The Association of MCP-1 Level and *MCP-1* -2518 A/G and *CCR2* 190 G/A Gene Polymorphisms with COPD and Pulmonary Hypertension

MCP-1 düzeyi ve *MCP-1* -2518 A/G ve *CCR2* 190 G/A Gen Polimorfizmlerinin KOAH ve Pulmoner Hipertansiyon ile İlişkisi

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ABSTRACT

Objective: Chronic obstructive pulmonary disease (COPD) is a chronic inflammatory disease characterized by obstructed airflow in the lungs. Pulmonary hypertension (PH) is a common complication of COPD and it is associated with pulmonary vascular remodelling. Monocyte chemoattractant protein-1 (MCP-1/CCL2) is one of the key chemokines associated with migration of monocytes and macrophages. Both MCP-1 and its receptor CCR2 have been declared to be involved in various diseases. We aimed to research a possible association of MCP-1 level, *MCP-1* -2518 A/G and *CCR2* 190 A/G polymorphisms with COPD and pulmonary hypertension in this study.
Material and methods: Eighty patients and eighty controls were included in the study. Serum MCP-1 levels were measured by ELISA method. Restriction fragment length polymorphism (RFLP) procedure was used to detect the genotypes of patients and control group.
Results: MCP-1 levels were found to be significantly higher in COPD patients than in healthy controls (P=0.001) and patients with COPD + PH had higher serum MCP-1 levels than COPD patients (P=0.005). No association was found between *MCP-1/CCR2* gene polymorphisms and patient groups (COPD and COPD + PH).
Conclusion: MCP-1 level seems to be associated with both COPD and pulmonary hypertension. Increased MCP-1 expression may most likely to be involved in the pathogenesis of these diseases.

Key Words: *MCP-1, CCR2,* COPD, pulmonary hypertension, polymorphism.

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ÖZET

Amaç: Kronik obstrüktif akciğer hastalığı (KOAH), akciğerlerde tıkalı hava akımı ile karakterize kronik enflamatuar bir hastalıktır. Pulmoner hipertansiyon (PH), KOAH'ın sık görülen bir komplikasyonudur ve pulmoner vasküler yeniden yapılanma ile ilişkilidir. Monosit kemoatraktan protein-1 (MCP-1 / CCL2), monositlerin ve makrofajların göçü ile ilişkili ana kemokinlerden biridir. Hem MCP-1 hem de reseptörü CCR2'nin çeşitli hastalıklarda etkili olduğu bildirilmiştir. Biz bu çalışmada, MCP-1 düzeyi, *MCP-1* -2518 A/G ve *CCR2* 190 A/G polimorfizmlerinin KOAH ve pulmoner hipertansiyon ile olası bir ilişkisini araştırmayı amaçladık.
Gereç ve yöntem: Seksen hasta ve seksen kontrol çalışmaya dahil edildi. Serum MCP-1 seviyeleri ELISA yöntemi ile ölçüldü. Hastaların ve kontrol grubunun genotiplerini tespit etmek için Restriksiyon Fragman Uzunluk Polimorfizmi (RFLP) metodu kullanıldı.
Bulgular: KOAH hastalarında MCP-1 düzeylerinin sağlıklı kontrollere göre anlamlı derecede daha yüksek olduğu (P = 0.001) tespit edildi ve KOAH + PH'lu hastalar da KOAH hastalarından daha yüksek serum MCP-1 düzeylerine sahipti (P = 0.005). *MCP-1/CCR2* gen polimorfizmleri ile hasta grupları arasında (KOAH ve KOAH + PH) bir ilişki bulunamadı.
Sonuç: MCP-1 düzeyi hem KOAH hem de pulmoner hipertansiyon ile ilişkili görünmektedir. Artmış MCP-1 ekspresyonu büyük olasılıkla bu hastalıkların patogenezinde rol oynamaktadır.

Anahtar sözcükler: *MCP-1, CCR2,* KOAH, pulmoner hipertansiyon, polimorfizm.

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INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a chronic condition characterized by progressive and irreversible airflow restriction caused by smoking or several occupational-environmental factors (1). Its main symptoms are respiratory distress, cough, sputum, and wheezing. Emphysema and chronic bronchitis are two main conditions in the course of COPD. The severity of COPD is explained based on forced expiratory volume 1 (FEV1) and the status of emphysema correlates with dyspnea (2). It is a multifactorial chronic inflammatory disease of the respiratory system predominantly associated with the lower respiratory pathways and the pulmonary parenchyma. Recently, the genetic mechanisms underlying the development of COPD have become the subject of comprehensive researches (3).

