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Elevated serum levels of interleukin-6 and CRP in chronic obstructive pulmonary disease

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ABSTRACT

Chronic Obstructive Pulmonary Disease (COPD) is one of the major causes of increased morbidity and mortality in the world and it is characterized by persistent progressive airflow limitation accompanied by enhanced inflammatory response with enhanced levels of inflammatory mediators. The aim of our study was to determine levels of IL-6 and CRP as biomarkers in clinical evaluation of COPD patients. This study included 71 individuals: 12 patients with COPD exacerbation, 20 patients with severe to very severe COPD, 20 patients with mild to moderate COPD and 19 healthy controls (smokers and non-smokers). Serum levels of IL-6 and CRP were evaluated by ELISA. The results showed that both IL-6 and CRP were significantly increased ($p<0.05$) in serum of COPD patients (5.08 ±2.36 pg/mL), (14.12 ±10.90 mg/L) re spectively as compared with healthy controls. COPD exacerbations showed also significant increased levels of IL-6 (7.35 ± 1.77 pg/mL) and CRP (30.39 ± 6. 54 mg/L) as compared to healthy controls and to stable COPD stages. This indicates the systemic inflammation associated with COPD and suggests the possibility to use IL-6 and CRP as diagnostic biomarkers for COPD and to evaluate the inflammatory response in COPD patients.

Key words: COPD, Exacerbation, Inflammatory mediators, Interleukin-6, CRP.

INTRODUCTION

Chronic Obstructive Pulmonary Disease (COPD) is the fifth cause of morbidity and mortality in the developed world [1] and according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) guidelines, COPD will become the third cause of death worldwide by 2020. As defined by the GOLD, “COPD is characterized by persistent airflow limitation that is not fully reversible and usually progressive and associated with an enhanced chronic inflammatory response in the airways and the lung to noxious particles or gases”[2].

In order to diagnose COPD, an assessment of lung function parameters is required using spirometer which classifies COPD into four stages (table 1). Furthermore, a faster decline in lung function accompanied by increased respiratory symptoms including dyspnea, cough, sputum volume and purulence, is associated with COPD exacerbation [3], which is defined as “a sustained worsening of the COPD patient’s condition from the stable state and beyond normal day-to-day variations that is acute in onset and may warrant additional treatment” [4].

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

Stage I	Mild	$FEV_1/FVC < 0.70$
		$FEV_1 \geq 80\%$ predicted
Stage II	Moderate	$FEV_1/FVC < 0.70$
		$50\% \leq FEV_1 < 80\%$ predicted
Stage III	Severe	$FEV_1/FVC < 0.70$
		$30\% \leq FEV_1 < 50\%$ predicted
Stage IV	Very severe	$FEV_1/FVC < 0.70$
		$FEV_1 < 30\%$ predicted or $FEV_1 < 50\%$ predicted plus chronic respiratory failure

The main pathologic characteristics of COPD are chronic bronchitis and lung emphysema which are results of chronic inflammation and structural changes that affect the airways of COPD patients [5].

COPD is associated with systemic inflammation and this association has been evaluated in COPD patients, where activation of circulating inflammatory cells and increased levels of pro-inflammatory cytokines and acute-phase reactants were shown as well as increased oxidative stress[6]. While C-reactive protein is the most studied biomarker in COPD population, also many other inflammatory markers have been evaluated such as IL-6, IL-8, TNF-α and others[7]. Their increased levels in both stable COPD and exacerbation highlight their important role in the inflammatory response seen in COPD, especially with the relation between inflammation intensity and disease severity[8]. The importance of studies evaluating these inflammatory biomarkers is in improving COPD diagnosis, COPD progression and trying to identify effectiveness of anti-inflammatory therapy in COPD[9].

The aim of this study was to evaluate serum levels of interleukin-6 (IL-6) and CRP in COPD patients at different stages of the disease and in exacerbations, as markers for the inflammatory response accompanying COPD in all stages and in attempt to use these inflammatory biomarkers in supporting the diagnosis of COPD.

EXPERIMENTAL SECTION

Study Subjects:71 individuals (49 men and 22 women) participated to this study and all of them signed an informed consent. They were divided into five groups: healthy non-smokers volunteers (n=9), smokers not suffering from COPD (n=10), mild to moderate COPD patients (n=20), severe to very severe COPD patients (n=20) and patients with COPD exacerbations (n=12). Exclusion criteria included the use of immune-modulatory drugs (e.g. steroids) within the past 14 days, history of asthma, autoimmune diseases, lung diseases or any cardiopulmonary comorbidity. Lung functions parameters (FEV_1, FVC and FEV_1/FVC) were measured using a spirometer.

Sampling: Blood samples were collected and after centrifugation, serum was acquired and aliquots were stored at -20^0C until assays were done.

