

Correlation of Eosinophil Derived Neurotoxin with airway resistance in asthmatics

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Abstract

Objective: To determine the correlation of EDN with lung function tests (FVC, FEV1, FEV1/FVC and FEF25-75%) in asthmatics and to compare these with matched controls.

Methods: This study was carried out at Army Medical College & Military Hospital, Rawalpindi, Pakistan. Forty four asthmatic patients and equal number of matching controls were selected. Lung function tests were done by using compact spirometer. Severity of asthma was graded according to the American Thoracic Society (ATS) "Asthma severity code and classification chart." Venous blood was used for estimation of eosinophil count while serum was used for estimation of EDN by ELISA. Correlation between EDN and various lung functions were calculated.

Results: The asthmatic patients had significantly more eosinophil count ($p < 0.001$) and Serum EDN levels ($p < 0.001$) than the controls. A significant correlation ($r = 0.934$, $p < 0.001$) was found between eosinophil count and EDN. A significant correlation between absolute eosinophil count and some of the lung functions [% predicted FEV1 ($r = -0.908$, $p < 0.001$) and FEV1/FVC ($r = -0.830$, $P < 0.001$)] was found. A similar significant correlation was found between EDN and some of the lung functions [% predicted FEV1 ($r = -0.855$, $p < 0.001$) and FEV1/FVC ($r = -0.814$, $P < 0.001$)]. Absolute eosinophil count and EDN increased significantly ($p < 0.001$) with increasing severity of asthma.

Conclusion: Serum EDN has significant correlation with changes in lung functions and severity of asthma (JPMA 60:97; 2010).

Introduction

There are many theories for pathophysiology of asthma. 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

Many blood cells have been implicated in the pathophysiology of asthma. During airway inflammation, increased number of activated cells (e.g. eosinophils and neutrophils) associated with inflammation are found in bronchial mucosa, bronchoalveolar lavage fluid, sputum and blood.^{2,3} Both eosinophils and neutrophils contain various mediators in their intracellular granules. When such cells are activated, various types of harmful mediators are released into

the immediate environment. A lot of research work is going on involving both eosinophil and neutrophil. However eosinophil has an advantage that it simultaneously fits in both the allergic and inflammatory theories of asthma.⁴

Eosinophilic inflammation is a feature of asthma. Total eosinophil count reflects asthmatic activity and is useful for regulating steroid dosage and for early detection of exacerbations. Eosinophils are currently regarded as the 'effector' cells responsible for much of the pathology of asthma. Eosinophil-mediated damage to the respiratory epithelium is a major pathogenic mechanism in asthma. However, serological markers to indicate eosinophil activation in this process are not fully defined.⁵ The eosinophil, its chemoattractants {interleukin-5, eotaxin etc} and its granular

products (Major Basic Protein, Eosinophil Cationic Protein and Eosinophil Derived Neurotoxin) top the list of under research chemical markers for asthma.

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.^{6,7} 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).^{8,9} 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.^{10,11}

Eosinophil-derived neurotoxin (EDN), also called eosinophil protein X (EPX), has been suggested to be a useful marker of eosinophilic inflammation. Initially it was thought that eosinophil protein X (EPX) and eosinophil-derived neurotoxin (EDN) are two separate proteins. Later, it was reported that EDN and EPX have similar molecular weight and both possess neurotoxic and helminthotoxic activities. These results indicated that EDN and EPX had virtually identical properties and were the same protein.¹² The molecular weight of the unreduced protein was 23,000 and after reduction 19,000. The EPX content was estimated to be on average 10 micrograms in normal eosinophils.¹³ It is a protein of ribonuclease A (RNase A) superfamily that has developed biological properties related to the function of eosinophils.¹⁴ It is highly basic, exhibits strong ribonuclease activity and leads to exfoliation of respiratory epithelium. It has been implicated in immunity to parasites and pathophysiology of chronic allergic responses.¹⁵ It has an ability to induce ataxia, paralysis, and central nervous system cellular degeneration in experimental animals (Gordon phenomenon).¹⁶

A lot of work has been done on some of the eosinophil products specially serum eosinophil cationic protein (ECP) and urinary eosinophil derived neurotoxin (EDN, also called eosinophil protein 'X' or EPX) in relation with asthma. Almost no work has been done on serum EDN in relation to asthma as the attention of researchers was mainly towards the urinary EDN/EPX. Reliable ELISA kits for assessment of EDN levels in serum, plasma and urine have been developed very recently. We planned this work to correlate the eosinophil granular protein; EDN, in serum with airway resistance in asthmatic patients with an idea to identify importance, of this activation marker of eosinophils in diagnosis and management of asthma.

