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Oxidant and antioxidant balance in stable COPD patients and during acute COPD exacerbations

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Abstract

The oxidant-antioxidant imbalance is an important factor in the pathogenesis of COPD and yet there is a lack of evidence about total antioxidant levels of COPD patients, which may provide valuable information about the prognosis of the disease. In this study, we aimed to evaluate and compare the oxidant-antioxidant systems in healthy controls and in COPD patients with a stable disease and during an acute exacerbation. The study was conducted with 42 COPD patients diagnosed by a pulmonologist and 21 healthy volunteers. Pulmonary function tests were performed, arterial blood gas; white blood cell, hemoglobin, hematocrit, platelet, neutrophil%, MCV, CRP, and sedimentation rate results were compared with total antioxidant status, total oxidant status levels, and oxidative stress index. There was a statistically significant difference between the three groups in FEV_1/FVC, FEV_1, FVC, pCO_2, pO_2, % SpO_2, white blood cell count, neutrophil %, CRP, and ESR values. In regard to oxidant-antioxidant system measurements there was no statistically significant difference in TAS levels, but there was a significant difference in TOS and oxidative stress index levels. Positive correlation between WBC and TOS was detected in the analysis performed and no correlation was found in other parameters. Our study showed that the measurement of TAS, TOS and OSI were meaningful parameters in the evaluation of oxidative imbalance. Oxidant stress was found to be significantly increased in all COPD patients, especially in patients with COPD exacerbations. Despite the increase in oxidative stress, no increase in antioxidant levels was observed in COPD patients.

Keywords: Antioxidant, COPD, exacerbation, oxidant

Introduction

Chronic Obstructive Pulmonary Disease (COPD) is a common, preventable and treatable disease that is characterized by persistent respiratory symptoms and airflow limitation that is due to airway and/or alveolar abnormalities usually caused by significant exposure to noxious particles or gases [1]. Oxidant-antioxidant imbalance is an important mechanism in the pathogenesis of COPD [2, 3]. There are multiple mechanisms in COPD that can cause oxidative stress. Inflammation, infections, hypoxia, smoking, lack of antioxidant response are the primary examples. [4, 5]. In the organism the rate of formation of free radicals and their detoxification rate are in an equilibrium, which is called oxidative equilibrium. This balance protects the cell from the negative effects of free radicals. If the balance is disrupted in favor of free radicals, the amount of free radicals in the cell increases. Adverse effects of free radicals on cell function and cell function is defined as 'oxidative stress' [6]. The lung is the organ most affected by oxidants. Oxidants damage the cell's genetic structure and ciliary function by damaging the structure of the extracellular matrix, biological membranes and DNA. They also affect enzymatic events, reduce surfactant activity, increase mucus production, and reduce the effectiveness of cytokines and proteases. [7, 8]. Interactions between oxidants, proteinases, inflammatory cells/mediators and triggering risk factors, insufficiency of protective mechanisms, antiproteinases and antioxidants due to multiple risk factors play a role in the development of COPD [9, 10]. Because several oxidants and antioxidants are likely to be involved in the pathogenesis of the inflammatory response in patients with COPD, we investigated total antioxidant levels (TAS), total oxidant levels (TOS) and oxidative stress index (OSI) to determine how oxidative and antioxidative status is affected by COPD. TAS, TOS and OSI show no significant differences in healthy adults. This suggests that these parameters may be used as a new biomarker for the assessment of oxidant stress in COPD. These parameters can be used as a viable test to detect and control oxidant-antioxidant imbalance earlier in COPD patients. In this study, we aimed to

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evaluate and compare oxidant-antioxidant systems in stable COPD, exacerbation periods in relation to healthy controls.

Materials and Methods

This was a single center cross-sectional prospective study carried out at The Bolu Abant Izzet Baysal University, Training and Research Hospital, Chest Diseases Service, data were collected between 07.2017-04.2018. Patients who decided to participate in the study signed an informed consent form, after getting the necessary information from the pulmonologist. 42 COPD patients who came to our clinic and were previously diagnosed by pulmonologists were included in the study, 21 of those were in a period of exacerbation (acute increase in dyspnea, cough or sputum volume/purulence). All patients were diagnosed according to GOLD criteria of FEV1/FVC <%70 of what is expected. 21 healthy volunteers were relatives of patient's and hospital personnel. COPD patients included in the study were ex smokers who quit at least 10 years before the study. Exclusion criteria for COPD group: pulmonary and extra pulmonary systemic inflammatory or other chronic or systemic diseases; rheumatologic or systemic inflammatory diseases, diabetes mellitus, liver failure, chronic renal failure, decompensated heart failure, hypertension, pregnancy, prerenal azotemia, active malignancy, obesity (BMI≥30 kg/m2) and being under the age of 18.

