



Matrix Metalloproteinases and Oxidative Stress in Patients with AECOPD

Akut Alevlenmeli Koah Hastalarında Matriks Metalloproteinazlar ve Oksidatif Stres

Oxidative Stress and AECOPD

Halef Okan Dogan¹, Hüseyin Aydın¹, Özlem Demirpençe¹, Fatma Mutlu Kukul Güven², Yusuf Kenan Tekin², Ali Şahin³
¹Department of Biochemistry, ²Department of Emergency Medicine, ³Department of Internal Medicine, Faculty of Medicine, University of Cumhuriyet, Sivas, Turkey

Özet

Amaç: Bu çalışmanın amacı akut alevlenmeli kronik obstruktif akciğer hastalığı (AAKOA) olanlarda oksidatif stres ile bazı matriks metalloproteinazlar (MMP-2 and MMP-9) ve doku metallopeptidaz inhibitör-1 (TIMP-1) arasındaki ilişkinin incelenmesidir. **Gereç ve Yöntem:** Çalışmaya 35 AAKOA hastası ve 35 sağlıklı kontrol dahil edildi. Çalışma popülasyonundaki bireylerin serum 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 grupları arasında TAS ve MMP-9 düzeyi yönünden anlamlı farklılık bulundu ($p < .001$). 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 görülmedi. **Tartışma:** Elde edilen sonuçlar göz önüne alındığında antioksidan tedavi yaklaşımlarının AAKOA hastalarında MMP-9 aracılı inflamasyonu azaltabileceği düşünülmektedir.

Anahtar Kelimeler

MMP-9; MMP-2; TIMP-1; Oksidatif Stres; AAKOA

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 metalloproteinase 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 exacerbated 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 ($p < .001$) 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 the oxidative status index ($r = 0.221$, $p < .001$). 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

DOI: 10.4328/JCAM.4718

Received: 24.06.2016 Accepted: 20.07.2016 Printed: 01.01.2017

J Clin Anal Med 2017;8(1): 64-7

Corresponding Author: Halef Okan Doğan, Department of Biochemistry, Faculty of Medicine, University of Cumhuriyet, Sivas, Turkey.

T.: +90 3462191010 E-Mail address: halefokan@gmail.com

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 endopeptidases 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, TOS, TAS, 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). TOS and TAS 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 metaaloproteinase-2, MMP-9: Matrix metaaloproteinase-9, TIMP-1: Tissue inhibitor of metalloproteinase, TAS: Total antioxidant status, TOS: Total antioxidant 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 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 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 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.

References

- Qaseem A, Wilt TJ, Weinberger SE, Hanania NA, Criner G, van der Molen T, et al. Diagnosis and Management of Stable Chronic Obstructive Pulmonary Disease: A Clinical Practice Guideline Update from the American College of Physicians, American College of Chest Physicians, American Thoracic Society, and European Respiratory Society. *Ann Intern Med* 2011;155:179-91.
- Mannino DM, Buist AS. Global burden of COPD: risk factors, prevalence, and future trends. *Lancet* 2007;370:765-73.
- Tuder RM, Petrache I. Pathogenesis of chronic obstructive pulmonary disease. *The J Clin Invest* 2012;122(8):2749-55.
- Bach PB, Brown C, Gelfand SE, McCrory DC. American College of Physicians-American Society of Internal Medicine; American College of Chest Physicians. Management of acute exacerbations of chronic obstructive pulmonary disease: a summary and appraisal of published evidence. *Ann Intern Med* 2001;134(7):600-20.
- Shah BH, Catt KJ. Matrix metalloproteinase-dependent EGF receptor activation in hypertension and left ventricular hypertrophy. *Trends Endocrinol Metab* 2004;15(6):241.
- Tan RJ, Fattman CL, Niehouse LM, Tobolewski JM, Hanford LE, Li Q, et al. Matrix metalloproteinases promote inflammation and fibrosis in asbestos-induced lung injury in mice. *Am J Respir Cell Mol Biol* 2006;35(3):289-97.
- Selman M, Ruiz V, Cabrera S, Segura L, Ramirez R, Barrios R, Pardo A, et al. TIMP-1, -2, -3, and -4 in idiopathic pulmonary fibrosis. A prevailing nondegradative lung microenvironment? *Am J Physiol Lung Cell Mol Physiol* 2000;279:562-74.
- Papakonstantinou E, Karakioulakis G, Batzios S, Savic S, Roth M, Tamm M, et al. Acute exacerbations of COPD are associated with significant activation of matrix metalloproteinase 9 irrespective of airway obstruction, emphysema and infection. *Respir Res* 2015;28:16:78.
- Cataldo D, Munaut C, Noel A, Franken F, Bartsch P, Foidart JM, et al. MMP-2- and MMP-9-linked gelatinolytic activity in the sputum from patients with asthma and chronic obstructive pulmonary disease. *Int Arch Allergy Immunol* 2000;123(3):259-67.
- Lagente V, Manoury B, Nenau S, Le Quement C, Martin-Chouly C, Boichot E. Role of matrix metalloproteinases in the development of airway inflammation and remodeling. *Braz J Med Biol Res* 2005;38:1521-30.
- Erel O. A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. *Clin Biochem* 2004;37:277-85.
- Erel O. A new automated colorimetric method for measuring total oxidant status. *Clin Biochem* 2005;38:1103-11.
- Yoshida T, Tuder RM. Pathobiology of cigarette smoke-induced chronic obstructive pulmonary disease. *Physiol Rev* 2007;87(3):1047-82.
- Macnee W, Rahman I. Oxidants and antioxidants as therapeutic targets in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 1999;160:58-65.
- Russell RE, Culpitt SV, DeMatos C, Donnelly L, Smith M, Wiggins J, et al. Re-

lease and activity of matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 by alveolar macrophages from patients with chronic obstructive pulmonary disease. *Am J Respir Cell Mol Biol* 2002;26(5):602-9.

