



## Assessment of Inflammatory Markers among COPD Patients and Healthy Smokers

Authors

Eman Omar Arram<sup>1</sup>, Noha Tharwat Abou El-Khier<sup>2</sup>, Safaa Mohamad EL-Ageery<sup>2</sup>

<sup>1</sup>Thoracic Medicine Department, <sup>2</sup>Medical Microbiology and Immunology Department, Faculty of Medicine, Mansoura University

### Abstract

**Background:** *The pathogenesis of COPD patients includes a variety of systemic inflammatory factors in response to environmental stimuli particularly among cigarette smokers.*

**Aim of the work:** *This study aimed to show the levels of serum inflammatory markers as interleukin (IL-8), Tumor necrosis factor- alpha (TNF- $\alpha$ ) and C-reactive protein (CRP) in stable Chronic obstructive pulmonary disease (COPD) patients, both healthy current smokers and non-smokers, and to show its relation with smoking load and the severity of airway obstruction.*

**Patients and Methods:** *The study was conducted on 70 cases divided into three groups; 40 COPD patients, 20 healthy current smokers and 10 healthy non smokers as a control., All cases age and sex characteristics were similar, complete history taking, general, local chest examination, pulmonary function tests (PFT), Serum levels of interleukin (IL-8), tumor necrosis factor-alpha (TNF- $\alpha$ ) and C-reactive protein (CRP) were measured in all groups.*

**Results:** *There were highly statistically significant difference (<0.001) as regards inflammatory cytokines IL8 and TNF- $\alpha$  and CRP among the three groups also as regard spirometer parameters. There were highly significant negative correlation between all markers and FEV1, while there were highly significant positive correlation between all markers and smoking load in COPD patients. There were highly significant negative correlation between all markers and FEV1 while significant positive correlation between only IL8 and pack-year index in healthy smoker group.*

**Conclusion:** *Plasma levels of inflammatory markers: IL8, TNF- $\alpha$  and CRP were high in COPD patients also the increase in serum inflammatory markers has a direct correlation with the severity of COPD and airway obstruction which conclude that markers of systemic inflammation have an important role in COPD pathogenesis.*

**Abbreviation:** *Chronic obstructive pulmonary disease (COPD), C-reactive protein (CRP), interleukin (IL-8), tumor necrosis factor- $\alpha$  (TNF-alpha), enzyme immunoassay (EIA), Forced expiratory volume in 1 second (FEV1).*

**Keywords:** *COPD, FEV1, IL8, TNF- $\alpha$ , CRP.*

### Introduction

Chronic obstructive pulmonary disease (COPD) is considered one of the major causes leading to

death<sup>[1]</sup>. COPD is characterized by reversible airway obstruction which is progressive and incomplete. It is associated with an abnormal

systemic inflammatory response of the lungs to particles or noxious gases<sup>[2-3]</sup>.

Pathogenesis of the majority of COPD systemic effects and systemic inflammation are due to chronic inflammation in the pulmonary tissue.<sup>[4]</sup>

Proinflammatory Cytokines are extracellular signaling proteins formed by various cells types in the body. In the last decade the role of inflammation and of proinflammatory cytokines in the COPD pathogenesis has been investigated. Several retrospective studies have postulated that active inflammation marked by increased serum levels of Tumor necrosis factor- alpha (TNF- $\alpha$ ), interleukin (IL-8), and C-reactive protein (CRP) was associated with the progression of COPD<sup>[5,6]</sup>.

An intense chronic inflammation of the lungs leads to progression of COPD<sup>[7]</sup>. Noxious particles that induce long-term tissue damage and acute inflammation induced by both contribute to chronic persistent inflammation. Some details regarding pathogenesis of COPD inflammation have been Known, a large defect in knowledge is present as regard role of the inflammatory cells in the progression of the disease<sup>[8]</sup>.

Higher prevalence of respiratory symptoms and lung function abnormalities, a greater annual rate of decline in FEV1 and a greater COPD mortality rate are present in cigarette smokers than nonsmokers. These differences between smokers and nonsmokers show direct correlation with the smoking severity (pack-years). Patients should be identified as early in the course of the disease as possible and certainly before the end stage of the illness when disability is substantial<sup>(1)</sup>.

IL-8 is considered a multifunctional chemokine involved in inflammation-mediated neutrophil infiltration and chemotaxis<sup>[9]</sup>. IL-8, also known as CXCL8, is a CXC chemokine that is a potent chemo attractant for neutrophils. In general, monocytes, tissue and alveolar macrophages, pulmonary epithelium, smooth muscles cells of the airway, eosinophils, fibroblasts and endothelial cells are its important sources<sup>[10]</sup>.

