

## Increased Serum Adhesion Molecules in Patients with Chronic Obstructive Pulmonary Disease and Secondary Erythrocytosis

### Kronik Obstrüktif Akciğer Hastalığı ve Sekonder Eritrositozu Olan Hastalarda Artmış Serum Adezyon Molekülleri

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#### Summary

**Objective:** Patients with COPD show increased levels of pro-inflammatory cytokines and adhesion molecules play an important role in this inflammatory response. The aim of this study is to investigate circulating adhesion molecules as intercellular adhesion molecule-1 (ICAM-1) and leukocyte-selectin (L-selectin) in patients with COPD associated with hypoxemic erythrocytosis.

**Method:** Forty-three patients with secondary erythrocytosis due to COPD and 10 healthy controls were included in this study. Serum samples from each subject were stored at -40°C until analysis. Commercial kits using quantitative sandwich enzyme immunoassay technique were used to measure serum levels of L-selectin and ICAM-1.

**Results:** Circulating ICAM-1 levels were found higher in patients with COPD and secondary erythrocytosis than the control group. L-selectin levels were not different between two groups. There was not any significant correlation between ICAM-1, L-selectin levels and hematocrit levels.

**Conclusion:** Increased ICAM-1 level seems to be related to chronic airway inflammation in COPD. (*Archives of Lung 2007; 8: 1-4*)

**Key words:** Chronic obstructive pulmonary disease, secondary erythrocytosis, ICAM-1, L-selectin

#### Özet

**Amaç:** KOAH'lı hastalarda proinflamatuvar sitokinlerde artış olmakta ve adezyon molekülleri oluşan inflamatuvar yanıtta önemli rol oynamaktadır. Bu çalışmada dolaşan adezyon molekülleri olan interseleüler adezyon molekül-1 (ICAM-1) ve lökosit-selektin'in (L-selektin) KOAH'lı hastalarda hipoksemik eritrositozla ilişkisini araştırmak amaçlandı.

**Metod:** Çalışmaya KOAH'a bağlı sekonder eritrositozu olan 43 hasta ve 10 sağlıklı kontrol alındı. Vakalardan alınan serum örnekleri -40°C'de saklandı. Kantitatif sandwich enzim immünassay tekniği ile serum L-selektin ve ICAM-1 düzeyleri ölçüldü.

**Bulgular:** KOAH'lı ve sekonder eritrositozu olan hastalarda kontrol grubuna göre serum ICAM-1 düzeyi yüksek saptandı. Serum L-selektin düzeyi için ise iki grup arasında fark yoktu. ICAM-1 ve L-selektin düzeyleri ile hematokrit düzeyleri arasında ise anlamlı bir ilişki bulunmadı.

**Sonuç:** Yüksek ICAM-1 düzeyi KOAH'da kronik hava yolu inflamasyonu ile ilişkili olabilir. (*Akciğer Arşivi 2007; 8: 1-4*)

**Anahtar Kelimeler:** Kronik obstrüktif akciğer hastalığı, sekonder eritrositoz, ICAM-1, L-selektin

#### Introduction

Chronic obstructive pulmonary disease (COPD) is a disease state characterized by airflow limitation that is not fully reversible. The airflow limitation is usually progressive and associated with an abnormal inflammatory response of the

lungs to noxious particles and gases (1). The inflammation is characterized by increased numbers of alveolar macrophages, neutrophils, cytotoxic T-lymphocytes, and the release of multiple inflammatory mediators (2). Several cytokines and cell adhesion molecules enhancing mainly a neutrophilic inflammation have been associated with COPD (3).

Cellular adhesion molecules, such as intercellular adhesion molecule-1 (ICAM-1), selectins and their leukocyte ligands are involved in intercellular activities of the relevant cell types. Patients with COPD show increased levels of pro-inflammatory cytokines and upregulation of surface adhesion molecules (4, 5). Adhesion molecules are prominently expressed in chronically inflamed lung (6).

There are three families of adhesion molecules named selectins, integrins, and immunoglobulin family (3). Leukocyte-selectin (L-selectin) is a member of selectin family. It is constitutively expressed on the surface of most leukocytes and it provides the free-flowing intravascular leukocytes to roll along the endothelium at sites adjacent to extravascular sources of chemokins and other chemoattractants. ICAM-1 is an adhesion molecule that is expressed on vascular endothelium and on immune and inflammatory cells and mediate the adhesion and transmigration of leukocytes to vascular endothelium (7-10). Different studies revealed different results about the status of ICAM-1 and L-selectin in COPD (11-13).

