

## CHRONIC OBSTRUCTIVE PULMONARY DISEASE: SYSTEMIC INFLAMMATION AND PULMONARY HYPERTENSION

### KRONİK OBSTRÜKTİF AKCİĞER HASTALIĞI: SİSTEMİK İNFLAMASYON VE PULMONER HİPERTANSİYON

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**Key words:** C-reactive protein, interleukin, COPD, pulmonary hypertension, systemic inflammation

**Anahtar sözcükler:** C-reaktif protein, interlökin, KOAH, pulmoner hipertansiyon, sistemik inflamasyon

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#### SUMMARY

Chronic Obstructive Pulmonary Disease (COPD) is a systemic disease associated with increase of inflammatory mediators in systemic circulation. However, it is not clear yet what the mediator is that potentially takes role in pulmonary hypertension (PH) forming secondary to COPD and leads to systemic inflammation. In this study, we examined the role of serum C-reactive protein (CRP), Tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), and interleukin-6 (IL-6) in COPD patients with and without PH.

65 COPD patients were studied. Pulmonary function test was measured by body plethysmograph. Pulmonary artery pressures (PAP) were evaluated by Dopler echocardiography. We divided the patients as with PH (PAP >35 mmHg) and without PH (PAP <35 mmHg). Serum TNF- $\alpha$ , IL-6 level was examined by ELISA and CRP level was examined nephelometrically by Immage (Beckman Coulter, the USA) analyzer.

While PH was present in 18 patients, it was not found in 47 patients. When COPD patients with

#### ÖZET

KOAH, sistemik dolaşımında inflamatuvar mediatörlerin artmasıyla ilişkili bir sistemik hastalıktır. Ancak KOAH'a sekonder oluşan pulmoner hipertansiyonda (PH) potansiyel olarak rol alan ve sistemik inflamasyon yapan mediatörün henüz ne olduğu açık değildir. Çalışmamızda PH olan ve olmayan KOAH hastalarında serum C-reaktif protein (CRP), Tümör nekrozis faktör- $\alpha$  (TNF- $\alpha$ ), interlökin-6 (IL-6)'nın sistemik inflamasyondaki rolünü araştırdık.

65 KOAH hastası çalışmaya alındı. Solunum fonksiyon testi body pletismograf ile ölçüldü. Pulmoner arter basınçları (PAB) transtorasik ekokardiyografi ile değerlendirildi. Hastaları PH olmayan (PAB <35 mmHg) ve olan (PAB >35 mmHg) olarak ikiye ayırdık. Nutrisyonel parametreler olarak Vücut Kitle İndeksi, Triceps Kalınlığı ölçüldü. Serum TNF- $\alpha$ , IL-6 düzeyi ELISA, CRP düzeyi ise otomatize Immage (Beckman Coulter, A.B.D.) analizörü ile nefelometrik olarak incelendi.

PH were compared with COPD patients without PH, no statistically significant difference was found among serum CRP, TNF- $\alpha$ , IL-6 levels. FEV1 (predicted %) value ( $44.1 \pm 12.6$ ) in COPD patients with PH was found lower than the ones without PH ( $FEV1=54.5 \pm 18.5$ ) as statistically significant. Blood gas analysis revealed significantly lower Pao<sub>2</sub> in patients with pulmonary hypertension compared to those without hypertension ( $p_{0.024}$ ). However, although 6-minute walking distance, which is an exercise test, was found less, no statistically significant difference was found.

Increasing systolic pulmonary artery pressure in COPD patients was not found associated with CRP, TNF- $\alpha$ , IL-6 levels. For this reason, more detailed studies with more patients are needed for clarifying the role of inflammatory mediators such as CRP, TNF- $\alpha$ , IL-6 in PH pathogenesis developing in COPD patients.

