doi • 10.5578/tt.20219904

Tuberk Toraks 2021;69(1):30-38

Geliş Tarihi/Received: 08.06.2020 • Kabul Ediliş Tarihi/Accepted: 10.03.2021

Association of plasma netrin-1 level with inflammatory balance and comorbidities in patients with acute exacerbation of COPD

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Cite this article as: Kerget B, Afşin DE, Kerget F, Koçak AO, Aksakal A, Kızıltunç A, et al. Association of plasma netrin-1 level with inflammatory balance and comorbidities in patients with acute exacerbation of COPD. Tuberk Toraks 2021;69(1):30-38.

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ABSTRACT

Association of plasma netrin-1 level with inflammatory balance and comorbidities in patients with acute exacerbation of COPD

Introduction: Chronic obstructive pulmonary disease (COPD) is a common lung disease characterized by airflow restriction and systemic inflammation. Netrin-1 is a protein mainly produced in the central nervous system and has proven anti-inflammatory activity. The aim of this study was to determine netrin-1 level and its relationship with comorbidities in patients with acute exacerbation of COPD.

Materials and Methods: The study included 232 patients aged over 40 years who were divided into 3 groups: Group 1: ex-smokers (≥ 20 pack-years) with COPD hospitalized for COPD exacerbation ($n=142$), Group 2: current-smokers (≥ 20 pack-years) without COPD ($n=30$), Group 3: a control group comprising healthy non-smokers ($n=60$). Plasma netrin-1 levels were measured using commercial enzyme-linked immunosorbent assay (ELISA) kit.

Results: There were significant differences in forced expiratory volume in 1 second (FEV_1), forced vital capacity (FVC), FEV_1/FVC , C-reactive protein (CRP), and plasma netrin-1 levels between patients with acute exacerbation of COPD and current smokers without COPD, healthy controls ($p=0.001$ for all). Netrin-1 levels at discharge were lower in COPD patients with diabetes mellitus (DM) compared to nondiabetic COPD patients ($p=0.01$). Weak correlation was observed between netrin-1 level at admission and FEV_1 , FVC, partial pressure of oxygen, and CRP levels ($r=0.394$, $p=0.01$; $r=-0.366$, $p=0.01$; $r=-0.19$, $p=0.05$; $r=0.306$, $p=0.01$). Netrin-1 level at admission was also moderately correlated with smoking history (pack-years) ($r=0.579$, $p=0.01$).

Conclusion: Netrin-1 was elevated in acute exacerbation of COPD and may be an important element in inflammatory balance. Patients with both COPD and DM were found to have lower netrin-1 levels at discharge after resolution of the acute exacerbation.

Key words: COPD; coronary artery disease; diabetes mellitus; netrin-1

ÖZ

KOAH akut alevlenme hastalarında plasma netrin-1 düzeyinin inflammatuar denge ve komorbiditeler üzerine etkisi

Giriş: Kronik obstrüktif akciğer hastalığı (KOAH) hava akım kısıtlaması ve sistemik inflamasyon ile karakterize yaygın bir akciğer hastalığıdır. Netrin-1 ön planda santral sinir sisteminden sentezlenen anti-inflamatuar etkinliği tespit edilmiş bir proteindir. Çalışmamızda netrin-1 düzeyinin KOAH akut alevlenme ve komorbiditelerle ilişkisini tespit etmeyi amaçladık.

Materyal ve Metod: Çalışmamıza 40 yaş üzeri 3 grupta 232 hasta dahil edildi. Grup 1: 20 paket/yıl üzeri sigara içmiş, ex-smoker ve KOAH akut alevlenme nedeniyle hastanede yatan hastalar ($n=142$), grup 2: aktif sigara içen ve KOAH olmayan hastalar, grup 3 ($n=30$) non-smoker sağlıklı grup ($n=60$). Hasta ve sağlıklı gruplardan 5 cc periferik venöz kan alındı. Plazma netrin-1 seviyesi enzim bağli immünosorbent (ELISA) yöntemiyle ölçüldü.

