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Original Article

Changes in serum eotaxin and eosinophil cationic protein levels, and eosinophil count during treatment of childhood asthma

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Background and Purpose: Increased serum levels of eotaxin are related to the severity of asthma in adults. There are limited data on the effects of oral corticosteroids and inhaled corticosteroid therapy on serum levels of eotaxin and eosinophil cationic protein (ECP) and peripheral blood eosinophil counts (ECs) in pediatric asthma patients. We investigated prospectively the changes in eotaxin and ECP serum levels and peripheral blood ECs after administering oral corticosteroids and then inhaled corticosteroids plus long-acting beta2 agonist treatment in pediatric patients.

Methods: Serum samples of 20 pediatric patients with mild-to-moderate asthma were collected before treatment, after 5-7 days of oral prednisolone treatment, and after 1-2 months of inhaled fluticasone plus salmeterol treatment. Peak expiratory flow was used as the outcome index.

Results: Serum eotaxin levels remained the same after oral prednisolone treatment, but decreased after subsequent inhalation treatment compared with the end of oral steroid treatment (64.7 ± 22.6 vs 85.7 ± 36.8 pg/mL, $p<0.001$). The EC and serum ECP levels declined soon after oral steroid treatment, rebounding to initial levels during inhalation treatment. The decrease in ECP level was positively correlated with the decrease in ECs with oral steroid treatment ($r^2 = 0.28$, $p=0.016$). There was no correlation between changes in eotaxin levels and peak expiratory flow.

Conclusions: Our data suggest that the serum eotaxin level, not peripheral blood EC or serum ECP level, declines during inhaled fluticasone plus salmeterol treatment and might serve as a surrogate marker of T helper 2 residual activity in pediatric asthma.

Key words: Asthma; Chemokines; Child; Eosinophils; Glucocorticoids

Introduction

Eosinophil accumulation in peripheral blood and tissue is a hallmark feature of atopic disorders, including asthma [1], allergic rhinitis, and eczema [2], and is frequently observed during acute exacerbation of asthma in either patients [3] or animal models [4]. The eosinophils migrate along the concentration gradient of chemoattractants, enter the pulmonary circulation, marginate to the vessel wall and subsequently enter the interstitial spaces. Several mediators, including lipid mediators, bacterial products, and chemokines, have been identified as eosinophil chemoattractants [5]. Eotaxin, an 8.4-kDa CC chemokine, has been implicated in allergen-induced eosinophil responses in the lung [6-8]. Eotaxin is also associated with status asthmatics or severe asthma in adults [9] and is secreted from endothelial cells [10,11], fibroblasts [12], macrophages, ciliated and non-ciliated bronchial epithelial cells, smooth muscle cells, chondrocytes [11], and eosinophils [10]. The receptor of eotaxin (CCR3) is expressed on T helper 2 (Th2) cells when co-localized with eosinophil.

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This suggests that eotaxin/CCR3 represents a novel mechanism of T-lymphocyte recruitment [13].

Eosinophil cationic protein (ECP) is secreted by activated eosinophils. Elevated serum ECP levels are associated with recurrent wheezing, and higher serum ECP levels are associated with current asthma and more severe atopy in children [14,15]. Serum ECP concentrations during acute exacerbations in asthma patients are significantly elevated as compared with those during clinical remission [16]. Several reports have shown higher ECP levels in the serum of asthma patients than in normal subjects [17,18]. It is of interest to measure the serum eotaxin and ECP levels as well as peripheral blood eosinophil counts (EC) in pediatric asthma patients during attacks and after treatment. This study was designed prospectively to investigate the eotaxin and ECP serum levels and peripheral blood EC in pediatric asthma patients on oral corticosteroids, and the levels after administration of inhaled corticosteroids plus long-acting beta2 agonist (LABA) treatment.