Assays: Serum levels of IL-6 and CRP were determined by using Enzyme-Linked Immunosorbent Assay (ELISA) kits. The IL-6 Human ELISA Kit (abcam, UK) utilizes an antibody specific for human IL-6 coated on a 96-well plate. The hsCRP ELISA kit (DRG, USA) utilizes a unique monoclonal antibody directed against a distinct antigenic determinant on the CRP molecule. Absorbance is measured at 450 nm.

Statistical analysis: data were analyzed using Excel (2007) and SPSS version 13.0.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 analysis of variance (ANOVA) with Bonferroni post-hoc test correction was used. The area under ROC curve was concluded to determine the diagnostic value for IL-6 and CRP. P-value <0.05 was considered significant.

RESULTS AND DISCUSSION

Serum levels of IL-6in COPD patients and control group:
The mean of IL-6 serum levels was significantly higher in COPD patients(5.08 ±2.36 pg/mL) than control group (0.47 ±0.26 pg/mL), ($p<0.05$), figure (1).

Serum levels of IL-6 were 7.35 ± 1.77 pg/mL in ECOPD, 4.55±2.03 pg/mL in COPD III-IV, 4.25 ±2.17 pg/mL in COPD I-II, 0.58±0.27 pg/mL in healthy smokers and 0.34 ±0.19 pg/mL in healthy non-smokers, figure (2). Statistically significant differences were found between healthy non-smokers and COPD I-II, healthy non-smokers and COPD III-IV, healthy non-smokers and ECOPD, healthy smokers and COPD I-II, healthy smokers and COPD III-IV, healthy smokers and ECOPD and also between ECOPD and COPDI-II, ECOPD and COPD III-IV, table (2).