Bulgular: Çalışmamızda FEV_1 , FVC, FEV_1/FVC , CRP ve plasma netrin-1 düzeyinde KOAH akut alevlenme ile gelen hastalarla, kontrol grubu ve aktif sigara içen grup arasında istatistiksel olarak anlamlı düzeyde farklı idi (tüm parametreler için $p=0,001$). KOAH akut alevlenme ile gelen ve diyabetes mellitusu (DM) olan hastalarda taburculuk sırasında yalnızca KOAH gözlenen hastalara nazaran daha düşük netrin-1 düzeyi tespit edildi ($p=0,01$). Netrin-1'in başvuru anındaki düzeyinin FEV_1 , FVC, PaO_2 ve CRP düzeyi ile yapılan korelasyon analizinde düşük düzeyde korelasyon gözlemlendi (sırasıyla $r=-0,394$, $p=0,01$, $r=-0,366$, $p=0,01$, $r=-0,19$, $p=0,05$, $r=0,306$, $p=0,01$). Netrin-1'in başvuru anındaki düzeyi ile sigara içme süresi (paket/yıl) ile yapılan korelasyon analizinde ise pozitif yönlü orta düzeyde korelasyon gözlemlendi ($r=0,579$, $p=0,01$).

Sonuç: KOAH akut alevlenmede artış gösteren netrin-1 inflammatuar dengenin sağlanmasında önemli olabilir. KOAH ile birlikte DM gözlenen hastalarda ise akut alevlenme tablosu geriledikten sonra taburculuk esnasında daha düşük plasma netrin-1 düzeyi tespit edildi.

Anahtar kelimeler: KOAH; koroner arter hastalığı; diyabetes mellitus; netrin-1

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is characterized by persistent airflow restriction associated with systemic findings. Low-grade systemic inflammation is observed in addition to the inflammatory process in the lungs and is considered the main cause of comorbidities in COPD. This systemic inflammation may be a result of the inflammation present in the lungs, or the pulmonary inflammation may be a manifestation of the systemic disease. Smoking, lung hyperinflation, tissue hypoxia, and skeletal muscle dysfunction have been proposed as possible factors in the pathogenesis of systemic inflammation in COPD. Disruption of the inflammation/anti-inflammation equilibrium is the main factor implicated in acute exacerbation and comorbidities of COPD (1,2).

Netrin-1 is a 50–75 kD laminin-like protein. When it was discovered in the central nervous system, it was

found to be involved in preventing chemotropic and cell apoptosis. Studies have also demonstrated its importance in neovascularization, cell adhesion, and tumorigenesis. The expression of netrin-1 is associated with infection and inflammatory factors. Netrin-1 receptor UNC5B is highly expressed in leukocytes. When it binds to UNC5B, netrin-1 prevents migration and accumulation of leukocytes in the vascular lumen. This reduces the synthesis of chemotropic and proinflammatory cytokines (3,4). Netrin-1 inhibits the migration of macrophages, which play an important role in atherosclerotic plaque stabilization. In studies of coronary artery disease (CAD), low netrin-1 levels were associated with increased susceptibility (5). Netrin-1 is also believed to be an anti-inflammatory regulator of inflammatory balance, smoking-related endothelial dysfunction and the atherosclerotic process that occurs in the damaged endothelium were found to increase netrin-1 level (6). Although studies in diabetes mellitus (DM) have shown that high

netrin-1 levels were associated with greater susceptibility to diabetes, they have yielded no data regarding the exact mechanism (7).

In the present study, we aimed to determine whether netrin-1 levels are associated with acute exacerbation of COPD and comorbidities that are important risk factors for COPD exacerbation.

MATERIALS and METHODS

Study Population

The study included 150 patients aged over 40 years who presented to the emergency clinic of our hospital and were admitted for acute exacerbation of COPD (GOLD 2019 group D) (1), 30 current smokers (at least 20 pack-years) without COPD who presented to our smoking cessation outpatient clinic, 60 healthy non-smokers with normal lung function were included as the control group. Eight COPD patients admitted for acute exacerbation died due to hypoxic respiratory failure and were excluded from the study.

Acute exacerbation of COPD was defined as an episode in which baseline dyspnea, cough, and/or sputum changed beyond normal day-to-day variations and required treatment with increased bronchodilator therapy or systemic steroids, antibiotics, and/or emergency room visit or hospital admission. Only patients with moderate and severe exacerbations were included in this study. Exclusion criteria included recent history of pulmonary embolism, cerebral aneurysm, hemoptysis, pneumothorax, nausea/vomiting, or thoracic, abdominal, or ocular surgery; smoking within the last 6 months; and presence of malignancy, hyperthyroidism, cystic fibrosis, angioedema, acute exacerbation of congestive heart failure, and tachycardia (heart rate $\geq 120/\text{min}$).