Body mass index, smoking history, pulmonary function tests, arterial blood gas, blood hemogram and biochemistry results were recorded. Measurement of different oxidant/antioxidant molecules separately is time-consuming, labor-intensive and costly and require complicated techniques so in this study the measurement of TAS, TOS and OSI were preferred. Serums were separated and stored in -80ºC freezer until analysis for total TAS and TOS. The levels were measured with Rel Assay commercial kit (Rel Assay Diagnostics, Gaziantep, Turkey) using Architect C8000 otoanalyser (Abbott, Chicago, IL, USA) in accordance with the manufacturer's instructions. The data were compared by comparing the COPD exacerbation and stable groups with each other and with the healthy control group. We determined serum TAS level using an automated measurement method based on the bleaching of the characteristic color of a more stable 2,20-azino-bis(3-ethylbenzthiazoline 6-sulfonic acid) radical cation by antioxidants. The results were expressed in mmol Trolox equivalents/L. For TOS, oxidants present in the sample oxidize the o-dianisidine–ferrous ioncomplex to ferric ions. The oxidation reaction is enhanced by glycerol, which is abundant in the reaction medium. Ferric ions form a coloured complex with xylenol orange in the acidic medium. Colour intensity measured spectrophotometrically is related to the total amount of oxidant molecules present in the sample The assay is calibrated with hydrogen peroxide, and the results are expressed as micromolar hydrogen peroxide equivalents per litre (mmol H_2O_2 equiv./lt) When calculating the oxidative stress index (OSI), expressed as a percentage of the ratio of TOS levels to TAS levels, the mmol value of the TAS test unit was converted to µmol unit as in the TOS test. The results were expressed as "arbitrary unit" (AU) and calculated according to the following formula:

$$\text{OSİ} = \text{TOS (µmol H2O2 equiv./lt)} \div \text{TAS}\left(\text{mmol Trolox equiv.}_{\text{lt}}\right) \text{x}$$

In calculating the sample size G Power 3.1. program was used. In accordance with the data obtained from the previous studies, it was calculated that a sample of 63 people was needed to detect a difference in the medium effect size (d = 0.5) between the three groups with one-way ANOVA at 80% power at p = 0.05 [11]. In descriptive statistics with numerical variables: mean, standard deviations were measured, where assumptions were met; where the assumptions are not met, the median and smallest-maximum values were measured; categorical variables are given as numbers and percentages. The normality assumption required for the use of parametric tests was examined by Shapiro-Wilks test. In examining whether there is a difference between the groups; one-way analysis of variance was used when the assumptions were met, and Kruskal-Wallis test was used when the assumptions were not met. Regression analysis was used to determine whether there is a difference between the groups in order to make an adjustment to the variables that are thought to be affected by the age variable. Significance level was taken as p <0.05. Analyzes were performed using SPSS v.21.

Results

Because all the patients (COPD exacerbation and stable COPD) who admitted to our outpatient clinic and met the appropriate criteria for our study were male; the healthy control group also consisted of only male subjects. Demographic characteristics are given in Table 1.

Table 1. Demographic characteristics

	n	Mean age (year)	BMI (kg/m²)
COPD exacerbation	21	69.9±10.4	25.1±4.6
Stable COPD	21	61.4±9.8	26.1±5.2
Control	21	63.0±8.3	27.2±2.9
All participants	63	64.8±10.1	26.1±4.4
p		0.013	0.298

There was a statistically significant difference between FEV1 / FVC ratios between the three groups (p <0.000). However, no statistically significant difference was found between COPD exacerbation and stable COPD patients (p = 0.282 (Table 2).

Table 2. Spirometry results

	FEV1/ FVC	FEV1 (%, lt)	FVC (%, lt)
COPD exacerbation	51 (33-66)	38.1±15.3	58.8±17.3
Stable COPD	62 (37-68)	54.0±21.2	73.0±22.4
Control	82 (71-97)	100.7±13.5	100.9±16.4
p	<0.000	0.000	<0.000

There was a statistically significant difference in pCO2 (p = 0.002), pO2 (p <0.000) and saturation values (p <0.000) between the three groups (Table 3).