16. Pratico D, Basili S, Vieri M, Cordova C, Violi F, Fitzgerald GA. Chronic obstructive pulmonary disease associated with an increase in urinary levels of isoprostane F2a-III, an index of oxidant stress. *Am J Respir Crit Care Med* 1997;158:1709-14.

17. Belvisi MG, Bottomley KM. The role of matrix metalloproteinases (MMPs) in the pathophysiology of chronic obstructive pulmonary disease (COPD): a therapeutic role for inhibitors of MMPs? *Inflamm Res*. 2003;52(3):95-100.

18. Pinamonti S, Muzzoli M, Chicca MC, Papi A, Ravenna F, Fabbri LM, et al. Xanthine oxidase activity in bronchoalveolar lavage fluid from patients with chronic obstructive lung disease. *Free Radic Biol Med* 1996;21:147-55.

19. Linder R, Rönmark E, Pourazar J, Behndig A, Blomberg A, Lindberg A. Serum metalloproteinase-9 is related to COPD severity and symptoms - cross-sectional data from a population based cohort-study. *Respir Res* 2015; 21;16:28.

20. Beeh KM, Beier J, Kornmann O, Buhl R. Sputum matrix metalloproteinase-9, tissue inhibitor of metalloproteinase-1, and their molar ratio in patients with chronic obstructive pulmonary disease, idiopathic pulmonary fibrosis and healthy subjects. *Respir Med* 2003;97:634-9.

21. Higashimoto Y, Yamagata Y, Iwata T, Okada M, Ishiguchi T, Sato H, et al. Increased serum concentrations of tissue inhibitor of metalloproteinase-1 in COPD patients. *Eur Respir J* 2005;25:885-90.

22. Kwiatkowska S, Noweta K, Zieba M, Nowak D, Bialasiewicz P. Enhanced exhalation of matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 in patients with COPD exacerbation: a prospective study. *Respiration* 2012;84(3):231-41.

23. Mercer PF, Shute JK, Bhowmik A, Donaldson GC, Wedzicha JA, Warner JA. MMP-9, TIMP-1 and inflammatory cells in sputum from COPD patients during exacerbation. *Respir Res* 2005;22:6:151.

24. Baraldo S, Bazzan E, Zanin ME, Turato G, Garbisa S, Maestrelli P, et al. Matrix metalloproteinase-2 protein in lung periphery is related to COPD progression. *Chest* 2007;132(6):1733-40.

25. Chen Y, Chen P, Hanaoka M, Droma Y, Kubo K. Enhanced levels of prostaglandin E2 and matrix metalloproteinase-2 correlate with the severity of airflow limitation in stable COPD. *Respirology* 2008;13(7):1014-21.

26. John G, Shizu H, Elliott WM, Xing Li, Chan B, Wright C, et al. The role of MMP2 in persistent inflammation in Chronic Obstructive Pulmonary Disease (COPD). *FASEB J* 2009;23:13.

27. Corry DB, Kiss A, Song LZ, Song L, Xu J, Lee SH. Overlapping and independent contributions of MMP2 and MMP9 to lung allergic inflammatory cell egression through decreased CC chemokines. *FASEB J* 2004;18(9):995-7.

28. Bernardo MM, Fridman R. TIMP-2 (tissue inhibitor of metalloproteinase-2) regulates MMP-2 (matrix metalloproteinase-2) activity in the extracellular environment after pro-MMP-2 activation by MT1 (membrane type 1)-MMP. *Biochemical Journal* 2003;374:739-45.

29. Kameda K, Matsunaga T, Abe N, Hanada H, Ishizaka H, Ono H. Correlation of oxidative stress with activity of matrix metalloproteinase in patients with coronary artery disease. Possible role for left ventricular remodeling. *European Heart Journal* 2003;2180-5.

30. Pustovrh MC, Jawerbaum A, Capobianco E, White V, Martinez N, Lopez-Costa JJ. Oxidative stress promotes the increase of matrix metalloproteinases-2 and -9 activities in the feto-placental unit of diabetic rats. *Free Radical Research* 2005;12:1285-93.

31. Yang ZZ, Zou AP. Homocysteine enhances TIMP-1 expression and cell proliferation associated with NADH oxidase in rat mesangial cells. *Kidney Int* 2003;63(3):1012-20.

32. Kenney MC, Chwa M, Atilano SR, Tran A, Carballo M, Saghizadeh M, et al. Increased levels of catalase and cathepsin V/L2 but decreased TIMP-1 in keratoconus corneas: evidence that oxidative stress plays a role in this disorder. *Invest Ophthalmol Vis Sci* 2005;46(3):823-32.

How to cite this article:

Dogan HO, Aydin H, Demirpençe Ö, Kukul Güven FM, Tekin YK, Şahin A. Matrix Metalloproteinases and Oxidative Stress in Patients with AECOPD. *J Clin Anal Med* 2017;8(1): 64-7.