

Research Article



Role of Eotaxin in Chronic Obstructive Pulmonary Disease (COPD)

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ABSTRACT

Eotaxin is a chemokine which characterized by eosinophil chemotactic and activating effects. Eosinophil takes part in airway inflammation in chronic obstructive pulmonary disease (COPD). The aim was to determine eotaxin values as a biomarker in clinical evaluation of COPD patients, and to compare its levels with IL-8 and ECP levels. This study included 35 patients with exacerbation of COPD, 30 patients with stable COPD, and 23 healthy controls. Automated eosinophil count was done. Serum Eotaxin IL-8, and ECP were assessed by ELISA. Our results showed an increase in the serum levels of Eotaxin, IL-8, ECP, and Eosinophil percentage in exacerbation COPD patients [(479.484±231.331 pg/ml) ($p<0.0001$); (76.949±19.550 pg/ml) ($p<0.0001$); (48.185±17.476 ng/ml) ($p<0.0001$); (3.388%±2.002) ($p<0.0001$) respectively] compared to healthy controls. Serum levels of Eotaxin, IL-8, ECP and Eosinophil percentage in stable COPD patients increased – but less than their increase in exacerbation COPD patients – [(318.295 ± 144.882 pg/ml) ($p<0.0001$); (50.854 ± 11.217 pg/ml) ($p<0.001$); (30.675±12.688 ng/ml) ($p<0.0001$); (2.01%±1.506) ($p>0.05$) respectively] compared to healthy controls. This indicates the important role of eotaxin in the inflammatory process of the disease, and the association between eotaxin concentrations and COPD severity. Therefore eotaxin could might be used as a biomarker in the clinical evaluation of COPD.

Keywords: Eotaxin, COPD, Exacerbation, Airway Inflammation, IL-8, Eosinophilic Cationic Protein.

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is the forth cause of morbidity and mortality in the world and represents a substantial economic and social burden. It is a preventable and treatable disease with some significant extra pulmonary effects that may contribute to the severity in individual patients. Its pulmonary component is characterized by airflow limitation that is not fully reversible. The airflow limitation is usually progressive and associated with an abnormal inflammatory response of the lung to noxious particles or gases.¹

The pathological hallmarks of COPD are destruction of the lung parenchyma, which characterizes emphysema, inflammation of the peripheral airways, which characterizes bronchiolitis, and inflammation of the central airways which characterizes chronic bronchitis. Emphysema will contribute to the airflow limitation by reducing the elastic recoil of the lung through parenchymal destruction, as well as by reducing the elastic load applied to the airways through destruction of alveolar attachments. On the other hand, bronchiolitis will contribute to the airflow limitation by narrowing and obliterating the lumen and by actively constricting the airways.

Interestingly, in peripheral airway epithelium of patients, goblet cell hyperplasia was associated with an increased number of neutrophils.² As neutrophil elastase is a remarkably potent secretagogue, the location of neutrophils within the epithelium may be crucial for the activation of the secretory function of goblet cells.

Cigarette smoke, one of the causal factors of COPD, stimulates IL-8 release from bronchial epithelial cells and alveolar macrophages. IL-8 is a potent neutrophil activator, inducing neutrophil chemotaxis and increasing neutrophil adherence to endothelial and epithelial cells. The numerous functions of IL-8 in COPD have led to the proposal that the difference between patients with COPD and 'healthy smokers' could be related to the amount of IL-8 produced and released by airway epithelial cells.³

In COPD airway wall eosinophilia has been observed at a level similar to that found in asthma, with an associated increase in eotaxin expression by airway sub-epithelial and epithelial cells.⁴

Increasing concentrations of CCL11/eotaxin were associated with increasing severity of airflow obstruction. Eotaxin is present in the serum of patients with COPD and the percentage fall in forced expiratory volume in one second (FEV1) correlated with eotaxin levels.⁵

Eotaxin is a stimulus for eosinophils, inducing eosinophil migration in vitro and accumulation in vivo. It is a potent eosinophil chemoattractant which signals via CCR3, a chemokine receptor highly expressed on eosinophils.⁶ Thus it is involved in both the mobilization of eosinophils and their progenitors from the bone marrow into the blood and in their subsequent recruitment into lung tissue.

Furthermore this chemokine combined with the elevated levels of MCP-1 found in COPD to cause increased monocyte migration into the airways thus resulting in the increased macrophages in the tissues associated with COPD.⁷



MATERIALS AND METHODS

Study Subjects

88 individuals (74 men and 14 women) took part in this study, and they were divided into three groups:

Group A : 35 patients (mean age 65.37) with chronic obstructive pulmonary disease during exacerbation, from Assad University hospital and Al-Mowasa University Hospital.

