Original Article

Endotrophin levels in clinically-stable chronic obstructive pulmonary disease

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Abstract

Aim: Chronic obstructive pulmonary disease (COPD) is characterized by chronic inflammation and permanent airflow limitations due to stenosis of the small airways. The role of the extracellular matrix (ECM) structure and its reshaping is gaining importance. Endotrophin is a division product formed during construction of type VI collagen. The aim of this study was to research the correlation between endotrophin and the stable COPD pathophysiology.
Materials and Methods: The study included 90 clinically-stable COPD patients and 38 participants without COPD. The demographic data, respiratory function tests and endotrophin levels of patients were recorded and statistically evaluated.
Results: Endotrophin levels were significantly high in COPD patients compared to healthy controls (p<0.001). Though there was a positive correlation between endotrophin and CRP (r=0.229, p=0.031), there was no correlation with FEV-1, FVC, mMRC dyspnea score, 6-minute walking test distance and GOLD degree.
Conclusion: Endotrophin was identified to be high in clinically stable COPD cases. This elevation appears to be associated with continuing inflammation in COPD. This correlation may guide prognosis studies for COPD and ensure the opportunity to develop new treatment strategies targeting endotrophin.

Keywords: CRP; chronic obstructive pulmonary disease; endotrophin; extracellular matrix; inflammation

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is one of the most common lung diseases around the world. It is characterized by chronic inflammatory and progressive airflow obstruction. COPD is due to a combination of factors like airflow obstruction, chronic inflammation, excessive mucus secretion, reshaping of the small airways and bronchoconstriction (1). There is a need to better understand these complicated mechanisms resulting in COPD.

In pulmonary diseases, the role of changes to extracellular matrix (ECM) structure is gaining importance. ECM is a macromolecular structure ensuring both physical support of tissues and shaping cell behavior in health and sickness due to signals given to cells (2). Identification of abnormalities in extracellular matrix components is important to develop new therapeutic approaches for COPD and to monitor disease progress.

Type VI collagen is a collagen found at the interface between the basal membrane of the lungs and the interstitial matrix, comprising a microfilament network providing structural support and additionally elasticity (3,4). Endotrophin occurs during formation of type VI collagen with the separation of the C-5 area at the carboxy tip of the alpha 3 chain. Endotrophin is necessary for creation of microfibrils in type VI collagen. It is thought to be an important tool for signaling in type VI collagen (5,6). Additionally, it is reported to play a role in processes like inflammation, angiogenesis, profibrosis and epithelial-mesenchymal transition (EMT) (7,8).

According to mechanisms playing a role in COPD pathophysiology, there are few studies about structural changes in the extracellular matrix. Studies examined ECM components during COPD flare-ups and identified increased destruction-construction cycles (9-10).

There are few studies performed about endotrophin (11-15). Most of these studies associated endotrophin with

COPD flare-ups. However, there is a need to identify and understand the stable COPD-endotrophin association.

In our study with this aim, we focused on the endotrophin levels of stable COPD patients. Investigating endotrophin levels in healthy controls, just as in pathologic conditions like physiological and stable COPD, will assist in understanding the role played by endotrophin in more detail.

MATERIALS and METHODS

Participants
This prospective study included 90 COPD patients who were clinically stable attending the internal medicine and respiratory diseases outpatient clinic and 38 healthy individuals. The demographic data, spirometry values, dyspnea scores and CRP levels of participants were recorded.

Patients with malignancy, obesity, diabetes mellitus, psychiatric disorders or chronic inflammatory disease, and receiving corticosteroid treatment or diet supplementation were excluded from the study

Ethics committee permission was obtained from the local ethics committee (ethics committee decision dated August 2020 and numbered 514.10).

Assessment Tools and Study Design
COPD diagnosis was made according to the Global Strategy for the Diagnosis, Management and Prevention of Chronic Obstructive Pulmonary Disease (GOLD) criteria. According to the GOLD criteria, individuals with FEV1/FVC <70% after bronchodilator on spirometric measurements were diagnosed with COPD.

According to expected FEV-1 percentage in measurements determined with respiratory function tests, patients were classified as ≥80% GOLD 1 (mild), 50-79% GOLD 2 (moderate), 30-49% GOLD 3 (severe) and <30% GOLD 4 (very severe).

According to GOLD criteria for COPD clinical staging, severity of airflow limitation (GOLD 1-4), number of flare-ups during the last year, hospitalizations, dyspnea scale (mMRC) and symptom assessments (CAT) were determined. According to these criteria, every patient was classified clinically as GOLD stage A, B, C or D. GOLD A: Gold 1-2, exacerbations per year ≤1, mMRC 0-1, CAT<10; GOLD B: Gold 1-2, exacerbations per year ≤1, mMRC 0-1, CAT<10; GOLD C: Gold 3-4, exacerbations per year ≥1, mMRC ≥ 2, CAT>10; and GOLD D: Gold 3-4, exacerbations par year ≥1, mMRC ≥ 2, CAT>10 (16).

