ORIGINAL ARTICLE

Relationship between serum γ-glutamyltransferase levels and acute exacerbation of chronic obstructive pulmonary disease

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KEY WORDS

γ-glutamyltransferase, chronic obstructive pulmonary disease, exacerbation, oxidative stress

ABSTRACT

INTRODUCTION γ-glutamyltransferase (GGT) is a plasma membrane enzyme, which is involved in antioxidant glutathione resynthesis.

OBJECTIVES The aim of the study was to compare the serum levels of GGT (which is considered a novel marker of oxidative stress) between patients with stable chronic obstructive pulmonary disease (COPD) and those with acute exacerbation of COPD, and the relationship of GGT with inflammation.

PATIENTS AND METHODS The study involved 132 patients with exacerbated COPD and normal function of the liver and biliary tract (mean age, 66.6 ±10.1 years; men, 88.6%) and 147 patients with stable COPD (mean age, 65.4 ±8.8 years; men, 87.1%). Serum GGT and C-reactive protein (CRP) levels were measured and compared between the groups.

RESULTS Serum GGT levels in patients with exacerbated COPD were significantly higher than in those with stable COPD (30 U/l; interquartile range [IQR], 18.8 vs. 25 U/l; IQR, 16; $P < 0.001$]. Serum CRP levels were significantly higher in patients with exacerbated COPD compared with those with stable COPD (34 mg/l; IQR, 58.3 vs. 16 mg/l; IQR, 24.6; $P < 0.001$). A significant positive correlation was observed between GGT activity and CRP levels ($r = 0.27$, $P = 0.002$). The GGT level of 29 U/l was set as a cutoff value of acute exacerbation with the specificity of 70.1% and sensitivity of 62.8% (95% confidence interval, 0.6–0.71; area under the curve, 0.66; standard error, 0.032; $P < 0.001$).

CONCLUSIONS Our study indicates that serum GGT levels as the marker of oxidative stress increase during exacerbated COPD and correlate with CRP levels. The measurement of GGT activity may be useful in the evaluation of exacerbated COPD.

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INTRODUCTION Chronic obstructive pulmonary disease (COPD) is a preventable and treatable disease associated with abnormal inflammatory response of the lungs to hazardous particles or gases, particularly those found in cigarette smoke. Patients with COPD frequently experience exacerbations that have a negative effect on the quality of life,[1,2] pulmonary function,[3,4] and prognosis.[5] Moreover, episodes of exacerbation generate substantial social and financial costs. Therefore, effective prevention is important both for the patients and healthcare providers. Exacerbations of COPD are associated with the increased levels of various inflammatory markers, including neutrophils and macrophages in the airways as well as cytokines (particularly interleukin [IL] 6 and IL-8).[6] Pathologically, COPD exacerbations appear as "inflammatory flare-ups", regardless of the causative factor (bacterial or viral infections, air pollution, etc).[7]

The presence of low-grade systemic inflammation with the elevated circulating levels of tumor necrosis factor-α (TNF-α), IL-6, IL-8, and C-reactive protein (CRP) has been shown to be correlated with restricted airflow in COPD, particularly in exacerbations.[8,9] Oxidative stress is

TABLE Characteristics of the study population

Characteristics	Stable COPD (n = 147)	Acute exacerbation (n = 132)	*P*
sex, male/female	140/7	117/15	NS
age, y	65.4 ±8.8	66.6 ±10.0	NS
BMI, kg/m^2	26.1 ±5.2	25.6 ±5.5	NS
total smoking time, pack-years	42.8 ±28.9	46.0 ±26.0	NS
FVC, ml	3137.9 ±903.5	2107.6 ±790.4	<0.001
FEV_1, ml	1755.2 ±612.3	1138.3 ±500.2	<0.001
FEV_1, %	61.7 ±18.4	44.6 ±16.1	<0.001
FEV_1/FVC	56.0 ±11.4	55.3 ±15.4	NS
PaO_2, mmHg	68.5 ±9.4	58.3 ±15.4	<0.001
$PaCO_2$, mmHg	35.1 ±4.6	42.2 ±13.0	<0.001
O_2 saturation, %	93.9 ±2.74	86.2 ±12.6	<0.001
hypertension, n (%)	28 (19)	23 (18.2)	NS
diabetes, n (%)	18 (12.2)	17 (12.9)	NS
heart failure, n (%)	14 (9.5)	12 (9)	NS
coronary artery disease, n (%)	25 (17)	24 (18.2)	NS
BUN, mg/dl	23.3 ±13.8	18.3 ±8.0	0.004
creatinine, mg/dl	0.93 ±0.22	1.12 ±0.95	NS
AST, U/l	23.9 ±6.8	24.3 ±7.9	NS
ALT, U/l	25.3 ±5.6	25.8±8.2	NS
total bilirubin, mg/dl	0.76 ±0.14	0.81±0.23	NS

