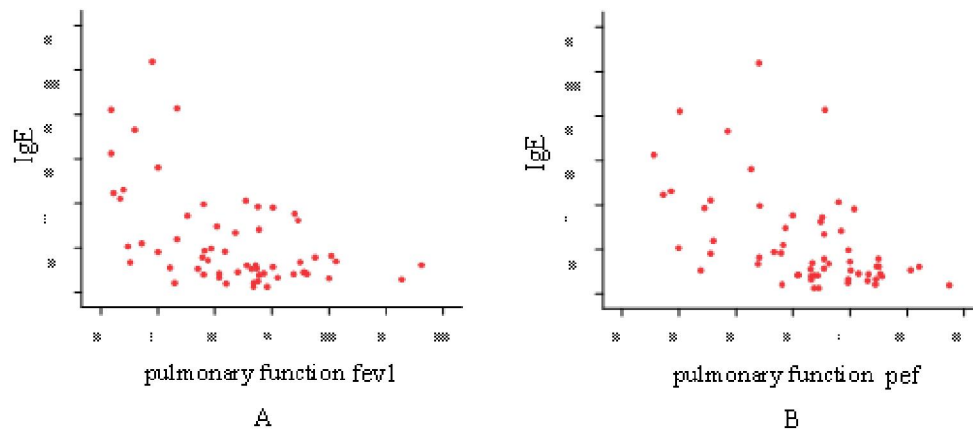


Fig. 2: **A.** Relationship of serum total IgE with FEV1. **B.** Relationship of serum IgE with PEF

FEV1: Forced expiratory volume in 1 second; PEF: Peak expiratory flow

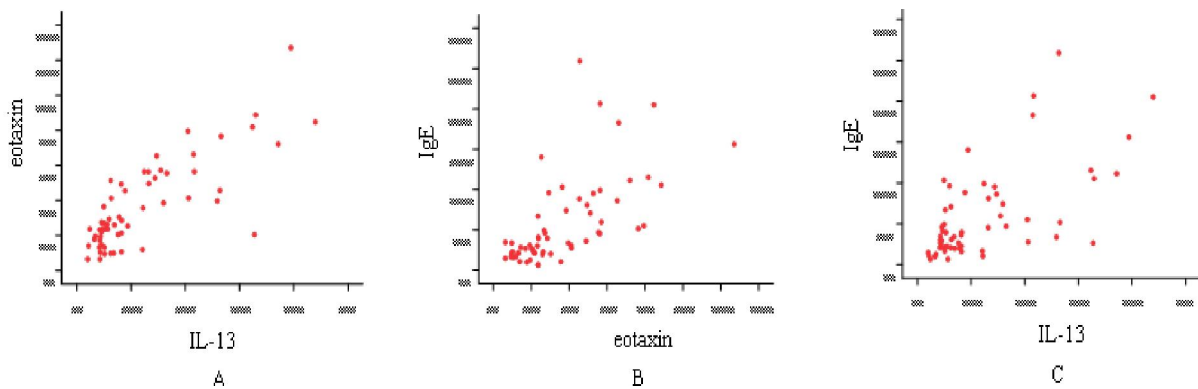


Fig. 3: **A.** Relationship of serum eotaxin with serum Interleukin 13; **B.** Relationship of serum total IgE with serum eotaxin; **C.** Relationship of serum total IgE with serum Interleukin 13

Discussion

The serum eotaxin and IL-13 levels in the asthma group during the acute and the clinical remission periods were significantly higher than those during the clinical remission period, as well as those of the healthy control group. These findings are consistent with those of previous studies^[8,9]. The serum eotaxin and IL-13 levels in the asthma group during the acute period were significantly increased, which declined during remission but they remained higher than those of the healthy controls. These results indicate that eotaxin and IL-13 are inflammatory factors involved in the pathogenesis of asthma in children during clinical remission. Hoffmann et al^[10] found that 6 weeks of inhaled corticosteroid and long-acting beta2 agonist inhalation therapy alleviated the clinical symptoms of asthmatic children and decreased the serum eotaxin levels. Their findings indicate that serum eotaxin is a potential marker for the severity of asthma in children and the effectiveness of treatment.

Our results indicate that serum eotaxin and IL-13 levels are negatively correlated with FEV1 and with PEF during the acute and clinical remission periods of asthma in children. The improvement in pulmonary function was accompanied by a reduction in serum eotaxin and IL-13 levels, which is consistent with previous studies^[11-14]. These findings suggest that eotaxin and IL-13 levels reflect the changes in pulmonary function among asthmatic children and could be an objective indicator for the severity of asthma, which would facilitate diagnosis and the assessment of pulmonary function.

