CORRELATION OF EOSINOPHIL CATIONIC PROTEIN WITH SEVERITY OF ASTHMA

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Background: Activated eosinophils play an important role in the pathogenesis of bronchial asthma. Upon activation eosinophils release their granular proteins. Eosinophil Cationic Protein (ECP) is a highly basic protein of ribonuclease-A family that is released from matrix of eosinophil granules. In the recent past a number of studies have shown increased levels of ECP in serum and induced sputum of asthmatics. We carried out this study to find out correlation of serum ECP with severity of asthma. **Methods:** This study was carried out on 44 asthmatics and 44 matched controls at Department of Physiology, Army Medical College, Rawalpindi from June 2002 to December 2003. Lung function tests were done using spirometer (Vitalograph-Compact) and severity of asthma was graded into four classes, mild intermittent, mild persistent, moderate persistent and severe persistent. Serum was used to assess ECP by ELISA. Statistical correlation between ECP and severity of asthma as well as lung function tests was calculated. **Results:** The asthmatics as compared with the controls had significantly more serum ECP. Serum ECP increased significantly with increase in severity of asthma (from mild intermittent to severe persistent). Serum ECP was negatively correlated with FEV1 and FEV1/FVC($r=-.823$ and $r=-.772$, $p<.001$ respectively). There was a significant positive correlation between serum ECP and severity of asthma ($r=0.947$ $p<0.001$) **Conclusions:** We conclude from this study that serum ECP can be used as a useful laboratory investigation for severity of asthma.

Keywords: Asthma, Eosinophil, Eosinophil Cationic Protein, ECP

INTRODUCTION

Pathogenesis of asthma has always remained a mystery. A lot of hypotheses have been suggested that propose totally different mechanisms at the biological level. These can be grouped into allergic, inflammatory, neurogenic, and physical mechanisms with current evidence in favour of a combination of allergic and inflammatory mechanisms.[1]

Eosinophils play an important role in the inflammatory events of allergic asthma. An early observation was that eosinophils accumulate in the asthmatic lung, but its significance was not recognized.[2] This relationship has focused research efforts on elaborating potential mechanisms through which eosinophils may contribute to tissue injury and oxidative modification of biological targets in asthma.[3]

It has been found that activated eosinophils play an important role in the pathogenesis of bronchial asthma.[4] Upon activation eosinophils undergo degranulation causing epithelial damage in the airway, desquamation and increased airway hypersensitivity.[5] It has been suggested that eosinophils may contribute to airway hyper responsiveness in asthma through the effects of eosinophil derived granular proteins in the bronchial epithelium.[6]

Eosinophil specific granules are membrane-bound and contain a number of highly cationic basic proteins that have been implicated in the tissue damage observed in asthma and similar allergic conditions. Each granule

comprises of rectangular or square crystalline-like core surrounded by a less electron-dense matrix.[7,8] The core of the granule contains almost exclusively Major basic protein (MBP) whereas the matrix contains three other eosinophilic basic proteins, Eosinophilic cationic protein (ECP), Eosinophil peroxidase (EPO) and Eosinophil derived neurotoxin (EDN).[9,10] It is thought that these granule-derived products having potent cytotoxic properties against bronchial epithelial cells and pneumocytes may be largely responsible for the damage associated with eosinophil infiltration in bronchial mucosa in asthma.[11-13]

ECP was first purified from human myleoid cells in 1971 and identified as an eosinophil granule protein in 1975.[14] ECP levels in biological fluids are an indicator of eosinophil-specific activation and degranulation and are currently used for the clinical monitoring and diagnosis of inflammatory disorders.[15] The first study about ECP published in 1977 reported that the serum concentration of 'eosinophil' cationic protein was correlated ($p<0.001$) to the number of eosinophil granulocytes in peripheral blood. It was suggested that quantitation of 'eosinophil' cationic protein in serum might be useful in the study of eosinophil granulocyte turnover and function in vivo.[16] In the next year the same authors[16] reported eosinopenic effect of beta-2-adrenergic drugs, salbutamol and terbutaline. They reported that these drugs were able to decrease serum ECP concentration.[17] The same team then performed inhalation challenge test in 12 patients with bronchial asthma. The subsequent variation in blood eosinophils and serum ECP was followed up. Uniform patterns in both parameters were seen suggesting active participation of the eosinophil leucocyte in allergic inflammation.[18]

