Eosinophilic cationic protein: is it useful in assessing control of childhood asthma?

M. Zedan,[1] A. Settin,[2] F. El-Chennawi,[3] T. El-Desouky,[1] N. Nasef[2] and A. Fouda[2]

البروتين اليوزيني الكاتيوني: هل هو مفيد في تقييم السيطرة على الربو عند الأطفال؟

مجدي زيدان، أحمد ستّين، فرحة الشناوي، طارق الدسوقي، نهاد ناصف، أشرف فودة

الخلاصة: قامت هذه الدراسة بتقييم مستويات اليوزينات في الدم المحيطي، والبروتين اليوزيني الكاتيوني s-ECP، بوصفهما واسمَيْن اثنَيْن من واسمات السيطرة على الربو. وقد شملت الدراسة 38 طفلاً مصاباً بالربو، منهم 16 طفلاً تمَّ إحكام السيطرة على المرض لديهم كلياً و22 طفلاً تمَّ إحكام السيطرة على المرض لديهم جزئياً. وقد قورن هؤلاء المرضى مع مجموعة من الأطفال الأصحاء الذين يماثلونهم من حيث العمر والجنس وعددهم 16 طفلاً. ووجد الباحثون أن لدى المصابين بالربو مستويات من عدد اليوزينيات ومن البروتين اليوزيني الكاتيوني أعلى مما هو عليه الأمر عند الأطفال الأصحاء. وأن لدى الأطفال الذين تمت السيطرة على المرض لديهم جزئياً، مستويات من الواسمَيْن أعلى بمقدار يُعْتَدُّ به إحصائياً مما هو عليه الأمر لدى الأطفال الذين تمت السيطرة لديهم كلياً على المرض. ولم يظهر في الأطفال المصابين بالربو الذين تمت السيطرة على المرض لديهم كليّاً أيَّة تغيرات يُعتدُّ بها إحصائياً في كلا الواسمَيْن مقارنةً بالأطفال الأصحاء. كما لاحظ الباحثون وجود ترابُط سلبي بين درجة السيطرة على الربو، وبين كلٍّ من تعداد اليوزينيات (معامل الترابط $r = -0.60$) ومستوى البروتين اليوزيني الكاتيوني (معامل الارتباط $r = -0.75$). واستنتج الباحثون أن كلاً من تعداد اليوزينيات والبروتين اليوزيني الكاتيوني قد يساعد على تقييم مدى السيطرة على الربو.

ABSTRACT This study evaluated peripheral eosinophil and serum eosinophilic cationic protein (s-ECP) levels as markers of asthma control. A total of 38 children with asthma (16 controlled and 22 partially controlled) were compared with 16 age- and sex-matched healthy children. Total asthma cases had higher eosinophil counts and s-ECP levels than healthy children and partially controlled asthmatics had significantly higher levels of both markers than controlled asthmatics. Controlled asthma cases showed non-significant changes in both parameters versus healthy children. A negative correlation was noted between degree of asthma control and both eosinophil counts and s-ECP levels ($r = -0.60$ and -0.75 respectively). s-ECP as well as peripheral eosinophil count may be helpful in the assessment of asthma control.

Utilité de la protéine cationique de l'éosinophile pour l'évaluation du contrôle de l'asthme chez l'enfant

RÉSUMÉ Cette étude portait sur les niveaux obtenus par le dosage sérique et dans le sang périphérique de la protéine cationique de l'éosinophile en tant que marqueurs du contrôle de l'asthme. Au total, 38 enfants souffrant d'asthme (contrôlé pour 16 d'entre eux et partiellement contrôlé pour 22 autres) ont été comparés à 16 enfants en bonne santé de même sexe et de même âge. Tous les cas d'asthme présentaient un comptage des éosinophiles et un dosage sérique de la protéine cationique de l'éosinophile supérieurs à ceux des enfants en bonne santé ; dans les cas d'asthme partiellement contrôlé, les niveaux des deux marqueurs étaient nettement supérieurs à ceux des cas d'asthme contrôlé. Les cas d'asthme contrôlé n'ont révélé aucun changement significatif des deux paramètres par rapport aux enfants en bonne santé. Une corrélation négative a été observée entre le degré de contrôle de l'asthme d'une part, et le comptage des éosinophiles et le dosage sérique de la protéine cationique de l'éosinophile d'autre part ($r = -0,60$ et $-0,75$ respectivement). Le dosage sérique et dans le sang périphérique de la protéine cationique de l'éosinophile peuvent être utiles pour évaluer le contrôle de l'asthme.