There was no statistically significant difference between hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV) and platelet levels (PLT) of the study groups. Among the three groups, white blood cell count (WBC) (p=0.020) and neutrophil % ratio (p<0.000) were statistically significant (Table 4).

Table 3. Blood gasses

	pH	pCO2 (mm/Hg)	pO2 (mm/Hg)	%SpO2
COPD exacerbation	7.43±0.04	38.8±5.1	48.1±7.1	89.8 (75.4-95.1)
Stable COPD	7.42±0.028	37.7±4.7	58.0±9.2	93.8 (78.4-97.7)
Control	7.44±0.031	34.0±3.1	70.8±8.9	96.0 (93.8-98.4)
p	0.260	0.002	<0.000	<0.000

Table 4. Total blood analysis

	HGB (gr/dL)	HCT (%)	WBC (K/uL)	Neutrophil %	MCV (fL)	PLT (K/uL)
COPD exacerbation	13.9±1.8	41.1±5.7	9.02 (4.02-28.6)	70.8±9.8	87.1±7.8	256.5±90.4
Stable COPD	14.2±1.6	42.7±4.5	8.03 (1.90-9.97)	60.7±8.5	86.3±8.7	231.1±48.5
Control	14.6±0.9	44.2±2.8	6.68 (4.86-10.5)	59.6±8.5	89.2±4.8	264.7±58.6
p	0.309	0.294	0.020	<0.000	0.431	0.281

There was a statistically significant difference between C-Reactive Protein (CRP) (p <0.000) and erythrocyte sedimentation rate (ESR) (p= 0.007) levels of the study groups (Table 5). When the COPD exacerbation and stable groups were compared, there was a statistically significant difference between CRP (p=0.026) levels and no statistically significant difference between ESR (p=0.091) levels.

Table 5. Biochemical parameters

	CRP (mg/ L)	ESR (mm/h)
COPD exacerbation	25. 0 (0.1-282.0)	31 (1-90)
Stable COPD	4.0 (0.1-25.8)	14 (2-42)
Control	1.1 (0.1-12.4)	10 (3-24)
p	<0.000	0.007

Among the parameters evaluated for the oxidant/antioxidant mechanisms, TAS levels (mmol/L) were 1.592 in COPD exacerbation group, 1.664 in stable COPD group, and 1.481 in the healthy control group. There was no statistically significant difference between three groups (p=0.144) (Table 6).

Table 6. Oxidative measurements

	TAS (mmol /L)	TOS (µmol/L)	OSI
COPD exacerbation	1.592±0.337	6.765±3.262	0.447±0.248
Stable COPD	1.664±0.219	5.00±1.251	0.306±0.073
Control	1.481±0.292	4.714±0.861	0.331±0.092
p	0.124	0.004	0.012

TOS (µmol/L), another parameter for the evaluation of oxidant-antioxidant mechanisms, was 6.765 in COPD exacerbation group, 5.00 in stable COPD group, and 4.714 in healthy control group. There was a statistically significant difference between three groups (p=0.004) (Table 6, Figure 1). There was a statistically significant difference between TOS (µmol/L) levels of exacerbation group and stable group (p=0.022).