Inclusion criteria for the COPD group were smoking cessation ≥ 6 months earlier, smoking history of ≥ 20 pack-years, and COPD group D classification according to the GOLD 2019 guidelines (FEV_1/FVC ratio < 0.7 in postbronchodilator pulmonary function test [PFT], mMRC score ≥ 2 , CAT score ≥ 10 , and ≥ 2 exacerbations or ≥ 1 hospital admission in the last year). The control group consisted of health care workers employed in our hospital who met the inclusion/exclusion criteria and agreed to participate in the study.

Blood samples were obtained from patients hospitalized for COPD exacerbation twice to measure netrin-1 level at admission and discharge, while

blood sampling was performed only once for the other patients. Diagnosis of CAD and DM in COPD patients was confirmed from their hospital records.

Treatment for COPD Exacerbation

Patients who presented with acute exacerbation of COPD received short-acting beta2 agonist+anticholinergic and methylprednisolone 0.5 mg/kg for the duration of their inpatient treatment in addition to the nasal oxygen therapy used during the maintenance phase. The patients received oral macrolide therapy for 5 days starting from the day of admission (5). Of the 142 patients hospitalized for COPD exacerbations, 78 were using long-term oxygen therapy at home. Another 19 were given long-term oxygen therapy based on evaluation at discharge. Nine of the 30 patients with diabetes mellitus were using an oral antidiabetic, while 21 were using insulin therapy. For patients using insulin, abnormal fasting blood glucose levels due to systemic steroid therapy were corrected by adjusting their insulin dosage. Patients using oral antidiabetic drugs who had abnormal fasting blood glucose levels due to systemic steroid were switched to insulin therapy. In addition, 9 patients whose blood glucose could not be controlled with oral antidiabetic drugs despite discontinuing steroid therapy were also prescribed insulin therapy and those with persistent high blood glucose during follow-up were transferred to the endocrinology department for blood glucose regulation.

Pulmonary Function Testing

The age, height, and weight of the patients were measured and recorded. Patients were instructed to avoid cigarettes for 24 hours, alcohol for 4 hours, strenuous exercise for 30 minutes, and heavy food for 2 hours before testing. They were asked to wear loose-fitting clothing that allowed unrestricted movement of the chest and abdomen during testing. BTPS correction was done based on room air pressure and barometric pressure. The expected maneuver was explained to the participants and repeated until 3 acceptable spiograms were obtained. Tests that met the reproducibility and acceptability criteria were included in the study. The same technician performed all pulmonary function tests using a Plusmed MIR Spirolab III device.

Laboratory Measurements

Venous blood samples were collected from all patients and separated into serum and cellular fractions within 2 hours by centrifugation at $3.000 \times g$ for

10 minutes. The serum was collected and clarified by centrifuging again at $10,000 \times g$ for 15 minutes. Serum samples were stored at -80°C until analysis. HbA1c levels were measured enzymatically using a chemistry analyzer (Olympus AU5400; Chemical Ltd., Tokyo, Japan). High-sensitivity C-reactive protein (hs-CRP) was determined using an enzyme-linked immunosorbent assay (ELISA) kit (R&D systems, Minneapolis, MN, USA).

Plasma Netrin-1 Measurement

After 15 minutes of semi-supine rest, blood samples were obtained from an antecubital vein into tubes containing ethylenediaminetetraacetic acid (EDTA) to prevent coagulation. Plasma samples were obtained and stored at -80°C until analysis. Netrin-1 was measured by ELISA (USCN ELISA, Wuhan, China).

Statistical Analysis

The study data were analyzed using Statistical Package for Social Sciences (SPSS) v20.0 software. Categorical variables were expressed as numbers and percentages while numerical variables were expressed as mean and standard deviation. Kolmogorov–Smirnov test was used to assess whether the data showed normal distribution. Friedman test and Bonferroni-corrected Wilcoxon test were used for numerical data with non-normal distribution in comparisons of repeated measures within groups, and Mann–Whitney U test was used to compare numerical data with non-normal distribution between the groups. Netrin-1 levels were compared between the groups using one-way ANOVA with Tukey test as post hoc analysis. Pearson correlation analysis was used to identify correlations between Netrin-1 levels and FEV_1 , FVC, partial pressure of oxygen, CRP, HbA1c and smoking history. A p value < 0.05 was considered statistically significant.

