ORIGINAL ARTICLE

Association Between Serum ECP levels and FEV1 in Asthma

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ABSTRACT

Background: Pathogenesis of asthma has always remained a mystery. A lot of hypotheses have been suggested that propose totally different mechanisms at the biological level. It has been found that activated eosinophils play an important role in the pathogenesis of bronchial asthma. Upon activation eosinophils undergo deregulation causing epithelial damage in the airway, desquamation and increased airway hypersensitivity.
Objective: To determine the association between serum Eosinophils Cationic Protein (ECP) and FEV1 and the effect of allergen exposure on ECP serum and sputum levels.
Patients and Methods: Serum ECP was determined in asthmatic patients by using enzyme linked immunosorbent assay and compared to control. For evaluation of the effect of natural allergen exposure on serum and sputum concentration of ECP, 20 patients from asymptomatic asthmatic patients and 20 healthy control subjects were included. At the time of enrollment in the study venous blood and sputum samples were collected for ECP determination. Following season exposure also sputum and venous blood collected to determine ECP.
Results: There was highly inverse correlation between serum ECP and FEV1 predicted percent ($P<0.0001$) asthmatic patients the study indicated that natural allergens exposure (post spring season) of asthmatic patients cause a significant ($P<0.05$) increase in serum and sputum ECP. T
Conclusion: Serum ECP can be used in the diagnosis of asthma and as marker for clinical and functional seriousness of asthma. In addition, it may be used for monitoring of disease severity and response to treatment and disease control. Natural allergen exposure was an important risk factor that may lead to inflammatory reaction and subsequent asthma exacerbation.
Key words: Asthma, ECP, Natural allergen.

INTRODUCTION

Asthma is a chronic inflammatory and allergic disease that involves local and systemic inflammatory reactions. The squeal of these changes is the affection of pulmonary function[1]. Serum ECP has a significant negative correlation with FEV_1 and FEV_1/FVC[2]. This reflects that a rise in serum ECP is a true representative of increased airway resistance found in asthmatics. This relationship of ECP has been reported by a number of other studies[3-9].

A number of studies have reported a little beyond the simple correlation of serum ECP and lung functions, as they reported the parallel improvement in lung functions and decrease in serum ECP after intervention[10-12]. There are only a few studies that have reported non significant relationship between lung function tests and serum ECP[13-18].

One of the very few Western studies of ECP, carried out in United Kingdom, very strongly concluded that serum ECP did not relate to any measure of asthma control. It had no association with current symptoms and had only a weak relationship with physiological measures. However this study found a significant, inverse correlation between FEV1and sputum ECP[19].

The value of correlation coefficient for relationship of ECP and lung function tests is very high in one study, probably because of a sample size that is larger than most of the studies reviewed[2]. A number of studies support this finding of a significantly positive correlation between serum ECP and severity of asthma, however only few of them have actually correlated serum ECP with the four recognized severity categories of asthma. Most of these studies have correlated serum ECP with acute attack and silent period[2, 20, 21].

MATERIALS AND METHODS

Patients: Serum ECP was determined in 139 asthmatic patients, 73 (52.5%) of them **were** symptomatic and 66 (47.5%) were asymptomatic. Ninety one (65.5%) patients of the total (139) were with mild asthma and 48 (34.5%) were with moderate asthma. In addition, 81 (58.3%) patients were from urban and 58 (41.7%) patients were from rural areas. Of the total symptomatic patients, 48 (65.8%) were

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with persistent asthma {21 (28.8%) persistent atopic and 27 (37%) persistent non atopic} and 25 (34.2%) were with intermittent asthma. In addition, 50 healthy non asthmatic individuals were included in the study as control.

The diagnosis of asthma was performed by specialist physician and was established according to National Heart Blood and Lung Institute / World Health Organization (NHLBI/WHO) workshop on the Global Strategy for Asthma[22]. Subjects were considered atopic by positive skin tests to at least one common aeroallergen. Patients were excluded if they were smokers, if they had respiratory infection within the month preceding the study, a rheumatological illness, malignancy, diabetic, heart failure, history of venous embolisms, coronary heart disease and liver or kidney diseases.

At enrolment, they all underwent full clinical examination, pulmonary function test, and blood sampling. Sputum samples were collected from patients when indicated. Normal volunteers were also enrolled in the study as a healthy control. None of them had any previous history of lung or allergic disease and were not using any medication. They had a normal lung function test (FEV1 > 80%) and negative skin allergy test. General stool examination was performed for all patients and control to exclude parasitic infections.

Asthma severity was classified according to the National Heart Blood and Lung Institute / World Health Organization (NHLBI/WHO) workshop on the Global Strategy for Asthma[22], and patients were classified as intermittent, persistent (mild, moderate or severe) asthmatics. The severity of asthma was evaluated in a prospective manner, with documented clinical events, lung function values and treatment in the year preceding the study, as previously recommended[22]. Acute asthma exacerbation was defined as dyspnea and wheezing with or without increased coughing[1]. The patients were recruited from the outpatient clinic of the Asthma and Allergy Centre in Tikrit. Their age range from 34 to 76 years (58.3±9.4 years). Patient was considered symptomatic when symptoms present at the time of clinical evaluation at time of study enrollment. While assigned as asymptomatic if asthma symptoms absent at the time of enrollment. All asymptomatic patients were considered as mild asthma. The sampling performed during the period from December 2004 to May 2005. All samples collected at morning following overnight fasting.