Group B : 30 patients (mean age 63.36) with stable chronic obstructive pulmonary disease who had not been hospitalized for the exacerbation of the illness for the past two month at least.

Group C : 23 healthy subjects (mean age 62.78) as a control group. All of them were lifelong nonsmokers, with normal spirometry and with no history of atopy.

Serology

Sera from patient with COPD and healthy individual were stored at -80° C until the assay.

Assays

The levels of CCL11/Eotaxin in sera were determined by sandwich Elisa kit (RayBio®, USA). This assay employs an antibody specific for human eotaxin coated on a 96-well plate. The concentration of eotaxin in the sera is presented as relative value compared to OD of the stander serum. Also the levels of IL-8 and ECP in sera were determined by Elisa, and Automated eosinophil count was done.

Statistical Analysis

Data were analyzed using Excel (2007), SPSS version 19. Results were expressed as mean \pm SD. Test was used to evaluate the relation between the variants, correlation between two variable was performed using Spearman correlation, P-value less than 0.05 was regarded as significant. The area under ROC curve were concluded to determine the diagnostic value for eotaxin.

RESULTS

Serum titer of Eotaxin for COPD patients and control group

The average level of eotaxin were significantly higher in patient with COPD during exacerbation ($X \pm SD$: 479.5 \pm 231.3 pg/ml) than stable COPD patients ($X \pm SD$ =318.3 \pm 144.9 pg/ml) and control group ($X \pm SD$ =166.5 \pm 104.9 pg/ml), ($P < 0.01$ and $P < 0.0001$ respectively), Figure (1).

Serum Eotaxin sensitivity and specificity for patient's evaluation between exacerbation and stable COPD

Serum eotaxin sensitivity and specificity for some cut-offs values and the results were as shown in table 1.

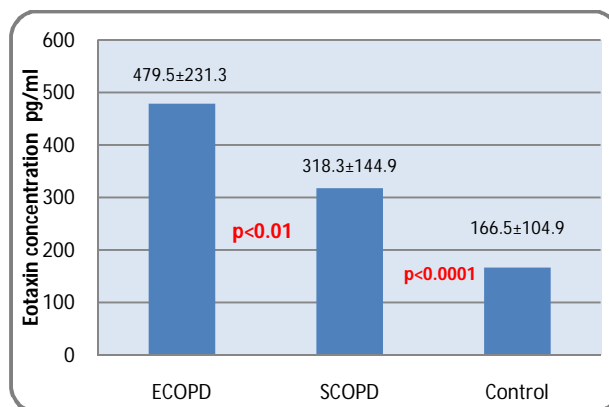


Figure 1: levels of eotaxin in the study groups

Table 1: Eotaxin sensitivity and specificity for patient's evaluation between exacerbation and stable COPD at some cut-offs values.

Cut-off	220.7	303.3	390	425.5	577.8
Sensitivity	85.30%	73.50%	67.60%	55.90%	38.20%
specificity	32.30%	48.40%	61.30%	77.40%	96.80%
PPV	60%	62.5%	68.57%	73.10%	92.80%
NPV	66.7%	60%	63.33%	58.97%	56.86%

At the cut-off value 577.8 pg/ml we got a very good specificity (96.80%) which gave us a good differentiation between exacerbation and stable COPD patients (high specificity helps in the differentiation between the two patient groups). In our study this eotaxin concentration was the best value that represent the threshold between exacerbated and stable COPD patients. The area under ROC curve was 0.703.

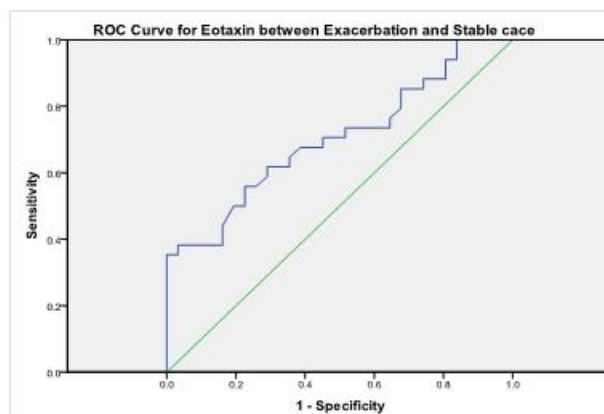


Figure 2: Serum Eotaxin sensitivity and specificity for patient evaluation between exacerbation and stable COPD

Serum Eotaxin sensitivity and specificity for differentiation between COPD patients and healthy controls

Serum eotaxin sensitivity and specificity for some cut-offs values and the results were as shown in table 2.