Dyspnea score used the modified Medical Research Council (mMRC) survey. The COPD assessment test (CAT) was performed to assess health status. Patients with CAT total score ≥10 were assessed as symptomatic. The six-minute walking test (6MWT) was used to test functional capacity and tolerance of physical exercise (17).

In addition to routine blood tests, additional blood samples were taken from the volunteers in a biochemistry tube, kept at room temperature for half an hour, centrifuged at 4000 rpm for 10 minutes, and the serum obtained was stored at -80 °C until analysis.

The sera were thawed at room temperature. An enzyme-linked immunosorbent assay (ELISA) kit (Sunred Biological Technology Catalogue 201-12-9305) was used for the measurement of serum endotrophin levels. The analytical (linear) detection range was 1.5ng/ mL-300ng/mL, the minimal detection limit was 1.398ng/ mL,The reported intraassay and interassay coefficient of variations (CVs) were 10% and 12%, respectively, for the endotrophin assay kit.

Statistical Analysis
Statistical analysis was performed using the SPSS version 20.0 (IBM Corp., Armonk, NY, USA). Descriptive data are expressed in mean ± standard deviation (SD) for continuous variables and in number and percentage (%) for categorical variables. An independent sample t-test was performed for the comparison of parametric data between two independent groups. The Mann-Whitney U test was used for the comparison of non-normally distributed variables. The Spearman correlation test was used to examine the independent variables affecting serum endotrophin levels. A p value of <0.05 was considered statistically significant.

RESULTS

Endotrophin levels of stable COPD patients were identified to be high compared to healthy individuals with values of 103.5 (42.8-73.8) and 28.9 (8.0-24.4), respectively (p=0.005) (Table 1).

Table 1. Comparison of demographic data, respiratory functions and endotrophin levels in COPD patients and control group

	COPD patient group	Control group	p
Age (years)	58.58 9	59.4 9.3	0.205
BMI (kg/m²)	25.1 3.0	26.7 5.1	0.084
Gender n (%)			
Female	18 (20%)	17 (45%)	
Male	72 (80%)	21 (55%)	0.030[b]
Endotrophin (ng/mL)	103.5 (42.8-73.8)	28.9 (8.0-24.4)	0.005[a]
CRP (mg/dL)	3.4 1.7	2.0 1.2	0.001
FEV1 (l)	1.8 0.77	3.1 0.32	<0.001
FVC (l)	2.6 0.81	3.6 0.41	<0.001
FEV1/FVC (%)	67 10	87 4.7	<0.001
6 MWT (m/s)	0.8 0.20	1.4 0.14	<0.001
CAT score	58/32	None	None
MMRC dyspnea scale	2 (1-4)	None	None

BMI; body mass index, FEV1; Forced expiratory volume in first second, FVC; forced vital capacity, 6 MWT;6 min walking test, CAT; COPD assessment test (ratio of symptomatic to asymptomatic), mMRC; modified Medical Research council dyspnea scale a: Mann Whitney U test b : chi square test, others used Student T test. Data are given as mean ± standard deviation unless otherwise stated

The CRP levels of stable COPD patients were identified to be higher than healthy individuals with values of 3.4±1.7 and 2.0±1.2, respectively (p=0.001) (Table 1).

The ages (p=0.205) and BMI values (p=0.084) were similar in the control group and COPD group. Of patients with COPD, 18 were women (%20) and 72(%80) (Table 1).

As expected, COPD patients had lower FEV1, FVC and FEV1/FVC values compared to healthy individuals (p<0.001). Additionally, the 6MWT was lower in the COPD group (p<0.001) (Table 1).

There was a positive correlation identified between endotrophin and CRP (r = 0.229, p = 0.031) (Table 2).

There was no correlation identified between endotrophin with FEV1, FVC, FEV1/ FVC values and 6MWT, CAT dyspnea and exercise scores (p >0.05) (Table 2) .

When GOLD values are compared within the COPD group and with the control group, endotrophin levels were higher in GOLD A+B and C+D groups compared to the control group (p<0.005) (Figure 1).

Endotrophin levels were similar in GOLD A+B and GOLD C+D groups (p>0.005) (Figure 1).

There was a positive correlation identified between endotrophin and CRP (Figure 2).

Table 2. Correlation table for endotrophin with respiratory functions and demographic data										
Endotrophin		**FEV1**	**FVC**	**FEV1/FVC**	**6MWT**	**CAT**	**mMRC**	**CRP**	**COPD duration**	**BMİ**
	r	-0.057	-0.075	-0.107	-0.153	0.043	0.022	0.229	0.075	-0.144
	p	0.596	0.487	0.318	0.153	0.690	0.841	0.031*	0.506	0.288