## INTRODUCTION

Pulmonary hypertension will develop in a significant number of patients with COPD over the course of their disease and they will experience increased morbidity and mortality as a result (1,2). In patients with mild to moderate COPD, histopathologic studies have shown inflammatory infiltrates in pulmonary arterial walls (3). And also several inflammatory proteins play an important role in pulmonary artery physiology and in regulation of pulmonary artery pressure (4-7). Levels of inflammatory proteins such as CRP, TNF- and IL-6 are increased in systemic circulation in such patients. However, the potential role of systemic inflammation in the pathogenesis of pulmonary hypertension secondary to COPD has not yet been established (8-10). The aim of the present study was to investigate the degree of systemic inflammation, and the relationship between levels of systemic inflammatory proteins such as CRP, TNF- , IL-6 in COPD patients with and without pulmonary hypertension.

PH 18 hastada mevcut iken 47 hastada saptanmadı. PH olan KOAH hastaları ile PH olmayan KOAH hastaları karşılaştırıldığında serum CRP, TNF- $\alpha$ , IL-6 düzeyleri arasında istatistiksel olarak anlamlı fark bulunmadı. PH olan KOAH'lılar da FEV1 değeri PH olmayanlara göre istatistiksel olarak anlamlı derecede daha düşük bulundu. PH olan hastalarda parsiyel oksijen basıncı PH olmayanlara göre daha düşük saptandı.

KOAH hastalarında artan sistolik pulmoner arter basıncı serum CRP, TNF- $\alpha$ , IL-6 düzeyleri ile ilişkili bulunmadı. Bu nedenle KOAH hastalarında gelişen PH patogenezinde serum CRP, TNF- $\alpha$ , IL-6 gibi inflamatuvar mediatörlerin rolü konusunun açıklığa kavuşturulması için daha detaylı ve hasta sayısının artırılarak yapılacak olan çalışmalara ihtiyaç vardır.

## MATERIALS AND METHODS

### Subjects

Patients with a diagnosis of COPD, determined according to the American Thoracic Society guidelines (11), were consecutively recruited to the study in a hospital setting. Exclusion criteria were respiratory disorders other than COPD, pulmonary embolism, left ventricular systolic or diastolic dysfunction, malignancy, systemic autoimmune disorders, infectious diseases and severe endocrine, hepatic, renal diseases. The study had local ethics committee approval and all subjects gave written consent to participate in the study.

### Pulmonary function tests

Pulmonary function was evaluated with body plethysmography all testing was performed according to the European Respiratory Society standards with patients in a sitting position at the same time of the day by the same technician.

### Pulmonary arterial pressure (Ppa)

Mean and systolic Ppas were assessed by Doppler echocardiography. Continuous Doppler

wave assessment of the peak velocity of the tricuspid regurgitation jet as well as pulsed Doppler recording of the time to peak velocity curves of pulmonary artery blood flow and right ventricular out flow tract were used to assess Ppas. Tricuspid regurgitant flow was identified by color flow Doppler techniques. The accuracy of Doppler echocardiography in the assessment of Ppa may not be so high in patients with COPD. A study (12) from the consensus symposium on pulmonary hypertension that was held in Venice, Italy, in 2003 suggested that a systolic Ppa of 35 mm Hg represents the cutoff value for pulmonary hypertension when assessed by Doppler echocardiography. Therefore, we have divided patients into those without pulmonary hypertension (systolic Ppa < 35 mm Hg) and those with pulmonary hypertension (systolic Ppa  $\geq$ 35 mm Hg). Transthoracic echocardiography also was used to assess systolic and diastolic left ventricular function.

#### **Measurement of CRP, TNF- $\alpha$ , and IL-6**

In all patients, peripheral venous blood samples from the antecubital vein were collected between 6:00 and 10:00 am after 10 h of fasting and abstention from the use of oxygen. Serum was separated from blood cells by centrifugation. All samples were stored at -70°C until analyzed. High-sensitivity serum CRP levels were assessed by chemiluminescent immunoassay. The analytical sensitivity of this CRP assay is 0.1 mg/L. Serum TNF- $\alpha$  and IL-6 levels were measured using commercially available enzyme-linked immunosorbent assay kits. At the time of the collection of venous blood samples, an arterial blood sample was obtained by puncturing the radial artery for blood gas analysis.