ECP is a protein of the ribonuclease A (RNase A) superfamily that has developed biological properties related to the function of eosinophils.[19] It is highly basic and has been implicated in immunity to parasites and pathophysiology of chronic allergic responses.[20] It leads to exfoliation of respiratory epithelium.[21] ECP also stimulates mast cell degranulation, inhibits T-cell activity, and shortens the coagulation time. ECP has been also found at increased levels in (late phase) bronchoalveolar lavage fluid from allergen challenged asthmatics. ECP in sputum may be used to estimate the severity of bronchial inflammation and obstruction in asthmatics as well as to monitor asthma drug therapy.[22]

Eosinophilic inflammation is a feature of asthma but serological markers to indicate eosinophil activation in this process are not fully defined.[23] A lot of effort is being put in to detect reliable serological markers for asthma. Eosinophil granular proteins are amongst the top candidates along with interleukins. If any relationship of these serological markers is confirmed with severity of asthma, it will open avenues for research on pharmaceutical agents to block this axis of pathogenesis in asthma. We carried out this study to determine correlation between the severity of asthma and serum ECP levels.

MATERIAL AND METHODS

This study was carried out at the Department of Physiology, Army Medical College, Rawalpindi from June 2002 to December 2003. The patients were taken from the Department of Pulmonology, Military Hospital, Rawalpindi. A total of 44 asthmatics were included in the study by non-probability (convenience) sampling. Fourty Four (44) age, Body Mass Index (BMI) and gender matched controls were also included for comparison.

The inclusion criteria were adult asthmatics of all severities of asthma with an acute exacerbation of asthma and showing reversibility of more than 10 % FEV1 in 15 minutes after 2 puffs of salbutamol inhalation. The exclusion criteria were history of parasitic infestations, presence of skin diseases, smoking, infectious exacerbations, use of steroids in any form, age above 60 years and regular exercise. Similarly conditions known to interfere with ECP levels were carefully excluded. They included allergic rhinitis, Parasitic infestations, S. haemato-bium, Vernal

kerato-conjuctivitis, Anisakis simplex, Dermatitis herpeti-formis, Toxocara, Atopic Dermatitis, Multiple Sclerosis, Uremia, Schizophrenia, CNS tumors, Ischemic heart disease. Exclusion criteria were observed for the controls as well.

Pulmonary function tests were done using Compact Spirometer (Vitalograph®). The functions used for analysis were Forced Expiratory Volume in first second (FEV_1), Forced Vital Capacity (FVC), FEV/FVC % and Forced Expiratory Flow Rate (FEF_{25-75}%). The severity of asthma was categorized into four classes Severe persistent, Moderate Persistent, Mild Persistent and Mild Intermittent based upon signs and symptoms as per American thoracic Society Asthma severity code and classification chart.[24]

Serum drawn from venous blood was used to estimate ECP by ECP ELISA kit supplied by Medical and Biological Laboratories Co., LTD. (MBL) Naka-ku Nagoya, Japan. (CODE No. 7618E). It is the quantitative assay kit for the measurement of human ECP level in serum and urine by sandwich ELISA method. It detects human ECP with a minimum detection limit of 0.125 ng/ml and does not cross-react with EDN.

The data were entered into statistical package SPSS *version* 10. Descriptive statistics were used to calculate Means and standard deviations of all the variables. Correlation coefficient was calculated to determine correlation between serum ECP and severity of asthma

RESULTS

The asthmatic and control groups were age, gender and BMI matched, therefore there was non significant difference between these parameters as shown in table-1. The number of asthmatics in the four categories was different as the sampling was non probability sampling. The distribution of subjects in the four categories is given in table-2. Table-3 shows a significant difference between the lung function tests of cases and controls, reflecting correct selection of cases and healthy controls.