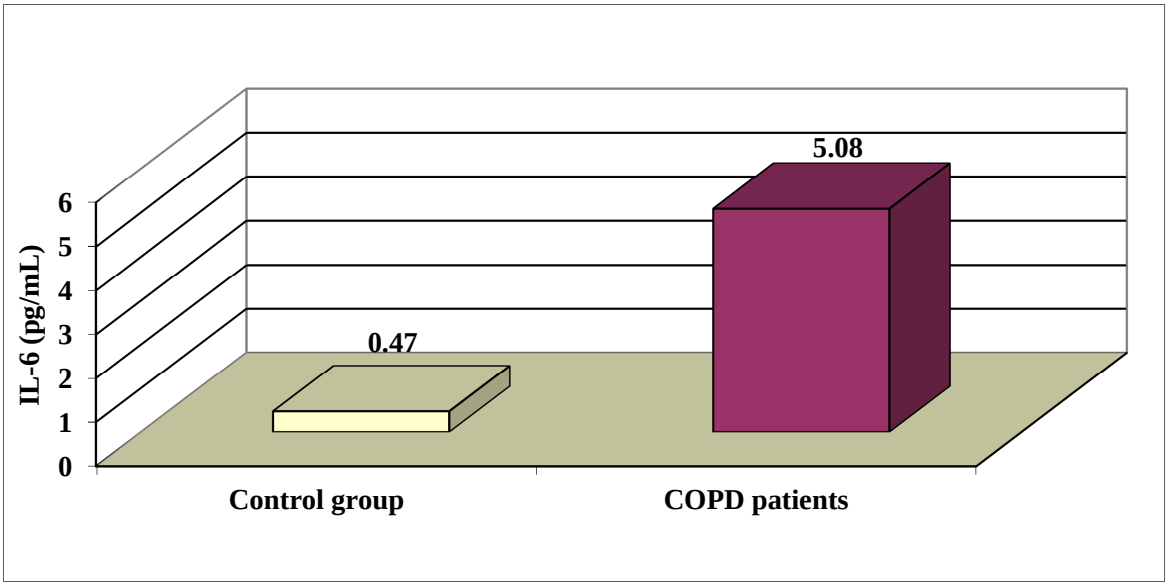

Figure 1: Serum levels of IL-6 in COPD patients and control group

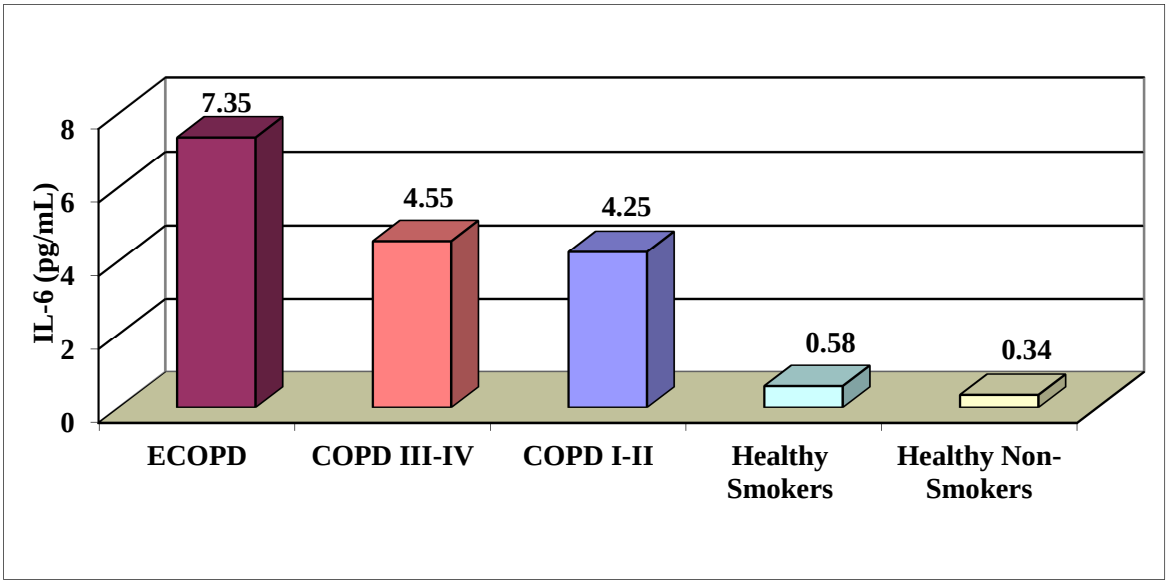

Figure 2: Serum levels of IL-6 in the 5 studied groups

Serum IL-6 sensitivity and specificity for differentiation between COPD patients and healthy controls:
Some cut-offs values of serum IL-6 sensitivity and specificity were shown in table (3). The best proportionality between sensitivity which was 96.2 % and specificity which was 100 % was given at the cut-off value 1.1395pg/mL which showed anexcellent differentiation between COPD patients and control group. In our study, this was the best value that represents the threshold (diagnostic value) between COPD patients and control group. The area under ROC curve was 0.995, figure (3).

Table 2: Significance of differences of means of IL-6 levels between the 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-value	Statistically significant differences
		COPD II-IV	2.81	0.64	0	**Yes**
		COPD I-II	3.1	0.64	0	**Yes**
		Healthy smokers	6.77	0.75	0	**Yes**
	ECOPD	Healthy non-smokers	7.01	0.77	0	**Yes**
Concentration of **IL-6**		COPD I-II	0.29	0.55	1	No
		Healthy smokers	3.96	0.68	0	**Yes**
	COPD III-IV	Healthy non-smokers	4.2	0.7	0	**Yes**
		Healthy smokers	3.67	0.68	0	**Yes**
	COPD I-II	Healthy non-smokers	3.91	0.7	0	**Yes**
	Healthy smokers	Healthy non-smokers	0.24	0.81	1	No

Table3: IL-6 sensitivity and specificity of differentiation between COPD patients and healthy controls at some cut-offs values

Cut-Off	Sensitivity	Specificity
0.7165	0.981	0.789
0.7265	0.981	0.842
0.744	0.981	0.895
0.8185	0.981	0.947
0.9915	0.962	0.947
1.1395	**0.962**	**1**
1.262	0.942	1
1.5145	0.923	1
1.892	0.904	1
2.3285	0.885	1
2.574	0.865	1

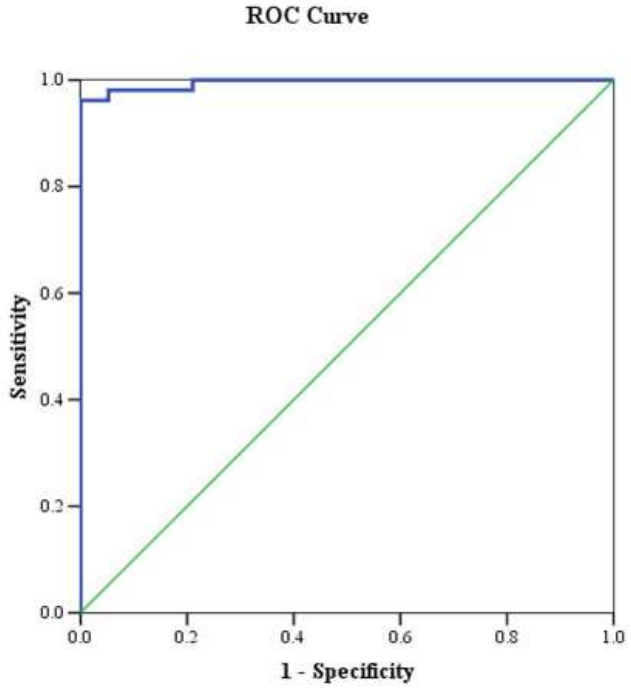

Figure 3: ROC curve for IL-6 between COPD patients and control group

Serum IL-6 sensitivity and specificity for differentiation between ECOPD and COPD I-II:

Some cut-offs values of serum IL-6 sensitivity and specificity was shown in table (4). The best proportionality between sensitivity which was 100% and specificity which was 55% was given at the cut-off value 4.121pg/mL which showed a moderate differentiation between ECOPD and COPD I-II. In our study, this was the best value that

represents the threshold (diagnostic value) between ECOPD and COPD I-II. The area under ROC curve was 0.846, figure (4).