Patients and Methods

This study was carried out at Department of Physiology, Army Medical College, Rawalpindi, Pakistan. A total of 88 subjects were included in the study. These comprised of 44 asthmatics and 44 matching controls. The asthmatic patients were taken from Pulmonology Department, Military Hospital, Rawalpindi, whereas, matched individuals from the general population (matched for gender, age, height, weight) served as the control group. The sampling technique was convenience (non probability) sampling.

The asthmatic patients were selected by observing the inclusion and exclusion criteria verified by a pulmonologist. We included adult men and women between 18-60 years of age, presenting with all severities of asthma with FEV1 reduced to < 80 % of the predicted, FVC normal or reduced than the predicted, FEV1 reduced to < 70% of the predicted based on American Thoracic Society scale for asthma severity.¹⁷ We included both known asthmatics having acute exacerbation or those reporting for the first time showing reversibility of more than 10 % FEV1 in 15 minutes after 2 puffs of salbutamol inhalation.

Patients with suspected infectious exacerbations, using steroids in any form, regularly exercising subjects/athletes, aged subjects (above 60 years), smokers and those with history of conditions known to interfere with eosinophil derived neurotoxin (EDN) levels namely allergic rhinitis, Parasitic infestations, *S. haematobium*, Vernal keratoconjunctivitis, *Anisakis simplex*, Dermatitis herpetiformis, *Toxocara*, Atopic Dermatitis, Multiple Sclerosis, Uraemia, Schizophrenia, CNS tumours or Ischaemic heart disease were excluded.

Pulmonary function tests of all the subjects were recorded by compact spirometer (Vitalograph® Ltd, England). Three readings were taken in all the cases except in patients who were very sick. Lung functions recorded were; Forced Expiratory Volume (FEV1), Forced Vital Capacity (FVC), FEV/FVC %, Forced Expiratory Flow Rate (FEF25-75%). Percentage of predicted value for all these tests was used for statistical analysis.

Venous blood was used for eosinophil count and serum extraction. The serum was stored at -20°C until estimation of EDN by using EDN ELISA kit supplied by Medical and Biological Laboratories Co., LTD. Naka-ku Nagoya, Japan. (Code No.7630). This is a quantitative assay kit for human EDN level in serum and urine by sandwich ELISA method. It detects human EDN with a minimum detection limit of 0.62 ng/ml and does not cross-react with ECP.

The data were analyzed by statistical package SPSS

version 14. Descriptive statistics were used to calculate Mean and standard deviation of all the variables. Paired sample 't' test was used to compare all the variables between asthmatics and matched controls for statistical significance using SPSS. Correlation coefficient was calculated to determine correlation between Eosinophil Derived Neurotoxin and spirometric values.

Results

A total number of 88 subjects (male and female) between 18-60 years of age were included in the study. Out of the 44 asthmatics (age; 34.89 ± 12.17 years) included in the study 12 (27.3%) had 'mild intermittent', 16 (36.4%) 'mild persistent', 10 (22.7%) 'moderate persistent' while 06 (13.6%) had 'severe persistent' asthma on ATS asthma classification. The mean age of matched controls was 36.27 ± 9.78 years. The male to female ratio in asthmatics and matched controls were 31:13.

The results of this study are summarized in tables 1-

Table-1: Lung function tests (% of predicted values) of asthmatics and controls. (The values are given as mean \pm SD).