[1]Allergy, Respiratory and Clinical Immunology Unit; [2]Department of Paediatrics; [3]Clinical Pathology and Immunology Unit, Faculty of Medicine, University of Mansoura, Mansoura, Egypt (Correspondence to M. Zedan: magdyzedan@mans.edu.eg).

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Introduction

Bronchial asthma is a chronic inflammatory disorder of the airways in which many inflammatory cells have been found to play a role, particularly mast cells, eosinophils and T-lymphocytes [1]. Immunohistochemical techniques have identified higher levels of the CD4+ subset of T-lymphocytes as well as eosinophils in the airways of patients with asthma than in non-asthmatic subjects [2].

The association between eosinophilia and asthma was observed shortly after eosinophils were discovered. In patients with asthma, eosinophils are present in increased numbers in the blood [3], sputum [4] and bronchoalveolar lavage fluid [5]. After activation, eosinophils can release granulocyte-derived proteins, the most toxic of which are eosinophilic cationic protein (ECP) and major basic protein [6].

Clinical research has suggested an emerging clinical usefulness of eosinophil granule proteins as serological makers in the assessment and management of asthma, of which ECP has been most widely characterized and researched [7,8]. We hypothesized that the degree of eosinophilic expression in the blood and the serum ECP (s-ECP) level may be correlated with the degree of asthma control. Accordingly the aim of our work was to evaluate the levels of asthma control in relation to serum eosinophil counts and s-ECP levels.

Methods

This was a case–control, cross-sectional study of children attending a hospital in Mansoura, Egypt.

Sample

The cases were 38 children with atopic asthma who were newly presenting to the Allergy and Respiratory Unit at the University of Mansoura Children's Hospital, Egypt, from 2002 to 2006. They were defined as asthmatic by the frequency of day and night asthma symptoms and the results of pulmonary function tests (PFT) and as atopic from positive skin prick tests. All were new asthma patients who had not previously received asthma controller medication. A control group of 16 healthy children matched by age and sex was chosen from among attendees at outpatient clinics who came for routine vaccination or regular check-ups.

According to the degree of severity of asthma on presentation, patients were given asthma controller medication based on the 2006 Global Initiative for Asthma (GINA) guidelines for asthma management [9]. Patients received inhaled corticosteroids (fluticasone-HFA/metered dose inhaler) 100 μg daily plus short-acting ß-2 agonists (salbutamol inhaler) as rescue medication.

After receiving controller treatment for 1 month, patients were categorized into controlled and partially controlled cases based on GINA criteria [9]. Controlled cases were those who had a frequency of daytime asthma symptoms or use of rescue medication twice or less/week; suffered no limitation of activities, no nocturnal symptoms and no asthma exacerbations; and had normal PFTs. Partially controlled cases were those who had a frequency of daytime symptoms or use of rescue medications more than twice/week; suffered any restriction of activities, nocturnal symptoms or asthma exacerbations; and PFTs showing forced expiratory volume in 1 s (FEV1) < 80% predicted.

Only atopic asthmatic patients with a positive skin test were included in the study. Patients with negative skin prick test, those on controller medications that did not comply with GINA guidelines (particularly oral corticosteroids) or who presented with severe exacerbation were excluded from the study.

Informed written consent was obtained from all participants before inclusion in the study, which was approved by Mansoura institutional review board.

Data collection

Skin-prick tests were performed only on the asthma cases at initial assessment to differentiate atopic from non-atopic asthmatics using various antigens, including 2 types of house-dust mite, cat and dog epithelial cells and mould and pollen antigens (Omega), together with negative (saline) and positive (0.5% histamine hydrochloride) controls. Wheal size was measured after 15 minutes [10]. A positive reaction was defined as a wheal larger than 3 mm [11]. Children were considered atopic if they had at least 1 positive skin-prick test response.