The serum TlgE levels in the asthma group during the acute period were significantly higher than those during clinical remission and those of the asthma group during both periods were significantly higher than those of the healthy controls. However, the changes in serum TlgE levels in the asthma group were not correlated with FEV1 and PEF (Fig. 2). Nevertheless, the changes in serum TlgE levels among asthmatic children were closely related to the changes of asthma.

TlgE is commonly used to reflect immune status. The high TlgE levels in this study may be because the blood tests coincided with the wormwood pollen allergy season in Baotou, and most of the symptoms were caused by wormwood pollen allergy. Although the skin prick tests could help during diagnosis, we chose not to use them because the skin prick test itself would aggravate the symptoms.

The serum IL-13, eotaxin and TlgE levels in children with bronchial asthma were positively correlated with each other in the acute and clinical remission periods (Fig. 3), which is consistent with previous studies^[4,16]. Serum TlgE, IL-13 and eotaxin interact with each other during the pathogenesis of bronchial asthma and cause the corresponding pathophysiologic changes. Previous methods for diagnosing bronchial asthma focus on signs and symptoms. The results of this study show that monitoring serum eotaxin, IL-13 and TlgE levels would reflect their relationships, which are helpful in investigating the pathophysiology of bronchial asthma, as well as guiding its clinical diagnosis and assessment.

The current study is a preliminary investigation

on the roles of eotaxin, IL-13 and IgE in asthma. Furthermore, it only includes children 5 years to 12 years old and the sample size is relatively small. Further studies should include a larger sample and children under 5 years old. Moreover, comparative studies in asthmatic children regarding the changes in cytokine levels before and after standardized treatment should be performed to determine their clinical significance.

Conclusion

Serum TIGe, IL-13 and eotaxin influence each other during exacerbation of bronchial asthma and influence the corresponding pathophysiologic changes. Serum IL-13 and eotaxin could be used as markers for evaluating the severity of bronchial asthma.

Acknowledgment

We are grateful for the funding support from the Technology Bureau of Baotou.

Conflict of Interest: None

References

1. Brand S, Kesper DA, Teich R, et al. DNA methylation of TH1/TH2 cytokine genes affects sensitization and progress of experimental asthma. *J Allergy Clin Immunol* 2012;129(6):1602-10.
2. Robinson DS. The role of the T cell in asthma. *J Allergy Clin Immunol* 2010;126(6):1081-91.
3. Park HJ, Lee CM, Jung ID, et al. Quercetin regulates Th1/Th2 balance in a murine model of asthma. *Int Immunopharmacol* 2009;9(3):261-7.
4. Batra J, Rajpoot R, Ahluwalia J, et al. A hexanucleotide repeat upstream of eotaxin gene promoter is associated with asthma, serum total IgE and plasma eotaxin levels. *J Med Genet* 2007;44(6):397-403.
5. Lee JH, Moore JH, Park SW, et al. Genetic interactions model among Eotaxin gene polymorphisms in asthma. *J Hum Genet* 2008;53(10):867-75.
6. Holgate ST. A look at the pathogenesis of asthma: the need for a change in direction. *Discov Med* 2010;9(48):165-71.
7. The Subspecialty Group of Respiratory Diseases, The Society of Pediatrics. Chinese Medical Association Diagnosis, prevention and treatment guidelines of children bronchial asthma. *Chin J Pediatrics* 2008;46(10):745-53.
8. Chu YT, Chiang W, Wang TN, et al. Changes in serum Eotaxin and eosinophil cationic protein levels, and eosinophil count during treatment of childhood asthma. *J Microbiol Immunol Infect* 2007;40(2):162-7.
9. Kanabar V, Page CP, Simeock DE, et al. Heparin and structurally related polymers attenuate eotaxin-1 (CCL11) release from human airway smooth muscle. *Br J Pharmacol* 2008;154(4):833-42.
10. Hoffmann HJ, Nielsen LP, Harving H, et al. Asthmatics able to step down from inhaled corticosteroid treatment without loss of asthma control have low serum eotaxin/CCL11. *Clin Respir J* 2008;2(3):149-57.
11. Griffin MJ, Chen E. Perceived control and immune and pulmonary outcomes in children with asthma. *Psychosom Med* 2006;68(3):493-9.
12. Delgado J, Barraneo P, Quirce S. Obesity and asthma. *J Investig Allergol Clin Immunol* 2008;18(6):420-5.
13. Zietkowski Z, Tomasiak-Lozowska MM, Skiepkowski R, et al. Eotaxin-1 in exhaled breath condensate of stable and unstable asthma patients. *Respir Res* 2010;11:110.
14. Griffin MJ, Chen E. Perceived control and immune and pulmonary outcomes in children with asthma. *Psychosom Med* 2006;68(3):493-9.
15. Kabesch M, Schedel M, Carr D, et al. IL-4/IL-13 pathway genetics strongly influence serum IgE levels and childhood asthma. *J Allergy Clin Immunol* 2006;117(2):269-74.