Özet
Amaç: Bu çalışmanın amacı akut alevlenmeli kronik obstruktif akıshastalığı (AAKOAH) olanlarda oksidatif stres ile bazı matriks metalloproteinazlar (MMP-2 ve MMP-9) ve doku metallopeptidaz inhibitör-1 (TIMP-1) arasındaki ilişkinin incelenmesidir. 

Gereç ve Yöntem: Çalışmaya 35 AAKOAH hastası ve 35 sağlıklı kontrol dahil edildi. Çalışma popülasyonundaki bireylerin sıvınlardaki MMP-2, MMP-9, TIMP-1, total antioksidan (TAS) ve total oksidan durumları (TOS) belirlendi. 

Bulgular: Hasta ve kontrol grubunda MMP-9 düzeyleri sırasıyla 201.19±12.91 pg/mL ve 189.32±13.01 pg/mL olarak bulundu. TAS düzeyleri ise sırasıyla 20.27±2.99 μmol trolox equiv/l ve 17.69±2.96 μmol trolox equiv/l olarak bulundu. Hasta ve kontrol grubları arasında TAS ve MMP-9 düzeyi yönünden anlamlı fark bulundu (<.001). Bununla birlikte TIMP-1 düzeyi hastalarda kontrol grubuna göre daha düşük düzeydeydi (p=.035). MMP-9 ile TAS (r=0.484, p=.001) ve oksidatif status indeksi (r=0.221, p=.001) arasında pozitif korelasyon saptandı. Bununla birlikte MMP-2 ve TIMP-1 ile TAS ve TOS arasında herhangi bir korelasyon görülmemiş. 

Tartışma: Elde edilen sonuçlar göz önune alındığında antioksidan tedavi yaklaşımlarının MMP-9 aracılı inflamasyonu önlenmesinde önemi bulunabileceğini düşünülmektedir.

Anahtar Kelimeler
MMP-9; MMP-2; TIMP-1; Oksidatif Stres; AAKOAH

Abstract
Aim: The aim of this study was to determine the relationship between oxidative status, matrix metalloproteinase-9 (MMP-9), matrix metalloproteinase-2 (MMP-2) and tissue metallopestidase inhibitor-1 (TIMP-1) in patients with acute exacerbation chronic obstructive pulmonary disease (AECOPD).

Material and Method: The study included a total of 35 patients with acute exacerbation COPD and 35 healthy nonsmoker controls. We assessed the concentrations of MMP-2, MMP-9, TIMP-1, total antioxidant status (TAS), and total oxidant status (TOS) in the study population. Results: MMP-9 concentrations were found as 201.19±12.91 pg/mL and 189.32±13.01 pg/mL in patients and controls, respectively. TAS concentrations were found as 20.27±2.99 μmol trolox equiv/l and 17.69±2.96 μmol trolox equiv/l in patients and controls, respectively. Significant differences were found between patients and controls in terms of MMP-9 and TAS (<.001) concentrations. We also found lower TIMP-1 concentrations in patients (p=.035) than controls. We also found correlation between MMP-9, TAS (r=0.484, p=.001) and oxidative status index (r=0.221, p=.001) among patients and controls, respectively. Significant differences were found between patients and controls in terms of MMP-9 and TAS (<.001) concentrations. We also found lower TIMP-1 concentrations in patients (p=.035) than controls. We also found correlation between MMP-9, TAS (r=0.484, p=.001) and oxidative status index (r=0.221, p=.001) among patients and controls, respectively. However, we did not find any significant correlation between MMP-2, TIMP-1, and oxidative stress markers (TAS and TOS).

Discussion: It is thought that the antioxidant treatment approaches could be beneficial for AECOPD patients for preventing the MMP-9 related inflammation.

Keywords
MMP-9; MMP-2; TIMP-1; Oxidative Stress; AECOPD
Introduction

Chronic obstructive pulmonary disease (COPD) is a preventable and treatable disease characterized by airflow limitation. This disease is characterized by cough, sputum production, and dyspnea [1]. The most important risk factor for COPD is cigarette smoking [2]. Various causative factors, such as inflammation, extracellular matrix destruction, and oxidative stress also constitute the pathophysiology of COPD [3]. Acute exacerbation chronic obstructive pulmonary disease (AECOPD) is defined as the deterioration period characterized by an increase in shortness of breath, decrease in daily performance, increase in the quantity of sputum, and change in its color, cough, and fever for a stabilized case [4]. Matrix metalloproteinases (MMPs) are endoproteinases involved in the regulating of extracellular matrix (ECM) turnover [5]. To date, 26 human MMPs have been identified in mammals [6]. The inhibition of MMPs is controlled by tissue metallopeptidase inhibitor (TIMPs) families, which are produced by different cell types [7]. Despite the investigations that have demonstrated the activities of MMP-9, MMP-2 and TIMP-1 in COPD [8-9], the exact mechanism of the MMPs and TIMP-1 alteration in COPD remains unclear [10].