Data are presented as mean ± standard deviation or number (percentage).

Conversion factors to SI units are as follows: for creatinine – 88.4, BUN – 0.357, total bilirubin – 17.1.

Abbreviations: ALT – alanine transaminase, AST – aspartate transaminase, BMI – body mass index, BUN – blood urea nitrogen, COPD – chronic obstructive pulmonary disease, FEV_1 – forced expiratory volume at 1 second, FVC – forced vital capacity, NS – non-significant, PaO_2 – partial pressure of oxygen in the arterial blood, $PaCO_2$ – partial pressure of carbon dioxide in the arterial blood

an important factor in the pathogenesis of COPD. Antioxidant molecules act as a defense against constant oxidative challenge. Pro-oxidant and antioxidant molecules are maintained at an appropriate level through a balance mechanism within the lung cells. Glutathione, originating from intracellular γ-glutamyltransferase (GGT), is the most important nonprotein sulphydryl that plays the key role in cellular antioxidant defense.[10] Although, the relationship between cellular GGT and serum GGT levels remains unknown, an increase in GGT activity can be a response to oxidative stress, marking an increased transport of glutathione into the cells.[11] GGT and CRP levels are elevated in several diseases characterized by chronic inflammation (such as diabetes, hypertension, and coronary artery disease). Moreover, serum GGT levels are increased in a number of diseases that are known to have oxidative stress in the pathogenesis. This may indicate that GGT levels can be evaluated as a response to oxidative stress and may be used as an inflammatory marker.[11-13]

The aim of the study was to compare serum GGT levels between patients with stable COPD and those with acute exacerbation of COPD, and its relationship with inflammatory activity.

PATIENTS AND METHODS

Study population

The study involved 279 patients aged 30 years and older with the diagnosis of COPD according to the criteria established by the Global initiative for Chronic Obstructive Lung Disease.[14] All patients were current or previous cigarette smokers with a smoking history of 10 pack-years or longer. Patients with a history of chronic alcohol consumption, hepatobiliary disorders, or any other acute diseases were excluded from the study.

The study protocol was approved by the Inonu University Ethical Committee, and written informed consent was obtained from each patient.

The study population consisted of 147 patients with stable COPD (without exacerbation or pneumonia within the previous 1 and 2 months, respectively) and 132 patients with acute exacerbation. Exacerbation was defined as an acute worsening of the patient's condition from the stable state to beyond normal day-to-day variations, presenting with any of the following: worsened dyspnea, increased sputum volume, and change in sputum color, and requiring a modification in regular medication.

Study design

Epidemiological (age, sex) and clinical (comorbidities, smoking status, COPD status, forced expiratory volume in 1 second [FEV_1], chronic oxygen therapy, previous steroid and antibiotic therapy) data were obtained and blood samples were collected from each patient for tests including serum GGT and CRP levels and arterial blood gases at the time of hospital admission and inclusion to the study. The body mass index (BMI) was calculated for both groups.

Biochemical measurements

Blood samples were drawn after 12-hour fasting. Glucose, creatinine, and arterial blood gases were determined by standard laboratory methods. The activity of GGT was measured by the Abbott-Architect autoanalyzer (Abbott, United States) using the original kits. CRP levels were measured using Bio B (Roche Diagnostics, Mannheim, Germany) by means of turbidometry. The reference range for CRP was 0–7 mg /l.

