SERUM CYSTATIN C LEVELS IN COPD: POTENTIAL DIAGNOSTIC VALUE AND RELATION BETWEEN RESPIRATORY FUNCTIONS

SERUMSKI NIVOI CISTATINA C U HRONIČNOJ OPSTRUKTIVNOJ BOLESTI PLUĆA: POTENCIJALNA DIJAGNOSTIČKA VREDNOST I ODNOS IZMEĐU RESPIRATORNIH FUNKCIJA

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Summary

Background: The aim of this study was to determine the level of serum cystatin C (CysC) in patients with Chronic Obstructive Pulmonary Disease (COPD) during exacerbation and stable periods and to investigate its potential diagnostic value and the relationship between CysC levels and the pulmonary function test (PFT).
Methods: One hundred twenty-six patients with COPD (68 in stable periods, 58 during exacerbation periods) and 50 healthy subjects were included in the study. PFT, body mass index (BMI), white blood cell counts, C-reactive protein (CRP), serum urea and creatinine levels were evaluated in both groups of patients. CysC levels were measured in all participants.
Results: Serum CysC levels were statistically higher in both COPD groups than the control group (p<0.001 for both) although there was no statistically significant difference between COPD groups (p>0.05). CysC levels showed negative correlation with forced expiratory volume in 1 second (FEV$_1$) and a positive correlation with C-reactive protein (CRP) levels in patients with stable COPD. There was a positive correlation between serum CysC levels and serum urea, creatinine, CRP levels in patients with COPD exacerbation (r=0.333, p=0.011; r=0.260, p=0.049; r=0.414, p<0.01 respectively). When stable COPD and control groups were evaluated, serum CysC had an area under the curve (AUC) in the receiver operating characteristic (ROC) curve of 0.951 (0.909–0.994 95% CI: p<0.001).

Kratak sadržaj

Uvod: Cilj ove studije bio je da se odredi nivo cistatina C (CysC) u serumu pacijenata sa hroničnom opstruktivnom bolešću pluća (HOBP) tokom perioda pogoršanja i u stabilnoj fazi bolesti i da se ispita njegova potencijalna dijagnostička vrednost kao i odnos između nivoa CysC i testa plućne funkcije (PFT).
Metode: Sto dvadeset šest pacijenata sa HOBP (68 u stabilnoj fazi, 58 tokom perioda pogoršanja) i 50 zdravih subjekata je uključeno u ovu studiju. PFT, indeks telesne mase (ITM), ukupan broj leukocita, C-reaktivni protein (CRP), nivoi uree i kreatinina u serumu određeni su u obe grupe pacijenata. Nivoi CysC izmereni su kod svih učesnika.
Rezultati: Serumski nivoi CysC bili su statistički viši u obe grupe sa HOBP nego u kontrolnoj grupi (p<0,001 za oba) iako nije bilo statistički značajne razlike između grupa sa HOBP (p>0,05). Nivoi CysC bili su u negativnoj korelaciji sa FEV$_1$ i u pozitivnoj korelaciji sa nivoima C-reaktivnog proteina (CRP) kod pacijenata sa stabilnom HOBP. Nađena je pozitivna korelacija između serumskih nivoa CysC i uree, kreatinina, nivoa CRP u serumu kod pacijenata sa pogoršanjem HOBP (r=0,333, p=0,011; r=0,260, p=0,049; r=0,414, p<0,001). Prilikom poređenja grupe sa stabilnom HOBP i kontrolne grupe, serumski CysC imao je površinu ispod ROC krive od 0,951 (0,909–0,994 95% CI: p<0,001).
Zaključak: Naši rezultati pokazuju da su nivoi CysC bili povišeni u obe grupe sa HOBP. Povišeni nivoi CysC možda su povezani sa smanjenjem plućne funkcije i inflamacijom kod

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Conclusions: Our results showed that CysC levels increased in both COPD groups. Increased CysC levels may be related with lung function decline and inflammation in COPD patients. In addition, CysC levels may be a potential indicator for the diagnosis of COPD.

Keywords: COPD, cystatin C, pulmonary function

obolelih od HOBP. Pored toga, nivoi CysC mogu biti potencijalni pokazatelj za dijagnozu HOBP.