BMI: body mass index, FEV1: Forced expiratory volume in first second, FVC: forced vital capacity, 6 MWT: 6 min walking test, CAT: COPD assessment test (ratio of symptomatic to asymptomatic), mMRC: modified Medical research council dyspnea scale. CRP. C-reactive protein, COPD: Chronic Obstructive pulmonary disease
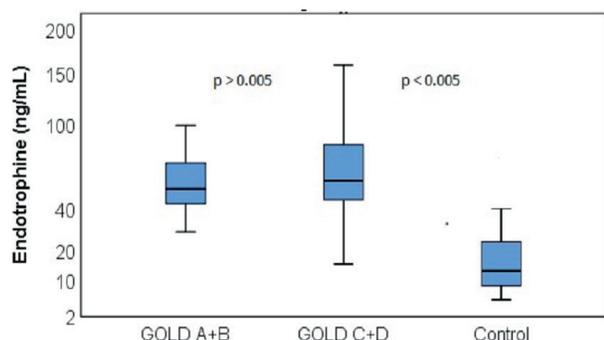
GOLD: Global initiative for chronic obstructive lung disease, p<0.005 statistically significant

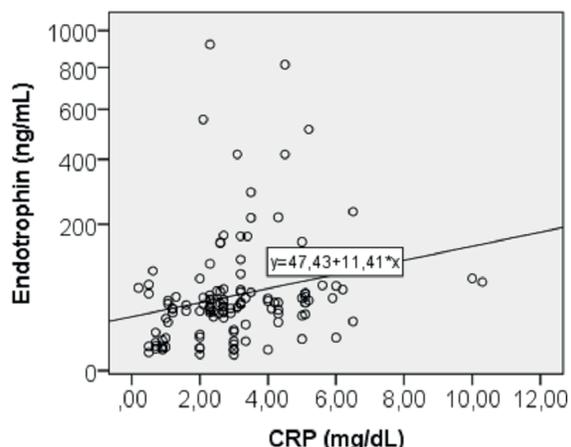**Figure 1.** Comparison of endotrophin levels in COPD patients and control group

Figure 2. Scatter plot of endotrophin with CRP

DISCUSSION

COPD is characterized by chronic inflammation causing changes to ECM components in lung cells (13).

In our study we found endotrophin levels in clinically-stable COPD patients were higher than healthy controls (Table 1). Endotrophin activates endothelial cell migration, encourages macrophage infiltration of damaged tissue and stimulates fibrosis (7,8). At the same time, it was shown to stimulate TGF-production (18). With these mechanisms, endotrophin may play an important role in fibrosis development and maintenance in the COPD pathogenesis. When we examine the literature, Jannia et al. identified increased serum endotrophin levels in stable COPD patients compared to a healthy group, similar to our study (13,14). Another study by the same team showed high endotrophin levels were associated with increased mortality in COPD patients (12). Endotrophin reduced during flare-up periods of the disease and was identified to increase in stable COPD stages (11,14). These studies showed a significant degree of variation of type VI collagen destruction and formation in stable and flare-up periods of the disease. The high endotrophin in stable COPD patients compared to the healthy group indicates type VI collagen formation. Endotrophin may increase with the aim of tissue repair in the lungs during stable COPD. This increase may cause initiation of an irreversible pathophysiological process in the lungs with the profibrotic and proinflammatory effects of endotrophin in advancing periods.

We did not identify a correlation between GOLD degrees, showing disease severity, with endotrophin (Figure 1).

There was no correlation between endotrophin with FEV-1, FVC, 6MWT, CAT dyspnea and exercise scores. There are studies compatible with our results in the literature. Jannie et al. did not identify a correlation between endotrophin with GOLD degrees, FEV-1 and MRC dyspnea scores in stable COPD patients (13). The same team did not find a correlation between endotrophin with FEV-1, FVC, mMRC dyspnea scale and exercise parameters like 6MWT during COPD flare-ups (14). The lack of difference between GOLD early and late stages and high identification compared to the control group show that endotrophin levels undergo changes from the early period. The reason for the lack of correlation between dyspnea scores, FEV-1 and FEV-1/FVC with endotrophin may be that mostly type I and III collagen are found in lung tissue and these are fibrillary collagens protecting the structure of pulmonary tissue (1,14). Collagen type VI is found relatively less compared to other collagens (15). For this reason, variations in endotrophin levels representing type VI collagen formation may not cause airflow limitations.

In our study, CRP was higher in the stable COPD group compared to the healthy group (Table 1). Additionally, there was a positive correlation identified between endotrophin and CRP (Figure 2). CRP is a biomarker showing tissue injury, presence of inflammation and severity of infection (19). In stable COPD, there is stable low-level continuing inflammation, independent of flare-ups. Increased CRP in stable COPD is reported to be a biomarker predicting flare-ups and poor prognosis (19-21). The inflammatory effect of endotrophin may act through macrophage activation and increased TGF-β production. In the literature, Şengül et al. stated that endotrophin correlated with CRP may play a role in inflammation in type 2 diabetic patients (22). The positive correlation between increased endotrophin and CRP in stable COPD patients leads to consideration that endotrophin has effects on inflammation, especially in COPD.

LIMITATIONS

The limitations of our study are its cross-sectional plan and lack of prognostic information.

CONCLUSION

In conclusion, endotrophin was identified to be high in clinically stable COPD patients. There was no correlation between endotrophin and GOLD degrees. This situation leads to consideration that endotrophin may be a biomarker increasing from the early period. The correlation of endotrophin with CRP may indicate a role in continuing inflammation in COPD. There is a need to perform more studies both at molecular level and clinically to determine the full role of endotrophin in stable COPD.

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