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The relationship of eosinophil cationic protein / eosinophil count ratio and disease severity in allergic asthma patients

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Abstract

Finding an applicable clinical biomarker in body fluids can be useful in asthma management. This study was conducted to support the investigations of probable immunological changes in asthmatic patients that may be useful therapeutic indicators. 61 matching patients and 23 apparently healthy individuals were included. Significant elevation in the level of eosinophil cationic protein (ECP) in asthmatics was found as compared with healthy controls, so as among asthma severity categories. The receiver operating characteristic curve analysis determined 29 ng/ml of serum ECP as the typical cut-off value of significant denotation for differentiation between asthmatic patients and healthy individuals, significant positive linear correlation between both ECP level and also ECP/Eosinophil ratio with clinically estimated asthma severity were observed. The median of serum total IgE and rate of 100 IU/ml overlapped serum total IgE individuals were significantly higher in asthmatics than healthy controls and so as among asthma severity categories, whereas no significant correlation between total serum IgE level and asthma severity was found. According to the results, determination of serum ECP can be considered as a diagnostic tool to distinguish asthma cases, besides it reflects the severity of the disease significantly, whereas calculation of ECP/Eo ratio may be an effective marker in reflecting asthma severity and the activity of eosinophils during the inflammation which may be useful in asthma control and in the assessment of the disease severity as well as the response to anti-inflammatory therapy, but it has no benefit for diagnosing asthma as no significant differences were recorded between asthmatics and healthy controls.

Keywords: Asthma, eosinophil cationic protein, ECP/Eo.

Introduction

Asthma is defined as a heterogeneous disease with a long-term inflammatory disorder of the airways as well as variant remodeling where several inflammatory cells take a part (1,2). This inflammation is linked with different manifestations like wheeze, cough, bronchial hyper responsiveness and bronchoconstriction in response to different types of specific stimuli such as allergens, viruses and exercise (3).The discovery of immunoglobulin (E) helped to understand the mechanism of occurrence of allergy, which is the main cause of the pathogenesis of allergic asthma (4). However, this was not enough alone to comprehend the pathophysiology of this disease, as studies still confirm the role of many inflammatory cells and cytokines secreted by inflammatory as well as airway structural cells (5,6).

It has become known that the eosinophils are among the most prominent inflammatory cells which involve in mediating the immune response during asthma (7), there are many evidences that indicate the distinctive role of these cells in the bronchial epithelial tissue damage due to their ability to release many inflammatory mediators and cytokines that contribute to recruitment and activation of cells at the site of inflammation, among the most important of these mediators is the eosinophil cationic protein ECP (8).

ECP exists in the matrix part of the eosinophil within specific granules and it forms one of their main components, it is highly cationic protein that belongs to the ribonuclease superfamily and has a highly toxic effect on the airways epithelial cells (9,10).

Study (11) was one of the first published studies on ECP, the study suggested that the quantitative estimation of ECP concentration in serum may be advantageous in assessing the activity of eosinophils *in vivo*. Afterwards,

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several studies were done to determine the levels of ECP in serum, plasma, sputum, saliva and bronchoalveolar lavage fluid (BALF) for allergic patients, but estimation of serum ECP was considered to be the most useful biomarker by Koh *et al*. as it combines ease and efficiency (12–15).

Direct assessment of asthma associated airway inflammation by biomarkers can have a positive impact on both asthma diagnosis and therapy strategies, thus estimation of ECP levels in body fluids of asthma patients can be a useful biomarker for the eosinophils activity, and so can take a part in assessing disease severity and estimating response to anti-inflammatory therapy (12,13,16).

Moreover, global studies have indicated that determination of the ratio of serum ECP to eosinophils count ECP/Eo may give a better accurate indication of eosinophils activity during asthma, as it represents the amount of ECP per cell and so gives a comparable picture about eosinophils activation status among patients (13,17,18).

In view of the scarcity of local studies concerned with this aspect, the current study aimed to determine the level of ECP and other biomarkers in the serum of Iraqi asthma patients and studying the advantage of these markers in diagnosis and assessment of the disease severity.

Materials and Methods

Subjects

In this observational case-control study, the patient group included 61 patients with asthma who visited AL-Zahra Consultant Center for Allergy and Asthma in Baghdad. All of the subjects gave informed consent prior to their participation in the study and then submitted to a general questionnaire to find out if they are eligible to be enrolled in the study. Patients aged 13-60 years were selected from newly diagnosed cases came to the center for the first time, or from patients who had discontinued treatment with steroids and antihistamines for a period that allowed for conducting the study tests without interference according to a specialist physician instructions. The disease was diagnosed by the specialist based on the patient's medical history, clinical examination and some common laboratory tests, and then the patients were classified into four groups of asthma severity and subjected to a questionnaire (2) Patients with any bacterial or parasitic infections were excluded from the study; this was verified by the specialist physician as well as via performing some tests such as general urine and stool.