In order to determine the effect of confounding factors on serum ECP concentration, the persistent asthmatic (48 patients) classified in to atopic asthmatic (21 patients) and non atopic asthmatic (27 patients). In addition, individuals with lower respiratory tract infection (10 patients with pneumonia), 10 healthy non asthmatic non atopic and 10 non asthmatic atopic subjects were included as control groups for comparison.

For evaluation of the effect of natural allergen exposure on serum and sputum concentration of ECP, 20 patients from asymptomatic asthmatic patients and 20 healthy control subjects were included. However, 8 patients and 10 healthy control individuals were defaulted from the study, and thus only 12 and 10 were included in the analysis for asthmatic and control respectively. At the time of enrollment in the study venous blood and sputum samples were collected for ECP determination. Following season exposure (Next May), also sputum and venous blood collected to determine ECP. The study was approved by the ethics committee of Tikrit University College of Medicine, and written consent was obtained from all participating subjects.

Skin Prick Test: The skin prick tests were performed for all patients and control and evaluated in accordance with European Academy of Allergy and Clinical Immunology subcommittee on allergy standardization and skin tests using standards allergen panel (Stallergen, France).

Lung Function Test: Computerized Spiro meter (Autosphiror, Discom-14, Chest Corporation, and Japan) was used for measurement of FEV1 predicted percent of the patients at their enrollment in the study and when indicated according to studies design.

Sputum Collection: Sputum was induced only when it could not be produced spontaneously. Sputum induction was performed as described before [1].

Determination of Serum Eosinophils Cationic Protein: Serum ECP determined by ELISA kit (MBL MESCACUP ECP TEST) from Medical and Biological Laboratories Co, LTD, and Japan.

Statistical Analysis: The values are reported as mean ± SD and 95% confidence interval. For statistical analysis between groups paired t test was used. Pearson test was used for correlation analysis. The levels of each marker were compared between the study groups and control group, using SPSS computer package. P values of < 0.05 were considered significant.

RESULTS

Correlation between Serum ECP and FEV1 in Asthma: There was high significant inverse correlation between serum ECP and FEV1 predicted percent in asymptomatic ($r = -0.75$, $P<0.0001$) and symptomatic
($r = -0.54$, $P<0.0001$) asthmatic patients (Table.1). In addition, the same patterns of correlation were achieved in intermittent ($r = -0.82$, $P < 0.0001$) and

persistent (r = - 0.63; P< 0.0001) asthmatic patients. The correlation was with less significance when persistent asthmatic subdivided into atopic (r = - 0.54; P< 0.02) and non atopic (r = - 0.50; P<0.01) persistent asthmatic. In mild asthmatic patients group the correlation was highly significant (r =-0.73; P<0.0001), also in moderate asthmatic patients the correlation was highly significant (P<0.0001), but with lower r value (r =-0.63).

There was a significant inverse correlation between serum ECP and FEV1 predicted percent in urban (r= -0.91; P<0.0001) and rural (r= -0.65; P<0.0001) asthmatic patients. The correlation was significant in patients with pneumonia (r= -0.95; P< 0.0001), in non asthmatic non atopic (r= -0.72; P<0.05) and non asthmatic atopic (r= -0.95; P< 0.0001) individuals. For all asthmatic patients there was a highly significant (P<0.0001) inverse correlation (r=-0.50) between serum ECP and FEV1 predicted percent.

Influence of Natural Allergen Exposure on ECP in Asthma: The study indicated that natural allergens exposure of asthmatic patients cause a significant (P<0.05) increase of serum ECP from 26.41 µg/l (± 12.82) at baseline to 42.66 µg/l (± 16.8) at post season exposure. For control group there were no significant changes after season exposure from that at baseline (Table.2). Furthermore, serum ECP in asthmatic group was significantly higher than that for control group for both baseline (P<0.001) and post season (P<0.0001) values and the difference was more prominent in post season.

Sputum ECP was significantly higher (P<0.001) at post season (438.39 µg/l ± 163.21) as compared to baseline values (216.97 µg/l ± 97.52). However, there were no significant changes following season exposure in sputum ECP of control group. Sputum ECP was significantly higher in asthmatic as compared to control group from both baseline values and postseason values (P<0.0001). An interesting finding was that sputum ECP level was 10 times higher than serum ECP.

Table:1. Correlation between FEV1 predicted percent and serum eosinophil cationic protein in different groups.

Group	r value	P value <
Asymptomatic	-0.75	0.0001
Symptomatic	-0.54	0.0001
Intermittent	-0.82	0.0001
Persistent	-0.63	0.0001
Persistent atopic	-0.54	0.02
Persistent non atopic	-0.50	0.01
Mild	-0.73	0.0001
Moderate	-0.63	0.0001
Urban	-0.91	0.0001
Rural	-0.65	0.0001
Pneumonia	-0.95	0.0001
Non asthmatic non atopic	-0.73	0.05
Non asthmatic atopic	-0.95	0.001
All asthma	-0.50	0.0001

The (–) mark mean inverse correlation

Table:2. Effect of natural allergens exposure on eosinophil cationic protein in asthmatic patients.