Chemokines have an important role in innate and adaptive immunity and they are involved in many physiological and pathological processes such as inflammation, cell proliferation, apoptosis, tumor metastasis and host defense (4). The human monocyte chemoattractant protein-1 (MCP-1) is a member of the C–C motif chemokine family and after secretion from cells, it binds to its receptor called C-C chemokine receptor type 2 (CCR2) (5). *MCP-1* gene is located on chromosome 17 (17q11.2) and encodes a protein of 76 amino acids with a molecular weight of approximately 13kDa (6). CCR2 with two isoforms called CCR2A and CCR2B, is the specific receptor of MCP-1 also known as C-C motif chemokine ligand 2 (CCL2) (7). MCP-1 is produced by a lot of cell types, such as fibroblasts, endothelial, epithelial, smooth muscle, mesangial, astrocytic, and monocytic cells and it has been shown to be elevated in tissue sections in ex-smokers with COPD (8). Chronic obstructive pulmonary disease is characterised by inflammation of the large and small airways and MCP-1 released by neutrophils may play a role in the pathogenesis of COPD (9).

A negative correlation was reported between sputum MCP-1/CCL2 levels and FEV1 and high levels of both CCR2 and MCP-1 mRNA were detected in bronchial epithelium of COPD patients (10). It has been reported that the *MCP-1* - 2518 A/G and *CCR2* 190 G/A polymorphism (also known as V64I) are associated with different inflammatory diseases and cancer (4, 11). Bai et al. Suggested that *MCP-1* - 2518 A/G and *CCR2* 190G/A gene polymorphisms are associated with elevated susceptibility to COPD in the Chinese population and these polymorphisms are new risk factors for COPD (12).

The structural remodeling of the pulmonary vasculature against to hypoxia in pulmonary hypertension is characterized by medial and adventitial thickening. MCP-1 stimulates monocyte infiltration of the injured vessel wall and may contribute the proliferation of smooth muscle cells in pulmonary and systemic hypertension (13).

In light of these data, we aimed to research the level of MCP-1 and the distribution of *MCP-1* and *CCR2* gene polymorphism in COPD and COPD with pulmonary hypertension.

MATERIAL AND METHOD

Study population

Our study was approved by Sivas Cumhuriyet University Clinical Research Ethics Committee and supported by Sivas Cumhuriyet University Scientific Research Projects Unit (CUBAP). Eighty patients who applied to Sivas Cumhuriyet University Faculty of Medicine Department of Chest Diseases between 2017-2018 and eighty healthy controls were included in the study. To be younger than 18 years of age, another systemic disease, severe smoking, alcohol use, mental disability, malignancy and the presence of acute infection were exclusion criteria for the patients. The control group was chosen from individuals who did not have any systemic disease or mental disorder and who were not smoking and using alcohol.

MCP-1 level

The patient and control bloods were taken to the biochemistry tube and centrifuged. ELISA method was used to measure serum MCP-1 levels in both patients and healthy control population.

Genotyping

Peripheral blood samples from COPD patients and healthy controls were taken to K_3EDTA tubes. DNA isolation was performed from 200 µl peripheral blood in the EZ1 Advanced XL (Qiagen) DNA isolation apparatus using EZ1 DNA Blood kit (Qiagen). A total of 25 µl PCR mixture (12.5 µl PCR master mix, 1 µl of each primer, 9.5 µl bidistilled water and 1 µl DNA) was prepared for the amplification of each DNA samples. The nucleotide sequence of the primers used to amplify the region of the A-2518G polymorphism was as follows: Forward 5'- TCT CTC ACG CCA GCA CTG ACC-3' Reverse 5'- GAG TGT TCA CAT AGG CTT CTG-3'. The amplification reactions were carried out in a thermal cycler as described below: Initial denaturation phase at 95°C for 3 minutes and 35 cycles of a process (denaturation phase at 95°C for 45 seconds, annealing phase at 55°C for 45 seconds, elongation phase at 72°C for 45 seconds) and final elongation phase at 72°C for 5 minutes. Primers for the region of *CCR2* G190A polymorphism was as follows: Forward 5'-CAT TGC AAT CCC AAA GAC CCA CTC-3' and reverse 5'-TTG GTT TTG TGG GCA ACA TGA TGG-3'. The amplification reactions were performed as described below: Initial denaturation phase at 94°C for 10 minutes, 40 cycles of a process (denaturation phase at 94°C for 1 minute, annealing phase at 61°C for 1 minute, elongation phase at 72°C for 1 minute) and final elongation phase at 72°C for 10 minutes. Fragments amplified with PCR were digested with FastDigest PvuII restriction endonuclease (Thermo Scientific) for *MCP-1* -2518 A>G polymorphism and FastDigest BseJI restriction endonuclease (Thermo Scientific) for *CCR2* G190A polymorphism. Digestion products were electrophoresed on a 2% agarose gel. DNA fragments were stained with ethidium bromide and genotyping was performed under UV transilluminator. We evaluated 234 bp band as AA, 159 bp and 75 bp as GG and 234 bp, 159 bp, and 75 bp was evaluated as AG genotype for *MCP-1* -2518 A>G polymorphism. In terms of *CCR2* G190A polymorphism, a single 173 bp band was evaluated as GG genotype, 149 bp and 24 bp as AA genotype, and the bands with 173 bp, 149 bp, and 24 bp were evaluated as GA genotype.