RESULTS

Mean age of the COPD, current-smoker and control groups was 58.6 ± 9.1 , 55.8 ± 8.6 and 57.8 ± 7.9 years, respectively. The COPD group included 89 males (62.6%) and 53 females (37.4%), current-smoker group included 16 males (53.3%) and 14 females (46.7%) and the control group included 35 males (58.3%) and 25 females (41.7%). Demographic and laboratory data, pulmonary function test results, and hospital length of stay of the COPD, current-smoker, and control groups are presented in Table 1. Netrin-1 level was significantly higher in patients with acute

COPD exacerbation compared to the current-smoker and control groups ($p = 0.02$, $p = 0.001$) and in the current-smoker group compared to the control group ($p = 0.02$). When post-exacerbation netrin-1 levels of the COPD group were compared with the other two groups, the current-smoker group had the highest level but there was no significant difference between the groups ($p = 0.87$, $p = 0.212$).

Tables 2 and 3 show comparisons of these parameters between patients with COPD alone and subgroups of COPD patients with CAD and DM. There was no significant difference between COPD patients with and without CAD in terms of netrin-1 levels at admission or discharge ($p = 0.097$, $p = 0.352$). COPD patients with and without DM showed no significant difference in admitting netrin-1 level ($p = 0.84$) but netrin-1 level at discharge was significantly lower in COPD patients with DM ($p = 0.01$).

Netrin-1 level at admission showed weak correlation was observed between admitting netrin-1 level and FEV_1 , FVC, partial pressure of oxygen, and CRP levels ($r = 0.394$, $p = 0.01$; $r = -0.366$, $p = 0.01$; $r = -0.19$, $p = 0.05$; $r = 0.306$, $p = 0.01$). (Figures 1,2). There was no correlation between netrin-1 at discharge and HbA1c level ($r = 0.097$, $p = 0.55$).

Netrin-1 level at admission showed moderate positive correlation with smoking history (pack-years) ($r = 0.579$, $p = 0.01$) (Figures 3).

DISCUSSION

In our study, patients presenting with acute exacerbation of COPD had a higher plasma netrin-1 level than the control group and current-smokers without COPD group. When COPD patients with CAD and DM were compared, plasma netrin-1 level was not associated with CAD but was lower in patients with DM. We also observed that current smokers without COPD had significantly higher netrin-1 level than the control group.

COPD is an inflammatory lung disease characterized by irreversible airflow restriction. Smoking is the leading cause of COPD in developed countries, whereas biomass exposure is a more important etiology in developing countries (5). Neutrophils play an important role in the clearance of these particles and in the inflammation they cause. Cyclooxygenase (COX)-2 and its metabolite prostaglandin E_2 play an important role in neutrophil migration and proliferation during inflammation in the lung (6).

Table 1. Comparison of demographic, laboratory, and pulmonary function test data between patients hospitalized for acute exacerbation of COPD and the control group

	Ex smoker with acute exacerbation of COPD (n= 142)	Current-smokers without COPD (n= 30)	Control (n= 60)	p*	p**
Age (years)	58.6 ± 9.1	55.8 ± 8.6	57.8 ± 7.9	0.08/0.134	0.1
Gender (males, %)	89 (62.6%)	16 (53.3%)	35 (58.3%)	-	
FEV ₁ (L/%)	1.17 ± 0.16/44.3 ± 6	2.17 ± 0.4/80.4 ± 2.2	2.19 ± 0.1/83.2 ± 3.1	0.001	0.91
FVC (L/%)	1.94 ± 0.2/78.3 ± 8	2.42 ± 0.1/92.1 ± 8.2	2.45 ± 0.2/95.3 ± 7.1	0.001	0.93
FEV ₁ /FVC (%)	56.9 ± 7.5	84.6 ± 2.5	87.6 ± 6.5	0.001	0.85
sO ₂	77.7 ± 9.4	-	-	-	
pO ₂	48.9 ± 10.8	-	-	-	
pCO ₂	42.7 ± 9.5	-	-	-	
HCO ₃	27.4 ± 4.2	-	-	-	
Lactate	2.3 ± 1.1	-	-	-	
CRP	41.7 ± 34.1	4.1 ± 0.9	3.13 ± 0.5	0.001/0.001	0.103
Smoking history (pack-years)	32.5 ± 16.6	34.3 ± 18.9	-	0.07	
Netrin-1 (at admission for COPD) (pg/ml)	109.4 ± 52.4	86.6 ± 19.6	75.7 ± 19.5	0.02/0.001	0.02
Netrin-1 (at discharge for COPD) (pg/ml)	83.6 ± 35.6	-	-	0.87/0.212	
Length of stay (days)	8.1 ± 2.3	-	-		

p*: Comparison of netrin-1 levels of the control group, current smokers without COPD group and patients hospitalized for acute exacerbation of COPD

p**: Comparison of netrin-1 levels of the control group and current smokers without COPD group