ECP µg/l	Group [NO.] Asthma [12]			Group [NO.] Control [10]			P value <
	Mean	SD	95% CI	Mean	SD	95%CI	
Serum baseline	26.41	12.82	18.26-34.56	7.50	1.42	6.34-8.57	0.001
Serum postseason	42.66	16.80	31.98-53.34	11.21	2.71	9.17-13.25	0.0001
P value <	0.05			NS			
Sputum baseline	216.97	97.52	180.97-246.98	78.59	27.5	58.93-98.25	0.0001
Sputum postseason	438.39	163.2	398.19-478	94.63	33.5	70.9-118.36	0.0001
P value <	0.001			NS			

DISCUSSION

In accordance with other investigations[16,21,23-25], the present study results showed a significant inverse correlation between serum ECP and FEV1 predicted percent. Meanwhile, our results, however, do not agree with data from others[2,11,26,27], who could not find a significant correlation between serum ECP and pulmonary function. The lack of correlation is not surprising as it is possible that the kinetic of change in lung function may differ from those of changes in inflammatory parameters. In addition, treatment regimens used to control asthma symptoms in patents included in the studies affect serum ECP and pulmonary function test which may lead to lack of correlation.

The present study demonstrates inverse correlation between serum ECP levels and FEV1 predicted percent in asymptomatic and symptomatic asthma in adults and the correlation was more significant when the patients were asymptomatic. The correlation was good in asymptomatic patients because the inflammation was under control in these patients. A significant inverse correlation was achieved between FEV1 predicted percent and severity of the disease and the correlation was of more value in mild than moderate asthmatic patients. The same pattern was demonstrated in intermittent

asthmatic as compared to persistent asthmatic patients.

From the present study data it concluded that correlation was strong in asymptomatic, intermittent and mild asthmatic patients as compared to symptomatic, persistent and moderate asthmatic patients respectively. This may be explained on the basis that during symptomatic phase of the disease, more severe and longstanding patterns lead to a higher degree of eosinophils activation which interfere with other path physiologic and/ or inflammatory changes in active asthma and consequently affect serum ECP and lung function, which may be also affected by other mediators. In addition, patients receive treatment during the attacks and when the disease was chronic (persistent) and the treatment with corticosteroids and anti – inflammatory

Drugs lead to changes in both serum ECP and lung function. Their changes by treatment over time may not be the same, as lung function improved rapidly as compared to serum ECP which was a reflection of inflammation... The support for this explanation was the strong correlation between serum ECP and FEV1 in healthy control subjects as compared to asthmatic patients. Thus the correlation may reflect mechanisms associated with the pathogenesis and severity of asthmatic disorders.

The present study showed that in asthmatic patients, there was a significant increase in serum and sputum ECP levels was found during pollen season as compared with before season, indicating that there was a relationship between ECP and allergen exposure. However, in the control group there were no significant differences between baseline ECP levels and post season values. Montan et al [28] found that tear ECP was significantly elevated in allergen challenged eyes compared to contra lateral eyes. Tomassini et al [29] reported that persistent natural exposure to a sensitizing allergen is responsible for a measurable increase in serum ECP levels in patients with allergy. Furthermore, BAL ECP levels were higher in allergen challenged group compared with the group evaluated at baseline [30]. Later, Blay et al[31] show that the change in BAL and serum ECP levels was statistically significant compared to that in control group.

ECP levels increased in blood and sputum for both allergic asthma and allergic rhinitis following allergen challenge[32]. The present study results are in accordance with previous studies which found that in asthmatic patients, an increase in ECP occurs after natural exposure during pollen season[33,34] or during alternate stays at high and low altitudes with subsequent natural allergen exposure[35,36]. Thus serum and sputum ECP levels could be a useful marker for selecting allergic patients with eosinophils related activation to allergen exposure, and could be of great importance in the prevention of allergic disease. Also, the increase in serum and sputum ECP levels due to eosinophils activation precedes the occurrence of asthma symptoms and may thus be a marker of allergen exposure in allergic asthma.

However, both serum and sputum ECP were increased significantly following exposure; the magnitude of increase was 10 time higher in sputum than in serum. This may suggest that local inflammation was more predominant that systemic inflammation in asthma, especially during exacerbation. An important finding of this study was that FEV1 was influenced by serum ECP level in asthmatic. Thus serum ECP can be used in the diagnosis of asthma and as marker for clinical and functional seriousness of asthma. ECP marker was a reflection of the activation of the eosinophils indicating that eosinophils play a role in the pathogenesis of asthma. Atopy does not influence serum ECP levels in asthmatic individuals. Infection and atopy do cause rise in serum ECP in non asthmatic subjects but still the differences in serum ECP from that in asthmatic patients was highly significant. Natural allergen exposure was an important risk factor that may lead to inflammatory reaction and subsequent asthma exacerbation.

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