Statistical analysis

Statistical analysis was performed with SPSS version 22.0 (SPSS Chicago, IL, USA). Averages of body mass index were compared with Independent Sample T Test. Chi-square test or Fisher's exact test was used to compare the distribution of gene polymorphisms between patients and controls. P values of less than 0.05 were considered as statistically significant and all results were expressed within a 95% confidence interval.

RESULTS

Eighty COPD patients and eighty healthy controls were included in the study. Sixty two of the patients were male (77.5%) and eighteen were female (22.5%). The mean age of the patients was 66.03 ± 9.46. Fourty nine of the control group were male (61.3%) and thirty one were female (38.7%). The mean age of the control group was 48.90 ± 12.01. The patients were diagnosed by physical examination, pulmonary function tests and chest X-ray. The body mass index of the patients was significantly higher than the control group (P=0.002)(Table 1).

Table 1. The characteristics of the study groups.

Condition	Patients	Controls
Age	66.03 ± 9.46	48.90 ± 12.01
Sex		
Male	62	49
Female	18	31
BMI	27.2 ± 6.25	24.5 ± 3.99

ELISA method was used for the detection of MCP-1 levels and the levels of MCP-1 in COPD patients were found to be statistically higher than in healthy controls (P=0.001/Table 2). In the second stage, MCP-1 levels were compared between COPD patients and patients with COPD + PH, and patients with COPD + PH had significantly higher serum MCP-1 levels compared to COPD patients (P=0.005/Table 3).

Table 2. The comparison of MCP-1 levels in the patients and control group.

Data	Patients (COPD)	Healthy controls	
Number (n)	80	80	
Mean MCP-1 level	415.69 ng/l	220.43 ng/l	P= 0.001
Standard deviation	59.137	20.615	

Table 3. The comparison of MCP-1 levels in the patients with COPD and COPD+PH.

Data	COPD	COPD+PH
Number (n)	49	31
Mean MCP-1 level	392.52 ng/l	438.95 ng/l
Standard deviation	33.117	70.372

P= 0.005

RFLP method was used for the determination of genotypes in the research of gene polymorphism and firstly, patients with COPD and controls were compared in terms of *MCP-1 and CCR2* gene polymorphism (Table 4,5).

Following this, patients with COPD + PH and those with only COPD complaints were screened for these polymorphisms (Table 6,7). No significant difference was found between COPD patients and control group and COPD patients and COPD + PH patients in terms of related gene polymorphisms.

Table 4. *MCP-1* -2518 A/G gene polymorphism in patients (COPD) and control group.

***MCP-1* -2518 A/G**	**COPD**	**Control**	**P value**	
Genotype				
AA	34	35		
AG	38	39		
GG	8	6	0.85 p>0.05	
Alleles				Odds Ratio: 0.918
A	106	109		
G	54	51	0.72 p>0.05	

Table 5. *CCR2* 190G/A gene polymorphism in patients (COPD) and control group.

***CCR2* G190A**	**COPD**	**Control**	**P value**	
Genotype				
GG	56	56		
GA	20	22		
AA	4	2	0.68 p>0.05	Odds Ratio: 0.915
Alleles				
G	132	134		
A	28	26	0.88 p>0.05	

Table 6. *MCP-1* -2518 A/G gene polymorphism in COPD and (COPD + PH) groups.