Ključne reči: HOBP, cistatin C, plućna funkcija

Introduction

There is a prominent inflammatory response and protease/antiprotease imbalance in COPD. Also, persistent low-grade systemic inflammation, such as increased blood leukocytes, CRP, and inflammatory cytokines, are shown in COPD patients. COPD exacerbations are associated with both airway and systemic inflammation and increased inflammatory markers are most likely associated with lung function decline (1). The protease/antiprotease hypothesis suggests that the pathogenesis of COPD and emphysema is due to an imbalance between enzymes that degrade the extracellular matrix within the lung and proteins that protect against this proteolytic activity (2).

Cystatin C (CysC) is the most important inhibitor of endogenous cysteine proteases. CysC forms complexes with cathepsins and regulates protease secretion or leakage from the lysosomes of dying or diseased cells (3). Increased CysC level was found in patients with clinical or subclinical emphysema, inflammatory lung disease and chronic kidney disease (4–7). However, association between CysC levels and lung function and its potential diagnostic value in COPD are not fully understood.

We aimed to assess CysC levels in patients with acute exacerbation of COPD and compare them with the stable phase of COPD patients. Also, we aimed to determine the relation between CysC and several parameters (PFT, CRP, serum urea and creatinine) in COPD patients.

Materials and Methods

Study population

Ethical approval was obtained by the institutional review board (14062016-11/15). One hundred twenty-six patients with COPD (68 stable patients who were admitted to our outpatient clinics for control and 58 COPD patients during an exacerbation who were hospitalized in our clinic) were included in the case control study. Fifty age and sex matched healthy individuals who were admitted to our outpatient clinics and who had no respiratory disease were enrolled as a control group. COPD was diagnosed according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) guidelines and an exacerbation of COPD was defined as sustained (48 hours) worsening of dyspnea, cough, or sputum production leading to an increase in the use of mainte-

nance medications and/or supplementation with additional medications (8, 9). All COPD patients were current or ex-smokers. Chronic kidney disease, coronary artery disease, cancer, diabetes mellitus, acute inflammatory disease and thyroid dysfunction were considered as exclusion criteria (10). The body mass index (BMI) and pulmonary function tests (PFTs) were detected and leucocyte count, CRP, serum urea and creatinine analysis were measured in COPD patients. In addition, COPD patients were staged according to the »ABCD« assessment tool (11). Serum CysC levels were measured in all subjects. PFT was performed using a spirometry device (Ultima CPX 790705-205, St. Paul, MN, USA). Spirometric examination was conducted according to the European Respiratory Society (ERS) criteria. FEV_1 and forced vital capacity (FVC) are expressed as percentages of predicted values according to the prediction equations of the ERS (12).

Laboratory measurements

Complete blood cell counts were measured by an automated blood counter (ADVIA 2120i, Siemens Healthcare Diagnostics Inc., Tarrytown, NY, USA) CRP levels were determined by immunonephelometry (BNII Nephelometer System, Siemens Healthcare Diagnostics Products, Marburg, Germany). The urea and creatinine levels were measured with an automated analyzer (Advia 2400 Chemistry System; Siemens Healthcare Diagnostics, Inc.). Serum was separated by centrifugation at 3000 g, and stored at −80 °C within 1 hour of collection until analysis. Serum CysC was measured using the N latex Cystatin C test kit (ref OQNM17; Siemens, Marburg, Germany) in a nephelometer (BNII Dade Behring, Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany).

Statistical analysis

IBM SPSS Statistics 21 (Statistical Product and Service Solutions 21.0 version, authorization code: d91314f638c364094170; Armonk, NY, USA) Package was used for the statistical analysis. The results were given as mean ± SD. A p value <0.05 was considered statistically significant. One-Way Anova test was used for the comparison of multiple groups. Student's t-test was used for the comparison of two independent groups. X^2 test was carried out to compare gender distribution. Correlations between the variables were established by the Pearson's correlation.

Results

Demographic data and laboratory results of all subjects and parameters of PFTs of COPD patients are shown in *Table I*. No statistically significant difference was found among the three groups in terms of age ($p > 0.05$) and gender ($p > 0.05$, $X^2 = 2.371$).

The mean serum CysC values in both COPD groups were significantly higher than control group ($p < 0.001$, for both COPD group). There was no statistically significant difference in CysC values between COPD groups ($p > 0.05$) (*Figure 1*). Also, no statistically significant difference was observed in CysC values between patients with emphysema and chronic bronchitis in both COPD groups. In addition, current smoker COPD patients had increased levels of CysC when compared to ex-smoker COPD patients in the stable group (1.91 ± 3.07, 0.88 ± 0.28, $p < 0.05$) but there was no statistically significant difference in CysC values between stable COPD patients according to the »ABCD« assessment tool (26 (38.2%) of them »B« and 42 (61.8%) of them »D«; the mean CysC 1.05 ± 0.56, 1.33 ± 2.30, respectively, $p > 0.05$).