#### **Statistical Analysis**

The results are presented as the mean  $\pm$  SD

for all variables that were normally distributed.

Differences between the groups (ie, patients with vs those without pulmonary hypertension) were analyzed using a twotailed unpaired t test for normally distributed variables and a Mann-Whitney U test for nonnormally distributed variables. A p value of < 0.05 was considered to be statistically significant.

## **RESULTS**

### **Patient Characteristics**

Sixty – five patients (41 male and 24 female) with COPD were recruited to the study. Pulmonary hypertension was present in 18 patients and was absent in 47 patients. No differences were seen between patients with and without pulmonary hypertension in the demographic data or in the body mass index (Table 1). Also, no differences were observed in pulmonary function test results (except FEV<sub>1</sub>) between the two groups. Only FEV<sub>1</sub> (predicted %) value (44.1 $\pm$ 12.6) in COPD patients with PH was found lower than the ones without PH (FEV<sub>1</sub>=54.5 $\pm$ 18.5) as statistically significant. Blood gas analysis revealed significantly lower PaO<sub>2</sub> in patients with pulmonary hypertension compared to those without hypertension (p - 0.024). Although 6-minute walking distance, which is an exercise test, was found less in patients with pulmonary hypertension, no statistically significant difference was found (Table 2). The mean left ventricular ejection fraction was 56.8  $\pm$  6.9% in patients without pulmonary hypertension and 57.4  $\pm$  7.2% in patients with pulmonary hypertension. A clinical diagnosis of coronary artery disease in the medical history was present in 24 patients without pulmonary hypertension and in 10 patients with pulmonary hypertension.

**SYSTEMIC INFLAMMATION AND PULMONARY HYPERTENSION**

**Table 1.** Demographic data in COPD patients without and with pulmonary hypertension\*.

Variables	Systolic Ppa		p Value
	<35 mmHg	≥35 mmHg	
Patients No	47	18	
Men	31	11	0.432
Vomen	16	7	
Age yr	57.06 ± 13.45	62.05 ± 10.38	0.072
Duration of COPD, yr	17.50 ± 12.50	15.60 ± 12.80	0.674
Smoking History, pack/yr	34.90 ± 30.1	22.40 ± 20.6	0.184
BMI**, kg/m <sup>2</sup>	25.90 ± 5.50	23.90 ± 4.60	0.355

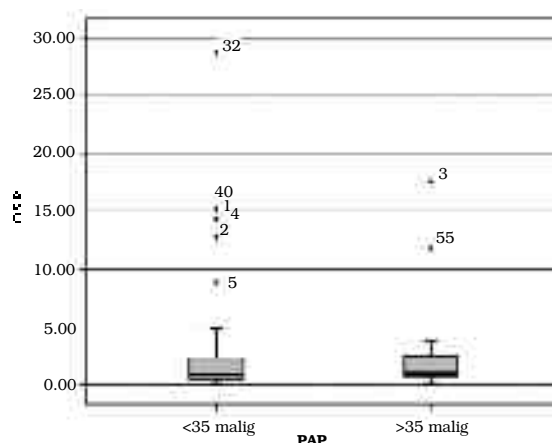
\*Values are given as the mean ± SD, \*\*BMI = Body Mass Index

**Table 2.** Pulmonary function parameters and arterial gas levels in COPD patients without and with pulmonary hypertension\*.

