Total antioxidant capacity as a marker in predicting severity of chronic obstructive pulmonary diseases

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Background The pathogenesis of chronic obstructive pulmonary disease (COPD) is multifactorial; oxidative stress is suggested to be one of the pathogenetic factors.

Objective The aim of this study was to assess the role of antioxidant status in pathogenesis of COPD and in predicting the severity of airway obstruction.

Patients and methods This case-controlled study was carried on 60 patients with COPD, and on 15 apparently healthy age-matched smokers and 15 apparently healthy age-matched nonsmokers, which served as control groups. Bronchoscopy with bronchoalveolar lavage was carried out for 10 COPD patients. Chest radiography, pulmonary function testing, and arterial blood gases were carried out for all groups. Serum level of total antioxidant (TAO) was also measured in all groups by using the enzyme-linked immunosorbent assay kit.

Results Serum TAO level was significantly reduced in COPD patients and healthy smokers compared with healthy nonsmokers ($P<0.001$, respectively); moreover, serum TAO level was significantly reduced in COPD compared with healthy smokers ($P<0.001$), and serum TAO was significantly reduced in severe and very severe COPD compared with mild and moderate COPD ($P=0.01$ and 0.006, respectively). TAO level significantly negatively correlated with each of $PaCO_2$ and HCO_3 in COPD patients, and it was significantly positively correlated with each of forced expiratory volume in 1 s/forced vital capacity and forced expiratory volume in 1 s %. TAO had a sensitivity and specificity of 86.67 and 93.33, respectively, as a biomarker for identification and predicting the severity of COPD with an area under the curve of 0.921.

Conclusion Serum TAO is a valuable biomarker in identifying and predicting the severity of COPD.

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Keywords: antioxidants, chronic obstructive pulmonary disease, pulmonary function tests

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Introduction

It is documented that chronic obstructive pulmonary disease (COPD) is a preventable and treatable disease. It is characterized by airflow limitation that is persistent and progressive. Noxious particles and gases exaggerate chronic inflammation in airways. The severity of COPD is potentiated by frequent exacerbation and comorbidities [1]. Pathogenesis of COPD is multifactorial and includes interaction between genetic and environmental factors. Environmental exposure such as cigarette smoking and air pollutants, in addition to reactive oxygen and nitrogen species from inflammatory cells in the lung, is associated with increased oxidant burden and oxidant damage of DNA and subsequent development of COPD [2,3]. Oxidant–antioxidant imbalance plays a key role in the pathogenesis of COPD [4]. Cigarette smoking, which plays a critical role in the pathogenesis of COPD, leads to generations of multiple chemical compounds, oxidants, and free radicals such as nitric oxide, superoxide anion, and hydroxyl radicals. Increased oxidative stress has been reported to be associated with derangement of pulmonary function [4]. Zeng *et al.* [5] observed increased expression of malondialdehyde, the product of lipid peroxidation in sputum of COPD patients, which further increased during acute exacerbation. Moreover, they observed a decreased antioxidant level in the sputum of COPD patients. Systemic manifestation of COPD and vascular complications such as coronary artery diseases may also be attributed to oxidant–antioxidant imbalance and systemic spillover of inflammatory mediators [6,7].

The objective of the present study was to determine the total antioxidant (TAO) status values in the patients who had been diagnosed with COPD to assess the role of antioxidants in its pathogenesis and to assess its correlation with the severity of COPD.

Patients and methods

This case-controlled study was carried out on three groups. The first group included 60 COPD patients diagnosed and classified on the basis of the criteria of the Global Initiative for Chronic Obstructive Lung Disease (GOLD) [1]. The second group included 15 age-matched and sex-matched apparently healthy smokers

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and the third group included 15 age-matched and sex-matched apparently healthy nonsmoker, both groups serving as control groups.

Patients and controls were enrolled from the outpatient clinic of a tertiary hospital. Informed written consents were obtained from all participants and the study was approved by the Ethics Committee of Faculty of Medicine.