Table-1: Age, height, weight and male: female ratio of asthmatics and controls

(The values of age, weight and height are given as mean±SD)

	Asthmatics (n=44)	Controls (n=44)
Age (years)	34.89 ± 12.17	36.27 ± 9.78
Height (cm)	168.18 ± 6.50	167.70 ± 3.99
Weight (kg)	67.39 ± 7.67	65.43 ± 9.24
Male : Female	31:13	31:13

None of the differences is statistically significant

Table-2: Frequency of asthmatics in various severity categories of asthma

Severity of asthma	Severity code	Cases	Percentage
Mild Intermittent	1	12	27.3
Mild Persistent	2	16	36.4
Moderate Persistent	3	10	22.7
Severe Persistent	4	06	13.6

Table-3: Lung function tests (% of predicted values) of asthmatics and controls

(The values are given as mean ± SD*)*

Lung Function Test	Asthmatics (n=44)	Controls (n=44)
FVC	80.34 ± 3.95*	91.77 ± 10.84
FEV_1	51.09 ± 12.90*	92.64 ± 12.62
FEV_1/FVC %	54.41 ± 10.33*	85.45 ± 8.00
FEF_{25-75}	43.23 ± 24.69*	100.11 ± 31.96

***:The difference is statistically significant at P < .001 on paired sample T test.**

The serum level of ECP in controls was 19.26±10.81 ŋg/ml while in the asthmatics group taken as a whole it was 34.98±26.54. This difference was statistically significant at p<0.001. However while considering the four categories of asthma separately it was found that serum ECP level was significantly more in the more severe categories as shown in table-4.

Table-4: ECP levels in different severity categories of asthmatics (n=44)

(The values are given as mean ±SD)

Severity of asthma	ECP ŋg/ml
Mild intermittent (n=12)	7.60±5.13
Mild Persistent (n= 16)	25.12±8.11
Moderate Persistent (n= 10)	56.12±10.22
Severe Persistent (n= 6)	80.80±9.56

Each value is significantly (p<0.001) more than the value of immediately lesser severity category.

Table-5 gives the values of correlation coefficient between serum ECP and lung functions. There is a highly significant negative correlation of serum ECP with FEV_1 (r = -0.823) and FEV_1/FVC (r = -0.772). There is a highly significant positive correlation between severity of asthma and serum ECP (r = 0.947).

Table-5: Correlation of serum ECP with lung function tests and severity of asthma in asthmatics and controls

Variable	Asthmatics (n=44)	Controls (n=44)

	(r)	Sig.	(r)	Sig.
FVC	-0.027	.863	-0.064	.678
FEV_1	-0.823**	.000	0.226	.081
FEV_1/FVC	-0.772**	.000	0.532**	.000
$FEF_{25-75\%}$	0.287	.059	0.349*	.020
Severity	.947**	.000	----	----

*** Correlation is significant at the 0.05 level (2-tailed).**

**** Correlation is significant at the 0.01 level (2-tailed).**

DISCUSSION

Over the last two decades and especially during last five years substantial research work has been carried out to determine changes in serum ECP levels due to different allergic and non-allergic diseases. As a result enough quality work is now available to bridge the link between eosinophil activity and phenomenon of allergy. Serum ECP is now closer to be declared as an established marker of allergy. However, there is not enough evidence to adapt it as an established diagnostic test for asthma severity. The main reason is that valid and reliable work on correlation of ECP with established diagnostic tests for asthma is scarce especially in adults. Here we have made an attempt to discuss our findings in comparison with contemporary studies to explore whether the eosinophil activity marker ECP has any potential to become an established test for the diagnosis of severity of asthma and to predict clinical response or otherwise.