Methods

Patients

The Institution Review Board of Kaohsiung Medical University Hospital approved the study protocol. Pediatric patients ranging in age from 5 to 15 years, who had suffered asthma attacks in the emergency room or outpatient clinics between January 2005 and December 2005, were included in the study. The children enrolled in this study fell into the categories of mild-to-moderately persistent asthma as defined by the National Asthma Education and Prevention Program, Expert Panel Report II, Guidelines for the Diagnosis and Management of Asthma [19]. The clinical severity of asthma was defined as mildly persistent if symptoms were observed more than once per week, peak expiratory flow (PEF) was >80% of the baseline value, and PEF variability was between 20% and 30%. The severity was defined as moderately persistent if symptoms occurred daily, PEF was between 60% and 80% of the baseline value, and PEF variability was >30%. None of the patients were febrile and none had been taking steroids for at least 1 month prior to enrollment in this study. After parental informed consent was obtained, patients were followed for 2 consecutive months.

Study design

The prospective cohort follow-up study included 1 week of oral steroid treatment (1 mg/kg/day prednisolone) with subsequent use of inhaled corticosteroids plus LABA (Seretide Evohaler [GlaxoSmithKline, Hong Kong], 50 μg fluticasone + 25 μg salmeterol, 1 blister twice a day). Serum levels of eotaxin and ECP and peripheral blood EC were measured in the acute stage before steroid treatment (0-2 days after asthma attack), after 5-7 days of oral steroid treatment (7-14 days after asthma attack), and in the convalescent stage with inhaled corticosteroids plus LABA treatment for more than 1 month. PEF was measured during the same period using a peak flow meter (Astech Co., Port Washington, NY, USA). Sera were stored at –80°C immediately after sampling. Serum ECP levels were measured by immunofluorescence, and serum eotaxin levels were measured by enzyme-linked immunosorbent assay (ELISA; Quantikine, Catalog Number: DTX00; R&D Systems, Minneapolis, MN, USA). This kit shows no cross-reaction with recombinant human monocyte chemotactic protein (MCP)-1, MCP-2, MCP-3, MCP-4, macrophage inflammatory protein (MIP)-1, MIP-1 (70-amino acid isoform) and RANTES (regulated on activation, normal T cell expressed and secreted). The sensitivity ranges from 7.8 pg/mL to 500 pg/mL. Those patients who failed to use oral steroids for at least 5 days, or those who used leukotriene antagonists during the follow-up period, were excluded. The control serum for eotaxin was chosen from a school-based study. We chose 20 students (10-11 years of age) whose International Study of Asthma and Allergies in Childhood questionnaire was negative for the purposes of control serum of eotaxin.

Statistical analysis

Statistical Package for the Social Sciences (SPSS) for Windows (Version 12.0, SPSS, Chicago, IL, USA) was used for all statistical analysis. Serum levels of eotaxin, ECP, and EC were analyzed by using the Wilcoxon signed-rank test. Differences were considered to be significant when $p<0.05$.

Results

Eighty three patients participated in this study. After follow-up, only 38 patients had completed the 5 to 7 days of oral prednisolone treatment. In all, 40 patients were excluded because they either underwent less than 5 days of oral steroid treatment or were unable to perform PEF tests, and 5 patients were lost to follow-up. Of the 38 patients, 25 completed the inhaled fluticasone plus salmeterol treatment; however, of the 25 patients,

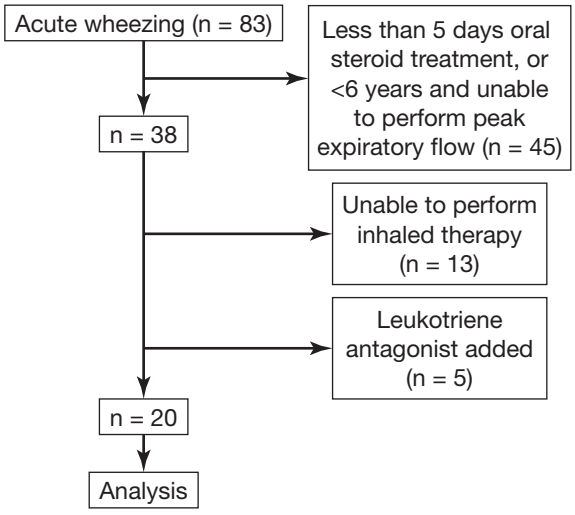

Fig. 1. Enrollment and retention characteristics of patients.