Table 4: IL-6 sensitivity and specificity of differentiation between ECOPD and COPD I-II at some cut-offs values

Cut-Off	Sensitivity	Specificity
3.0835	1	0.3
3.3005	1	0.35
3.5125	1	0.4
3.616	1	0.45
3.6965	1	0.5
4.121	**1**	0.55
4.8	0.917	0.55
5.178	0.833	0.55
5.442	0.833	0.6
5.744	0.833	0.65
5.8665	0.833	0.7

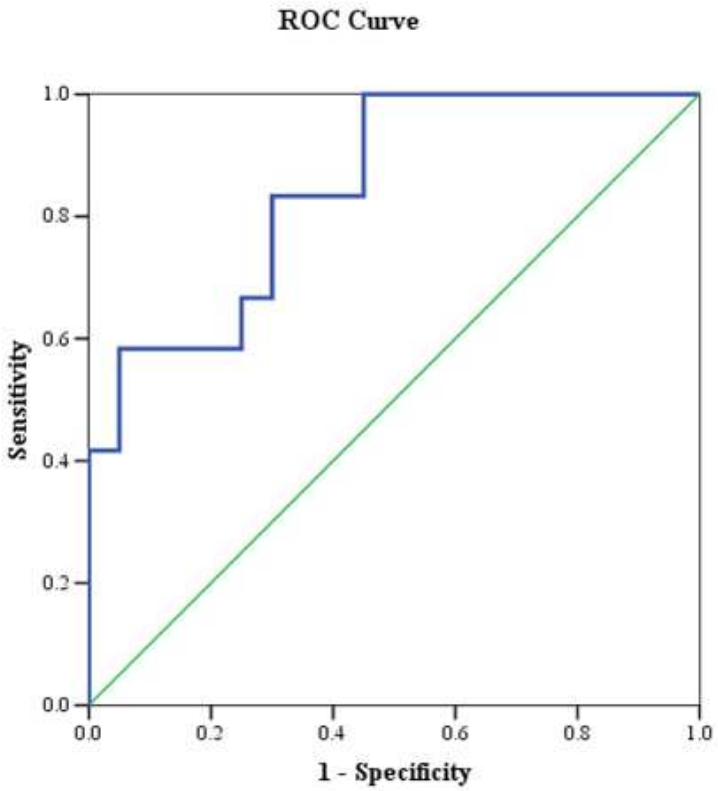

Figure 4: ROC curve for IL-6 between ECOPD and COPD I-II

Serum IL-6 sensitivity and specificity for differentiation between ECOPD and COPD III-IV:

Some cut-offs values of serum IL-6 sensitivity and specificity was shown in table (5). The best proportionality between sensitivity which was 83.3% and specificity which was 70% was given at the cut-off value 5.828pg/mL which showed a moderate differentiation between ECOPD and COPD III-IV. In our study, this was the best value that represents the threshold (diagnostic value) between ECOPD and COPD III-IV. The area under ROC curve was 0.842, figure (5).

Table 5: IL-6 sensitivity and specificity of differentiation between ECOPD and COPD III-IV at some cut-offs values

Cut-Off	Sensitivity	Specificity
4.611	0.917	0.3
4.7995	0.917	0.35
4.9885	0.917	0.4
5.3285	0.833	0.45
5.668	0.833	0.5
5.828	**0.833**	0.55
5.9035	0.75	0.55
6.0075	0.75	0.55
6.272	0.667	0.6
6.47	0.667	0.65
6.5075	0.583	0.7

