Original article

Assessment of plasma ECP levels in Saudi patients with chronic asthma as a marker of asthma exacerbation during sandstorms

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Abstract

Background and Aims

Eosinophils are a key player in allergic inflammation. ECP and IL-4 are essential mediators of allergic inflammation in the lungs and therefore can be used to help in the diagnosis and follow-up of allergic asthma. This study aimed to evaluate whether ECP and IL-4 levels in the plasma of asthmatic patients may reflect the clinical status of asthma exacerbations during sandstorms.

Methods

Thirty patients had allergic asthma, and sixteen normal controls were enrolled in this study. These subjects were divided into two groups. The first group included seventeen patients who had chronic symptoms of asthma but were controlled, and the second group included thirteen patients with chronic symptoms who developed exacerbations during sandstorms. Total IgE, ECP, and IL-4 levels were measured in the plasma of asthmatic patients and normal controls by using specific ELISAs.

الملخص

الخلفية:

تلعب خلايا الحمضات دور رئيسي في التهابات الحساسية، ويعد البروتين الحمضي الموجب ECP و IL-4 عاملان أساسيان للالتهاب التحسسي في الرئتين وبالتالي يمكن استخدامهما للمساعدة في تشخيص ومتابعة الربو التحسسي. تهدف هذه الدراسة إلى تقييم ما إذا كانت مستويات ECP و IL-4 في بلازما مرضى الربو قد تعكس الحالة السريرية للمرضى في تفاقم الربو أثناء العواصف الرملية.

طريقة البحث

في هذه الدراسة تم جمع ثلاثون عينة من مرضى يعانون من الربو التحسسي وستة عشر من المجموعة الضابطة من الأصحاء. تم تقسيم مرضى الربو إلى مجموعتين. المجموعة الأولى تحتوي على عدد سبعة عشر مريض يعانون من أعراض الربو المزمنة لكنهم تحت السيطرة، والمجموعة الثانية يبلغ عددها ثلاثة عشر مريض يعانون من أعراض الربو المزمنة وتفاقمت خلال العواصف الرملية. تم قياس إجمالي مستويات IgE، ECP و IL-4 في بلازما مرضى الربو من المجموعة الضابطة باستخدام طريقة المقايسة المناعية للأنزيم المرتبط الأليزا (ELISA) .

النتائج

أظهرت الدراسة مستويات IgE الكلية أعلى بكثير في المرضى الذين يعانون من الربو (متوسط = ٧٦٤ وحدة دولية/مل ، P= ٠،٠٠٠١) مقارنة بالمجموعة الضابطة (١١٣ وحدة دولية/مل) .

Results

Total IgE levels were significantly higher in patients with asthma (mean= 467 IU/ml, P = 0.0001) than in controls (113 IU/ml). IL-4 was not detected in patients with asthma or in controls. ECP levels were elevated significantly in patients with severe symptoms of asthma (mean= 3.28 ng/ml, P=0.0006) in comparison to those in normal controls or patients with controlled asthma (1.9 ng/ml, P=0.02). In addition, there was no significant increase in ECP levels in patients with controlled asthma relative to those in normal controls.

Conclusion

These findings suggest that ECP but not IL-4 may be a useful marker to evaluate the severity of asthma, follow and predict a response to steroid treatment.

Key words:

Asthma, IgE, ECP, IL-4, Eosinophils

لم يتم قياس الانترلوكين -٤ في المرضى الذين يعانون من الربو أو في المجموعة الضابطة . مستويات البروتين الحمضي الموجب ECP مرتفعة بشكل ملحوظ في المرضى الذين يعانون من اعرض الربو الحادة = ٣,٢٨ نانوجرام / مل ، P = 0.0006 (بالمقارنة مع المجموعة الضابطة أو المرضى الذين يعانون من الربو المتحكم به)٩.١ نانوجرام / مل 0.02 ، = P بالإضافة إلى ذلك، لم تكن هناك زيادة كبيرة من ECP في المرضى الذين يعانون من الربو تسيطر عليها نسبة إلى المجموعة الضابطة .