All enrolled participants were subjected to complete history and clinical examination, chest radiography for exclusion of other diagnosis, spirometry using ZAN300 (ZAN Messgerite GmbH Schlimpfhofer Strasse 14, D-97723, Oberthulba, Germany), which measured each of forced expiratory volume in 1 s (FEV_1%), FEV_1/forced vital capacity (FVC), and forced expiratory flow 25–75% predicted, and arterial blood gases using blood gases analyzers (Rapid Lab 850; CHI-RON/Diagnostics, Halstead, UK). Samples were obtained on room temperature by using radial artery puncture. Ten patients with COPD were subjected to bronchoscopy with bronchoalveolar lavage (BAL), and BAL samples were obtained. In addition, 5 ml venous blood samples were withdrawn from each participant by venipuncture, and then the blood was centrifuged for the determination of TAO capacity by using the enzyme-linked immunosorbent assay kit. The TAO capacity, both in serum and BAL fluid, was measured by using the total antioxidant assay kit purchased from Cayman's Chemical Company (1180 East Ellsworth Road, Ann Arbor, Michigan, USA). There was no separation of aqueous and lipid soluble antioxidants, and therefore the antioxidant activities of all constituents were assessed. The assay is dependent on the ability of antioxidants in the samples to inhibit the oxidation of ABTS (2,2′-azino-di-[3-ethylbenzthiazoline sulfonate]) to ABTS•–by metmyoglobin [8].

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (IBM Corp., Released 2011, IBM SPSS Statistics for Windows, Version 20.0, IBM Corp., Armonk, NY) software. The results were expressed as means±SD or frequencies. Independent Student's *t*-test was carried out for comparison between COPD patients and controls. One-way analysis of variance was used for comparison of continuous variables between more than two groups. Pearson's correlation analysis was used to evaluate the correlations between different parameters in each group. *P*-values less than 0.05 were considered statistically significant.

Table 1 Demographic data of the study groups

Variables	COPD (*N*=60)	Healthy smokers (*N*=15)	Healthy nonsmokers (*N*=15)	*P*-value
Age	60±6.01	59.13+8.17	60+6.06	NS
BMI	26.53±6.99	26.33+5.95	26.53+7.06	NS
Sex				
Males	52 (86.7)	13 (83.3)	12 (80)	NS
Females	8 (13.3)	2 (17.7)	3 (20)	NS
Smoking habits				
Nonsmokers	0	0	15 (100%)	<0.001*
Mild smokers	8 (13.4)	2 (13.33)	0	
Moderate smokers	22 (36.6)	6 (40)	0	
Heavy smokers	30 (50)	7 (46.66)	0	

Data are expresses as mean±SD or *n* (%). COPD, chronic obstructive pulmonary disease. *Significant.

Table 2 Functional and laboratory data of chronic obstructive pulmonary disease patients and controls

Variables	COPD (*N*=60)	Healthy smokers (*N*=15)	Healthy nonsmokers (*N*=15)	P_1	P_2	P_3
FEV_1/FVC%	57.88±8	80.67±4.73	83.8±5.95	0.000*	0.000*	0.02*
FEV_1%	32.27±15.23	108.27±20.71	110.53±18.45	0.000*	0.000*	NS
$FEF_{25–75}$%	14.03±9.06	90.67±29.61	87.6±28.36	0.000*	0.000*	NS
pH	7.42±0.04	7.44±0.03	7.42±0.03	0.032*	NS	NS
$PaCO_2$ (mmHg)	55.25±12.36	41.4±3.09	37.93±4.91	0.000*	0.001*	0.002*
PaO_2 (mmHg)	64.43±8.86	67.2±3.38	72.5±4.68	0.043*	0.000*	0.003*
HCO_3 (mEq/l)	34.93±7.09	23.13±3.02	26.93±4.46	0.000*	0.000*	<0.001*
SaO_2%	91.77±2.63	92.93±1.44	95.2±1.63	0.012*	0.000*	<0.001*
Serum TAO (mmol/l)	0.22±0.12	0.36±0.08	0.54±0.21	<0.001*	<0.001*	<0.001*
TAO in BAL (mmol/l)	0.13±0.05					

BAL, bronchoalveolar lavage; COPD, chronic obstructive pulmonary disease; $FEF_{25–75}$%, forced expiratory flow 25–75%; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; HCO_3, bicarbonate; $PaCO_2$, arterial partial pressure of carbon dioxide; PaO_2, arterial partial pressure of oxygen; SaO_2, arterial oxygen saturation; TAO, total antioxidant. P_1, COPD versus healthy smokers. P_2, COPD versus healthy nonsmokers. P_3, healthy smokers versus healthy nonsmokers. *Significant.