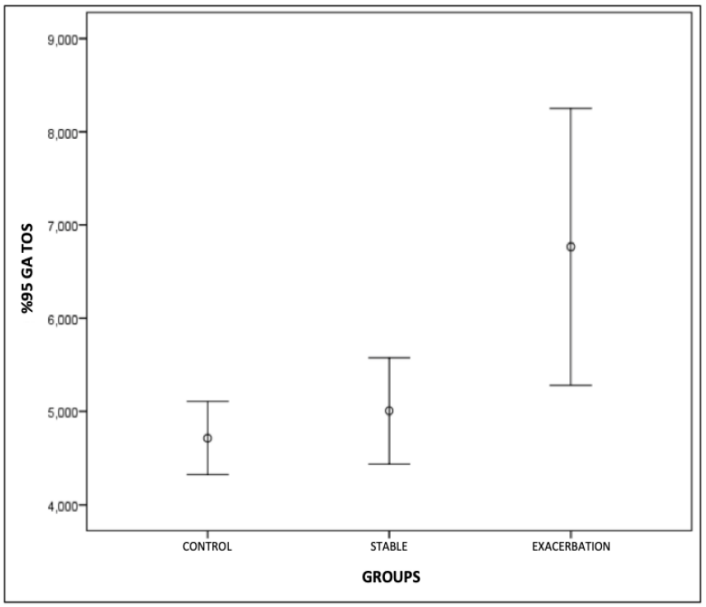

Figure 1. TOS levels

Results of oxidative stress index were 0.447 in COPD exacerbation group, 0.306 in stable COPD group and 0.333 in healthy control group. There was a statistically significant difference between three groups (p=0.012) (Table 6, Figure 2). When the oxidative stress index was evaluated between exacerbation group and stable group, a statistically significant difference was observed (p=0.015).

In our study, when compared with healthy controls, COPD patients showed statistically significant differences in CRP, ESR, WBC,% NEU, pO_2, pCO_2, SPO_2, and respiratory function tests. The correlation of these parameters with oxidative stress markers TAS, TOS and OSI was analyzed. A positive correlation was found between TOS and WBC (p=0.028). No correlation was found in the other parameters (p>0.05).

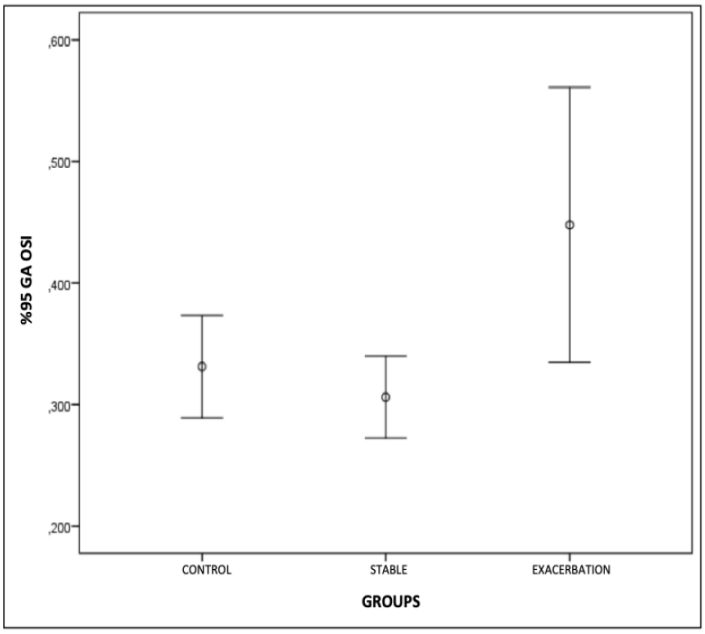

Figure 2. OSI levels

Discussion

In our study, it was found that TAS, TOS and OSI levels changed statistically significantly in COPD patients showing an oxidant-antioxidant imbalance. A significant increase in oxidant stress was found in all COPD patients, especially during exacerbations. Although there was an increase in oxidant stress, there was no increase in the antioxidant level in COPD groups. This may be the underlying mechanism the increased risk factor associated with many oxidative stress-related diseases such as pulmonary embolism, OSAS, ischemic heart disease, malignancies, cerebrovascular diseases, musculoskeletal disorders in COPD patients [12]. Measurement of total antioxidant status may provide more valuable information than individual antioxidant levels. Therefore, in determining the level of antioxidant mechanism of the body, total antioxidant capacity measurement (TAOC), which gives overall antioxidant value, is becoming more common than individual antioxidant levels. In our study, total oxidant/ antioxidant level measurement method developed by Erel was used which has been shown to be a practical and reliable method in many studies. Thus, with total antioxidant level to which total -SH, vitamin C, uric acid, vitamin E, bilirubin and many other antioxidants are contributing were measured accurately, as well as total oxidative stress caused by free radicals such as hydroxyl (OH-), hydrogen peroxide (H_2O_2), singlet oxygen (O_2-), lipid hydroperoxide (LOOH), and superoxide (O_2.) [13, 14].

Many studies of oxidative stress have shown that free oxygen radicals caused by defense mechanisms against exogenous toxic inhaled particles in smokers cause oxidative stress [15, 16]. In the case of smoking cessation, the decrease in lung function slowed and the cells of the endogenous mechanism causing oxidative stress in the lungs decreased six months after quitting [4]. For this reason, all volunteers who participated in our study had either never smoked or had a history of smoking but have not smoked in the last 10 years; thus our study was able to evaluate oxidative stress that is specific to COPD.