الخلاصة

تشير هذه النتائج إلى أمكانية استخدام PCE وليس LI-٤ كمؤشر لتقييم شدة الربو، متابعة والتنبؤ بالاستجابة للعلاج بالستيرويد

الكلمات المفتاحية:

الربو ، الجسم المضا -E البروتين الحمضي الموجب ، الأنترلوكين - ٤، خلايا الحمضات

Introduction

Allergic asthma is a chronic inflammatory disease of the lungs that is characterized by various symptoms, including coughing, wheezing, shortness of breath, and chest tightness. Asthma disease is considered one of the most common chronic diseases, with an increasing prevalence rate in Saudi Arabia[1]. Asthma exacerbations can be activated by several factors, such as respiratory tract infection, exercise, and exposure to allergens or irritants, such as detergents, smoke and dust [2]. During sandstorms, asthmatic patients are at high risk of developing severe symptoms [3].

Allergic asthma is known to be mediated by IgE, which can play an important role in allergic inflammation in the airways [4]. Asthma is a complex disease, and several immune and nonimmune cells and molecules contribute to the development of asthma. Along with other inflammatory

cells, eosinophils mediate a key role in asthma. Infiltration of eosinophils into the bronchial wall results in the release of inflammatory mediators, such as eosinophil cationic protein (ECP), and degranulation of this protein can cause injury to the airway epithelium, induce the production of histamine from mast cells, narrow the airway smooth muscle and enhance airway hyperresponsiveness[5]. Increased levels of ECP in serum, sputum and bronchoalveolar lavage fluid (BALF) have been observed in asthmatic patients relative to those in normal controls, and measuring this protein has been suggested for use as a marker to monitor and evaluate airway inflammation in asthma [6, 7].

Th2 cytokines, such as IL-4, have an essential role in the development of asthma. IL-4 is capable of inducing B cell class switching to IgE, and overproduction of this cytokine is linked with the development of asthma [8]. IL-4 exerts its biological function through binding to its receptor (IL-4R), which is expressed on the surface of various cell types [9]. Eosinophils express the receptor IL-4R, and their eosinophilic granulocytes have been shown to contain significant levels of IL-4[10]. Furthermore, IL-4 was shown to be important in facilitating infiltration of eosinophils into the airways, immunoglobulin E (IgE) production and increased airway responsiveness in a murine model of allergen-induced airway inflammation [11]. Targeting IL-4R with a humanized monoclonal antibody (dupilumab) in persistent eosinophilia patients is correlated with a reduction in exacerbations and enhancements in lung function and control of asthma [12, 13]

From the abovementioned results, factors that activate eosinophils may contribute to airway dysfunction and tissue remodeling in asthma and therefore are essential mediators in asthma pathology.

This study is designed to determine the airway inflammation in bronchial asthma through measurement of ECP and IL-4 in the plasma of asthmatic patients as possible markers for airway inflammation to evaluate clinical status during sandstorms.

Materials and Methods

Subjects:

Thirty patients with allergic asthma and sixteen normal controls were included in this study. Samples were collected from two groups of patients with asthma. The first group consisted of patients with chronic asthma that was controlled, and the second groups were asthmatic patients with chronic symptoms who developed severe exacerbations during sandstorms and were admitted to an emergency department at Alzulfi General Hospital in

the Kingdom of Saudi Arabia. All the patients were examined and diagnosed by a respiratory consultant at Al Zulfi General Hospital. The clinical characteristics of all subjects are shown in Table 1. Normal controls were recruited from healthy blood donors at the blood bank at Alzulfi General Hospital. Normal controls were selected and defined as those not having any of the following: asthma, wheezing, coughing, smoking, recent vaccination, respiratory tract infection, chronic diseases, history of allergy, allergic rhinitis, atopic dermatitis and usage of treatments, especially corticosteroids. All subjects in this study provided written informed consent, and the Majmaah University Institutional Review Board approved the study (MUREC-April.01/COM-206).