Tumor necrosis factor- alpha (TNF- $\alpha$ ) it is thought to play a critical role in the pathogenesis of COPD

by promoting and maintaining the expression and the release of various proinflammatory mediators that lead to tissue damage and remodeling. It is a powerful proinflammatory cytokine primarily produced by activated macrophages<sup>[11]</sup>.

There are two different hypotheses that have been proposed to explain the association between CRP and COPD. The first hypothesis is the effect of lung inflammation itself in COPD patients. It is extensively known that prolonged exposure to cigarette smoking cause lung injury and inflammation<sup>[12]</sup>. Once this process starts, lung inflammation persists even after smoking cessation<sup>[13]</sup>, thus resulting in an exponential systemic reaction, related to the severity of COPD<sup>[14]</sup>.

CRP was reported by some authors that its increase is secondary to increase of serum concentration of other pro-inflammatory cytokines (TNF- $\alpha$ , IL-8)<sup>[15]</sup>.

#### **We aimed in this study**

To show the levels of serum inflammatory markers as CRP, IL8 and TNF- $\alpha$  in stable COPD patients, both healthy current smokers and non smokers and to show its relation with smoking load and the severity of airway obstruction.

#### **Patients and Methods**

The study was conducted on 70 cases, Data collected in the outpatient clinics of Thoracic Medicine Department of Mansoura University Hospitals during period from January 2016 to the January 2018.

The cases were categorized into 3 main groups:

Group 1: included 40 COPD patients diagnosed as COPD according to GOLD 2017 (1).

Group 2: included 20 healthy current smokers

Group 3: included 10 healthy non smokers.

#### **Inclusion Criteria**

- 1) Age >40 years
- 2) Stable disease for at least 2 months

#### **Exclusion Criteria**

- 1) Patients with pulmonary disease other than COPD
- 2) Cardiac diseases, hepatic cirrhosis, end stage renal disease, malignancy.

### 3) Refuse study.

All cases age and sex characteristics were matching, complete history taking, general and local chest examination. Pulmonary function tests (PFT) Manufactured by medical equipment Europe -Hammelburg -Germany were performed in all cases according to American Thoracic Society Standards<sup>[16]</sup>.

Informed consent was obtained from all cases, the protocol of this research was approved by the ethical committee in the Faculty of Medicine, Mansoura University, with code number R/17.11.104.

### Serum Samples

5–10 mL venous blood samples were withdrawn in plain tube under aseptic conditions from patients and control. It was left to clot at room temperature for 30 minutes then centrifuged at 3000 RPM for 15 minutes, and the serum was separated and was quickly frozen at  $-70^{\circ}\text{C}$  and stored until processed.

#### IL-8:

IL-8 levels were measured by enzyme immunoassay (EIA) for the in vitro quantitative measurement of human IL-8 in serum (Boster Biological Technology Co., LTD; human IL-8 ELISA kit).

#### TNF- $\alpha$ :

TNF- $\alpha$  levels were measured by EIA (Thermo Fisher scientific Co.; TNF alpha Human ELISA Kit).

#### CRP:

Serum CRP level measurement was tested by the semi-quantitative latex agglutination test (Omega Diagnostics kits, UK) according to manufacturer's guide.

### Statistical Analysis

The collected data were coded, processed and analyzed using the SPSS (Statistical Package for Social Sciences) version 15 for Windows® (SPSS Inc, Chicago, IL, USA). Qualitative data was presented as number and percent. Comparison between groups was done by Chi-Square test.

Quantitative data was tested for normality by Kolmogorov-Smirnov test. Normally distributed data was presented as mean  $\pm$  SD. Student t-test was used to compare between two groups. F-test (One Way Anova) was used to compare between more than two groups. Pearson's correlation coefficient was used to test correlation between variables.

### Results

This study was conducted on 70 cases divided into 3 groups; 40 COPD patients, 20 healthy current smoker and 10 healthy non smokers as a control. Both patients and control groups were cross matched for age and sex

Table 1 presents the demographic data of the three groups as regards age and sex, pack-year smoking index.

The mean age among COPD patients was  $55.45 \pm 4.94$ , it was  $53.1 \pm 6.8$  in the healthy smoker group, while the mean age among healthy non smokers was  $52.6 \pm 7.47$ .

