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Research Article

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Elevated serum of ECP in acute exacerbations of COPD

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ABSTRACT

Chronic obstructive pulmonary disease (COPD) affects a large number of patients in the world and is associated with significant morbidity, disability and mortality. Exacerbations of COPD are a major cause of morbidity, mortality and hospital admission. During COPD exacerbations, airway inflammation shows some features of Eosinophilic inflammatory processes, with recruitment of eosinophils. The aim of our study was to investigate whether ECP, released by activated eosinophils, concentrations could be a useful marker in the diagnosis of COPD exacerbations. This study included 88 individuals: 30 patients with acute COPD exacerbations (ECOPD), 15 patients with mild to moderate stable COPD (COPD I-II), 15 patients with severe to very severe stable COPD (COPD III-IV) and 30 healthy controls (11 non-smokers and 17 healthy smokers). The levels of eosinophil cationic protein (ECP) was determined by ELISA. The results showed that serum ECP was significantly increased in COPD patients (31.314 ± 11.361 ng\ml) compared with healthy controls (6.579 ± 2.685 ng\ml). COPD exacerbations showed also significant increased levels of serum ECP (39.195 ± 10.246 ng\ml) compared to stable COPD I-II (20.722 ± 3.343 ng\ml) and stable COPD III-IV (26.144 ± 5.799 ng\ml). But there was no significant differentiation among stable COPD groups (P=0.311). This indicates the systemic inflammation associated with COPD and suggest the possibility to use serum ECP as a diagnostic biomarker and to evaluate the inflammatory response in COPD exacerbations.

Key words: COPD, serum ECP, Acute Exacerbation, inflammation.

INTRODUCTION

COPD, a common preventable and treatable disease, is characterized by persistent airflow limitation that is usually progressive and associated with an enhanced chronic inflammatory response in the airways and the lung to noxious particles or gases. Exacerbations and comorbidities contribute to the overall severity in individual patients [1].

A clinical diagnosis of COPD should be considered in any patient who has dyspnea, chronic cough or sputum production, and a history of exposure to risk factors for the disease[2]. Spirometry is required to make the diagnosis; the presence of a post-bronchodilator $FEV_1/FVC < 0.70$ confirms the presence of persistent airflow limitation and thus of COPD[3].The severity of COPD is classified in terms of FEV_1into 4 stages[1],table(1).

Table 1: Classification of COPD (adapted from the gold guidelines 2015)

Stage I	**Mild**	**FEV1 ≥ 80% predicted.**
Stage II	**moderate**	**FEV1 ≤ 80% predicted.**
Stage III	**Severe**	**30% ≤ FEV1 ≤ 50% predicted.**
Stage IV	**very severe**	**FEV1< 30% predicted or FEV1 < 50% predicted + chronic respiratory failure.**

FEV1: Forced expiratory volume in the first second, FVC: Forced vital capacity

COPD is frequently complicated by acute exacerbations[4], defined as “an acute event characterised by a worsening of the patient’s respiratory symptoms that is beyond normal day-to-day variations and leads to change in

medication."[1]Frequent exacerbations may themselves result in decreased lung function and could thereby increase disease severity and mortality[5]. During COPD exacerbations, airway inflammation shows some features of asthmatic inflammatory processes, with recruitment of eosinophils and recovery of significant amounts of ECP in bronchial lavage fluid (BAL)[6]. Eosinophil cationic protein (ECP) is a potent cytotoxic secretory protein with bactericidal and antiviral properties. ECP is released by activated eosinophils and regarded as a marker of eosinophilic inflammation[7].

The aim of this study was to evaluate the serum levels of ECP in COPD stages and in COPD exacerbation, as a marker for the inflammatory response accompanying COPD in all stages and in attempt to use this inflammatory biomarker in the diagnosis of COPD exacerbations.