The mean CRP, leucocytes levels were significantly higher in the exacerbation group than in stable COPD ($p < 0.001$ for both) (*Table I*). The mean FEV_1 levels were significantly lower in the exacerbation group than stable COPD ($p < 0.05$).

Significant negative correlation was found between FEV_1 and CysC levels and positive correlation between CysC and smoking (pack/year) and CRP levels in the stable COPD group ($r = -0.421$,
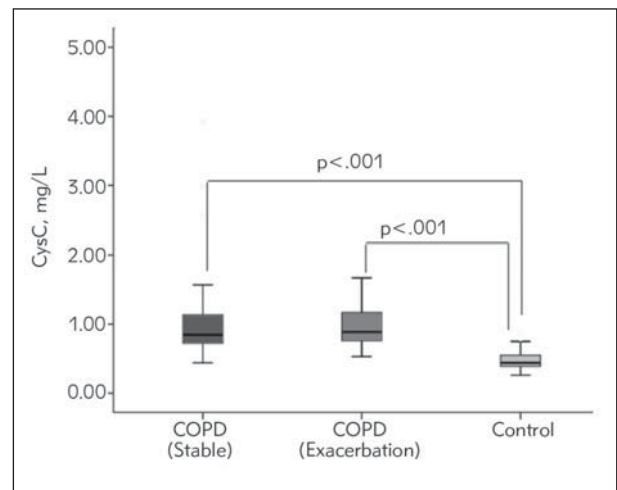

Figure 1 Serum CysC levels in the study population.

Table I Demographic data, functional parameters, and laboratory results of all subjects.

Parameters	COPD (Stable) (n= 68)	COPD (Exacerbation) (n= 58)	Control (n= 50)
Age (years)	64.19 ± 10.6	65.12 ± 6.93	60.12 ± 11.77
Gender (male/female)	60/8	48/10	41/9
Current/ex/never smoker	23/45/–	13/39/–	12/15/23
Smoking (pack/years)	20.35 ± 10.7^d	19.06 ± 10^e	13.63 ± 7.02
FEV_1 (%)	45.34 ± 16.11^c	39.67 ± 15.07	NA
FVC (%)	$69.81 \pm 19.46b$	52.02 ± 13.43	NA
FEV_1/FVC (%)	56.49 ± 10.78	54.29 ± 9.98	NA
CysC (mg/L)	1.01 ± 0.56^a	0.97 ± 0.29^a	0.47 ± 0.13
Leucocyte ($\times 10^3/\mu L$)	7174 ± 2508^b	10753 ± 4985	NA
CRP (mg/L)	14.70 ± 18.89^b	43.09 ± 51.32	NA
BMI (kg/m^2)	25.67 ± 4.82	25.86 ± 7.0	NA
Urea (mmol/L)	14.44 ± 7.06	16.33 ± 7.01	NA
Creatinine (mmol/L)	0.07 ± 0.02	0.07 ± 0.02	NA

CRP; C-reactive protein, FEV_1; forced expiratory volume in 1 second, FVC; forced vital capacity, BMI; body mass index, N/A = not available, CysC; cystatin C

[a] $p < 0.001$, compared with healthy controls, [b] $p < 0.001$, [c] $p < 0.05$, compared with COPD patients with exacerbation, [d] $p < 0.01$, compared with healthy controls, [e] $p < 0.05$, compared with healthy controls
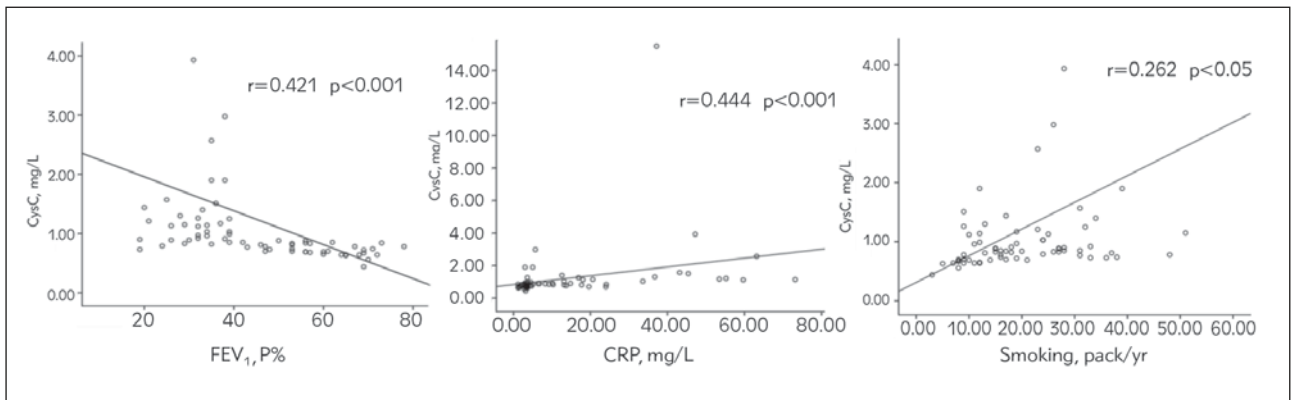
Figure 2 Correlations between CysC and forced expiratory volume in 1 s (FEV₁) and C-reactive protein (CRP) and smoking in stable COPD patients.