5 were excluded because leukotriene antagonist was included mid-way during the therapy regimen. Therefore, only 20 patients completed the 5 to 7 days of oral prednisolone therapy, and later used inhaled fluticasone plus salmeterol purely as maintenance therapy (Fig. 1).

PEF during the treatment course

All the participants took the PEF tests while in the acute stage, after oral steroid treatment, and during the convalescent stage while receiving inhaled steroids plus LABA treatment. All PEF data were transformed to the predicted value according to their age, body weight, and body height. The predicted PEF improved gradually during treatment with oral steroids and inhaled steroids plus LABA (p<0.001) [Fig. 2].

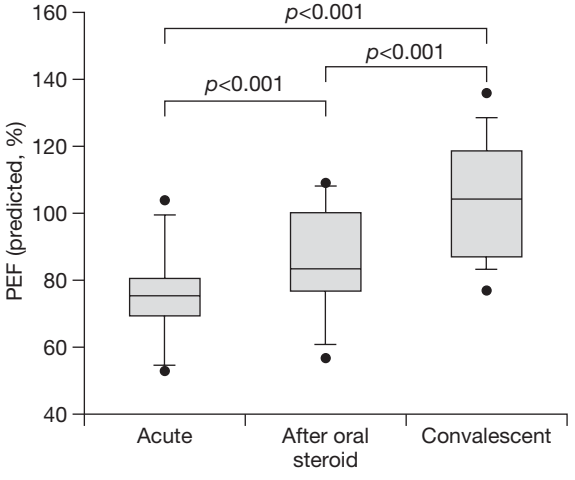

Fig. 2. Box plot showing peak expiratory flow (PEF) improvement after oral steroids and inhaled steroids plus long-acting beta2 agonist treatment. The boundary of the box closest to zero indicates the 25th percentile, a line within the box marks the median, and the boundary of the box farthest from zero indicates the 75th percentile. Error bars above and below the box indicate the 90th and 10th percentiles, respectively. Dots above or below are outliers (Wilcoxon signed-rank test, p values are indicated).

Serum eotaxin levels after treatment with inhaled steroids plus LABA

The serum eotaxin levels of asthma patients were higher than those of controls in the acute stage (p=0.027), after oral steroid treatment (p<0.001), and during the convalescent stage (p<0.001). The serum levels of eotaxin did not decrease during oral steroid treatment, but significantly decreased during the convalescent stage with inhaled steroids plus LABA treatment compared with the end of oral steroid treatment (64.7 ± 22.6 pg/mL vs 85.7 ± 36.8 pg/mL, p<0.001)[Fig. 3]. The changes in serum eotaxin levels did not correlate with the changes in PEF (r^2 = 0.104, p=0.16) [Fig. 4].

Serum ECP levels and blood EC during treatment with inhaled steroids plus LABA

The peripheral blood EC decreased after oral steroid treatment (compared with the acute situation (218 ± 127/mm^3 vs 415 ± 315/mm^3, p=0.04), but rebounded during the convalescent stage of inhaled steroids plus LABA treatment (393.59 ± 180.47/mm^3, p<0.001) [Fig. 5]. The changes in serum ECP levels were similar to the changes in EC (Fig. 6). The decline in the ECP

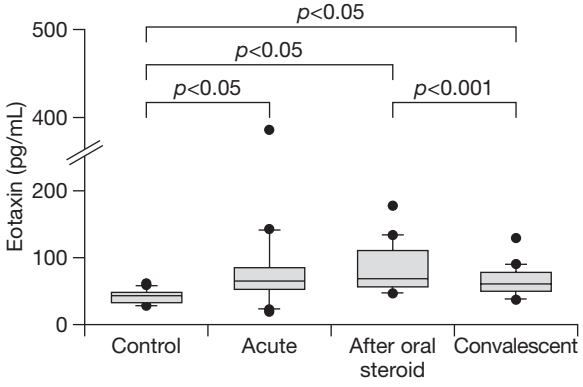

Fig. 3. Serum eotaxin levels after oral steroid treatment and during inhaled steroids plus long-acting beta2 agonist treatment. The control serum was collected from students who were negative for eotaxin as reported in the International Study of Asthma and Allergies in Childhood questionnaire, and their serum eotaxin levels were significantly lower than that of asthmatic patients (Wilcoxon signed-rank test, p values are indicated).