Secondary erythrocytosis is a compensatory mechanism against tissue hypoxia in hypoxemic COPD patients. However, only a fraction of hypoxemic COPD patients develop erythrocytosis. The mechanisms underlying this observation are not understood yet. Hypoxemia can activate circulating white blood cells (WBCs) and enhance WBC-endothelial cell interactions thus affect cellular adhesion molecules (14). The aim of this study is to investigate circulating adhesion molecules ICAM-1 and (L-selectin) in patients with COPD associated with hypoxemic erythrocytosis.

## Materials and Methods

### Patients

Forty-three patients with COPD associated with secondary erythrocytosis were recruited from the outpatient clinics of Atatürk Chest Diseases and Chest Surgery Education and Research Hospital and Hacettepe University Medical Faculty Department of Internal Medicine. Selection of COPD patients was based on the definition provided by Global Initiative for Chronic Obstructive Lung Disease (GOLD) (1). Patients with stable airflow limitation measured by spi-

**Table 1. Arterial blood gas analysis of COPD patients**

PaO <sub>2</sub> (mm Hg)	43.6±7.92
PaCO <sub>2</sub> (mm Hg)	54.28±12.66
SaO <sub>2</sub> (percent)	73.26±10.44

**Table 2. Hematocrit, L-Selectin and ICAM-1 levels of the study groups**

Variables	Group 1 (n=45)	Group 2 (n=10)	p
Hematocrit (%)	56.5±4.0 (range: 51.2-69.0)	40.5±4.2 (range: 33.3-45.3)	<0.0001
L-Selectin	10.7±1.7 (range: 8-19.7)	10.0±1.0 (range: 8.7-11.7)	0.16
ICAM-1	20.8±4.0 (range: 11.7-30.3)	14.6±1.0 (range: 13.1±16.0)	<0.0001

rometry as a forced expiratory volume in one second (FEV<sub>1</sub>) of <70% of the predicted value and bronchodilator reversibility of <10% predicted in FEV<sub>1</sub> were included into the study. All COPD patients were stable during the last month and were not using inhaler or systemic corticosteroids. All COPD patients had hypoxia, hypercapnia with a hemotocrit level higher than 50% along with normal erythropoietin levels (Table 1). The patients with pneumonia, bronchiectasis, and lung cancer were excluded from the study. As cigarette smoking has been shown to affect adhesion molecules, the control group was chosen from the healthy smokers (n=10) without any pulmonary symptoms and having normal FEV<sub>1</sub> and FVC results by spirometry .

### L-Selectin and ICAM-1 measurements

Venous blood samples (6 ml) were collected from the antecubital veins and centrifuged. Serum samples were stored at -40°C until the analysis of L-Selectin and ICAM-1. L-Selectin and ICAM-1 levels were measured with commercial kits using quantitative sandwich enzyme immunoassay technique (R&D Systems Inc. Minneapolis, USA).

### Statistical Analysis

Statistical analysis was carried out using SPSS programme version 12.0 (SPSS Inc. Chicago IL, USA). Mann Whitney-U was used to compare differences in means of continuous variables. Spearman rank correlation coefficient test was used to examine the association between variables. The results were expressed as mean ± SD. A p value less than or equal to 0.05 was considered significant.

## Results

Group 1 included 43 patients (38 males/5 females, mean age: 65.5±10.6 years, range: 37-83) with secondary erythrocytosis due to COPD. Group 2 included 10 healthy smokers (6 males/4 females, mean age: 46.8±18.3 years, range: 27-79). Mean smoking history of group 1 and 2 were 41.0±16.7 and 39.0±24.6 pack-years respectively (p>0.05). The comparison of L-Selectin and ICAM-1 levels of the study groups are seen in Table 2. L-Selectin levels were not different between the study groups. But ICAM-1 levels was significantly higher in Group 1 (p<0.0001) compared to Group 2 (Figure 1). There was not any significant correlation between L-Selectin, ICAM-1, and hematocrite levels (p=0.311 and p=0.665 respectively).

## Discussion

This is the first study investigating the expression of adhesion molecules in serum of COPD patients with secondary

erythrocytosis. We demonstrated that circulating ICAM-1 levels were higher in patients with hypoxemic erythrocytosis compared to controls. But circulating L-selectin levels were not different. And there was not any correlation between ICAM-1, L-selectin and hematocrite levels.

COPD is defined as a disease state characterized by chronic inflammation that leads to fixed narrowing of small airways and alveolar wall destruction. Much of the recent research has focused on the nature of this inflammatory response. Histopathological studies of COPD showed a predominant involvement of peripheral airways and lung parenchyma (15). Recruitment and activation of these inflammatory cells are important events in the pathogenesis of COPD and the expression of several adhesion molecules such as LFA-1, Mac-1, and L-selectin on the neutrophil surface, and ICAM-1 on the endothelium play a central role in these processes (16). Increased circulating ICAM-1 concentrations in our hypoxemic COPD patients seems to be related to the chronically inflamed lung. Hypoxemia of obstructive sleep apnea was previously shown to modulate the expression of proinflammatory mediators and adhesion molecules (17). Therefore, hypoxemia could further complicate interactions of inflammation and adhesion molecules in COPD patients (18).