Table 2. Comparison of demographic, laboratory, and pulmonary function test data between patients with COPD and COPD+CAD

	COPD (n= 80)	COPD+CAD (n= 62)	p
Age (years)	56.7 ± 5.6	58.2 ± 9.2	0.04
FEV ₁ (L/%)	1.17 ± 0.15/43.6 ± 6.2	1.16 ± 0.17/45.3 ± 5.8	0.539
FVC (L/%)	1.96 ± 0.21/79.1 ± 8.8	1.92 ± 0.18/77.3 ± 6.9	0.234
FEV ₁ /FVC (%)	55.4 ± 8.1	58.7 ± 6.3	0.008
sO ₂	77.1 ± 11.2	78.7 ± 6.2	0.262
pO ₂	49.9 ± 12.5	47.8 ± 8.2	0.231
pCO ₂	40.5 ± 6.4	45.6 ± 11.8	0.001
HCO ₃	26.8 ± 3.4	28.3 ± 4.9	0.032
Lactate	1.9 ± 0.84	2.6 ± 1.3	0.001
CRP	40.7 ± 39.5	43.1 ± 25.8	0.697
Smoking history (pack-years)	32.6 ± 17.5	32.5 ± 15.5	0.967
Netrin-1 (at admission for COPD) (pg/ml)	102.9 ± 43.2	117.6 ± 61.8	0.097
Netrin-1 (at discharge for COPD) (pg/ml)	81.1 ± 35.4	86.8 ± 35.9	0.352
Length of stay (days)	8.1 ± 2.2	7.9 ± 2.3	0.592

Studies have shown that in acute exacerbation of COPD, production of cytokines is increased, especially interleukin (IL)-1 β , which in turn increases

COX-2 activity. COX-2 products may promote an inflammatory response that leads to excessive production of cytokines such as IL-6, IL-8, and IL-10 via

Table 3. Comparison of demographic, laboratory, and pulmonary function test data between patients with COPD and COPD+DM

	COPD (n= 108)	COPD+DM (n= 34)	p
Age (years)	60.1 ± 7.2	59.3 ± 6.6	0.231
FEV ₁ (L%)	1.17 ± 0.16/44.3 ± 6.3	1.15 ± 0.15/45.1 ± 5.5	0.651
FVC (L%)	1.94 ± 0.2/78.7 ± 8.6	1.94 ± 0.19/77.4 ± 5.9	0.995
FEV ₁ /FVC (%)	56.4 ± 7.8	58.3 ± 6.4	0.15
sO ₂	77.8 ± 10.4	77.5 ± 5.5	0.831
pO ₂	50.2 ± 11.7	45.1 ± 6.1	0.014
pCO ₂	43.5 ± 10.2	40.3 ± 6.5	0.09
HCO ₃	27.8 ± 4.1	26.7 ± 4.6	0.261
Lactate	2.2 ± 1.1	2.4 ± 1.2	0.365
CRP	40.5 ± 36.3	45.7 ± 25.4	0.354
Smoking history (pack-years)	32.2 ± 16.4	33.7 ± 17.6	0.649
Netrin-1 (at admission for COPD) (pg/ml)	109.8 ± 50.4	107.7 ± 59.3	0.84
Netrin-1 (at discharge for COPD) (pg/ml)	87.6 ± 38.9	70.9 ± 16.7	0.01
Length of stay (days)	7.9 ± 2.3	8.3 ± 1.7	0.485

Figure 1. Correlation analysis between netrin-1 and FEV₁, FVC.

lymphocytes (7,8). Some of these cytokines can disrupt cell repair by preventing apoptosis, as well as make it difficult to control inflammation.

Netrin-1 is an intracellular secreted soluble protein and the earliest isolated member of the netrin family. This protein can be secreted by many tissues but is produced mainly in the central nervous system (4). Studies have shown that netrin-1 inhibits inflammation in renal ischemia-reperfusion injury, intestinal diseases, diabetic nephropathy, and corneal diseases (3, 9-11). Infection and inflammatory diseases play an important role in netrin-1 expression and its receptor UNC5B is highly expressed in leukocytes.