Statistical analysis

The statistical analysis was performed using SPSS v. 16.0 (SPSS Inc. Chicago, Illinois, United States) and the baseline characteristics of participants were presented as the mean ± standard deviation and number (%). Data were compared using the independent-sample *t* test for continuous data, the χ^2 test for categorical variables, and the Mann-Whitney *U* test for nonparametric data with an irregular distribution. Correlations between GGT activity and the other

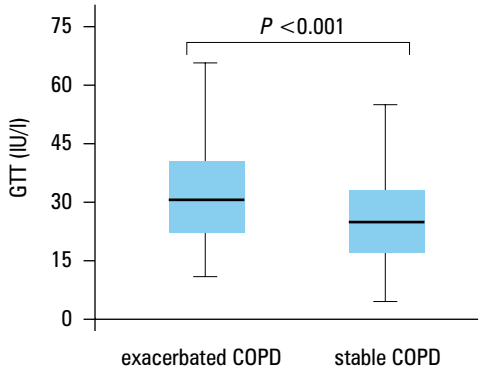

FIGURE 1 Comparison of serum γ-glutamyltransferase (GGT) levels between stable and exacerbated chronic obstructive pulmonary disease (COPD)

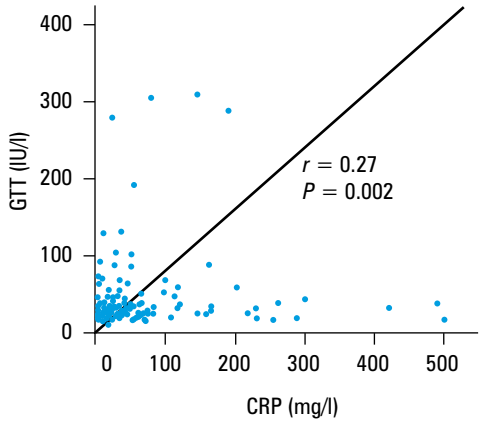

FIGURE 2 Correlation between serum γ-glutamyltransferase (GGT) and C-reactive protein (CRP)

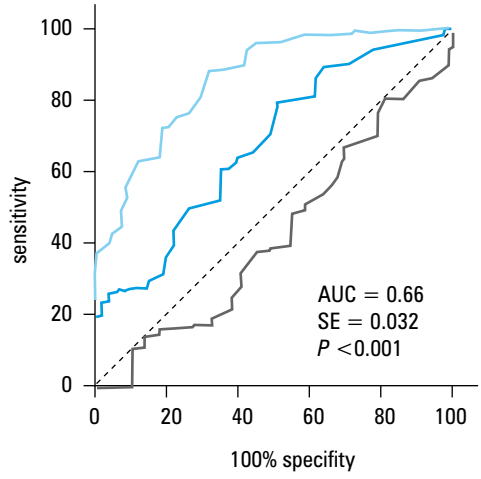

FIGURE 3 Receiver operating characteristic curves for γ-glutamyltransferase values to determine acute exacerbation of chronic obstructive pulmonary disease
Abbreviations: AUC – area under the curve, SE – standard error

variables were evaluated by the Pearson and Spearman rank correlation tests where appropriate. The receiver operating characteristic (ROC) curves were generated for GGT values to determine acute exacerbation. A *P* value of less than 0.05 was considered statistically significant.

RESULTS A total of 279 patients were included in the study and classified into the group with stable COPD (n = 147) and the group with exacerbated COPD (n = 132), as shown in TABLE 1. There was no significant difference between the groups in terms of age, sex, comorbidities (diabetes, coronary arterial diseases, heart failure), BMI, serum creatinine, FEV_1/forced expiratory volume (FEV) ratio, and the total duration of smoking.

FEV_1 was measured at baseline in all patients. Clinical examination and spirometry confirmed COPD in all patients. All spirometric and blood gas parameters, except the FEV_1 / FVC ratio, were worse in the group with exacerbated COPD (TABLE 1).