Pack-year smoking index in COPD group was  $39.4 \pm 7.69$  while in healthy smoker was  $19.8 \pm 4.47$  which is highly statistically significant difference between both ( $<0.001$ ).

Table 2 shows statistically significant difference ( $<0.001$ ) in spirometer parameters among the three groups, FEV1/FVC%, FEV1 AND FVC were  $51.35 \pm 6.69$ ,  $49.2 \pm 11.46$  and  $60.05$  in COPD patients VS  $83.1 \pm 3.74$ ,  $82 \pm 4.68$  and  $87.3 \pm 5.77$  in healthy current smoker while  $85.8 \pm 5.39$ ,  $84.6 \pm 3.44$  and  $94.8 \pm 7.32$  in healthy non smokers.

Table 3 shows statistically significant difference ( $<0.001$ ) between the three groups as regards inflammatory cytokines IL8 and TNF- $\alpha$  and CRP. The results as regard inflammatory cytokines (IL8 and TNF- $\alpha$ ) showed: IL8 was  $97.7 \pm 19.89$  pg/ml in COPD patients vs  $40.3 \pm 4.43$  pg/ml in second group while it was  $20.2 \pm 4.18$  pg/ml in non smokers group

Also, TNF- $\alpha$  level was  $79.65 \pm 21.31$  pg/ml in COPD group vs  $30.4 \pm 6.16$  pg/ml in healthy current smoker while the level in third group was  $15.2 \pm 3.91$  pg/ml

While inflammatory marker level CRP was 21.45 ±5.73mg/l in first COPD group vs 8.5±3.71 mg/l in second group while it was 3.2±1.23 mg/l in third group.

Table (4) presents correlation between both (FEV1 and pack- year smoking index) and inflammatory markers IL8,TNF-αand CRP in COPD patients; there were highly significant negative correlation between all markers and FEV1, while there were highly significant correlation between all markers and smoking load.

Table (5) presents correlation between both (FEV1 and pack-year smoking index) and inflammatory markers IL8, TNF-α and CRP in healthy smoker group; there were highly significant negative correlation between all markers and FEV1 while significant positive correlation between IL8 and pack-year index.

Fig (1) shows spirometer parameters in all three groups and Fig (2) shows levels of inflammatory markers in all three groups.

**Table (1):** Demographic data of the three groups

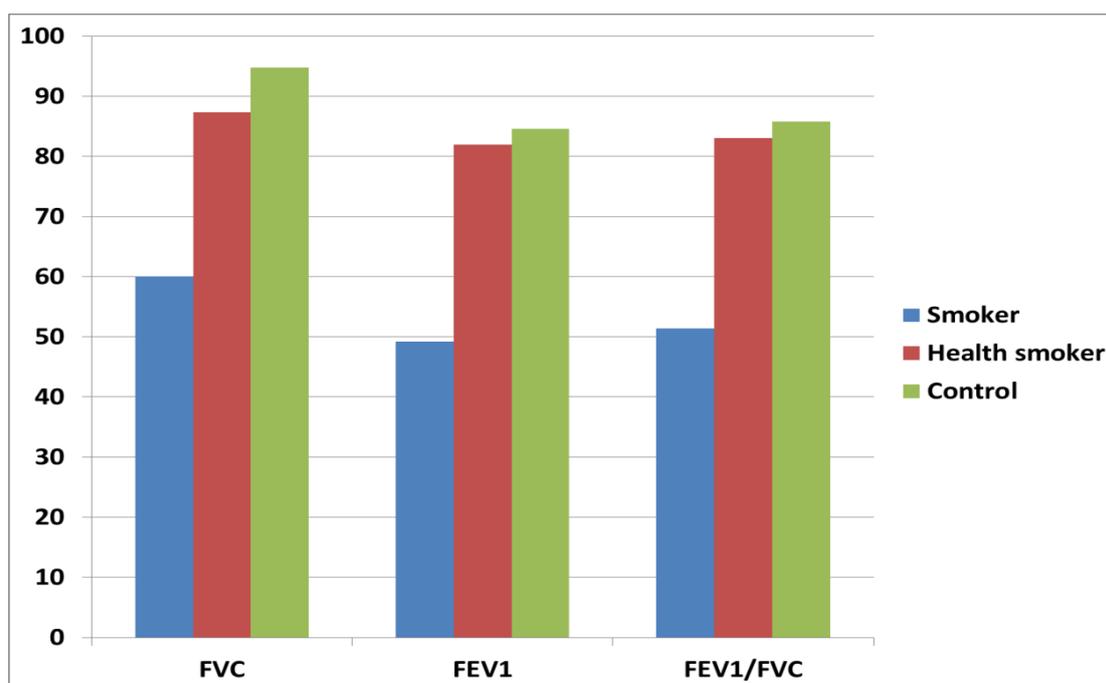
	Smokers (n = 40)	Healthy smokers (n = 20)	Control (n = 10)		P
Age	55.45 ± 4.94	53.1 ± 6.8	52.6 ± 7.47	F=1.585	0.213
Pack/year	39.4 ± 7.69	19.8 ± 4.47		t=12.456	< 0.001*
Sex				χ <sup>2</sup> = 0.847	0.655
Male	36 (90%)	18 (90%)	8 (80%)		
Female	4 (10%)	2 (10%)	2 (20%)		