At the cut-off value 235.3 pg/ml we got a good sensitivity (73.80 %) which gave us a good Differentiation between COPD patients and healthy individuals (high sensitivity helps in the detection of the largest proportion of

patients). In our study this eotaxin concentration was the best value that represent the threshold between COPD patients and healthy controls. The area under ROC curve was 0.872.

Table 2: Eotaxin sensitivity and specificity of differentiation between COPD patients and healthy controls at some cut-offs values.

Cut-off	161.9	235.3	292.2	381.2
Sensitivity	87.70%	73.80%	64.60%	56.90%
specificity	65.20%	78.30%	87.00%	95.70%
PPV	87.69%	90.56%	93.33%	97.36%
NPV	65.21%	51.42%	46.51%	44.00%

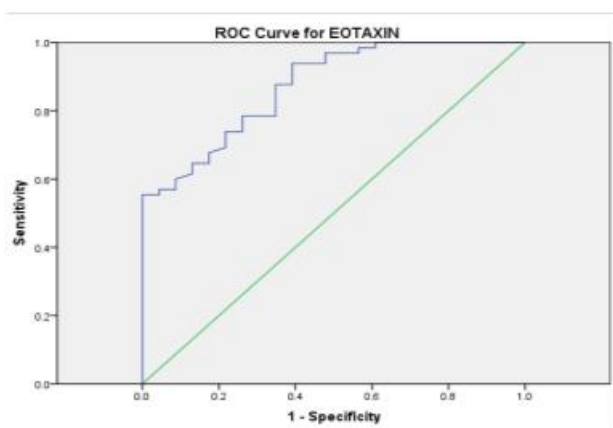


Figure 3: Serum Eotaxin sensitivity and specificity for differentiation between COPD patients and healthy controls

The diagnostic value of serum eotaxin when shared with other parameters to assess the patient's condition between stable and exacerbated disease

The diagnostic value of serum eotaxin in the patient evaluation is between exacerbation and stable COPD at the 577.8 pg/ml cut-off, when shared with IL-8 at its 64 pg/ml cut-off or with ECP at its 47.1 ng/ml cut-off. We had got results shown in table 3.

Table 3: The diagnostic value of eotaxin when shared with other parameters to assess the patient's condition between stable and exacerbated disease

	sensitivity	specificity	PPV	NPV
Eotaxin+IL-8	77.1%	90%	90%	77.1%
Eotaxin+ECP	65.7%	83.3%	82.1%	67.7%

The diagnostic value of serum eotaxin when shared with other parameters for the differentiation between COPD patients and healthy controls

The diagnostic value of serum eotaxin in the differentiation is between COPD patients and healthy individuals at the 235.3 pg/ml cut-off, when shared with IL-8 at its 44.3 pg/ml cut-off or with ECP at its 16.7 ng/ml cut-off. We had got results shown in table 4.

Table 4: The diagnostic value of eotaxin when shared with other parameters for the differentiation between COPD patients and healthy controls

	sensitivity	specificity	PPV	NPV
Eotaxin+IL-8	92.3%	60.9%	87%	73.7%
Eotaxin+ECP	98.5%	65.2%	88.9%	93.7%

Correlations between serum eotaxin and the rest of the studied parameters in patients with Chronic Obstructive Pulmonary Disease

A strong and statistically significant correlation was observed between serum levels of eotaxin and the eosinophil percentage in the exacerbation group (P-value <0.0001, $R = 0.779$).

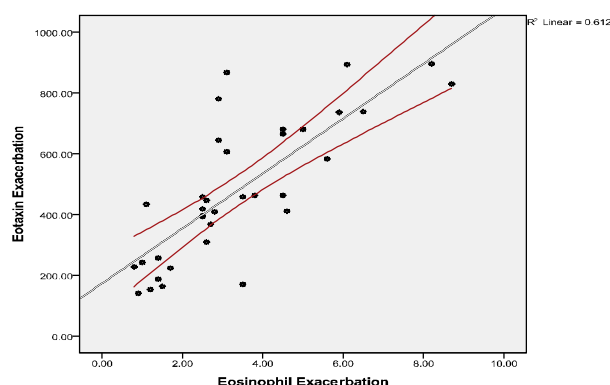


Figure 4: linear regression between serum eotaxin and eosinophil percentage in group A.