Oxidative stress can be determined by measuring the concentrations of total antioxidant capacity. Total oxidant status (TOS) and total antioxidant status (TAS) are often used to estimate the overall antioxidant status [11,12]. Previous studies have shown that imbalances in oxidant and antioxidant status are predisposing factors in the pathogenesis of COPD following long-term exposure to cigarette smoking [13]. Alveolar macrophages and activated neutrophils appear to be the critical cells for releasing reactive oxygen substances, superoxide radicals, and hydrogen peroxide [14]. These inflammatory cells also release MMP-2, MMP-9, and TIMP-1 [15]. However, several studies have focused on the effects of the oxidative stress and MMPs in COPD [16-18]. To the best of our knowledge, no study that investigates the relation between the MMPs and oxidative stress in patients with AECOPD is found in the literature. The aim of this study was to compare serum values of MMP-9, MMP-2, TIMP-1, MMP-9/TIMP-1 ratio, TAS, TOS, and oxidative stress index (OSI) in patients and controls. We also evaluated the relationship between MMPs and oxidative stress. This study may contribute to the understanding of the roles of the MMPs and oxidative status in the pathogenesis of AECOPD.

Material and Method

Patients:

This study included 35 patients who applied to Cumhuriyet University Medicine Faculty, Department of Emergency Medicine and were diagnosed with AECOPD [23 males and 12 females; with ages 18-74 years (mean age: 47 ± 11.13)] and 35 healthy nonsmoker controls [21 males and 14 females; with ages 24-75 years (mean age: 45 ± 12.56)]. All of the patients included in the study had a history of smoking. Patients had several symptoms, such as shortness of breath, cough, and increase in the quantity of sputum and change in its color. The exclusion criteria for patients were pregnancy, the history of alcohol consumption, the diagnosis of type 2 diabetes mellitus, cardiovascular disorder, nephropathy, and asthma. Patients received standard treatment with nebulized β-agonist (5 mg salbutamol), an anticholinergic (500 μg ipratropium bromide) every six hours, controlled oxygen therapy, and oral or intravenous antibiotics at the judgment of the admitting physician. Blood samples were taken at first admission. Specimens were collected in anticoagulant-free, gel-containing tubes. The protocol was approved by the ethical committee of Cumhuriyet University Medical Faculty. Blood gas analysis results were obtained from the laboratory information system.

Biochemical analysis:

Serum levels of MMP-2 and MMP-9 were measured using a commercially available enzyme immunoassay kit (Ray Biotech, USA). TAS and TOS were measured using a commercially available colorimetric kit (Rel Assay Diagnostic, Turkey). The oxidative stress index (OSI) is defined as the ratio of the TOS to TAS levels. OSI, which is the indicator of the oxidative stress degree, was used as a parameter to assess redox status. Blood gas analysis was performed using an autoanalyser (Radiometer,USA).

Statistical analysis:

Data normality was assessed by the Shapiro-Wilk test. A two-sided independent samples t-test was used to compare the differences between MMP-2, MMP-9, TIMP-1, TAS, TOS, and OSI. Chi-square analysis was used to compare the differences of categorical variables. Pearson correlation coefficients were calculated to see the relationship between MMP-2, MMP-9, TIMP-1, TAS, TOS, and OSI. Analyses were performed using IBM SPSS software (release 20.0, IBM, SPSS Inc., Chicago, IL, USA), considering P < 0.05 as statistically significant.

Results

Mean pH, pO2, pCO2 , SO2 and MetHb values were 7.40 ± 0.7, 43.20 ± 11.46 mmHg, 50.33 ± 12.74 mmHg, 79.64% ± 15.45 and 1.02% ± 0.14 in the patients, respectively. Mean levels of MMP-9, MMP-2, TIMP-1, MMP-9/TIMP-1, TAS, TOS and OSI in patients and controls are shown in Table 1. We found correlation between MMP-9, TAS (r=0.484, p<0.001) and OSI (r=0.747, p<0.001). However, we did not find any significant correlation between MMP-2 [TAS (r=0.183, p=0.130), TOS (r=0.122, p=0.316), and OSI (r=0.221, p=0.660)], TIMP-1 [TAS (r=0.17, p=0.889), TOS (r=0.131, p=0.278) and OSI (r=0.096, p=0.430)] and oxidative status.