Lung Function Test (% of predicted values)	Asthmatics (n=44)	Controls (n=44)
FVC	$80.34 \pm 3.95^*$	91.77 ± 10.84
FEV1	$51.09 \pm 12.90^*$	92.64 ± 12.62
FEV1/FVC %	$54.41 \pm 10.33^*$	85.45 ± 8.00
FEF25-75	$43.23 \pm 24.69^*$	100.11 ± 31.96

*The difference is statistically significant at $P < 0.001$ on paired sample t test.
(FVC: Forced vital capacity, FEV1: Forced expiratory volume in first second, FEF: Forced expiratory flow rate)

Table-2: Eosinophil count and EDN levels in different severity categories of asthmatics (n=44). (The values are given as mean \pm SD).

Severity of asthma	Eosinophil count Cells/ μ l	EDN n/ml
Mild intermittent (n=12)	292.08 ± 69.35	27.04 ± 16.52
Mild Persistent (n= 16)	367.56 ± 48.61	42.47 ± 13.32
Moderate Persistent (n= 10)	510.70 ± 85.00	63.64 ± 17.74
Severe Persistent (n= 6)	684.00 ± 75.58	89.73 ± 9.97

(EDN: Eosinophil Derived Neurotoxin).

4. Table-1 shows comparison of Lung function tests in asthmatics and controls. Table 2 compares eosinophil count and serum EDN values in asthmatics. Each value were significantly ($p < 0.001$) higher than the value in the category of asthmatics with immediately lesser severity. That is, the eosinophil count and EDN levels increased significantly ($P < 0.001$) as the severity of asthma got amplified. However, IgE of moderate persistent is significantly less ($P < 0.001$) than IgE of mild persistent category.

Table-3 presents the values of correlation

Table-3: Correlations of serum EDN with eosinophil counts and lung function tests in asthmatics and controls.

Variable	Asthmatics (n=44)		Controls (n=44)	
	Correlation	Significance	Correlation	Significance
Eosinophil Count	0.934*	0.000	0.809*	0.000
FVC	0.107	0.489	-0.054	0.727
FEV1	-0.855*	0.000	0.183	0.234
FEV1/FVC	-0.814*	0.000	0.395*	0.008
FEF25-75%	-0.197	0.199	0.270	0.077

*Correlation is significant at the 0.01 level (2-tailed).
(FVC: Forced Vital capacity, FEV1: Forced Expiratory Volume in first Second, FEF: Forced Expiratory Flow rate).

Table-4: Correlation of severity score of asthma with eosinophil counts, serum EDN and lung function tests in asthmatics.

Variable	Pearson Correlation	Significance
Absolute Eosinophil count	0.879*	0.000
EDN	0.814*	0.000

*Correlation is significant at the 0.01 level (2-tailed)
(EDN: Eosinophil Derived Neurotoxin).

coefficients (and statistical significance) of serum EDN with absolute eosinophil counts and lung function tests in the asthmatics and controls. The data revealed highly significant direct correlation between EDN levels and eosinophil count while EDN levels had strong ($P < 0.001$) inverse correlation with FEV1 and FEV1/FVC in asthmatics. Table-4 presents the correlation and statistical significance of severity of asthma with absolute eosinophil count and serum EDN in the asthmatics.

Discussion

Eosinophil Cationic Protein (ECP) and Eosinophil Derived Neurotoxin (EDN) are two serological markers of eosinophil origin that have been proposed and evaluated as the markers of allergic manifestations like asthma. In the present study, the asthmatic patients had significantly higher eosinophil count and serum EDN than the matched controls. In addition, a significant positive correlation (0.934; $P = 0.00$) was found between eosinophil count and serum EDN levels.