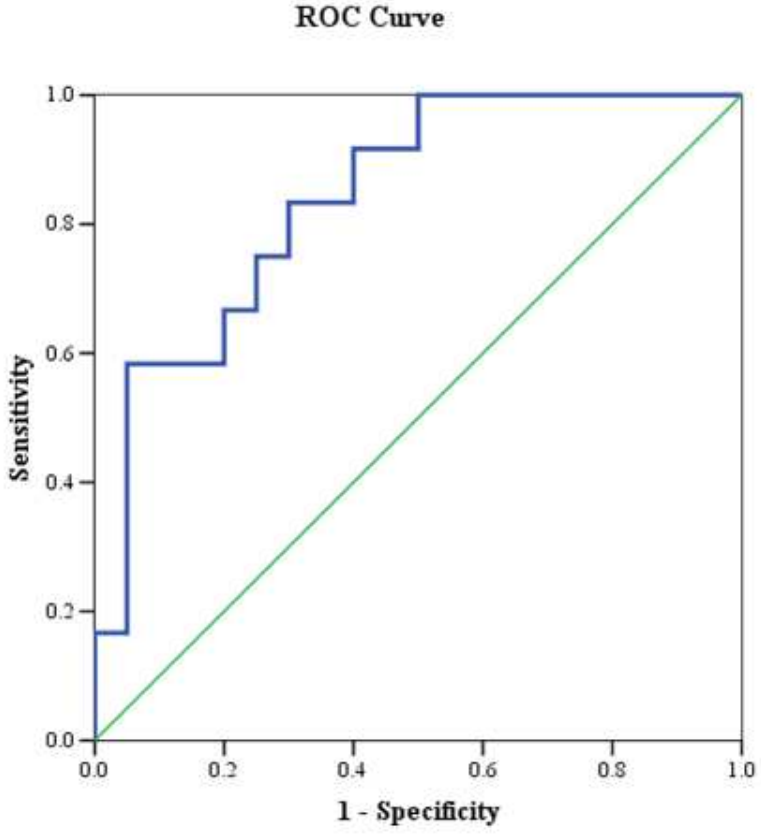

Figure 5: ROC curve for IL-6 between ECOPD and COPD III-IV

Serum levels of CRP in COPD patients and control group:
The mean of CRP serum levels was significantly higher in COPD patients (14.12 ±10.90 mg/L) than control group (2.39 ±2.75 mg/L), ($p<0.05$), figure (6).

Serum levels of CRP were 30.39 ± 6.54 mg/L in ECOPD, 10.53 ±6.27 mg/L in COPD III-IV, 7.95 ±5.82 mg/L in COPD I-II, 2.77 ±2.62 mg/L in healthy smokers and 1.97 ±2.99 mg/L in healthy non-smokers, figure (7). Statistically significant differences were found between healthy non-smokers and COPD III-IV, healthy non-smokers and ECOPD, healthy smokers and COPD III-IV, healthy smokers and ECOPD and also between ECOPD and COPDI-II, ECOPD and COPD III-IV, table (6).