\*Significant P < 0.05

**Table (2):** Pulmonary function of the three groups

	Smokers (n = 40)	Healthy smokers (n = 20)	Control (n = 10)	F	P
FEV1	49.2 ± 11.46	82 ± 4.68	84.6 ± 3.44	115.499	< 0.001*
FVC	60.05 ± 9.22	87.3 ± 5.77	94.8 ± 7.32	117.632	< 0.001*
FEV1/FVC%	51.35 ± 6.69	83.1 ± 3.74	85.8 ± 5.39	270.112	< 0.001*

\*Significant P < 0.05

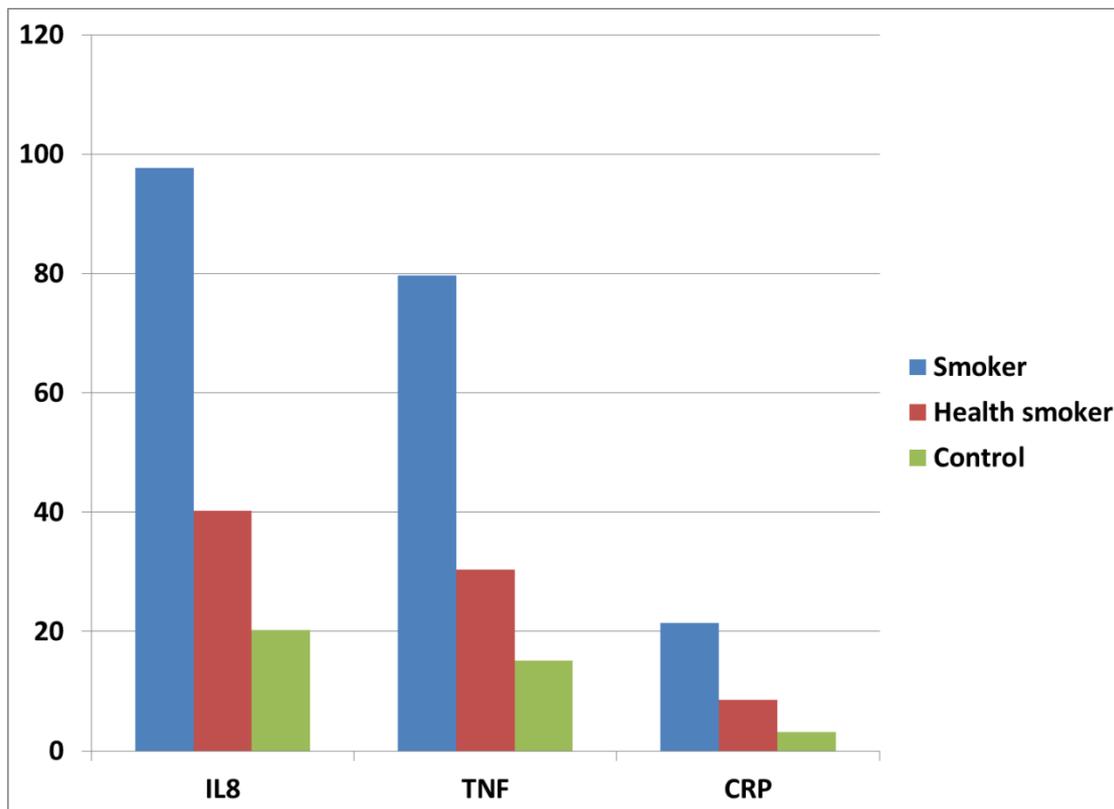


**Fig (1):** Spirometer parameters in all three groups

**Table (3):** Inflammatory cytokines IL8, TNF- $\alpha$  and CRP in the three groups

	Smokers (n = 40)	Healthy smokers (n = 20)	Control (n = 10)	F	P
<b>IL-8 (pg/ml)</b>	97.7 $\pm$ 19.89	40.3 $\pm$ 4.43	20.2 $\pm$ 4.18	153.517	< 0.001*
<b>TNF<math>\alpha</math> (pg/ml)</b>	79.65 $\pm$ 21.31	30.4 $\pm$ 6.16	15.2 $\pm$ 3.91	94.010	< 0.001*
<b>CRP (mg/L)</b>	21.45 $\pm$ 5.73	8.5 $\pm$ 3.71	3.2 $\pm$ 1.23	83.940	< 0.001*

\*Significant P < 0.05



**Fig (2):** levels of inflammatory markers in all three groups.

**Table (4):** Correlation between both (spirometer parameters and pack- year smoking index) and inflammatory markers IL8, TNF- $\alpha$  and CRP in COPD patients

	FVC		FEV1		FEV1/FVC		Back/year	
	R	P	r	P	r	P	r	P
<b>IL8</b>	-0.892	< 0.001*	-0.915	< 0.001*	-0.928	< 0.001*	0.876	< 0.001*
<b>TNF</b>	-0.902	< 0.001*	-0.951	< 0.001*	-0.947	< 0.001*	0.896	< 0.001*
<b>CRP</b>	-0.926	< 0.001*	-0.935	< 0.001*	-0.942	< 0.001*	0.864	< 0.001*

\*Significant P < 0.05

**Table (5):** Correlation between both (spirometer parameters and pack- year smoking index) and inflammatory markers IL8, TNF- $\alpha$  and CRP in health smoker group

	FVC		FEV1		FEV1/FVC		Back/year	
	r	P	r	P	r	P	r	P
<b>IL8</b>	-0.536	0.015*	-0.783	< 0.001*	-0.638	0.002*	0.472	0.036*
<b>TNF</b>	-0.569	0.009*	-0.749	< 0.001*	-0.687	0.001*	0.187	0.431
<b>CRP</b>	-0.254	0.281	-0.200	0.397	0.140	0.555	0.261	0.267

\*Significant P < 0.05

## Discussion

COPD is characterized by progressive expiratory airflow limitation resulting from an abnormal inflammatory response to noxious particles or gases<sup>[17]</sup>. Increased neutrophils, macrophages, T-lymphocytes, and increased cytokines including IL-6, IL-8, and TNF- $\alpha$  considered as an initial inflammatory response