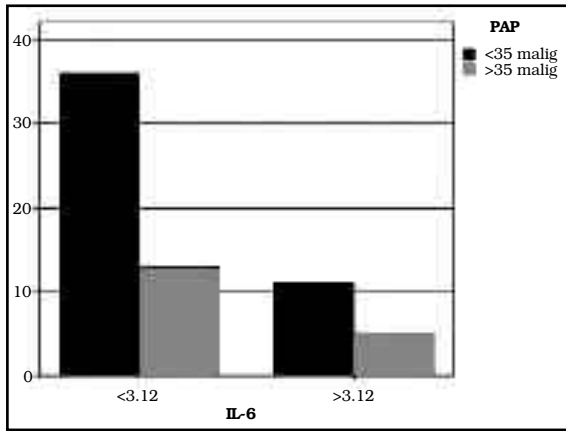
Variables	Systolic Ppa		p Value
	<35 mmHg	≥35 mmHg	
FEV1, % predicted	54.05 ± 18.50	44.17 ± 12.60	0.042*
FVC, % predicted	70.64 ± 20.30	65.06 ± 16.50	0.056
FEV1/FVC, %	65.00 ± 10.00	63.70 ± 9.40	0.549
RV, % predicted	83.30 ± 35.42	79.35 ± 22.94	0.507
TLC, % predicted	77.34 ± 19.92	78.32 ± 21.54	0.302
RV/TLC ratio, % predicted	110.92 ± 27.54	109.12 ± 27.51	0.784
PaO2 mmHg	68.25 ± 14.28	56.14 ± 10.82	0.024
PaCO2 mmHg	45.24 ± 20.16	47.24 ± 24.52	0.605
Six minute walking test	410.86 ± 109.63	400.64 ± 136.01	0.817

**CRP, TNF-α, and IL-6 in Patients With and Without Pulmonary Hypertension**

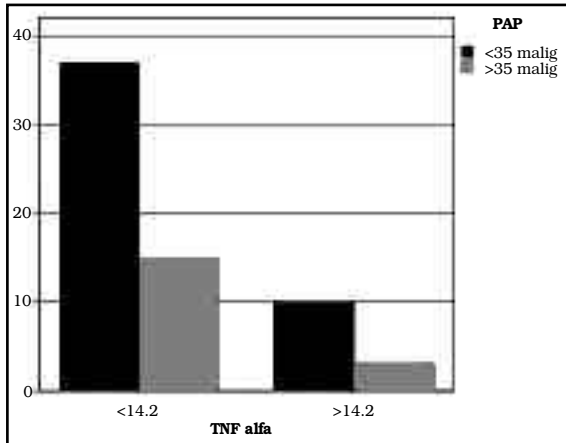
Serum CRP and TNF-α and IL-6 levels were higher in patients with pulmonary hypertension compared to those without pulmonary hypertension. But, no statistically significant difference was found among serum CRP, TNF-α, IL-6 levels between two groups (Fig 1,2,3).



**Figure 1.** Serum CRP Concentration in COPD patients without and with pulmonary hypertension.



**Figure 2.** Relationship between TNF- and pulmonary artery pressure.



**Figure 3.** Relationship between IL-6 and pulmonary artery pressure.

## DISCUSSION

The present study provides an observation on the potential significance of systemic inflammation in patients with COPD who have pulmonary hypertension. Our data demonstrate that COPD patients with pulmonary hypertension have higher serum CRP, TNF- and IL-6 levels compared to those with normal Ppa levels. But no statistically significant difference was found between two groups.

Although it has been known for years that inflammation plays a role in the airway

disease of COPD, the potential role of inflammation in the pathogenesis of vessel disease was not well-studied until the past few years. Seminal work by Peinado and colleagues (3) showed that the walls of small pulmonary arteries in COPD patients are commonly infiltrated with leukocytes, especially CD8-positive lymphocytes. It has been repeatedly reported that oxidative stress induced by cigarette smoke results in the local up-regulation of the synthesis of inflammatory cytokines (13). However, besides local inflammatory processes in lungs, systemic inflammation, as reflected by increases in several inflammatory markers in systemic circulation, is a key feature of COPD (8-10). Nevertheless, the potential role of systemic inflammation in pulmonary circulation in patients with COPD is not well-understood.