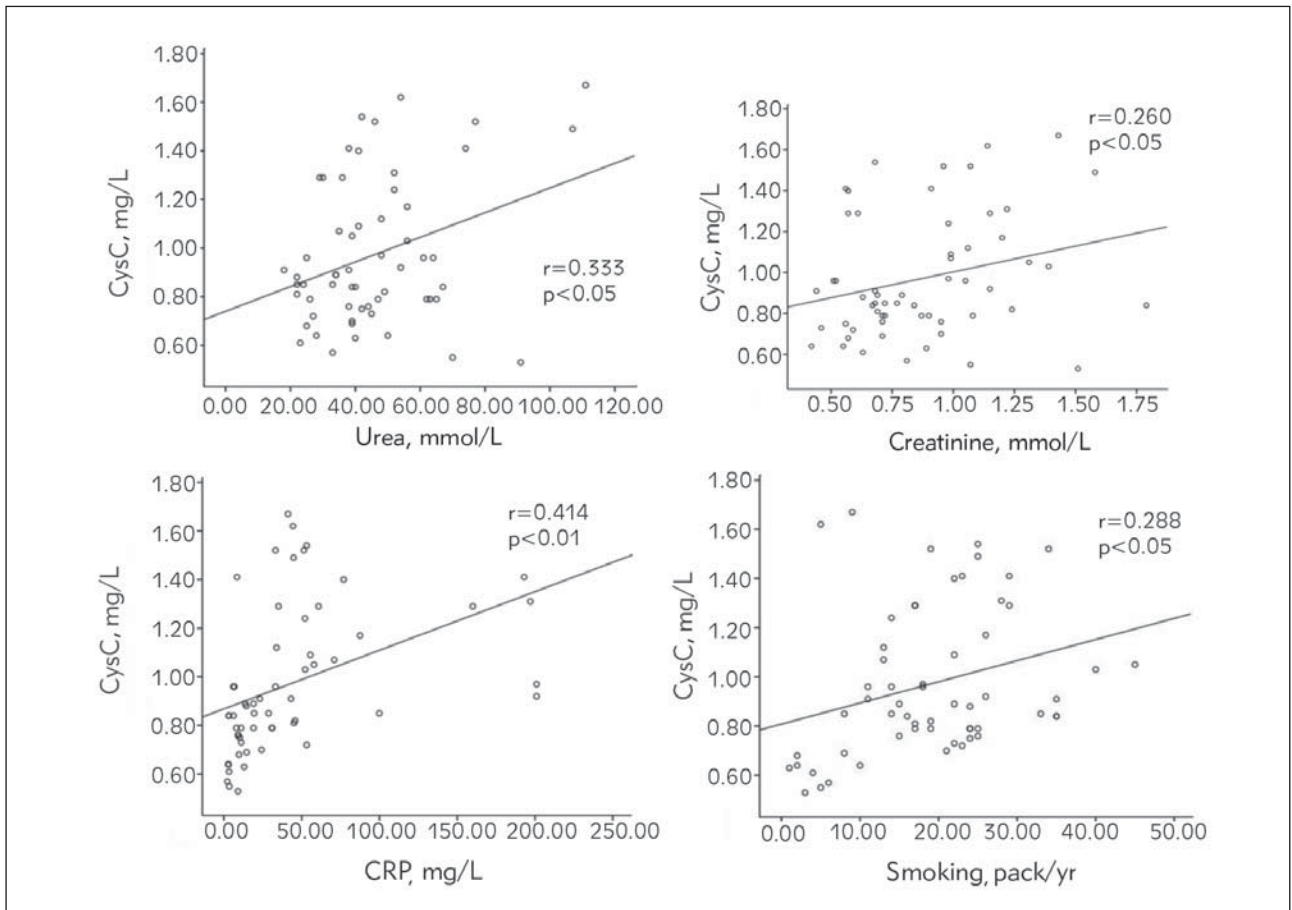**Figure 3** Correlations between CysC and urea, creatinine, CRP and smoking levels in exacerbation group.