EXPERMENTAL SECTION

Study subjects: 88 individuals (82 males and 6 females) participated to this study and all of them signed an informed consent. They were divided into five groups: healthy nonsmokers (NS, n=11), healthy smokers (HS, n=17), mild to moderate stable COPD patients (COPD I-II, n= 15), severe to very severe stable COPD patients (COPD III-IV, n= 15) and patients with acute COPD exacerbations (ECOPD, n=30). Exclusion criteria included the use of immune-modulatory drugs like steroids within the past 14 days, history of asthma, autoimmune diseases, allergic diseases, lung diseases, heart diseases and tumors. Lung functions parameters (FEV1, FVC and FEV1\FVC) were measured using a spirometer.

Sampling: blood samples were collected and after centrifugation, serum was acquired and aliquots were stored at -40°C until assays were done.

Assays: Serum levels ECP were determined by using enzyme-linked immunosorbent assay (ELISA) kit. Human RNase ELISA kit (Uscn life science Inc., PRC). Absorbance is measured at 450 nm.

Statistical analyses: data were analyzed using Excel (2013) and SPSS version 22. Results were presented as mean ± SD. Comparisons between the means of two independent groups were performed using student's t-test. To analyze the variables, one-way analyses of variance (ANOVA) with Bonferroni post-hoc test correction was used. The area under ROC curve was concluded to determine the diagnostic value for ECP. P value <0.05 was considered significant.

RESULTS AND DISCUSSION

Serum levels of ECP in COPD patients and control group:

The mean of ECP serum levels was significantly higher in COPD patients (31.314 ± 11.361 ng\ml) than control group(6.579 ± 2.685 ng\ml), (P<0.05), figure (1).

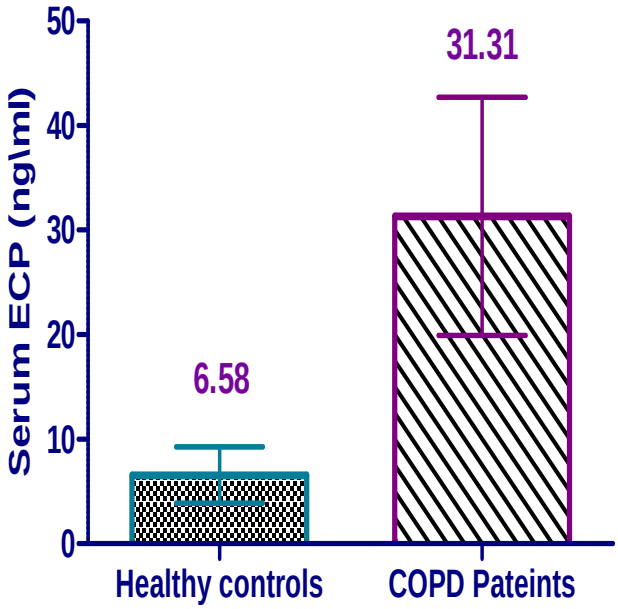

Figure (1): serum levels of ECP in COPD patients and healthy controls

Serum levels of ECP were 39.195 ± 10.246 ng\ml in ECOPD, 26.144 ± 5.799 ng\ml in COPD III-IV, 20.722 ± 3.343 ng\ml in COPD I-II, 7.729 ±2.560 ng\ml in healthy smokers (HS), 4.802 ± 1.805 in healthy non-smokers (NS), figure(2). Statistically significant differences were found between ECOPD group and other groups (COPD I-II, COPD III-IV, HS and NS) in our study. Statistically significant differences were found also between healthy groups and stable COPD (COPD I-II and stable COPD III-IV) groups. In contrast, statistically significant differences were not found between COPD I-II and COPD III-IV, nor between healthy smokers and healthy non-smokers, table (2).