Table 1. Mean levels of MMP-9, 2, TIMP-1, MMP-9/TIMP-1, TAS, TOS and OSI in patients and controls

|                      | Controls (n=35) | Patients (n=35) | P     |
|----------------------|----------------|----------------|-------|
| MMP-2 (pg/mL)        | 6028 ± 1022    | 6385 ± 1066    | .158  |
| MMP-9 (pg/mL)        | 189.32±13.01   | 201.19 ± 12.91 | < .001|
| TIMP-1 (pg/mL)       | 148.24±23.46   | 137.17±19.27   | .035  |
| MMP-9/TIMP-1         | 1.30±0.21      | 1.49±0.21      | < .001|
| TAS (μmol trolox equiv/l) | 17.69±2.96 | 20.27±2.99        | < .001|
| TOS (μmol H2O2 equiv/l) | 3.38±0.51     | 3.24±0.56      | .270  |
| OSI                  | 0.53±0.11      | 0.64±0.14      | < .001|

MMP-2: Matrix metalloproteinase-2, MMP-9: Matrix metalloproteinase-9, TIMP-1: Tissue inhibitor of metalloproteinase, TAS: Total antioxidant status, TOS: Total oxidant status, OSI: Oxidative stress index. Results are expressed as mean ± SD with 95% confidence intervals.
Discussion
Several studies have demonstrated the changes of oxidative status and the concentrations of MMPs in plasma, serum, and sputum of COPD patients with respect to healthy subjects. However, the relationship between oxidative stress and MMP concentrations has not been evaluated in patients with AECOPD. This study found that serum MMP-9, MMP-9/TIMP-1, and TAS concentrations were significantly greater in patients than in the healthy nonsmoker controls. However, TIMP-1 concentrations were lower in AECOPD patients. Additionally, a positive correlation was found between MMP-9, TAS, and OSI in AECOPD patients.

In the studies conducted by Papakonstantinou et al. [8], Linder et al. [19], and Beeh et al. [20], it was found that the concentration of MMP-9 and the MMP-9/TIMP-1 ratio were higher in AECOPD patients compared to the control subjects. Our results were in accordance with these studies. These findings support the hypothesis that MMP-9 may also contribute to the acute exacerbation pathogenesis of COPD. Several studies have shown an increased concentration of TIMP-1 in COPD and AECOPD patients [8, 20-22]. We found lower TIMP-1 concentrations in patients than healthy non-smoker controls. This result is in accordance with Mercer et al. [23]. We consider that the decreased TIMP-1 concentrations cause an increase in MMP-9 concentrations due to the decreased inhibition effect [7]. The ratio of MMP-9 to TIMP-1 has been correlated with clinical parameters and lung function [20]. The present study found a statistically significant difference in MMP-9/TIMP-1 ratio between patients and controls. We believe that the MMP-9/TIMP-1 ratio can be used as an additional prognostic value in combination with standardized risk factors for risk stratification in patients with AECOPD.

MMP-2 belongs to the same gelatinase family as MMP-9 [9]. Previous studies have reported increased MMP-2 expression and concentration in COPD patients [9,24,25]. However, different results have been reported about the role of MMP-2 in various studies [25,26]. Whereas no significant difference has been observed between AECOPD patients and the nonsmoker healthy controls in terms of serum MMP-2 concentrations. The lack of difference in MMP-2 level between AECOPD patients and the control group arises from the deficiency of MMP-2 for removing the inflammatory cells as compared to MMP-9, in inflammatory lung diseases as stated by Corry et al. [27]. One of the important limitations of this study was that we did not measure TIMP-2 concentration, which plays an important role in MMP-2’s inhibition [28]. Thus, the effect of TIMP-2 on MMP-2 levels could not be evaluated. Our findings suggested that MMP-2 might not contribute to the exacerbation of AECOPD, in contrast to MMP-9.

Previous studies have shown that oxidative stress contributes to the regulation of MMPs activity in the different physiologic processes [29-30]. In the current study, we examined the correlation between MMP-2, MMP-9, TAS, TOS, and OSI in AECOPD patients. Although we did not find any correlation between MMP-2 and oxidative status, we found a positive correlation between MMP-9, TAS and OSI. These findings implied that the oxidative status may play a critical role in the MMP-9 related damage in AECOPD patients. Additionally, we believe that the increased levels of MMP-9 may cause an increase in TAS level, thus decreasing the inflammatory effects of MMP-9 in AECOPD patients.

Although a correlation between TIMP-1 and oxidative stress in vitro studies was observed in studies reported by Yang et al. 2003 [31] and Kenney et al. 2005 [32], we did not find any correlation in patients with AECOPD. The different effects of oxidative status on TIMP-1 concentration in vitro and in vivo conditions might cause the aforementioned difference. This finding might indicate that the oxidative status does not have an important role on the TIMP-1 concentration in AECOPD patients. The other limitation of this study was that stable COPD patients were not included in the study. Additionally, we did not evaluate the correlations between MMP-9, MMP-2, TAS, TOS, OSI, and lung function.

In conclusion, we observed an increase in MMP-9 and a decrease in TIMP-1 concentrations in AECOPD patients compared to healthy controls. We also found a positive correlation between MMP-9, OSI, and TAS. Finally, it is suggested that antioxidant treatment approaches can be beneficial for preventing MMP-9 related inflammation in AECOPD patients.

Competing interests
The authors declare that they have no competing interests.

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