When netrin-1 binds to UNC5B, it prevents the migration and accumulation of leukocytes and macrophages within the vascular lumen (12). Netrin-1 exerts its anti-inflammatory activity by regulating the inflammatory response of leukocytes and macrophages by suppressing COX-2-mediated PGE2 production (13). In studies evaluating the relationship of netrin-1 with CAD, negative correlation was observed between low netrin-1 level and CAD prevalence. This finding was attributed primarily to the inability of netrin-1 to suppress the macrophage inflammatory response, which plays an important role in atherosclerotic plaque formation (14,15). Netrin-1 levels were found to be lower in newly diagnosed type 2

Figure 2. Correlation analysis between netrin-1 and PaO₂, CRP.

Figure 3. Correlation analysis between netrin-1 and smoking history (pack-years).

DM patients compared to a control group (16). Netrin-1, which is synthesized in the pancreas, was found to play an important role in the morphogenesis stage and low levels of netrin-1 in DM patients were associated with blood glucose dysregulation (17).

In our study, patients with acute exacerbation of COPD had higher netrin-1 levels at admission than at discharge. In fact, netrin-1 levels in the COPD patients at discharge did not differ significantly from those of the control group and current-smokers group. Netrin-1 level increased in correlation with decline in pulmonary function test parameters and lower PaO₂ in patients presenting with acute exacerbation of COPD. Significant increases in IL-4, IL-8, and IL-10 have been observed with reduction in pulmonary function test results (18). Considering that netrin-1 suppresses IL-6, IL-8, and IL-10 via the

COX-2 system, this may be interpreted as an effort to inhibit the inflammatory response associated with impaired pulmonary function. Furthermore, studies examining the relationship between hypoxia and netrin-1 have shown that hypoxia-inducible factor 1 is associated with increased netrin-1 synthesis (18). This finding can be said to confirm our current findings. In the present study, there was a positive correlation between netrin-1 level and smoking history. In previous studies, smoking was found to disrupt tissue oxygenation and increase endothelial dysfunction and atherosclerotic plaque formation in this region, and this was found to cause an increase in plasma netrin-1 level (6). The data obtained in our study are consistent with those of previous studies.

Netrin-1 level in patients with COPD and diabetes did not differ significantly at admission but was lower at discharge. This may be related to abnormal blood glucose levels in some DM patients with acute exacerbation of COPD caused by systemic steroid therapy and their previous glucose regulation therapy being inadequate. These findings confirmed the negative correlation between netrin-1 and fasting blood glucose level also demonstrated in previous studies. The fact that no significant correlation was detected between HbA1c and netrin-1 level may be attributed to HbA1c being an indicator of long-term blood glucose regulation, whereas our study had a short follow-up period. In the comparison of COPD patients with and without CAD, we observed no difference in netrin-1 levels at time of admission or discharge. However, the subgroup of patients with COPD and CAD had significantly higher age, PaCO₂, HCO₃⁻, and lactate levels. These findings indicate that clinical

course is more severe in patients with COPD and CAD who are older, have increased PaCO₂ (an indicator of impaired ventilation), and elevated lactate level (associated with mortality). A possible reason we did not observe a significant difference in netrin-1 level is that none of the CAD patients in our study showed any signs or symptoms of worsening CAD at admission or during follow-up, whereas all of the data from studies that reported an association with netrin-1 were obtained during periods of CAD instability. Therefore, by not contributing further to the existing inflammatory state of the COPD patients, CAD may not have increased their levels of netrin-1, which is considered primarily an anti-inflammatory mediator.

The main limitation of this study is that the current-smokers group was smaller than the COPD and control groups. Nevertheless, the fact that our findings were largely consistent with those of previous studies suggests that the results are generalizable.

CONCLUSION

In conclusion, netrin-1 may be an important biomarker for the control of the inflammatory response in COPD, especially during acute exacerbation. The main adverse effect of treatment for acute exacerbation of COPD is impaired blood glucose regulation, which it may bring about in DM patients by making previous glucose regulation treatment insufficient or decreasing netrin-1, an important regulator of blood glucose. Netrin-1 appeared to be unaffected in the presence of stable CAD, another important comorbidity in COPD. However, this relationship should be verified in studies of the protective effect of CAD in the acute stage.

Ethical Committee Approval: The approval for this study was obtained from Ataturk University Ethics Committee.

CONFLICT of INTEREST

The authors of this meta-analysis declare that they have no conflict of interest.

AUTHORSHIP CONTRIBUTIONS

Concept/Design: BK, DEA

Analysis/Interpretation: BK, FK, AOK, AK

Data Acquisition: BK, AA, FK

Writing: BK, FK

Clinical Revision: EYU, ÖA, MA

Final Approval: BK, MA

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