In our study asthmatic subjects taken as a group show an increased level of serum ECP as compared with controls. However, looking at the cases individually we found that some patients with undeniable evidence of asthma had normal ECP values. This reflects that, in some cases, the eosinophilic inflammation may not be a predominant relevant factor for their asthma.

The results closest to ours as far as ECP is concerned are those of Parra et al. In their adult asthmatic subjects mean ECP levels were 13.22 ± 1.11 ng/ml in controls (10) and 30.5 ± 2.38 ng/ml in patients (24). ECP levels were 24.23 ± 3.37 in mild, 31.69 ± 4.21 in moderate, and 37.61 ± 4.52 ng/ml in severe asthma.[25] In the study of Dal Negro et al on adult asthmatics mean serum ECP was 4.6 micrograms/l ± 0.6 SE in normal subjects and 22.4 micrograms/l ± 1.3 SE in asthmatics ($p < 0.001$).[26]

Di Lorenzo et al concluded from their study of 52 adult asthmatics that subjects with acute exacerbation of asthma show high serum ECP and more interestingly, the response to brochodilator was higher in patients with lower serum ECP levels.[27] Serum ECP levels in asthmatic patients were significantly higher than those in non-asthmatic patients in a study by Numao et al ($p<0.01$).[28] Serum values of ECP were significantly higher in asthmatics than in controls in a recent study by Zubovic et al.[29] Serum concentrations of ECP were significantly elevated in asthmatics in a study by Krug as compared with the controls.[30]

Kunkel et al found ECP in the acute asthmatic group to be significantly higher than the control group and the levels in the acute group decreased significantly ($p=0.004$) after one week on oral steroids.[31] A study by Badr-el-Din et al found that mean serum ECP level in all asthmatic

patients, during and after exacerbation, was significantly higher than the control group and was significantly higher during attacks than 2 weeks after the termination of exacerbation. [32]

Encouraged by the results of ECP in serum, many workers are trying other body fluids for its estimation. A lot of work has been done on presence and importance of increased ECP in induced sputum of asthmatics.[4,33] Similarly there are studies that have estimated ECP in the most relevant place that is, in bronchoalveolar lavage (BAL) fluid.[34,35]

Nevertheless none of these studies has identified any decision level for either eosinophil count, serum ECP or ECP/eosinophil ratio to discriminate atopic from non-atopic individuals.

We found that serum ECP has a significant negative correlation with FEV_1 and FEV_1/FVC. 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.

The results of Villa Asensi et al resemble our data except for the age of subjects.[36] Dal Negro et al in two articles have reported the correlation of serum ECP with a decrease in percent predicted FEV_1($r = 0.6$)[26,37] In a study conducted in Kuwait on adult asthmatics, there was a negative correlation between sECP and FEV_1(% predicted).[6] Wever et al reported the significant negative correlation of sECP with lung functions. The strongest correlation was with FEV_1/FVC ratio ($r=-0.61$, $P < 0.001$).[38] Motojima et al reported a significant inverse correlation between serum ECP concentration and %FEV_1.[39] Koller et al documented a strong correlation between the levels of eosinophil proteins and variables of pulmonary function.[40] FEV_1 and FEV1/FVC were significantly correlated with the levels of ECP in the study of Jang et al.[41]

A number of studies have reported a little beyond the simple correlation of sECP and lung functions, as they reported the parallel improvement in lung functions and decrease in sECP after intervention. [27,42,43]

There are only a few studies that have reported non significant relationship between lung function tests and serum ECP. A unique study is that of Marks et al who reported that ECP levels were not related to impaired lung function.[44] Exactly similar is the study of Prehn et al who found no correlation between sECP any of the lung function parameters measured.[45] No correlation was found between the ECP level and FEV_1/FVC ratio in the study of Juntunen-Backman et al.[46]

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 ($r=-0.48$, $p=0.004$).[47]

The value of correlation coefficient for relationship of ECP and lung function tests is very high (near perfect linear negative correlation) in our study, probably because of a sample size that is larger than most of the studies reviewed.