***MCP-1* -2518 A/G**	**COPD**	**COPD+PH**	**P value**	
Genotype				
AA	20	14		
AG	25	13		
GG	4	4	0.65 p>0.05	Odds Ratio: 0.991
Alleles				
A	65	41		
G	33	21	0.97 p>0.05	

Table 7. *CCR2* 190 G/Agene polymorphism in COPD and (COPD + PH) groups.

***CCR2* G190A**	**COPD**	**COPD+PH**	**P value**	
Genotype				
GG	35	21		
GA	12	8		
AA	2	2	0.87 p>0.05	Odds Ratio: 0.813
Alleles				
G	82	50		
A	16	12	0.62 p>0.05	

DISCUSSION

COPD is a heterogeneous disease with the airflow limitation and it has a long clinical course on the basis of pulmonary structural changes, such as *devastation* of the lung *parenchyma* and reduction in the caliber of the small airways (14). It is associated with the inhalation of noxious agents, especially cigarette smoke and it is characterized by abnormal inflammatory reaction in the lung which results in progressive airway obstruction (15). COPD is a complex condition that is affected by genetic and environmental factors and their interactions (16). Characterization of blood biomarkers particularly for emphysema, which can express this heterogeneity may help to manage the COPD (17).

MCP-1 is the predominant phenomenon that promotes migration of peripheral blood monocytes from blood vessels into lungs. In combination with chemotactic function, MCP-1 also contributes to inflammation by promoting the expression of other inflammatory mediators (18). Wang et al. found that MCP-1 were positively correlated in acutely exacerbated chronic obstructive pulmonary disease patients, and were significantly higher than in control (19). Frankenberger et al. found an increased expression for MCP-1 in COPD (8). In a study by Eickmeier et al., MCP-1 was increased in COPD sputum (20). C-C chemokine receptor type 2 (*CCR2*) gene encodes a receptor for MCP-1. The binding of this receptor with MCP-1 on monocytes and macrophages mediates chemotaxis and migration induction. It was declared that CCR2 has a possible role in COPD development and progression (21).

The *MCP-1* is a polymorphic gene and a single-nucleotide polymorphism (-2518A/G, rs1024611) in the *MCP-1* gene distal regulatory region has been extensively studied (22). More than 10 prevalent single-nucleotide polymorphisms in the promoter region of the *MCP-1* gene have been studied and its – 2518 A/G polymorphism is one of the most common variants in this gene (23).

On the other hand, The *CCR2* 190 G/A (rs1799864) single-nucleotide polymorphism has been associated with the pathogenesis of certain diseases (24). *MCP-1* -2518 G/A polymorphism may affect the transcriptional activity and monocyte MCP-1 production (25). *CCR2* 190 G/A polymorphism converts valine to isoleucine at codon 64 (*CCR2* V64I). In some studies, it is stated that *MCP-1* -2518 A/G and *CCR2* 190 G/A gene polymorphisms may be effective in COPD formation (12). Liu et al. found no association between MCP1 -2518 gene polymorphism and COPD and this gene polymorphism and plasma MCP-1 levels in the Taiwanese men (26).

There are also studies on the association of MCP-1 with pulmonary hypertension. Schlosser et al. declared that the plasma levels of MCP-1 in the patients with pulmonary hypertension were significantly higher than healthy control participants (27). It was hypothesized that the MCP-1/CCR2 system plays complementary roles to promote lung macrophage activation during hypoxia exposure and the subsequent development of hypoxic pulmonary hypertension (28).

Our study revealed that MCP-1 levels were significantly increased in COPD patients and pulmonary hypertension. At the same time, MCP-1 levels was statistically higher in COPD + pulmonary hypertension patients than in only COPD cases. This situation confirms that MCP-1 levels are increased in both COPD and pulmonary hypertension. We could not find any significant difference between the patients and control groups and COPD and COPD + PH cases in terms of *MCP-1*-2518 G/A and *CCR2* 190 G/A gene polymorphisms. On the other hand, COPD patients were overweight than the control group in our study. This may be a general feature of COPD in terms of living standards and physical activity.

In conclusion, MCP-1 is involved in the pathogenesis of COPD and pulmonary hypertension and its expression increases during the disease process. *MCP-1* -2518 G/A and *CCR2* 190 G/A gene polymorphisms do not contribute to COPD and pulmonary hypertension. In this context, it can be thought that these polymorphisms also do not have a relationship with MCP-1 levels.

Conflict of interest
No conflict of interest was declared by the authors.

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