A statistically significant correlation was observed between serum levels of eotaxin and the Eosinophil percentage in the stable group (P-value <0.0001, $R = 0.681$)

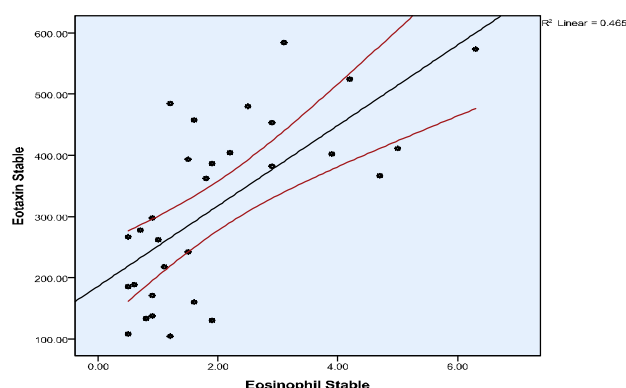


Figure 5: linear regression between serum eotaxin and eosinophil percentage in group B.

A statistically significant correlation was found between serum levels of eotaxin and ECP in exacerbation group (P-value <0.05, $R = 0.393$).

We did not find a statistically significant correlation between serum levels of eotaxin and IL-8 neither in exacerbation group (P-value = 0.743 > 0.05, $R = 0.058$), nor in the stable group (P-value = 0.651 > 0.05, $R = 0.086$).

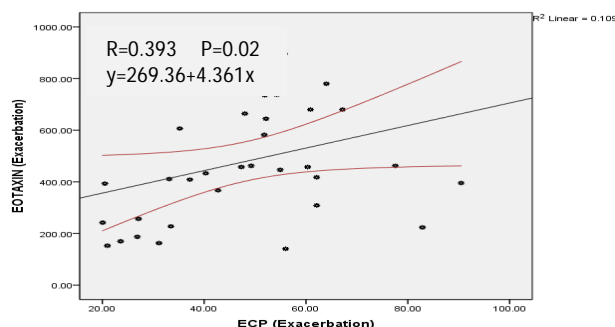


Figure 6: linear regression between serum eotaxin and ECP in group A.

Also we did not find a statistically significant correlation between serum levels of eotaxin and ECP in the stable group ($P = 0.408 > 0.05$, $R = 0.157$).

DISCUSSION

Our results showed a clear rise in the serum eotaxin levels of exacerbation group compared to stable and control groups ($P < 0.01$, $P < 0.0001$ respectively). And the levels of stable group were higher than control group ($P < 0.0001$). This changes in eotaxin concentrations demonstrated that the changing in eosinophils accumulation in the lungs may be an important factor in influencing the stability of the disease. Eotaxin -signals via CCR3 which is highly expressed on eosinophils- contributes to eosinophils recruitment into inflamed lung tissue during exacerbation⁸ and stable COPD⁹. These results were similar to D'Armiento JM et al., 2009¹⁰ that found significantly high levels of eotaxin in severe stages and exacerbations than the level in stable disease and controls, but they found no significantly difference between stable patients and healthy subjects. Conversely our result was similar to Jahnz-Rozyk K et al., 2000¹¹ which included 15 stable COPD patients ($X \pm SD = 286.0 \pm 101.4$ pg/ml), and 15 healthy controls ($X \pm SD = 109.6 \pm 56.1$ pg/ml), and the difference was statistically significant ($P < 0.0001$). Zhu J et al., 2001¹² also found a significant eotaxin increase in smoking patients with stable COPD than healthy non-smokers.

There were a significant correlations between serum levels of eotaxin and the eosinophil percentage in the stable ($P\text{-value} < 0.0001$, $R = 0.681$) and exacerbation ($P\text{-value} < 0.0001$, $R = 0.779$) groups. These correlations explain eosinophilic inflammatory process accruing in COPD patients by eotaxin recruitment and its specific chemotactic for these cells from circulation into lung tissue. Our result was similar to Fujimoto K et al., 2005¹³ which found that eotaxin as a eosinophil chemoattractant may contributes to eosinophilic inflammation during stable phase of COPD, and may plays a role in the increase in airway eosinophilic inflammation during an acute exacerbation.

There was a statistically significant correlation between serum levels of eotaxin and ECP in the exacerbation phase ($P\text{-value} < 0.05$, $R = 0.393$). This correlation refers to the potential role of eotaxin in ECP release from

eosinophils and subsequently the increase in its concentration in serum during exacerbation phase, ECP in his turn has a destructive effects on the airways thus contribute to the inflammatory process.

CONCLUSION

Serum Eotxin Levels were increased in COPD patients compared with healthy people, and it had a good diagnostic value in the differentiations between exacerbated COPD patients, stable COPD patients and controls. Combination between serological measure of eotaxin with IL-8 or ECP can increase the diagnostic value in the evaluation of the patient's condition and in differentiating between patients and healthy.

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