p<0.001, r=0.444, p<0.001, r=0.262, p<0.05 respectively) (*Figure 2*). Also, a significant positive correlation was observed between CysC levels and urea, creatinine, smoking (pack/year) and CRP levels in the exacerbation group (r=0.333, p<0.05; r=0.049, p<0.05; r=0.288, p<0.05; r=0.414, p<0.01, respectively) (*Figure 3*).

When stable COPD patients and control group were evaluated, CysC had an area under the curve (AUC) in the receiver operating characteristic (ROC) curve of 0.951 (0.909–0.994 95% CI: p<0.001). A CysC level of 0.69 mg/L was accepted as the *cut-off* value between stable COPD patients and control group. CysC had a sensitivity of 91% and specificity of 80% (ROC curve) (*Figure 4 A, B*).
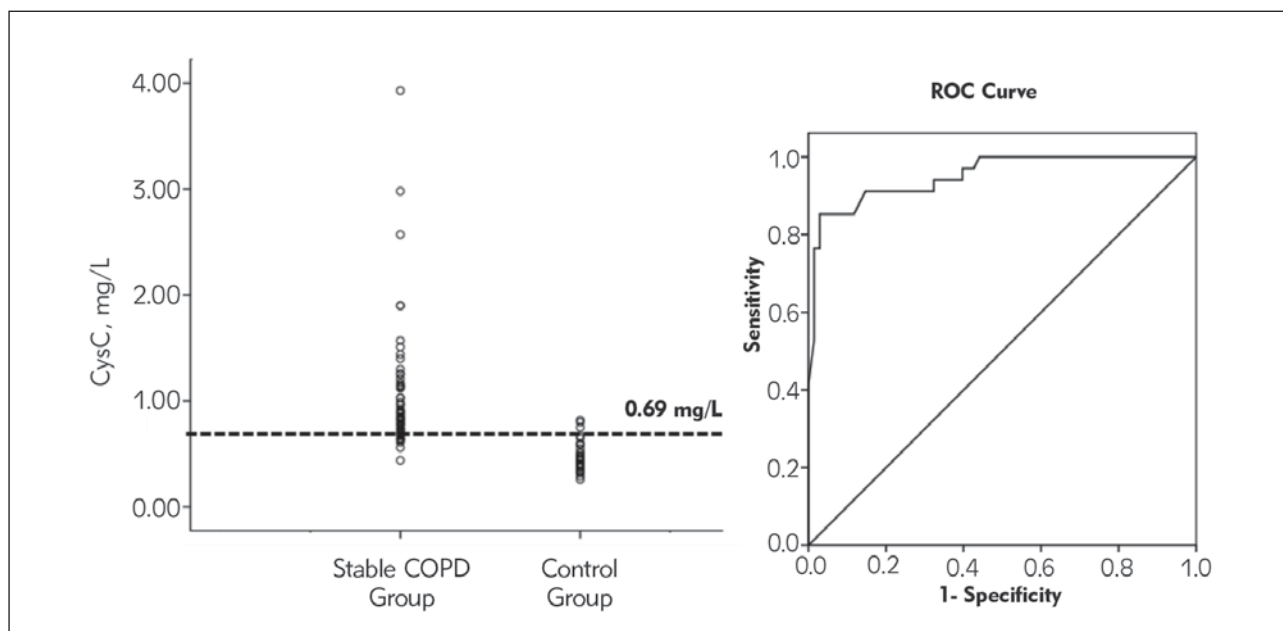
Figure 4 A; A cut-off level of CysC in stable COPD patients and control group. B; Receiver operating characteristic (ROC) curve indicating sensitivity and specificity of CysC to diagnose COPD.

Discussion

The main finding of our study was, CysC levels were significantly higher in both COPD groups than controls. There was a negative correlation between FEV_1 and CysC levels in the stable COPD group and a positive correlation between CysC and CRP levels in both COPD groups. Also, CysC had a sensitivity of 91% and specificity of 80% in the stable COPD group.