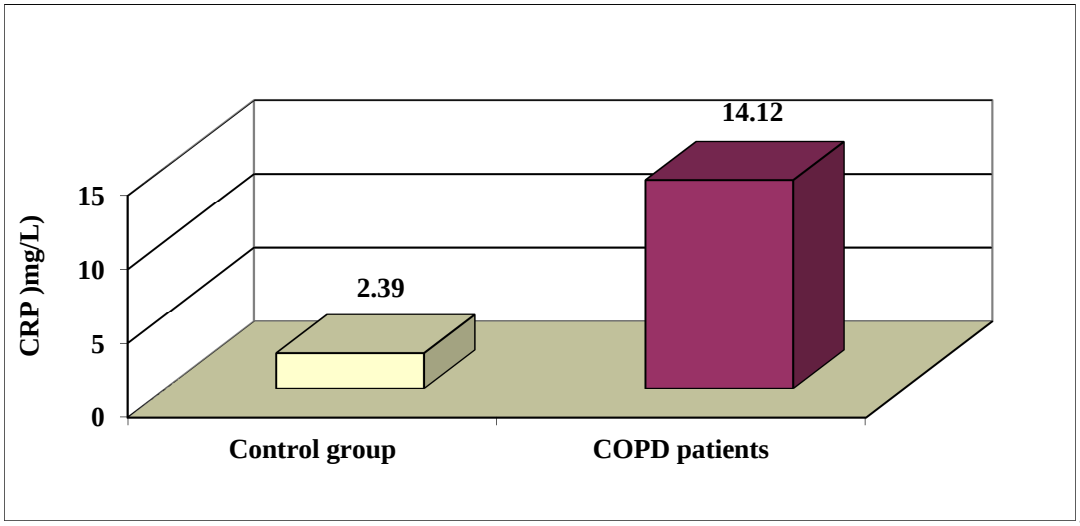

Figure 6: Serum levels of CRP in COPD patients and control group

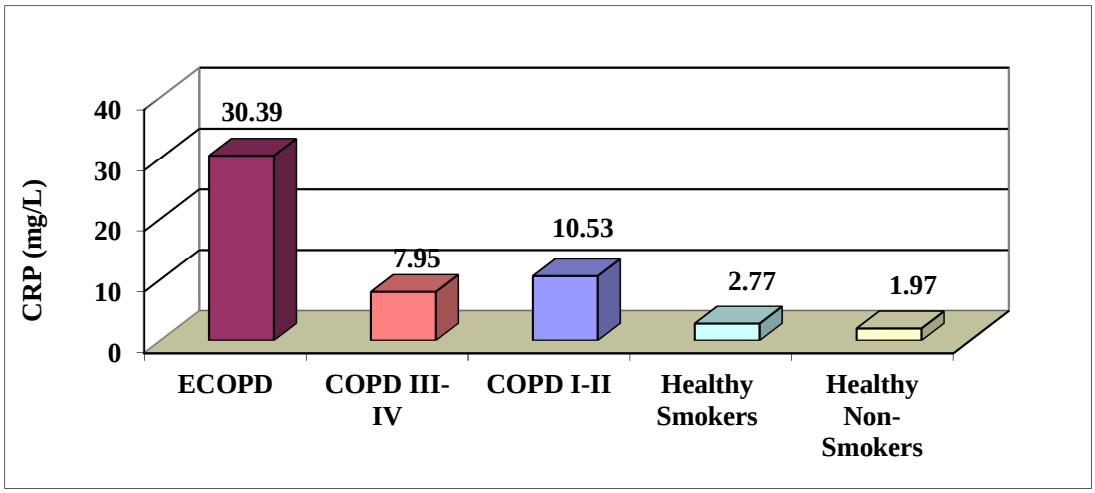

Figure 7: Serum levels of CRP in the 5 studied groups

Table 6: Significance of differences of means of CRP levels between the 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-value	Statistically significant differences
		COPD II-IV	19.86	2.01	0	**Yes**
		COPD I-II	22.44	2.01	0	**Yes**
		Healthy smokers	27.62	2.35	0	**Yes**
	ECOPD	Healthy non-smokers	28.43	2.43	0	**Yes**
Concentration of **CRP**		COPD I-II	2.58	1.74	1	No
		Healthy smokers	7.76	2.13	0.005	**Yes**
	COPD III-IV	Healthy non-smokers	8.57	2.21	0.002	**Yes**
		Healthy smokers	5.18	2.13	0.177	No
	COPD I-II	Healthy non-smokers	5.98	2.21	0.086	No
	Healthy smokers	Healthy non-smokers	0.8	2.53	1	No

Serum CRP sensitivity and specificity for differentiation between COPD patients and healthy controls:
Some cut-offs values of serum CRP sensitivity and specificity was shown in table (7). The best proportionality between sensitivity which was 86.5% and specificity which was 73.7 % was given at the cut-off value 2.6mg/L which showed agood differentiation between COPD patients and control group. In our study, this was the best value

that represents the threshold (diagnostic value) between COPD patients and control group. The area under ROC curve was 0.898, figure (8).

Table 7: CRP sensitivity and specificity of differentiation between COPD patients and healthy controls at some cut-offs values

Cut-Off	Sensitivity	Specificity
1.63	0.962	0.526
1.85	0.962	0.579
2	0.942	0.579
2.25	0.942	0.632
2.45	0.904	0.632
2.6	**0.865**	0.737
2.8	0.846	0.737
2.95	0.827	0.737
3.35	0.788	0.737
3.9	0.788	0.789
4.15	0.769	0.789

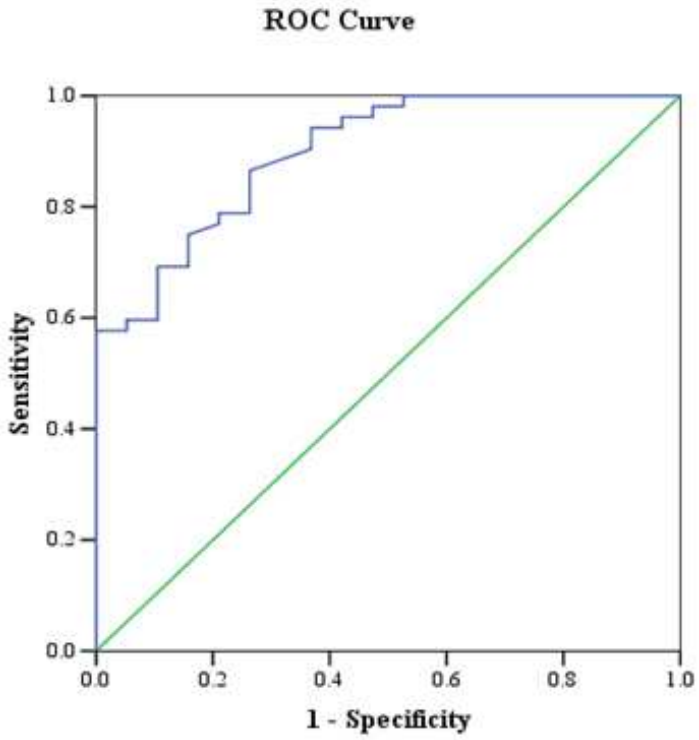

Figure 8: ROC curve for CRP between COPD patients and control group

Serum CRP sensitivity and specificity for differentiation between ECOPD and COPD I-II:

Some cut-offs values of serum CRP sensitivity and specificity was shown in table (8). The best proportionality between sensitivity which was 100% and specificity which was 100% was given at the cut-off value 18.3 mg/L which showed an excellent differentiation between ECOPD and COPD I-II. In our study, this was the best value that represents the threshold (diagnostic value) between ECOPD and COPD I-II. The area under ROC curve was 1.000, figure (9).