Morioka et al were pioneers amongst those who studied serum EDN levels. They compared EDN in serum, plasma and urine and reported that EDN levels in serum, plasma and urine of patients were significantly correlated with the number of peripheral blood eosinophils.¹⁸ Another study on serum EDN was conducted by Pronk-Admiraal et al, in which relationship between eosinophil concentration and serum eosinophil protein 'X' (EPX/EDN) concentration was investigated in 80 subjects. Higher eosinophil counts were found related with the increased serum EPX concentrations.¹⁹

In the study of Cottin et al, U-EPX was significantly

higher in patients with increased number of eosinophils. The limitation of this otherwise excellent study was that they made a generalized observation of increased U-EPX with increased eosinophil count, but the statistical correlation between the two was non significant.²⁰

Out of the very few studies available on serum, Chivato et al reported an increase in serum levels of EDN in asthmatics during the pollen season.²¹ In the study of Pronk-Admiraal et al atopic subjects (n=19) had a significantly higher EPX concentration and a significantly lower EDN/eosinophil ratio compared with nonatopic subjects (n=61).¹⁹

Among the studies opposing the importance of EDN, is the work by Labbe et al which found no significant difference in urinary EDN level in atopic asthmatics compared with nonatopic patients.²²

Wojnarowski et al found U-EPX levels of asthmatic and atopic children significantly higher than the healthy subjects. This study however, pointed out a decreased sensitivity of EDN due to a great overlap between asthmatics and controls.²³ In a study by Cottin et al on asthmatic children, U-EPX concentrations were significantly higher in all asthmatic children during attacks than those in the control group.²⁰ In a study by Lugosi et al U-EPX levels were increased in asthmatic children compared with controls. In addition, U-EPX levels were higher in symptomatic than in asymptomatic patients.²⁴ Severien et al also reported a similar increase in EDN in asthmatic children compared with controls.²⁵ Shirakawa et al found from their study of asthmatic children that urinary EPX (EDN) levels were high during acute asthma which decreased gradually with improvement of acute asthma, however, these levels of eosinophil derived proteins were significantly higher than those in non-atopic children even when the acute symptoms were over.²⁶

Despite finding of significant levels of EDN/EPX in urine of asthmatics by a number of studies, it appears logical that measurement of this blood based protein in serum may be more representative and easier than in urine as manifested by our assays in blood samples of asthmatic subjects.

Another objective of our study was to determine correlation of serum EDN with lung functions in both asthmatics and controls to establish a link with airway hyper responsiveness in asthmatics. It was found that FEV1 and FEV1/FVC had a significant negative correlation with eosinophil count while FVC and FEF25-75% did not show a significant correlation. This reflects that a rise in serum EDN is a true representative of increased airway resistance found in asthmatics. This also suggests that this parameter has significant diagnostic and prognostic value.

There is no previous work available mentioning the correlation of serum EDN with lung functions. However, a few studies are available that have calculated correlation of urinary EDN/EPX with lung function tests. The results of these studies are contradicting. Significant inverse correlation was found between lung functions and urinary EPX level in the study of Shirakawa et al.²⁶ In a cohort (follow up) study by Lugosi et al, U-EPX levels were significantly correlated with pulmonary functions on the baseline. During the follow-up period, changes in U-EPX values were significantly related to changes in pulmonary function.²⁴ However, Cottin et al found no significant correlation between U-EPX concentrations and PEF.²⁰

Looking at our own results and by the review of literature we infer that serum EDN may serve as an objective indicator for clinical activity in the asthma. It can be a useful addition in available diagnostic tests to assess prognosis and effect of medication. Serum EDN can serve as useful non invasive marker of airways inflammation and disease activity in acute and chronic asthma patients. It has a potential to serve as a marker for predicting and monitoring the clinical course of asthma.

Limitations of the study:

Our major limitation was that we could not exclude all the known causes of variation in serum EDN, we relied mainly on history which is never conclusive in most of the cases like parasitic infestations, atopic dermatitis, allergic rhinitis, Crohn's disease etc. Similarly there could be a chance of error in the history taken from the patients that they had not used any medicine before reporting to the hospital. The patients have a trend to hide medication on the way. We could not separate groups of atopic and nonatopic individuals due to difficulty in sampling. We did not exclude the patients with normal eosinophil count; the idea was to have a true reflective study of asthma as a whole and not just in the patients with eosinophilia.

Conclusion

This study concludes that serum Eosinophil Derived Neurotoxin has a significant correlation with FEV1 and FEV1/FVC in asthmatics. It is reflective of airway resistance however there is need for further studies to develop proper cutoff values.

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