Control Subjects

This group consisted of 23 healthy people, in line with the group of patients in terms of age and gender. Patients who did not have any history related to asthma were selected, and they were also confirmed to be free of symptoms and clinical signs of any other allergic and parasitic diseases in the same way as the patients' group.

Samples Preparation

Blood was collected from eligible patients & samples then transferred to Becton Dickinson serum separation tubes, BD Vacutainer®SST™ tubes (4ml) and EDTA tubes (2ml), for serum ECP, IgE & eosinophil count respectively. All collected specimen aliquots were processed in a standardized format. In summary, SST tubes samples were inverted gently five times to disperse and prompt the clotting activator. Thereafter, tubes were spun after 1hr of clotting time at 3000rpm for 15 min in a cooled centrifuge; serum was aliquoted into Eppendorf tubes and frozen at -20 C° for further analysis.

Estimation of ECP & total IgE serum levels

Levels of ECP in serum were measured by enzyme-linked immunosorbent assay (ELISA) technique using MESACUP ECP test kit (Cat. No. 7618E, MBL Co., Ltd., Japan) according to the manufacturer's instructions (19), it is a quantitative assay kit for the measurement of human ECP level in serum and urine by sandwich ELISA method which measures human ECP with a minimum detection limit of 0.125 ng/ml and does not crossreact with eosinophil derived neurotoxin EDN. For

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estimation of serums total IgE levels, an ELISA kit was used (EV 3840-9601 E, Euroimmun, Germany), and the test had been performed according to the manufacturer's instructions (20).

Statistical analysis

The statistical analysis of the study results was carried out using version 20 of the Statistical Package for Social Sciences (SPSS), $p<0.05$ was considered statistically significant. Significance was calculated by Mann-Whitney and Kruskal-Wallis between two groups and among groups respectively. Chi-Square test was used for variable that have categories. The optimal cut-off value for serum ECP was determined by Receiver Operating Characteristic (ROC) analysis of sensitivity and specificity at different cut-off values (21).

Results and discussion

The study outcomes revealed that the median of serum total IgE in asthmatic was 139 IU/ml, while this mean was 29 IU/ml in the control group, the difference was significant between the two groups ($p < 0.001$), table 1. On the other hand, serum total IgE levels showed no significant differences with the disease severity ($p=0.1$) as shown in table 2.

It was found that serum IgE levels that exceed 100IU/ml could be a good indicator of developing allergy (22). In line with this, our results revealed that 57.4% of asthmatics in this study have passed the mentioned value, while only 4.3% of the control group have concentration of IgE that is above 100IU/ml with a significant difference between the two groups ($p<0.001$), table3. In addition, this study also found a significant difference between the frequencies of asthma patients who have more than 100 IU/ml of IgE in their serum and the asthma severity categories,table 4.

The results of the present study were consistent with previous researches that had found a significant increase in serum IgE levels in asthmatic patients as compared with controls both locally (20,23,24) and worldwide (25,26). With regard to asthmatic patients with higher IgE levels that exceed 100IU/ml, this study results comes in parallel with other previous studies that linked this incline to atopy and associated allergic asthma, it was found that 60%-74% of asthmatics have IgE levels of more than 100IU/ml (22,27), which highlights its role in the pathogenesis of allergic asthma (20,24).

In relation to asthma severity and associated IgE levels, our result agrees with (28) who found that serum IgE levels do not differ significantly with the variation of asthma severity. Yet, (22) has found a significant relationship between asthma severity and IgE concentration (22), this variation may due to many factors like patients age range, gender, smoking status and environmental factors (29).

It will be recalled that asthma is an inflammatory disorder where many cells and components take part via several mechanisms. Consequently IgE role alone may not be the most essential that determines asthma severity. Thus, it could be stated that high levels of total IgE antibody can reflect the presence of the common allergic condition in many asthmatic patients, and may vary particularly in the severe cases of the disease.