Table 8: CRP sensitivity and specificity of differentiation between ECOPD and COPD I-II at some cut-offs values

Cut-Off	Sensitivity	Specificity
9.95	1	0.65
13.45	1	0.7
15.15	1	0.8
16.1	1	0.85
16.75	1	0.95
18.3	**1**	1
21.75	0.917	1
23.95	0.833	1
24.9	0.75	1
26.05	0.667	1
28.3	0.583	1

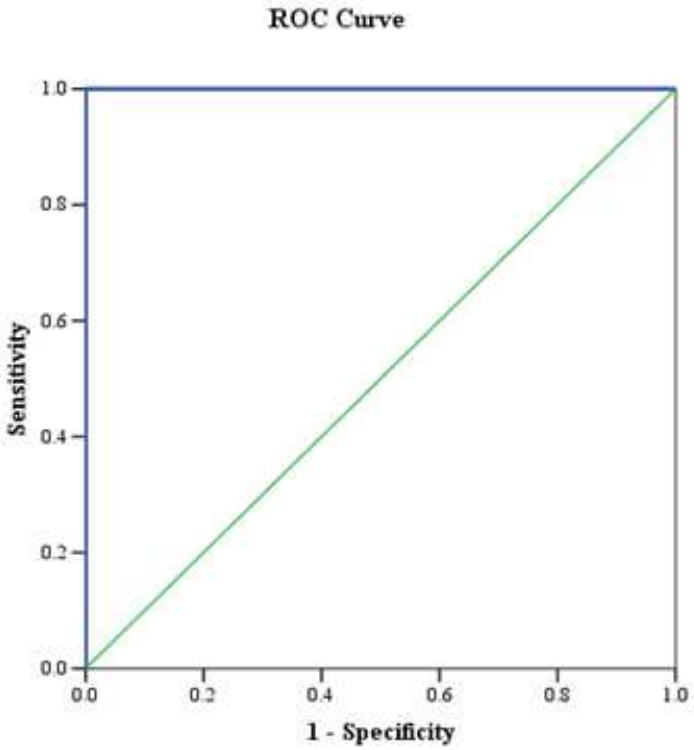

Figure 9: ROC curve for CRP between ECOPD and COPD I-II

Table 9: CRP sensitivity and specificity of differentiation between ECOPD and COPD III-IV at some cut-offs values

Cut-Off	Sensitivity	Specificity
16.3	1	0.7
16.55	1	0.75
16.7	1	0.8
16.95	1	0.85
17.35	1	0.9
18.6	**1**	**0.95**
19.65	0.917	0.95
21.8	0.917	1
23.95	0.833	1
24.9	0.75	1
26.05	0.667	1

Serum CRP sensitivity and specificity for differentiation between ECOPD and COPD III-IV:
Some cut-offs values of serum CRP sensitivity and specificity was shown in table (9). The best proportionality between sensitivity which was 100% and specificity which was 95% was given at the cut-off value 18.6 mg/L which showed an excellent differentiation between ECOPD and COPD III-IV. In our study, this was the best value that represents the threshold (diagnostic value) between ECOPD and COPD III-IV. The area under ROC curve was 0.996, figure (10).

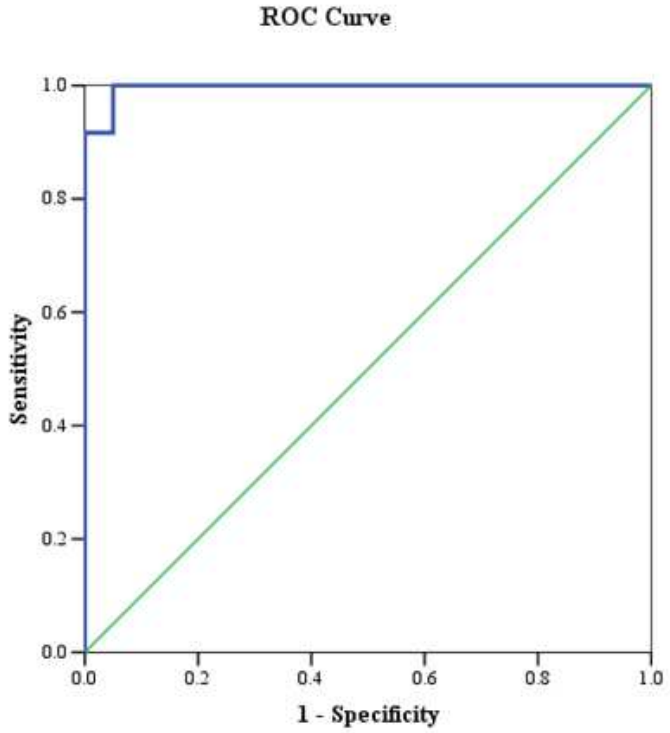

Figure 10: ROC curve for CRP between ECOPD and COPD III-IV

Our results showed a clear increase in the serum levels of IL-6 and CRP in COPD patients compared to control group ($p<0.05$)and significant increases were shown in ECOPD compared to healthy smokers and non-smokers and compared to stable COPD in all stages but not between stable COPD stages.