Pulmonary function tests such as FEV1, peak expiratory flow rates (PEF%, PEF25%, PEF50% and PEF75%) were done for both cases and controls at the initial assessment as part of diagnosis and after 1 month of controller medications as an evaluation tool for the degree of control. It was performed by a bodyplethysmograph (Master Screen Body) for measurement of static and dynamic pulmonary functions.

Blood samples were taken for complete blood count and determination of peripheral eosinophil counts for both cases and controls. The s-ECP assay was also done for both cases and controls (Immulite ECP, for use on the Immulite and Immulite1000 systems, Siemens) [12].

Statistical analysis

SPSS, version 12.0, was used for all analyses. Descriptive data included means and standard deviations (SD) in addition to median values. Non-parametric statistical tests were used including Mann–Whitney U-test for comparison of numerical variables and Spearman test for correlations. *P*-values < 0.05 was considered statistically significant.

Results

Background characteristics

During the study period 62 children newly presented with asthma to the

outpatient clinic of the Allergy and Respiratory Unit; 12 of them refused to participate in the study, 10 did not meet the inclusion criteria and 2 were lost to follow-up after starting controller medication. Thus, 38 newly presented asthmatic children were enrolled in the study (19 males and 19 females), with a mean age of 10.3 (SD 1.9) years. Based on their response to controller medication and GINA criteria they were divided into controlled (16, 42.1%) and partially controlled asthma cases (22, 57.9%). They were compared with the 16 healthy control children.

The PFTs showed significantly lower values in all parameters (FEV1, PEF%, PEF25%, PEF50%, PEF75%) in the total group of asthma cases compared with healthy children. Also, significantly lower PFT values were found for the same parameters in partially controlled compared with controlled asthmatics (Table 1).

Eosinophil levels

The total group of asthma cases had a significantly higher peripheral eosinophil count compared with the healthy control group [mean 627.4 (SD 103.4) versus 371.5 (SD 34.3) cells/mm^3 respectively] and also a higher s-ECP level than healthy children [mean 51.8 (SD 47.8) versus 13.8 (SD 3.26) ug/L respectively] ($P < 0.001$) (Table 2). The same was observed comparing the partially controlled asthma cases with the healthy children for peripheral eosinophil count [mean 854.2 (SD 92.1) versus 371.5 (SD 34.3) cells/mm^3 respectively] and s-ECP level [mean 56.2 (SD 57.2) versus 13.8 (SD 3.3) ug/L respectively] ($P < 0.001$).

On the other hand, there were non-significant differences comparing controlled asthma cases with healthy control children for both eosinophil count [mean 396.9 (SD 45.6) versus 371.5 (SD 34.3) cells/mm^3] and s-ECP level [mean 20.2 (SD 19.9) versus 13.8 (SD 3.26) ug/L respectively]. There were also significantly higher eosinophil counts in partially controlled asthma cases compared with controlled asthma cases [mean 854.2 (SD 92.1) versus 396.9 (SD 45.6) cells/mm^3] and s-ECP [mean 56.2 (SD 57.2) versus 20.2 (SD 19.9) ug/L respectively] ($P < 0.05$) (Table 2).

Testing the correlations of both eosinophil counts and s-ECP levels with degree of asthma control, we

Table 1 Background characteristics and respiratory parameters of the study subjects

Variable	Healthy controls (n = 16)	Total asthma cases (n = 38)	Partially controlled asthma (n = 22)	Controlled asthma (n = 16)
Males/females (no.)	6/2	19/19	10/12	9/7
Mean (SD) age (years)	10.8 (2.5)	10.3 (1.9)	10.0 (2.1)	10.0 (1.9)
Mild/moderate asthma (no.)	n/a	21/17	12/10	9/7
Mean (SD) duration of symptoms[a] (months)	n/a	7 (6)	7 (3)	8 (1)
Respiratory parameters	***Mean (SD)***	***Mean (SD)***	***Mean (SD)***	***Mean (SD)***
FEV1 (mL)	90.1 (11.5)	75.6 (11.2)[c]	62.5 (13.5)[c,d]	88.8 (9.7)
PEF% (mL)	87.7 (4.6)	75.3 (10.7)[c]	63.8 (18.3)[c,d]	87.7 (4.6)
PEF25% (mL)	83.7 (22.4)	62.7 (9.6)[c]	51.8 (14.4)[c,d]	73.7 (7.1)[b]
PEF50% (mL)	118.1 (35.2)	62.6 (13.7)[c]	48.4 (13.9)[c,d]	76.9 (13.6)[c]
PEF75% (mL)	111.1 (23.1)	51.6 (13.7)[c]	37.1 (17.8)[c,d]	66.3 (9.1)[c]