to harmful noxious particles or gases. The lungs are not the only organ develop inflammation. Many studies have shown elevated systemic levels of IL-6, IL-8, and TNF- $\alpha$  in patients with COPD. This may be due to release of mediators from the lungs. There is positive correlation between increase in these proinflammatory cytokines and severity of COPD and is believed to contribute to the systemic comorbidities associated with COPD, Hacievliyagil et al.<sup>[18]</sup> observed that higher concentrations of inflammatory cytokines, including IL-6, IL-8 and TNF- $\alpha$ , are reported in patients with more severe COPD compared with those with less severe COPD.

Cigarette smoking is the major risk factor in the development and progression of COPD. The degree of airway inflammation seems higher in COPD patients as compared to healthy non-smokers, irrespective whether these patients were current smokers or ex-smokers<sup>[19]</sup>.

It is difficult to know exact role of cigarette smoking in the pathogenesis of COPD, this is my attributed to many causes :First :many researches take sample that contain smokers and ex-smokers .Second :many researches recruit COPD patients with chronic bronchitis patients .Third: investigations of one inflammatory aspect or one type of sample (serum, sputum, broncho-alveolar lavage ,bronchial biopsies, peripheral airway) may be inadequate to get good opinion .Fourth: process of remodeling in COPD may continue lung inflammation independent of the effect of cigarette smoking<sup>[20]</sup>.

Systemic inflammation decreased when lung function improved and increased when lung function decreased .systemic inflammation leads to dysfunction in respiratory endothelium, leading

to infiltration of pulmonary vessels and lung parenchymal damage.<sup>[21]</sup>

We observed that COPD was associated with elevated IL-8 levels when compared with control groups However, Aaron et al.<sup>[22]</sup> indicated that IL-8 level was not associated with COPD pathogenesis. The inconsistent findings may be related to the inclusion of studies with different baseline characteristics and early stages of COPD that are insensitive to systemic inflammatory markers.