Table 1: Characteristics of asthmatic patients and clinical presentations

Categories	Normal control	Chronic asthma - controlled	Severe exacerbations
Number of subjects	16	17	13
Sex (male/female)	10/6	8/9	10/3
Mean age	35.05	34.17	39.66
Treatment usage			
LABA	-	17	12
SABA	-	17	6
ICS	-	15	8
Oral steroid	-	2	4
Disease duration	-	6 – 20 years	1 – 4 days
Clinical presentations			
Shortness of breath	No	No	Increased
Cough	No	Asymptomatic coughing	Continuous
Wheezing	No	Asymptomatic wheezing	Frequent and loud
Chest tightness	No	No	Present
Mucus production	Not present	Present	Increased
Respiratory tract infection	Not present	Not present	Not present
Speaking	Normal	Normal	Difficult
Mean heart rate per minute	96.04	95.07	110.03
Mean O2 saturation %	95.06	92.02	90.1

.ICS: inhaled corticosteroid; LABA: long-acting beta agonist; SABA: short-acting beta agonist.O2; oxygen

Isolation of Plasma from blood:

Vein puncture was conducted in the subjects of the study, including the normal controls and patients with asthma. Following collection, peripheral blood was transmitted directly to tubes containing the anticoagulant EDTA. After that, the blood was centrifuged at 1800 rpm for 5 minutes at room temperature. After centrifugation, plasma at the top layer was collected and then placed into aliquots in small tubes and frozen at -20°C.

Detection of total IgE, ECP and IL-4 protein levels:

A specific ELISA technique was used to measure protein concentration in the plasma obtained from normal controls and patients with asthma. Total IgE levels were measured using quantikine ELISA assays [Abcam, UK, cat# ab108650]. Human interleukin 4 (IL-4) cytokine [R&D, UK, and cat# DY204] and RNASE3 (ECP) [Abbexa, UK, cat# abx055137] were measured using quantikine ELISAs. ELISAs were carried out as described in the manufacturer's instructions for each assay. The lower detection limit for the IL-4 ELISA was 31 pg/ml and for ECP (1.5 ng/ml). The optical density (OD) was measured using a microplate reader (BioTek ELx800) at 450 nm. Software (KC Junior) was used to calculate the protein concentration for each protein of interest.

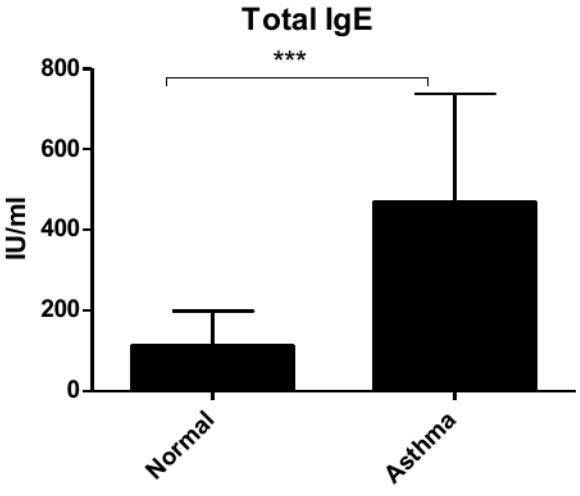
Statistics:

Data from the study are expressed as the mean ± standard deviation (SD), and an independent nonparametric sample test (Mann-Whitney U test) was used to calculate statistical significance using GraphPad Prism 6 software. P-values indicate significance (* for $p<0.05$, ** for $p<0.001$ and *** for $p<0.0001$).

Results

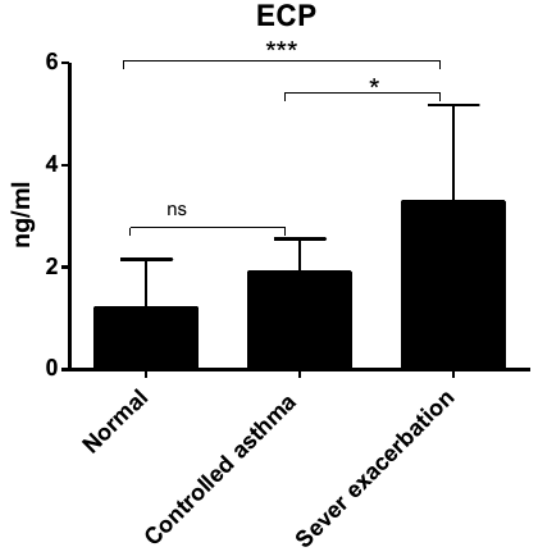
Total IgE levels were elevated significantly in all asthmatic patients [mean 467 IU/ml, P=0.0001] compared to those in normal controls [113 IU/ml] (figure 1).