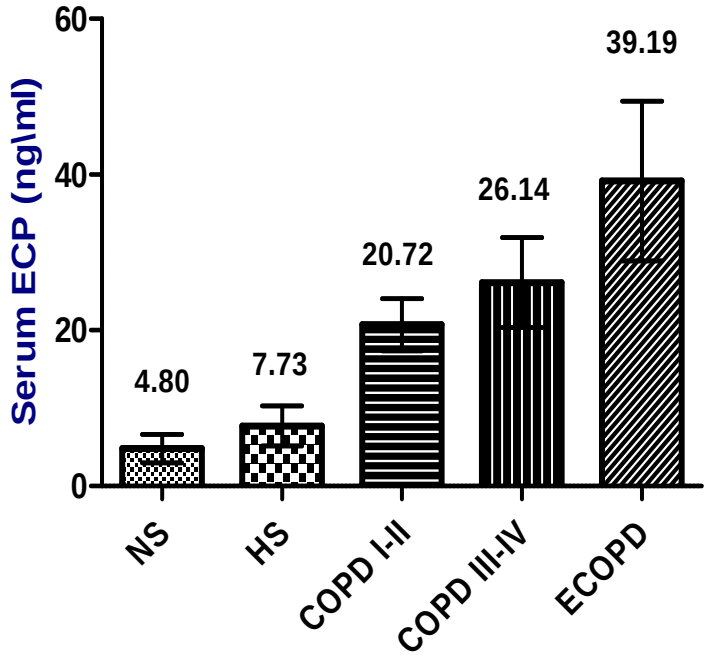

Figure (2): serum levels of ECP in study groups

Serum ECP sensitivity and specificity for differentiation between COPD patients and healthy controls:
Some cut-offs value of serum ECP sensitivity and specificity were shown in table (3). The best proportionally between sensitivity which was 1% and specificity which was 1% was given at the cut-off value 11.884 ng\ml which showed an excellent differentiation between COPD patients and healthy controls. In our study, this was the best value that represents the threshold (diagnostic value) between COPD patients and healthy controls. The area under roc curve was equals 1, figure (3).

Table (2): Significance of differences of means of ECP levels between 5 studied groups using the Bonferroni post-hoc test correction

The studied parameter	Group (I)	Group (J)	difference between two means (I-J)	standard error	P-vlaue	statistically significant differences
Serum levels of ECP	ECOP	COPD III-IV	13.05	2.14	< 0.001	Yes
		COPD I-II	18.47	2.14	< 0.001	Yes
		HS	31.47	2.06	< 0.001	Yes
		NS	34.39	2.39	< 0.001	Yes
	COPD III-IV	COPD I-II	5.42	2.47	0.311	No
		HS	18.41	2.4	< 0.001	yes
		NS	21.34	2.69	< 0.001	yes
	COPD I-II	HS	12.99	2.4	< 0.001	yes
		NS	15.92	2.69	< 0.001	yes
	HS	NS	2.93	2.62	1	No

Serum ECP sensitivity and specificity for differentiation between ECOPD and stable COPD III-IV:
Some cut-offs value of serum ECP sensitivity and specificity were shown in table (3). The best proportionally between sensitivity which was 83.3% and specificity which was 86.7% was given at the cut-off value 30.779 ng\ml which showed a good differentiation between ECOPD and stable COPD III-IV. In our study, this was the best value that represents the threshold (diagnostic value) between ECOPD and stable COPD III-IV. The area under roc curve was equals 0.889, figure (4).

Serum ECP sensitivity and specificity for differentiation between ECOPD and stable COPD I-II:
Some cut-offs value of serum ECP sensitivity and specificity were shown in table (3). The best proportionally between sensitivity which was 96.7% and specificity which was 1% was given at the cut-off value 30.779 ng\ml which showed an excellent differentiation between ECOPD and stable COPD III-IV. In our study, this was the best value that represents the threshold (diagnostic value) between ECOPD and stable COPD I-II. The area under roc curve was equals 0.998, figure (5).