Table 1: Difference in serum total IgE levels between asthmatics and controls

Serum Total IgE IU/ml	Asthmatics	Controls
Range	**(5-889)**	**(5-118)**
Median	**139**	**29**
Number	**61**	**23**
(Mann-Whitney) p<0.001		

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Table 2: Difference in serum total IgE levels among different clinically assessed disease severity groups

Serum total IgE	Mild intermittent	Mild persistent	Moderate persistent	Severe persistent
Range	(5-853)	(12-437)	(15-380)	(21-898)
Median	125	51	204	484
Number	34	10	9	8
(Kruskall-Wallis)p=0.1 non-significant				

Table 3: Difference in the frequency distribution of asthma patients and controls with serum total IgE >100IU/ml

	Total number	>100IU/ml	%
Asthmatics	61	35	57.4
Controls	23	1	4.3
($\chi 2$) p<0.001			

Table 4: Difference in frequency distribution among asthma patients with total IgE levels greater than 100 IU/ml from different clinically assessed severity groups

Severity	Total number	>100IU/ml number	%
Mild intermittent	34	18	52.9
Mild persistent	10	3	30.0
Moderate persistent	9	7	77.8
Severe persistent	8	7	87.5
($\chi 2$)p<0.049			

The present study aimed to measure the concentration of ECP in the serum of Iraqi asthma patients and its relationship to disease severity. Serum ECP of asthmatic patients ranged between 2.5 to 185 ng/ml, while it varied between 2.4-40 ng/ml in the control group, the median of ECP concentration was 40 ng/ml and 15.5 ng/ml in the patient and control group respectively, the differences were significant between the two groups ($p < 0.001$), as shown in Table 5.These results agrees with earlier studies that found a significant increase in asthmatics serum ECP levels as compared with controls both in children (30,31) as well as in adult asthma patients (12,32) .

The results in table 6 reveals a significant increase in the median of ECP concentration which is directly proportional to the increase in the severity of the disease, where the median of serum ECP increased significantly from 33.25ng/ml in mild intermittent patients to 132.5 in severe persistent asthmatics category, these outcomes comes in parallel with previous researches where an association between asthma severity and serum ECP levels was found (19,33,34).

Table 5: Difference in ECP levels between asthmatics and controls

Serum ECP ng/ml	Asthmatics	Controls
Range	(2.5-185)	(2.4-40)
Median	40	15.5
Number	55	21
(Mann-Whitney) p<0.001		

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Table 6: Difference in serum ECP levels in different clinically assessed disease severity groups

Serum ECP ng/ml	Mild intermittent	Mild persistent	Moderate persistent	Severe persistent
Range	(2.5-67.5)	(9-65)	(7-170)	(32.5-185)
Median	33.25	38	55	132.5
Number	30	8	9	8
(Kruskall-Wallis)p=0.01				

The study was concerned with determining the cut-off value of serum ECP in order to identify the possibility of adopting it as a diagnostic marker to differentiate between asthmatics and healthy individuals, given that there is a lack in previous local studies in this regard. According to the ROC analysis, the concentration of 29 ng/ml was selected as the cut-off value that gave the highest specificity of the test 90% along with the highest sensitivity of 69%, by applying this value 18 out of 22 controls gave negative results for the test, while 37 individuals, which is equivalent to more than two-thirds of the 55 patients gave positive results for serum ECP test, Fig.1.

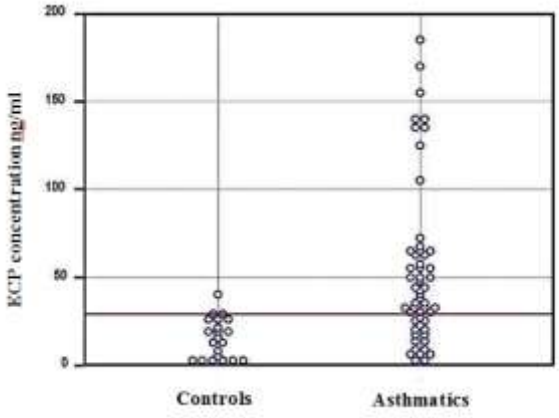

Figure 1: The application of ECP cut-off value of 29ng per mil (in red), sensitivity=69.1%, specificity = 90.5%

The ECP/Eosinophils ratio could reflect the intensity of these cells activation during asthma inflammation, since it represents the number of ECP molecules per peripheral eosinophil, which is considered as a potential indicator of eosinophil activation that may increase by the induction of ECP release during inflammation (17). In this context, there was an increase in this ratio between asthmatics and controls despite not reaching the significance (table 7), the study found a significant increase in this ratio according to the severity of the disease, table 8. These outcomes agrees with other studies results which showed a similar significant incline of this ratio in relation to asthma severity, and that it could be the most accurate and qualitative indicator than ECP or total eosinophil counts alone in indicating both eosinophil activity and assessment of asthma severity (17,35). To be mentioned, the non-significant difference of this ratio between the patients and control groups may due to that there was not an increase in the number of eosinophil in the blood of some patients, with a slight increase in serum ECP of some controls on the opposite side based on the test results. In addition, it was found that The ECP/Eo ratio has a wide range in healthy subjects (35).