[a]Duration of asthma symptoms before presentation.
[b]P < 0.05 versus healthy controls; [c]P < 0.001 versus healthy controls; [d]P < 0.001 versus controlled asthma cases (non-parametric statistics).
SD = standard deviation; n/a = not applicable; FEV1 = forced expiratory volume in 1 s; PEF% = peak expiratory flow.

Table 2 Eosinophil counts and serum eosinophilic cationic protein (ECP) levels of the study subjects

Variable	Healthy controls (n = 16) Mean (SD) [95% CI]	Total asthma cases (n = 38) Mean (SD) [95% CI]	Partially controlled asthma (n = 22) Mean (SD) [95% CI]	Controlled asthma (n = 16) Mean (SD) [95% CI]
Peripheral eosinophil count (cells/mm^3)	371.5 (34.3) [53.5–489]	627.4 (103.4) [221–1272][a]	854.2 (92.1) [467–1710][a,b]	396.9 (45.6) [89–734]
Serum-ECP level (µg/L)	13.8 (3.3) [7.9–19.7]	51.8 (47.8) [17.1–91.2][a]	56.2 (57.2) [33.3–95.9][a,b]	20.2 (19.9) [10.8–28.7]

SD = standard deviation; CI = confidence interval.
[a]P < 0.001 versus healthy controls; [b]P < 0.001 versus controlled asthma cases (non-parametric statistics).

Table 3 Non-parametric correlation of eosinophil counts and serum eosinophilic cationic protein (s-ECP) levels of asthma patients versus healthy controls

Variable	Spearman correlation coefficient (*r*)	*P*-value
Peripheral eosinophil count (cells/mm^3)	–0.60	< 0.001
Serum-ECP level (µg /L)	–0.75	< 0.001

found a significant inverse correlation in both parameters using the Spearman non-parametric correlation test (r = –0.60 and –0.75 respectively, P < 0.001). Thus, higher eosinophil counts and s-ECP were correlated with poorer asthma control, with a higher correlation for s-ECP than eosinophil count (Table 3).

Discussion

Direct measurement of airways inflammation using biological markers could potentially refine asthma management. This explains the current research interest in measuring levels of exhaled nitric oxide and eosinophil granule proteins especially s-ECP in asthma [13].

This study revealed that both peripheral eosinophil count and s-ECP levels were significantly higher in atopic asthmatics as a group than in healthy control subjects. On the other hand, both parameters were significantly higher among partially controlled asthma cases compared with healthy control children as well as controlled asthma cases. Interestingly, however, controlled asthma cases showed non-significant changes in the levels of both parameters versus healthy control children.

These higher levels of s-ECP and eosinophil counts in children with uncontrolled asthma may suggest that eosinophil-mediated inflammation is important to investigate in assessing asthma control and in deciding treatment regimens. This finding is supported by the evidence that eosinophils play an important role in the pathogenesis of asthma and that elevation of peripheral blood eosinophil count is a risk factor for the development of airway remodelling and irreversible changes in lung function [14]. This is also supported by the research of Lee et al. who reported that higher levels of s-ECP were associated with more severe exacerbation of asthma followed by a decrease in s-ECP levels with resolution of symptoms [15].

Our work also showed a significant inverse correlation between level of asthma control and both parameters, particularly s-ECP, implying that poorer control is expected with higher s-ECP levels. This will add to the work of Koh et al., who described a correlation between asthma severity and s-ECP level. Thus, considering that s-ECP has been widely investigated as a potential biomarker of airway inflammation, it may have a useful role to play as a control parameter in asthma guidelines [16].

In conclusion, despite the small sample size, this study has demonstrated that s-ECP and peripheral eosinophil counts may have clinical usefulness in assessing levels of asthma control and hence in refining asthma management.

Based on these findings, we recommend conducting a larger, randomized controlled trial to evaluate the correlation between s-ECP level and degree of asthma control and to obtain a cut-off point for s-ECP beyond which a patient may be considered uncontrolled.

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