COPD is an inflammatory disorder which involves accelerated lung function loss. Persistent inflammation in COPD may lead to the recruitment of inflammatory cells and increased protease activity in the lungs. Chronic cigarette smoking may decrease CysC levels, which is a cathepsin L inhibitor in the lungs, and this may cause emphysematous changes (13). Contrastly, increased levels of CysC in BAL and serum were determined in patients with emphysema especially in the smokers and inflammatory lung disease (6, 14, 15). Also, it has been shown that the level of CysC in the culture medium of alveolar macrophages from smokers was higher than in non-smokers (16). Another study showed a significant association between emphysema and serum CysC in a large representative US population. The emphysema group had significantly higher mean CysC levels compared to the normal controls. Authors concluded that any active or second-hand smoke exposure might contribute to increased serum CysC levels (5). In a previous study, a significant relation was found between inflammatory parameters such as interleukin-6, resistin, tumor necrosis factor, and CRP with CysC and it was thought that the elevation of CysC was secondary to the inflammatory processes in the lung (5, 17). Moreover, serum CysC has been found as a positive acute-phase reactant in COPD patients and might indicate systemic inflammation during the progression of COPD (18). In accordance with these studies, we also determined increased levels of CysC in both COPD groups compared with healthy controls and the level of CysC was positively correlated with smoking and CRP levels in stable COPD patients. These data suggest that elevated CysC levels may be associated with pulmonary inflammation caused by smoking.

It was shown that a significant relation between serum CysC level and lung functional parameters in stable COPD patients and a high CysC concentration might be a potential indicator of impaired lung function and measuring routinely CysC levels might improve the diagnosis and assessment of COPD severity (10, 19). Our results showed a significantly negative correlation between CysC levels and FEV_1 in stable COPD patients but there was no significant difference in the CysC levels when we staged COPD patients according to the »ABCD« assessment tool. So, we think that serum CysC may be used only to determine and follow up the severity of airflow limitation in these patients.

The diagnosis of chronic kidney disease in COPD patients may be useful for the prognosis of COPD patients. Recently, a meta-analysis result showed that there was a statistically significant relation between chronic renal failure and short-term mortality in hospitalized adults with acute exacerbation of COPD (20). Also, the relation between CysC levels and the comorbidities of renal dysfunction in patients with

COPD exacerbation was determined and CysC has been found an independent risk factor for hospital mortality in COPD exacerbation (21). CysC may reflect renal function in COPD patients (22–24). Our results showed that CysC levels were positively correlated with urea, creatinine and CRP levels in patients with COPD exacerbation. The mean creatinine concentration was within a normal range and below 2 mg/dL in our patients with COPD exacerbation. We think that CysC may be a good measurement of renal function in COPD exacerbation. Macrophages which are major inflammatory cells that produce cystatin C play an important role in the chronic inflammatory process in stable COPD, while neutrophils are majorly involved in acute inflammatory responses in patients with acute exacerbation (13, 25, 26). This may explain why the serum level of CysC was not significantly different in both COPD group.

Proteomic analyses for determining disease markers in various samples from COPD patients have been done in some studies. Serum levels of CysC may be a potential marker to determine the disease severity but to our knowledge, there is no data about the potential diagnostic value of CysC in COPD patients (10, 19). Our results determined that serum levels of CysC showed high sensitivity and specificity for the diagnosis of COPD. Because of the high sensitivity and specifity, CysC might be suitable as a marker for diagnosis of the disease.

Our study has several limitations. Primarily, the sample size in this study is relatively small. Second, we excluded COPD patients with comorbidities that also affect inflammatory responses. Third, CysC levels were only measured in sera. Also, systemic treatment used in the exacerbation period may affect CysC levels in these patients.

In conclusion, the level of CysC was increased in COPD patients. Increased CysC levels may be related with inflammation and the severity of airflow obstruction but not disease severity in stable COPD patients. In addition, cystatin C levels may be a potential indicator for the diagnosis of COPD.

Author contributions

All authors contributed to the writing of the manuscript and approved the final version of the manuscript for submission.

Idea and study design and writing of the manuscript: S.T, follow up of the patients and determination of COPD forms: F.D, collection of the blood samples: Ö.Ö, interpretation of data, editing of the manuscript: G.K, statistical analyses: M.T, biochemical analysis: D.K.

Conflict of interest statement

The authors stated that they have no conflicts of interest regarding the publication of this article.

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