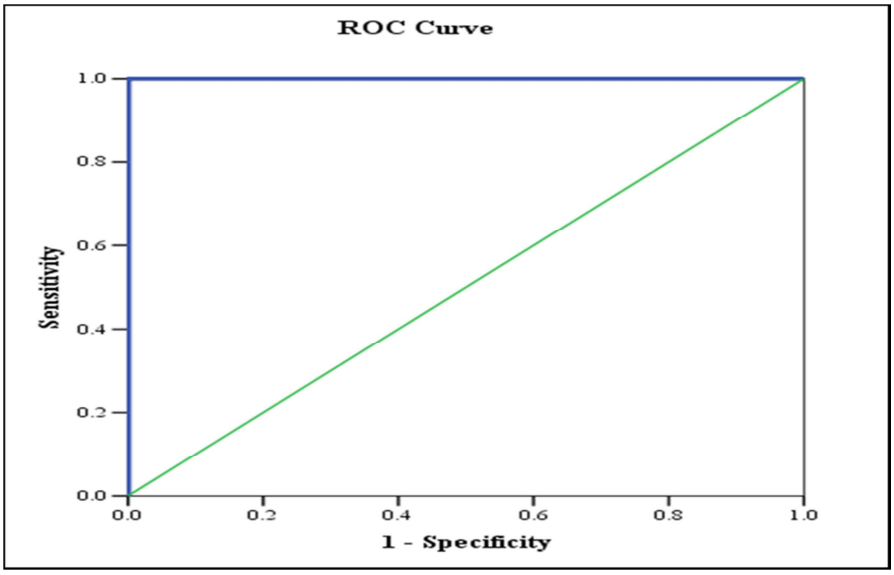

Figure (4): Roc curve for ECP between ECOPD and COPD III-IV

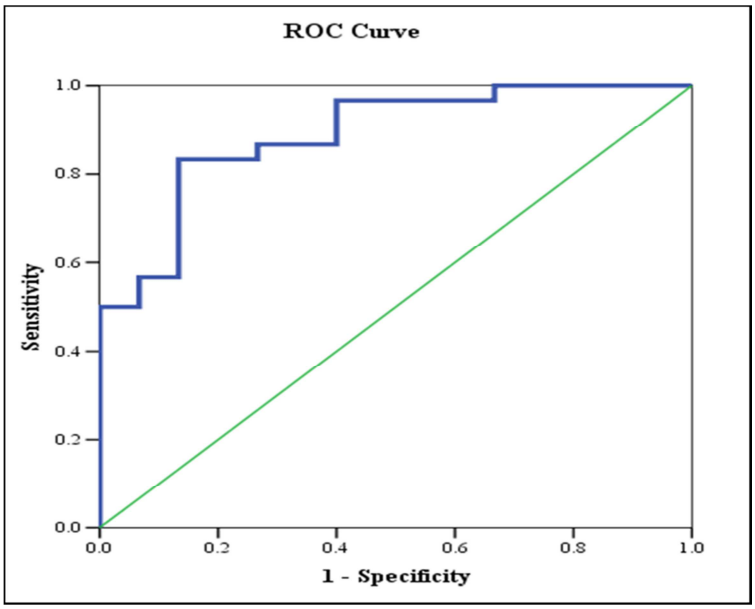

Figure (3): Roc curve for ECP between COPD patients and healthy controls

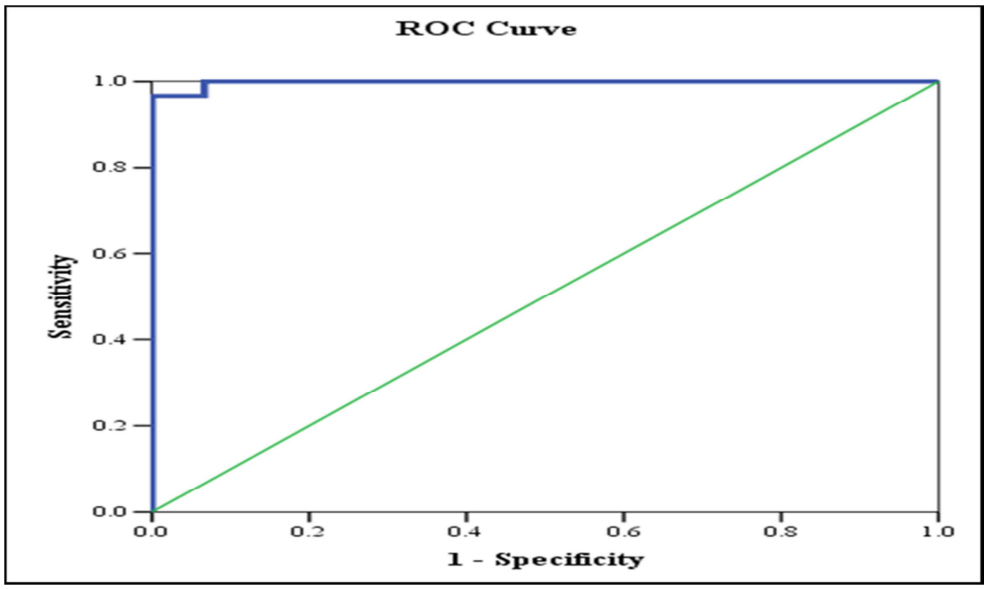

Figure (5): Roc curve for ECP between ECOPD and COPD I-II

Table (3): ECP sensitivity and specificity of differentiation among study groups.

COPD patients VS healthy controls			ECOPD VS stable COPD III-IV			ECOPD VS stable COPD I-II		
Cut-Off	**Specificity**	**Sensitivity**	**Cut-Off**	**Specificity**	**Sensitivity**	**Cut-Off**	**Specificity**	**Sensitivity**
9.277	0.786	1	28.388	0.600	0.867	22.697	0.733	1
9.910	0.821	1	28.617	0.667	0.867	23.631	0.800	1
10.011	0.857	1	29.176	0.733	0.867	24.252	0.867	1
10.219	0.929	1	30.030	0.733	0.833	24.642	0.933	1
10.895	0.964	1	30.560	0.800	0.833	25.469	0.933	0.967
11.884	**1**	**1**	**30.779**	**0.867**	**0.833**	**26.903**	**1**	**0.967**
13.636	1	0.983	31.291	0.867	0.733	27.933	1	0.933
16.057	1	0.967	31.897	0.867	0.667	28.947	1	0.867
17.647	1	0.950	33.263	0.867	0.567	30.250	1	0.833
18.226	1	0.933	34.556	0.933	0.567	31.291	1	0.733
18.594	1	0.917	35.021	0.933	0.5	31.897	1	0.667

Our results showed a clear increase in the serum levels of ECP in COPD patients compared to healthy controls ($P<0.05$) and significant increases were shown in ECOPD compared to stable COPD in all stages but not between Stable COPD stages. Also significant increases were shown in stable COPD groups compared to healthy controls (smokers and non-smokers). Significant differences were not shown between healthy smokers and healthy non-smoker.