Studies have demonstrated a relationship between chronic inflammation and increased oxidative stress triggered by excessive fat deposition in obese subjects [17, 18]. Therefore, BMI ≥30 kg / m^2 is among the exclusion criteria of our study. In addition, there was no statistically significant difference between the 63 participants in terms of BMI. In this way, it is aimed to make the data more reliable in the evaluation of oxidative stress that is specific to COPD.

Inal et al. investigated the alteration of lipid peroxidation and antioxidant enzyme activities with aging and found that antioxidant enzyme activities in erythrocytes changed and peroxidative damage increased with age [19]. In our study, statistically significant difference was found in the mean age of COPD group compared to the control group (p= 0.013). Regression analysis was performed separately for all the data evaluated within the scope of the study and statistically significant results were still obtained without the impact of age differences. Thus, we can say that the results of our study are specific to COPD independent of the age factor.

In many studies, the oxidant-antioxidant balance in COPD was deteriorated in favor of oxidants; significant increase in oxidant load was found in COPD patients [20-22]. In our study, it was found that there was a statistically significant increase in oxidative stress in COPD patients compared to healthy control group, and this finding was interpreted to be in accordance with the literature.

The continued increase in oxidant load may also be an indicator of chronic subclinical inflammation during stable periods [23].

Changes in antioxidant defense may be increased due to defensive response or decreased due to neutralization by oxidants. If the reserves are insufficient, there may be no change [24, 25]. In a study by Tuğ et al. oxidant-anti oxidant levels were evaluated in COPD patients during exacerbation and stable periods after an attack; the antioxidant response was unchanged against the oxidative stress, which increased during episodes [26]. Similarly, in our study, although there was an increase in oxidative stress when compared with healthy subjects in exacerbation and stable groups, no statistically significant difference was found in total antioxidant levels. In another study, a decrease in serum concentrations of antioxidant vitamins A and E was found in stable and exacerbated COPD patients; therefore, it has been suggested that increasing the antioxidant capacity of these patients with external antioxidants (such as vitamins A, C and E) may positively affect the clinic by reducing the oxidative load in COPD [27].

In many previous studies, smoking habits, comorbidities, obesity, age and gender inequalities were ignored; this makes it doubtful whether these results were specific for COPD. In the methodology of our study, all parameters that might be related to oxidative stress and which could affect the outcome of our study were included under the exclusion criteria. Patients and healthy individuals with these characteristics were not included in the study group. In the analysis, statistically significant differences were found in the evaluation of oxidant-antioxidant system between COPD groups and healthy subjects. However, there was no correlation between these statistically significant parameters and TAS, TOS and OSI levels. Evaluating the correlation between these parameters with larger population samples may give statistically significant results in future studies.

The results of our study suggest that TAS, TOS and OSI may be used as a biomarker for the evaluation of oxidative stress in COPD. However, the fact that the method is not automated, reliable but not easily applicable suggests that it is not yet available as a routine parameter. However, by eliminating these difficulties, TAS, TOS and OSI levels can be used as a viable test in order to detect and control oxidant-antioxidant imbalance earlier in COPD patients. As a result of studies related to more quantitative evaluations, more meaningful evaluations can be achieved by determining cut-off levels in mathematical analyzes and measurement criteria.

Conclusion

In our study, it was found that oxidant-antioxidant imbalance in COPD patients can be demonstrated by measuring TAS, TOS and OSI levels. There was a significant increase in oxidative stress in all COPD patients, but no increase in antioxidant response. Significant differences were found in the parameters of CRP, ESR, WBC,% NEU, pO_2, pCO_2,% SPO_2, , and respiratory function tests, but there was no correlation between these parameters and TAS, TOS and OSI levels. More robust studies are needed to evaluate this correlation more effectively. The fact that TAS, TOS and OSI levels do not show significant differences in healthy adults suggests that these parameters may be used as a new biomarker for the evaluation of oxidative stress in COPD.

Data Availability
The data sets used and/or analyzed during the current study can be obtained from the corresponding author on reasonable request.

Conflict of interest
The authors declare that they have no competing interest.

Financial Disclosure
All authors declare no financial support. T

Ethical approval
Study was approved by the Bolu Abant Izzet Baysal University Clinical Researches Ethics Committee with the protocol number 2017/77.

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