Figure 1: Plasma levels of total IgE. Total IgE levels were measured by using a specific ELISA. Total IgE levels were elevated significantly in asthmatic patients compared to those in normal controls. Data are expressed as the mean ± standard deviation (SD). P-values indicate significance .(for p<0.0001 ***)



ECP levels were elevated significantly in patients with severe symptoms of asthma (mean= 3.28 ng/ml, P =0.0006) in comparison to those in normal controls or patients with controlled asthma (1.9 ng/ml, P=0.02). In addition, there was no significant increase in ECP levels in patients with controlled asthma relative to those in normal controls (figure 2).

Figure 2: Plasma levels of ECP. ECP levels were measured by using a specific ELISA. ECP levels were elevated significantly in asthmatic patients with severe exacerbations compared to those in normal controls as well as those in patients with controlled asthma. Data are expressed as the mean ± standard deviation (SD). P-values indicate significance .(for p<0.05 and *** for p<0.0001 *)



Discussion

This study aimed to assess plasma levels of ECP and the IL-4 cytokine as possible markers in asthma execrations during sandstorms. Allergic asthma is characterized by elevated IgE levels. To further ensure that samples received were taken from allergic patients, total IgE levels were measured. As expected, a significant increase in the total IgE level was noticed in all asthmatic patients compared to that in normal controls (figure 1).

Several studies have established that in asthma, serum levels of ECP reflect the activity of eosinophil cells and the severity of disease [14, 15]. In the current study, ECP levels were increased significantly in all asthmatic patients who were on ICS treatment relative to those in normal controls, and patients with severe exacerbations had higher levels of ECP than patients with controlled asthma or normal controls (figure 2). However, it has been shown that ECP levels are reduced during treatment with ICSs and therefore can be used to evaluate the response to steroid treatment[16, 17].

Taken together, these findings suggest that high doses of ICSs are necessary to manage or prevent asthma exacerbations, as not all asthmatic patients respond equally to ICSs [18], and evaluation of ECP may reveal a useful marker to evaluate disease exacerbation during sandstorms. On the other hand, ECP does not necessarily represent a specific marker of asthma exacerbations, as this protein can be measured in several inflammatory conditions; therefore, assessment of ECP levels in patients

diagnosed with asthma may be used for further evaluation of the extent and severity of inflammation in asthma [19, 20].

Furthermore, IL-4 levels in plasma were found to be positively correlated with the lung function and severity of asthma and therefore can be used as a potential marker for the evaluation of disease [21]. However, in the current study, there was no detection of IL-4 in the plasma of either normal controls or patients with asthma. This result could be explained as follows: the lower detection limit of IL-4 in the ELISA was approximately 31 pg/ml; therefore, IL-4 was not detected either because its levels were very low or because IL-4 is not produced by healthy subjects. Additionally, ICSs have been reported to inhibit the gene expression of IL-4 [22], which may explain why there was no detection of IL-4 in asthmatic patients since the patients were using ICSs during the experiment. Thus, IL-4 measured in the plasma of asthmatic patients cannot be used as a marker to evaluate the severity of disease during exacerbation.

The limitations of the current study are that patients with high plasma levels of total IgE were not evaluated for further evidence of parasitic infection or allergic bronchopulmonary aspergillosis; therefore, a further study should determine whether increased levels of total IgE are a result of allergic reaction and not due to parasitic infection or allergic bronchopulmonary aspergillosis. Furthermore, the analyses of ECP and IL-4 in lung secretions, such as sputum or BALF, were outside the scope of this study, and therefore, subsequent studies should address the possibility that lung cytokine expression is a good indicator of disease activity.

In conlusion, IL-4 in plasma may not be a useful diagnostic biomarker to evaluate airway inflammation and monitor asthma severity during sandstorms, especially if the patients have used ICSs. However, ECP measured in the plasma may represent a useful diagnostic marker for asthma exacerbation.

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Conflict of interest

The author has no conflicts of interest to declare.

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