The increased levels of serum ECP are associated with the systemic inflammation accompanying the disease. The association between the increase of serum ECP levels and the severity of COPD especially in exacerbations was clear. We can explain the high levels of ECP in COPD exacerbations by the Eosinophilic inflammation of airways during exacerbations and the activation of eosinophils which release the ECP.

Our results were in agreement with Abdel Samea ER et al[8], 2011 who found significant high level of serum ECP in both stable and acute COPD exacerbation when compared to healthy subjects. Incontrast of us, Abdel Samea ER et al didn't find significant difference between stable COPD and acute COPD exacerbation groups.

Also, Our results were in agreement with Fiorini G et al[6], 2000, who found significant high level of serum ECP in both stable and acute COPD exacerbation when compared to healthy subjects and between stable COPD group compared to COPD exacerbations. Also we were in agreement with Yu JF et al [9], 2011 in china who found significant high level of serum ECP in COPD patients compared to healthy subjects.

CONCLUSION

In conclusion, we demonstrated an increased levels of serum ECP in COPD patients with an excellent diagnostic value in the differentiation between COPD patients and healthy people. Serum levels of ECP also had a good differentiation between COPD exacerbations and stable COPD III-IV. In addition to an excellent differentiation

between COPD exacerbations and stable COPD I-II. So serum ECP can be used as a biomarker for COPD Exacerbations diagnosis and for the evaluation of the inflammatory response in COPD.

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