A number of studies support our finding of a significantly positive correlation between serum ECP and severity of asthma, however only few of them have actually correlated sECP with

the four recognized severity categories of asthma. Most of these studies have correlated sECP with acute attack and silent period.

Serum ECP levels were found higher during asthma attacks and after 24 hours of attacks than those in stable condition (free of attacks for more than 7 days) in a study by Hasegawa et al.[48] Similarly serum ECP levels were significantly higher in patients who had asthma attack within 24 hours than those who did not, and were higher in the patients who had asthma attack almost everyday for the past two weeks than those who had not in the study of Miyoshi et al.[49]

Jang and Choi found that moderate to severe asthmatics had significantly higher levels of ECP compared to mild asthmatics. They concluded that sputum ECP level is closely related to the clinical status in asthmatics. Very much like our results, in this study FEV1, FEV1/FVC were significantly correlated with the levels of ECP.[50]

On the contrary Vanto et al found that serum ECP correlated very weakly with asthma severity (rs=0.21, P=0.046).[51] In the study of Ishigaki et al there was a significant correlation between asthma attack frequency and sECP level.[52] In the study of Zubovic et al serum-ECP was significantly higher in asthmatics than in controls and it correlated with the clinical severity of asthmatics.[29] In the study of Niimi et al serum ECP level correlated with all indices of disease activity examined including severity score.[53] Enthralled by finding of an excellent correlation between serum ECP and clinical severity of asthma Yoshizawa et al concluded that in adults with asthma serum ECP levels may be more closely related to clinical severity than are blood eosinophil counts.[54]

Amongst the notable studies of eosinophil activity markers in induced sputum Fujiomoto et al found that ECP levels were significantly positively correlated with the mean weekly total symptom scores (r=0.48). They reported that when the asthmatics were classified as having mild (n=12), moderate (n=14), or severe (n=10) asthma as evaluated by their symptoms and peak expiratory flow rate (PEFR), the ECP levels showed significant increases in accordance with the severity of asthma.[4] Another comprehensive study on induced sputum is that of Nakazawa et al who found significant correlations between the sputum ECP level and the mean weekly symptom scores and asthma scores.[55]

Looking at our own results and by the review of literature we infer that serum ECP may serve as objective indicator for clinical activity in the asthma. Our results point to a possible pathophysiological axis in asthma that is based upon altered airway resistance due to eosinophils and eosinophil activity markers. If it is confirmed that this axis has some role in pathophysiology of asthma then it will open doors to new pharmaceutical research targeted at developing anti-eosinophil activity drugs on the pattern of anti histamine drugs.

Eosinophilia is a common laboratory finding in asthmatics. Our study advocates the possible supplementation of serum ECP along with total peripheral eosinophil count as another objective parameter that might help in selecting the appropriate severity level in asthmatics. It can be a useful addition in available diagnostic tests, to assess prognosis, and to assess effect of drugs.The principal usefulness of the sECP may be for the individual follow up and control of each specific patient.

A number of studies encouraged by excellent results of ECP evaluation in induced sputum of asthmatics are advocating adaptation of ECP in induced sputum as a diagnostic parameter for severity of asthma. Collection of induced sputum and its processing is not as easy as is being projected. Induced sputum is unsafe to handle particularly

in countries with high prevalence of pulmonary tuberculosis. This will be impossible during epidemics of influenza or SARS. We are sure that our study and other similar studies will eventually establish the importance of serum ECP in diagnosis and management of asthma.

CONCLUSIONS

We conclude from this study that Eosinophil Cationic Protein has significant negative correlation with FEV_1 and FEV_1/FVC, but does not have significant correlation with FVC and $FEF_{25-75\%}$. In addition Eosinophil Cationic Protein has significant positive correlation with the severity of asthma based on clinical grading and lung function tests. It can be used as a marker of severity of asthma.

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