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Table 7: Difference in ECP/Eo ratio between asthmatics and controls

ECP/Eo (pg/cell)	Asthmatics	Controls
Range	(0.008-0.124)	(0.014-0.214)
Median	0.129	0.094
Number	55	21
(Mann-Whitney) p=0.06 non-significant		

Table 8: Difference in ECP/Eo in different clinically assessed disease severity groups

ECP/Eo (pg/cell)	Mild intermittent	Mild persistent	Moderate persistent	Severe persistent
Range	(0.008-0.488)	(0.038-0.625)	(0.016-0.394)	(0.192-0.648)
Median	0.095	0.095	0.138	0.381
Number	30	8	9	8
(Kruskall-Wallis)p=0.006				

Among different serum markers, the study could find that only ECP and ECP/Eo ratio have a positive correlation with asthma severity, table 9. This finding comes in parallel with earlier researches that reported a positive correlation between ECP levels and the severity of asthma, besides that ECP/Eo ratio was considered the best immunological marker for asthma severity (17); considering its importance in reflecting active eosinophils, which could better correlate with the severity of asthma, than each of these markers alone.

Table 9: Correlation coefficients between asthma severity and different markers

Immunological marker	r
Serum total IgE	0.16
Blood eosinophil count	0.01
Serum ECP	0.41**
ECP/Eo ratio	0.43**
**p<0.01	

Based on the above, it could be stated that the determination of the level of ECP in the serum can be used to detect the presence or absence of asthma, and then it reflects the severity of the disease significantly. However, the calculation of the ECP/Eo ratio could be considered as a better accurate and specific marker of peripheral activated eosinophils and it correlates with the severity of the disease better than ECP or circulating eosinophils individually, which can be a useful tool in assessing the disease severity and estimating the response to anti-inflammatory therapy in order to control asthma. Further studies with larger sample sizes are needed to draw a firm conclusion, besides applying these markers in asthma therapy and a follow up plan at periodic intervals for Iraqi allergic asthma patients.

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العلاقة بين نسبة ECP/ Eo وشدة المرض لدى مرضى الربو الأرجي

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الخلاصة

اجريت هذه الدرسة للتحري عن التغيرات المناعية المحتملة لدى مرضى الربو والتي قد يكون من الممكن الاستفادة منها للأغراض العلاجية ،١٦ مريضا و ٢٣ فردا من الاصحاء قد ضمتهم الدراسة، سُجل وجود ارتفاع معنوي في مستوى بروتين الحمضة الهابطي ECP لدى مرضى الربو بالمقارنة مع مجموعة السيطرة، كذلك الحال مع الزيادة في شدة المرض. اثبتت نتائج اختبار منحنى تشغيل المستقبل ROC ان تركيز ٢٩ نانوغرام\مل ل ECP في المصل يمثل القيمة فاصلة cut-off value ذات الأهمية الأستدلالية للتفريق بين مرضى الربو و الأصحاء، كما توصلت الدراسة الى وجود علاقة خطية موجبة ذات قيمة معنوية بين تركيز ECP و كذلك نسبة ECP\Eo مع الأختلاف في شدة المرض المقيمة سريرياً، كما لوحظ وجود ارتفاع معنوي في نسبة المرضى الذين يفوق تركيز الكلوبيولين المناعي (E) في مصولهم ١٠٠ وحدة عالمية\مل عنه لدى مجموعة السيطرة كما هو الحال بين المجاميع المختلفة من شدة المرض، لم يثبت وجود علاقة معنوية بين تركيز الكلوبيولين المناعي(E) الكلي و شدة المرض ، بناءً على ما تقدم يمكن القول أن تحديد مستوى ECP في مصل الدم يمكن أعتماده للكشف عن وجود أو عدم وجود أصابة بالربو ثم انه يعكس شدة المرض بشكل معنوي، غير أن حساب نسبة ECP/Eo يمكن أن يعُد مؤشراً ذا فائدة للتعبير عن شدة المرض وفعالية الخلايا الحمضة اثناء الإلتهاب و هو ما يمكن الأستفادة منه في متابعة الحالة المرضية و تحديد التحسن في شدة المرض و الإستجابة لمضادات الإتهاب اثناء العلاج وليس للأغراض التشخيصية حيث لا فروق معنوية مسجلة بين المرضى و الأصحاء.

الكلمات المفتاحية: الربو الأرجي ، بروتين الحمضة الهابطي ، التغيرات المناعية.