These increased levels of both IL-6 and CRP in COPD patients is associated with the systemic inflammation accompanying the disease which is shown by elevated levels of acute phase proteins (CRP) in blood and elevated levels of inflammatory cytokines after an overspill from the lungs. In our study, the association between the increase of these mediators and the severity of the disease especially with exacerbations was very clear. In addition, the high sensitivity and specificity of IL-6 and CRP serum levels allow us to determine the occurrence of COPD and to predict exacerbations in some cases.

Our results were in agreement with Malo et al, 2002 who found statistically significant differences between ECOPD and healthy non-smokers for both IL-6 ($p<0.05$) and CRP ($p<0.005$) serum levels[10] and also in agreement with the study of Valipour et al, 2008 which included 30 ECOPD patients, 30 stable COPD patients and 30 healthy controls and showed that IL-6 and CRP serum levels where higher in ECOPD than in stable COPD, which in turn are higher than in healthy controls with a statistically significant difference[11].

Our results were in accordance with Morales et al, 2010 who proved also increased levels of both IL-6 and CRP in COPD patients compared with healthy people[12] and with Garcia-Rio et al, 2010 who evidenced that this increase is associated with the stages of the disease, and thus with its severity[6]. Samy et al, 2010 [13]approved this and

Agusti et al, 2010who assumed that IL-6 and CRP serum levels increase with severity, but differences between stages were small and not consistent, furthermore, they showed that the increase of the biomarkers is not influenced by active smoking[14]. In 2012, Saldias et al evidenced also the increase of IL-6 and CRP serum levels in ECOPD with a study that included 120 patients with COPD exacerbations[15].

In contrast, our results were not similar to Ünver et al, 2014 who did not found statistically significant differences between COPD patients and healthy smokers and non-smokers in serum levels of IL-6 and CRP[16] and maybe it is due to the use of some anti-inflammatory drugs that affects the inflammatory response in patients.

In conclusion, we demonstrated increased serum levels of IL-6 and CRP in COPD patients including patients with exacerbation with an excellent diagnostic value given by IL-6 concentrations and a good one given by CRP in the differentiation between healthy people and COPD patients. Serum levels of IL-6 also had a moderate differentiation between ECOP and COPD I-II and COPD III-IV while CRP serum levels showed an excellent one between the same groups. Both IL-6 and CRP can be used as biomarkers for diagnostic purpose and for the evaluation of inflammatory response in COPD patients. Further investigations studying the effects of the use of anti-inflammatory drugs in COPD groups are also needed.

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REFERENCES

[1] G Viegi; F Pistelli;DL Sherrill et al,*Eur Respir J*, **2007**, 30(5), 993-1013.
[2] *Global Initiative for Chronic Obstructive Lung Disease.* . Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease. January **2015** [cited 2015 February 27].
[3] E Sapey; R.A. Stockley, *Thorax*, **2006**, 61(3), 250-8.
[4] J.A. Wedzicha; G.C. Donaldson,*Respir Care*, **2003,** 48(12),1204-13.
[5] S Hacker; C Lambers; K Hoetzenecker et al,*Clin Lab*, **2009**, 55(1-2), 31-40.
[6] F Garcia-Rio;M Miravitlles; JB Soriano et al, *Respir Res*, **2010,** 11(1), 63.
[7] M Dahl;J Vesto;P Lange P;SE Bojesen; A Tybjaerg-Hansen;BG Nordestgaard , *Am J Respir Crit Care Med*, **2007**, 157(3), 250-5.
[8] K Kostikas; AKoutsokera; S Papiris;KI Gourgoulianis; S Loukides, *Clin Exp Allergy*., **2008,** 38(4), 557-65.
[9] PJ Barnes;B Chowdhury; SA Kharitonov; H Magnussen; CP Page; D Postma; M Saetta , *Am J Respir Crit Care Med*, **2006**, 174(1), 6-14.
[10] O Malo; J Sauleda; X Busquets et al, *Arch Bronconeumol*, **2002,** 38(4), 172-6.
[11] A Valipour;M Schreder; M Wolzt et al;*Clin Sci (Lond)*, **2008**, 115(7), 225-32.
[12] S A Morales; DJ Dreyse;PO Díaz; PF Saldías; M Carrasco; BC Lisboa, *Rev. Med. Chill*, **2010,** 138(8), 957-64.
[13] N Samy;MD Abd El-Maksoud; A EL Khayyal; A Imam, *New York Science Journal*, **2010,** 6(3), 25-32.
[14] A Agustí; LD Edwards;SI Rennard et al,*PLoS One*, **2012,** 7(5).
[15] PF Saldias; PO Díaz; DJ Dreyse et al, *Rev Med Chil*, **2012**, 140(1), 10-8.
[16] R Ünver; F Deveci; G Kirkil; D Kaman, *ERJ*, **2014,** 44,3656.