Neutrophils are key cells in the inflammatory response that characterises COPD (19). Compared with healthy non-smokers, COPD patients have increased numbers of neutrophils, both in sputum and bronchoalveolar lavage fluid (20). Adhesion molecules mediate migration of leukocytes from the vascular compartment into inflamed tissue. They are expressed on the surface of endothelial cells and leukocytes and their expression is upregulated by pro-inflammatory cytokines (21-24). A study investigating the expression adhesion molecules on bronchial biopsies from nonsmokers, asymptomatic smokers, and smokers with chronic bronchitis and airway obstruction suggested an increased expression of E-selectin in ves-

sels and ICAM-1 on epithelial cells of patients with airway obstruction (25). The soluble isoforms of these adhesion molecules can be found in circulation, and have been increasingly recognized as markers of inflammation and endothelial activation (26). Production of proinflammatory cytokines by hypoxic endothelial cells may be followed by induction of endothelial-leukocyte adhesion molecule-1 (ELAM-1) and enhanced expression of ICAM-1 during reoxygenation (18), as we observed in the peripheral blood of our COPD patients.

Several clinical investigations have demonstrated the increased expression of ICAM-1 in COPD patients. Riise et al found that ICAM-1 levels were increased both in serum and bronchoalveolar lavage in COPD patients compared to controls (27). They suggested that high levels of circulating cell adhesion molecules may be associated with an upregulation of adhesion molecules on endothelial and epithelial surfaces in COPD. Recently Gerritsen et al demonstrated that ICAM-1 levels were higher during exacerbation and reduced with therapy (28). But in the study of Nougera et al, ICAM-1 levels were significantly lower in COPD patients compared to healthy controls. There was not any significant difference in L-selectin levels (19). P-selectin is another member of selectin family. Kunter et al investigated P-selectin in COPD and they found P-selectin level higher in patients with COPD than the controls. Also P-selectin level was found increased in the COPD patients who received standard treatment without steroid (29).

In bronchial biopsies of severe COPD cases there is an increased neutrophilic inflammation (20). Therefore, upregulation of adhesion molecules can be due to the enhanced neutrophilic inflammation. The subjects of the present study were all severe cases with hypoxemia. Hypoxia is a stimulus which induces endothelial cell synthesis and release of IL-1 alpha, resulting in an autocrine enhancement in the expression of adhesion molecules (18). Increased circulating levels of ICAM-1 in our COPD cases might be related to those pathobiological events. However, ICAM-1 and L-selectin levels were not found to be related with the development of secondary erythrocytosis in hypoxemic COPD patients.

Clinical efficacy of fenoterol in patients with obstructive lung disease had been suggested to include downregulation of adhesion molecule expression on airway epithelial cells (30). Therefore, increased expression of ICAM-1 may be either a "cause" or "effect" of airway inflammation in COPD. The cellular and molecular mechanisms that regulate the immunomodulatory functions of airways may offer new and important therapeutic targets in treating lung diseases (31). This hypothetical perspective based on the findings of our present study and previous researches should be tested in future investigations (6,30-32).

In conclusion, in this study, circulating ICAM-1 levels were found higher in patients with COPD and secondary erythrocytosis than the control group. Thus, increased ICAM-1 level seems to be related to chronic airway inflammation in COPD.

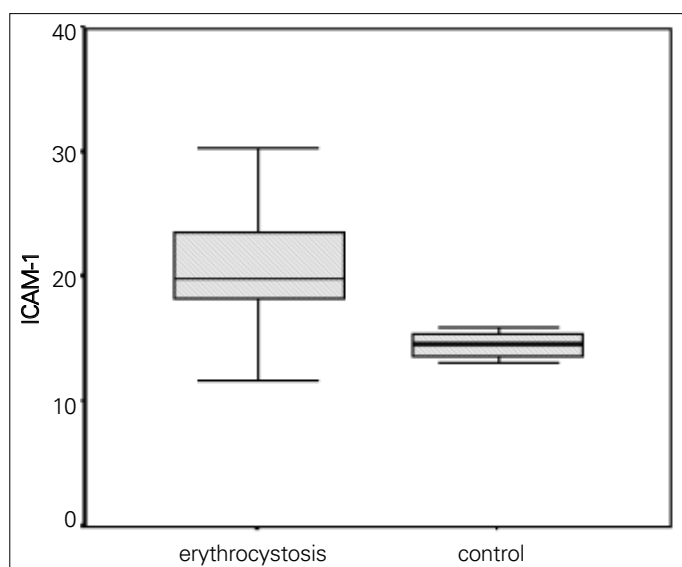


Figure 1. Mean ICAM-1 levels in the study groups

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