When compared between the groups, serum CRP levels were significantly increased in patients with exacerbated COPD (34 mg/dl; interquartile range [IQR], 58.3 vs. 16 mg/dl, IQR, 24.6; *P* <0.001]. Interestingly, patients with exacerbation had higher serum GGT activity than those with stable COPD (30 U/l; IQR, 18.8 vs. 25 U/l; IQR, 16; *P* <0.001) (FIGURE 1).

There were no correlations between GGT levels and FVC, FEV_1, FEV_1/FVC, PaO_2, PCO_2, O_2 saturation, and total duration of smoking. A significant positive correlation was found only between the GGT activity and CRP measurements ($r = 0.27$, $P = 0.002$) (FIGURE 2).

The cutoff value for serum GGT for the prediction of acute exacerbation was 29 U/l with the specificity of 70.1% and sensitivity of 62.8% (95% confidence interval, 0.6–0.71; area under the curve, 0.66; standard error, 0.032; *P* <0.001) (FIGURE 3).

DISCUSSION To our knowledge, this is the first study to report that serum GGT activity and CRP levels are positively correlated with one another and significantly increased in patients with exacerbated COPD compared with those with stable COPD. Moreover, we showed that the GGT level of 29 U/l may be used as a biomarker to determine the exacerbation of COPD with a moderate sensitivity and specificity.

In addition to chronic local inflammation, which causes structural alterations in the small airways and alveoli in COPD, the presence of systemic inflammation with low severity has also been confirmed although the exact mechanism remains unknown. In particular, there have been studies showing the correlation between restricted airflow and systemically elevated levels of cytokines such as TNF-α, IL-6, IL-8, and CRP.[8,9] In systemic inflammation, the circulating oxidants increase while the antioxidant capacity decreases. Antioxidants are found in the systemic circulation (glutathione, ascorbate) and the epithelial lining fluid (glutathione, mucin, ascorbic acid, ceruloplasmin). The production of glutathione by the bronchial epithelium cells has been shown particularly in smokers who are known to have increased oxidative stress.[15] The body receives support from the xenobiotic metabolizing enzymes to protect against oxidative stress. Glutathione S-transferase is one of those enzymes.

Free radicals play a critical role in inflammation and intensify inflammatory reactions at specific sites. Some mediators can activate intra- and extracellular pathways to elevate free radicals in multiple oxidative processes.[16] In clinical practice, GGT, the enzyme responsible for the extracellular catabolism of glutathione with an important role in

antioxidant defense systems, is a commonly used diagnostic test for liver diseases. GGT is considered a biomarker of oxidative stress due to its role in the degradation of antioxidant glutathione.[17] It has recently been reported that the upregulation of GGT in inflammation increases antioxidant defense probably due to leukotriene-induced inflammation.[18] Daubeuf et al.[19] reported that the expression of GGT mRNA is induced by 3 cytokines, interferon (IFN)-α, IFN-β, and TNF-α.

Consistent with the previous studies,[20-22] CRP levels were significantly elevated during exacerbation and have also been shown to be associated with the declining lung function and worsening of COPD.[23] Epidemiological studies have shown that reduced lung function is associated with a decrease in antioxidants. This is consistent with oxidative stress playing a direct or indirect role in the pathophysiology of COPD.[18] Serum CRP shows positive correlation with GGT,[24] indicating that there may be an underlying relationship between general inflammation and oxidative stress in exacerbated COPD.

A strong clinical relationship between CRP and GGT was initially described by Lee et al.[12] In their study, increased GGT as a marker of oxidative stress was found to be correlated with CRP in a large healthy population. Later, a number of other studies questioned the relationship between GGT and CRP, and reported a positive correlation[12,24]; however, so far, no study has been designed to measure serum GGT and CRP levels in patients with exacerbated COPD. Several pathological conditions can affect oxidative stress. Since there were no baseline differences in, for example, comorbidities or hepatic function between patients with stable and exacerbated COPD in our study, increased GGT levels in the exacerbated COPD group were thought to be the result of COPD exacerbation itself. Moreover, both GGT and CRP levels are increased and positively correlated with one another in exacerbated COPD, so it can be concluded that both inflammation and increased oxidative stress contribute to or are results of exacerbation.