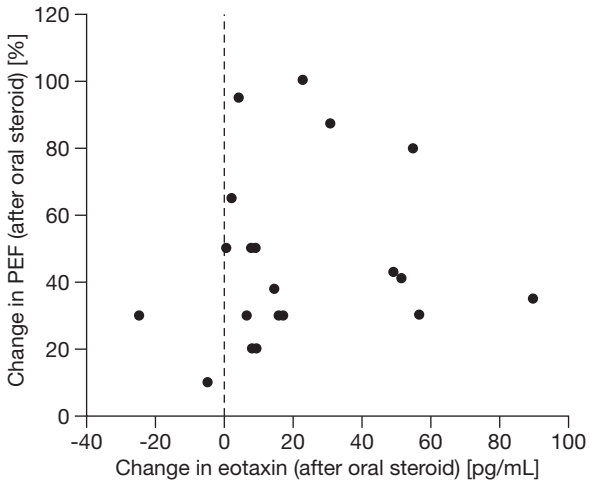

Fig. 4. Changes in serum eotaxin levels were not correlated with the changes in peak expiratory flow (PEF) during the convalescent stage (r^2 = 0.104, p=0.16).

level significantly correlated with the decline in EC during oral steroid treatment (r^2 = 0.28, p=0.016) [Fig. 7].

Discussion

Our data revealed that the serum eotaxin levels in the pediatric population were much lower than those in adults. The average serum level of eotaxin in acute atopic adults is approximately 250 to 1100 pg/mL [20]. Pediatric asthmatic patients may have only one-third to one-fourth concentration of the adult eotaxin level during their attacks. Those patients aged 10 to 11 years without any asthma symptoms had the lowest eotaxin

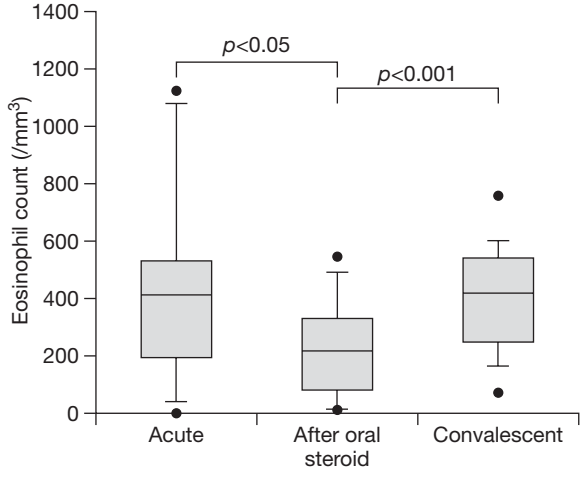

Fig. 5. Blood eosinophil count after oral steroid treatment and during inhaled steroids plus long-acting beta2 agonist treatment (Wilcoxon signed-rank test, p values are indicated).

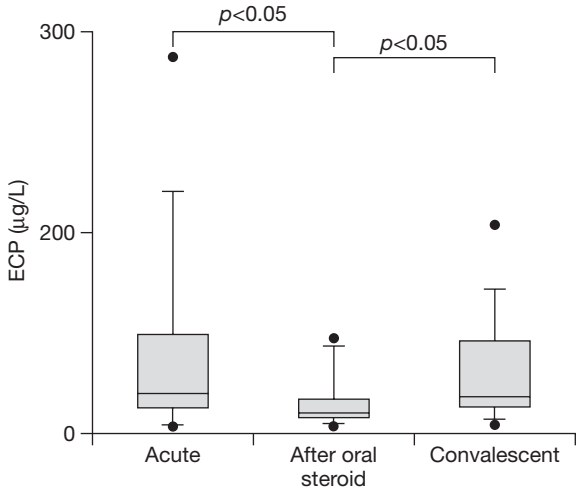

Fig. 6. Serum eosinophil cationic protein (ECP) levels after oral steroid treatment and during inhaled steroids plus long-acting beta2 agonist treatment (Wilcoxon signed-rank test, p values are indicated).

levels (42.04 ± 10.7 pg/mL) [Fig. 3]. To our knowledge, serial serum eotaxin levels from acute asthma attack to convalescence in a pediatric group have never been discussed.