Even in patients with primary pulmonary hypertension, systemic inflammation may play a role, as such patients have elevated levels of TNF- and IL-6 in the systemic circulation compared to individuals without pulmonary hypertension (14) these clinical observations are supported by in vitro models that demonstrate the synergistic effects of inflammation and hypoxia in down-regulating nitric oxide production and inducing endothelial dysfunction in the pulmonary vasculature (15).

The study by Joppa and colleagues (16) in this issue suggests that systemic inflammation may also play a major role in pulmonary hypertension in COPD patients. The authors carefully selected 43 consecutive patients with moderate to severe COPD (mean FEV<sub>1</sub>, 46% predicted), and performed a variety of clinical, physiologic, and biochemical measurements to determine the relationship between systemic inflammation and pulmonary arterial hypertension. They found that

patients with significant pulmonary hypertension had higher levels of circulating C-reactive protein (CRP) and TNF-. Furthermore, there was a significant linear relationship between serum CRP levels and systolic pulmonary artery pressure in these patients, further emphasizing the likely importance of systemic inflammation in COPD-related pulmonary hypertension.

Some studies (17,18) have suggested that CRP may have complex and direct modulatory effects on endothelial cells. These studies have suggested that CRP may contribute to endothelial dysfunction and may potentially lead to vascular remodeling. Nevertheless, CRP might promote similar pathologic processes in the pulmonary circulation as well.

At present, the prognostic value of CRP level is being evaluated in a prospective study (18) in patients with pulmonary arterial hypertension. In patients with COPD, CRP level was higher, but no statistically difference was found.

Elevated serum TNF- levels were observed in patients with pulmonary hypertension secondary to chronic thromboembolic disease (19). In another study, (20) however, no correlation was seen between TNF- levels and pulmonary vascular resistance in such patients. The findings of the present study showed higher TNF- levels in COPD patients with pulmonary hypertension compared to those with normal Ppa values. Further studies are needed to study this phenomenon in more detail.

Several reports have indicated the potential role of IL-6 in severe primary pulmonary hypertension (14) and pulmonary hypertension associated with connective tissue diseases (21). In another study, COPD patients with PH (mean pulmonary artery pressure (PAP), > 25 mm Hg) had lower PaO<sub>2</sub> and higher plasma IL-6 values than

those without PH; there were no differences in terms of pulmonary function test results or CT scan emphysema scores. Plasma IL-6 correlated with mean PAP (r -0.39; p<0.001) and was included in a multiple stepwise regression analysis, with mean PAP as the dependent variable (22). In the present study, circulating IL-6 levels did not differ between COPD patients with and without pulmonary hypertension.

There are limitations to this study. First, the value of TNF- $\alpha$  and IL-6 were not calculated as a numeric because of the device. For this reason we divided the patients into two groups according to TNF- $\alpha$  and IL-6. (TNF- $\alpha$  < 14,2 and TNF- $\alpha$   $\geq$ 14,2, IL-6 <3.12 and IL-6  $\geq$ 3.12) and therefore we could not give a numeric value.

The second limitation of the present study is the use of transthoracic echocardiographic indexes to classify pulmonary hypertension. Although several studies have revealed significant statistical correlations between systolic Ppa estimated by Doppler echocardiography and that measured by right heart catheterization in patients with cardiac diseases and pulmonary diseases, the estimation of systolic Ppa by echocardiography may be inaccurate in patients with advanced lung disease (23,24,25). Third, our analyses are based on single measurements of Ppa and inflammatory markers, which may not reflect these relationships over time.

In conclusion, our study showed that increased systolic pulmonary artery pressure in patients with COPD are not associated with higher serum levels of CRP, TNF- $\alpha$ , IL-6. For this reason, more detailed studies with more patients are needed to clarify the role of inflammatory mediators such as CRP, TNF- $\alpha$ , IL-6 in PH pathogenesis developing in COPD patients.

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