The most important limitations of our study are the small sample size and cross-sectional design, and thus our results cannot be generalized. Another limitation is the lack of comparison of lung function between the groups at baseline (when both stable), because most patients with exacerbated COPD were referred to our clinic from different hospitals. Therefore, the first contact with these patients was during the exacerbation period and we did not have any data on prior spirometric tests. Moreover, the study was conducted in patients with multiple comorbidities that could affect GGT. However, the distribution of comorbidities was similar between the groups. Yet another limitation is the lack of other markers of oxidative stress such as malondialdehyde, superoxide dismutase, and glutathione.

In conclusion, GGT may be the key marker of diseases associated with inflammation (and oxidative stress) such as the systemic pathophysiological process known as COPD. To our knowledge, the most important biomarker that increases in acute exacerbation of COPD is CRP,[20] and a significant correlation between GGT and CRP observed in patients with acute exacerbation in this study supports the use of GGT as a useful parameter to evaluate exacerbated COPD.

Further studies of systemic and local GGT functions and their relationship with short- and long-term clinical results may lead to a better understanding of the processes involved in COPD and help develop new treatment strategies.

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ARTYKUŁ ORYGINALNY

Związek między poziomem γ-glutamylotransferazy w surowicy i zaostrzeniem przewlekłej obturacyjnej choroby płuc

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SŁOWA KLUCZOWE

γ-glutamylotransferaza, przewlekła obturacyjna choroba płuc, stres oksydacyjny, zaostrzenie

STRESZCZENIE

WPROWADZENIE γ-glutamylotransferaza (GGT) jest enzymem błonowym i cytoplazmatycznym biorącym udział w odnowie zasobów glutationu mającego własności przeciwutleniające.

CELE Celem badania było porównanie poziomu GGT (uważanej za nowy wskaźnik stresu oksydacyjnego) w surowicy pacjentów ze stabilną przewlekłą obturacyjną chorobą płuc (POChP) i pacjentów z zaostrzeniem POChP, a także określenie jego związku z procesem zapalnym.

PACJENCI I METODY Do badania zakwalifikowano 132 pacjentów z zaostrzeniem POChP z normalną funkcją wątroby i dróg żółciowych (średni wiek: 66,6 ±10,1 roku; mężczyźni: 88,6%) oraz 147 pacjentów z POChP w stabilnym okresie choroby (średni wiek: 65,4 ±8,8 roku; mężczyźni: 87,1%). Dokonano pomiaru poziomu GGT i białka C-reaktywnego (CRP) w surowicy oraz porównano wyniki w obu grupach pacjentów.

WYNIKI Poziom GGT w surowicy pacjentów z zaostrzeniem POChP był istotnie wyższy niż u tych ze stabilną chorobą (30 j./l; przedział międzykwartylowy [*interquartile range* – IQR]: 18,8 *vs* 25 j./l; IQR: 16; p <0,001). Poziom CRP w surowicy był istotnie wyższy u pacjentów z zaostrzeniem POChP (34 mg/l; IQR: 58,3 *vs* 16 mg/l; IQR: 24,6; p <0,001). Obserwowano istotną dodatnią korelację między aktywnością GGT i poziomem CRP (r = 0,27, p = 0,002). Poziom GGT 29 j./l wyznaczono jako punkt odcięcia dla zaostrzenia POChP ze swoistością 70,1% oraz wrażliwością 62,8% (95% CI: 0,6–0,71; pole pod krzywą: 0,66; błąd standardowy: 0,032; p <0,001).

WNIOSKI Nasze badanie wskazuje, że poziom GGT w surowicy jako wskaźnika stresu oksydacyjnego wzrasta podczas zaostrzenia POChP i koreluje ze stężeniem CRP. Pomiar aktywności GGT może być przydatny w ocenie przebiegu zaostrzenia POChP.

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