The results of the present work are consistent with the study of Daldegan et al.<sup>[23]</sup> who found that serum IL-8 concentrations were higher in COPD patients than in patients with asthma or in healthy control individuals.

Also, Garcia-Rio et al.<sup>[24]</sup> found that COPD patients showed higher levels of IL-8 compared with controls, and serum concentrations were related to the severity of COPD, also we found higher significant negative correlation between IL8 and FEV1 in both COPD and healthy smoker. Also, Demirci et al.<sup>[25]</sup>, studied 23 COPD patients (Stage I), 15 (Stage II) and 12 (Stage III-IV). Ten healthy nonsmoking as control group. They found that as the stage of COPD increased, the levels of IL-8 increased.

The role of TNF- $\alpha$  in COPD is thought to be central to both lung and systemic inflammation<sup>[26]</sup>.

In the present study, serum TNF- $\alpha$  was significantly higher in COPD patients than both control groups, with also highly significant negative correlation between its level and FEV1 in both COPD and healthy smoker.

The results of this work are consistent with those of Takabatake et al.<sup>[27]</sup> and Bolton et al.<sup>[28]</sup>, who found that the serum level of TNF- $\alpha$  was higher in COPD patients than in control participants.

Our results agree with Abd El-Maksoud et al.<sup>[29]</sup>, Garcia-Rio et al.<sup>[30]</sup>, Xie et al.<sup>[31]</sup>, Ibrahim et al.<sup>[32]</sup>, and Abd El Aziz et al.<sup>[33]</sup>, who found that the concentrations of circulating TNF- $\alpha$  were significantly higher in patients with COPD in comparison with the control group, and their

levels increased according to the stage of the disease.

Bruno et al.<sup>[34]</sup> studied 90 individuals [subdivided into three equal groups: group I (control), group II (patients with COPD), and group III (patients with COPD and cardiovascular complications)], and they found no significant difference between the groups.

Five studies suggested that COPD was associated with lower or insignificant serum TNF- $\alpha$  levels<sup>[35-39]</sup>.

Our results showed also no significant correlation between its level and pack-year index in healthy smoker.

Yende et al.<sup>[40]</sup> and Amer et al.<sup>[41]</sup> explain the decrease in serum TNF- $\alpha$  in COPD patients by the relatively short serum half-life of TNF- $\alpha$ .

In the present study, there were a significant negative correlation between serum IL-8 and FEV1, FEV1/FVC (FEV1%), Kanazawa et al.<sup>[42]</sup>, Soler et al.<sup>[43]</sup>, Zhang et al.<sup>[44]</sup>, and Demirci et al.<sup>[25]</sup> found a negative correlation between IL-8 and FEV1.

In contrast to our results, Pinto-Plata et al.<sup>[45]</sup> and Akbulut et al.<sup>[46]</sup> found no correlation between the IL-8 value and FEV 1 and FEV1/FVC values.

Pinto-Plata et al.<sup>[47]</sup> and Amer et al.<sup>[41]</sup> found a significant negative correlation between TNF- $\alpha$  levels and FEV1. In contrast to our results, Abd El-Maksoud et al.<sup>[29]</sup> found no significant correlation between TNF- $\alpha$  and FEV1.

C-reactive protein (CRP) is a potential biomarker of systemic inflammation that is synthesized predominantly by the hepatocytes in response to tissue damage or inflammation<sup>[48]</sup>. Several previous studies have documented that CRP levels are increased in stable COPD patients<sup>[49]</sup>. In agreement to our results which shows highly significant increase in COPD patients than control groups, with highly significant negative correlation between its level and FEV1 and highly significant correlation between its level and smoking load in COPD group.

However, the results of 4 studies<sup>[50-53]</sup> showed no association between CRP and the risk of COPD,

our data presents non significant correlation between CRP level and pack year index in healthy non smoker.

### Conclusion

The results of this study showed higher plasma levels of inflammatory markers: IL8, TNF- $\alpha$  and CRP in COPD patients and it is clear that the increase in serum inflammatory markers has a direct correlation with the severity of COPD and airway obstruction which conclude that markers of systemic inflammation have an important role in COPD pathogenesis, however not all markers has relation with smoking load in healthy smoker, it gives an idea that after exposure to smoking: once the process of inflammation starts it persists, also remodeling in COPD may maintain the inflammatory process independent of cigarette smoking.

Other research studies with increase sample size are needed to reinforce our findings, to develop possible new designing for new therapeutic medication.

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