In this study, the peripheral blood EC and serum ECP levels decreased after 5 to 7 days of oral corticosteroid treatment. However, the serum eotaxin levels remained the same. These data suggest that eotaxin did not directly contribute to the decrease in EC. Park et al [21] used systemic steroids 2 mg/kg/day for 7 days in 7 adults with acute asthma, and found that interleukin (IL)-5, rather than eotaxin in the induced sputum, was effectively decreased by the inhibitory effect of steroids in acute exacerbation. Therefore, we deduced that the decrease in EC may be associated with the decrease in serum IL-5 levels or steroid-induced apoptosis as shown in other studies [21,22].

Decrease in serum ECP levels was significantly correlated with the decrease in peripheral blood EC (Fig. 7), which means that activated eosinophils decreased after oral steroid treatment. ECP is an important protein in eosinophils and is secreted only after eosinophil activation. Our data indicate that oral steroid treatment can effectively decrease both total EC and activated EC, which is in concordance with previous studies [23,24].

Surprisingly, EC and ECP serum levels reverted to initial levels when inhaled fluticasone plus salmeterol treatment was started. This indicates that inhaled therapy cannot halt the number and activity of peripheral blood eosinophils. However, the serum levels of eotaxin in

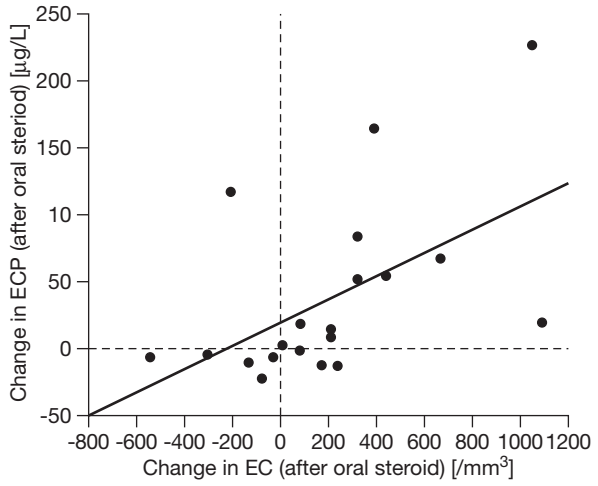

Fig. 7. Changes in serum eosinophil cationic protein (ECP) levels were positively correlated with the changes in peripheral blood eosinophil count (EC) after oral steroid treatment (r^2 = 0.28, p=0.016).

convalescence were significantly decreased. Van Rensen et al [25] demonstrated that treatment with inhaled steroids in asthmatic adults for 4 weeks leads to improvements in airway hyperresponsiveness and decreases EC in induced sputum. Our data provide some evidence that the decrease in eotaxin during inhaled fluticasone plus salmeterol treatment may play a role in decreasing pulmonary eosinophil recruitment.

There were at least 3 limitations to this study. First, the total number of patients that completed this study was small. Some patients refused to continue with the protocol because of the requirement for frequent blood sampling. In addition, performing PEF tests was difficult for patients younger than 6 years old. Furthermore, drug compliance was variable, especially in pediatric patients. Therefore, selection bias did exist in this study. Second, this study was designed as a follow-up study of one type of therapy in asthmatic patients. Therefore, confounding factors such as the effect of environment control or allergen avoidance could not be eliminated. Third, it is unknown whether the decrease in serum eotaxin levels was caused by the steroid component or the LABA component of the inhaled therapy. Further study should aim at using monotherapy with inhaled steroids only or inhaled LABA only instead of combined therapy, double-blinded with another control group, in order to control for these confounding factors.

In conclusion, our data suggest that serum eotaxin levels decline during inhaled fluticasone plus salmeterol treatment, but not during oral steroid treatment. The reduction in serum eotaxin levels is not correlated with the improvement in PEF. Thus, we could not use serum eotaxin levels to predict the improvement in PEF. We suggest that serum eotaxin levels, but not peripheral blood EC or serum ECP levels, might serve as a surrogate marker of Th2 residual activity in treating pediatric asthma.

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