Results

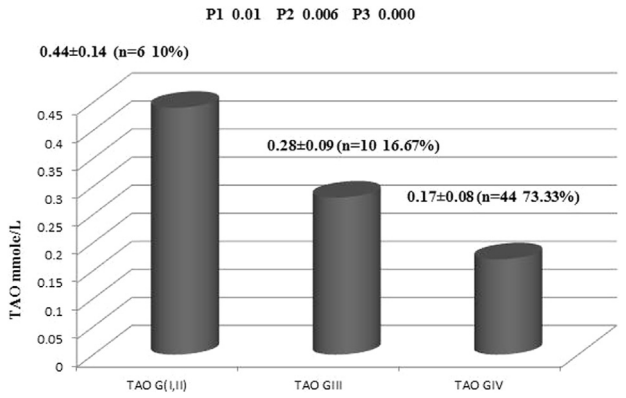
The demographic data of the studied groups demonstrated no significant difference regarding age, sex, and BMI (Table 1). The COPD patients presented with cough, sputum production, and dyspnea. Table 2 demonstrates that 90% (54 patients) of COPD patients had severe to very severe obstructive pulmonary function. The mean±SD FEV_1% for all COPD patients was 32.27 ±15.23; they also were hypoxemic and hypercapnic; $PaCO_2$ and PaO_2 were 55.25±12.36 and 64.43±8.86, respectively.

Moreover, Table 2 demonstrates that the total serum antioxidant (TAO) was significantly reduced in COPD patients compared with healthy smokers and healthy nonsmokers($P<0.001$ and 0.001, respectively), and also it was significantly reduced in healthy smokers compared with healthy nonsmokers ($P<0.001$). TAO in BAL fluid was 0.13±0.05 mmol/l.

Figure 1 demonstrated that TAO was significantly reduced in severe and very severe COPD compared with mild and moderate COPD (P=0.01 and 0.006, respectively).

Figures 2 and 3 showed a significant negative correlation between the TAO level and each of $PaCO_2$ and HCO_3 (r=–0.297, P=0.005; r=–0.405, P=0.000) in COPD patients; furthermore, the TAO level significantly positively correlated with each of FEV_1/FVC and FEV_1%, (r=0.39, P=0.000; r=0.33, P=0.002, respectively). By plotting the receiver operating characteristic curve, TAO had a sensitivity and specificity of 86.67 and 93.33, respectively, as a biomarker for identifying and predicting the severity of COPD with an area under the curve of 0.921 (Fig. 4).

Figure 1

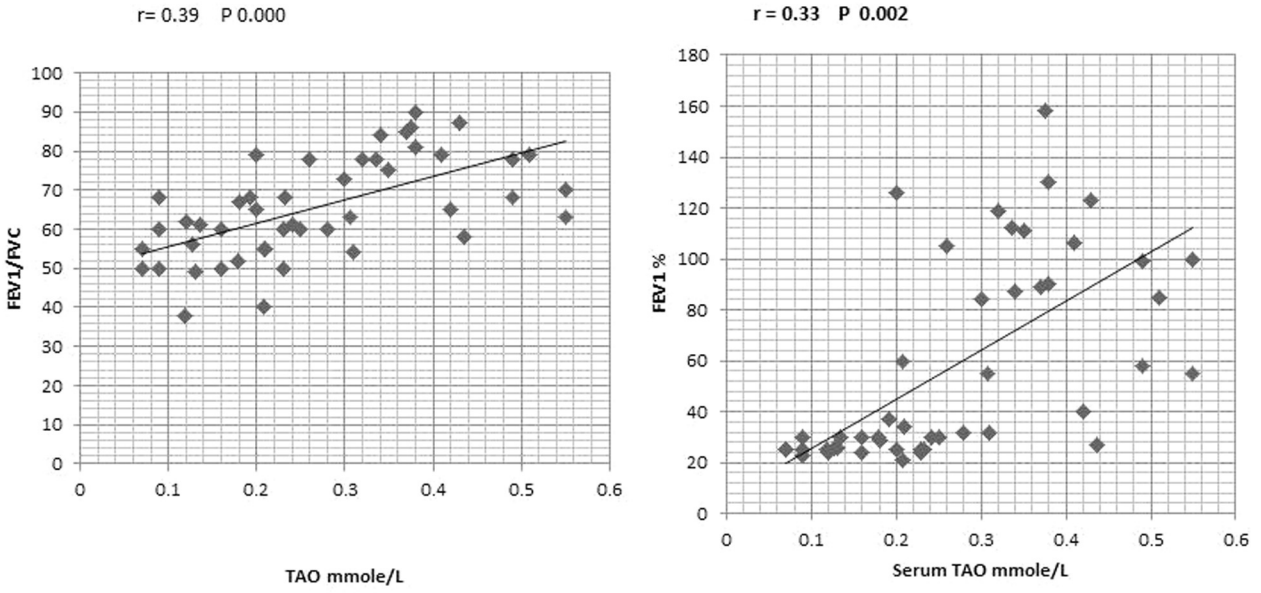


Total antioxidant (TAO) in serum (mmol/l) in chronic obstructive pulmonary disease patients (expressed as mean±SD). Values of TAO presented as mean±SD. P_1, GOLD I, II versus GOLD III; P_2, GOLD I, II versus GOLD IV; P_3, GOLD III versus GOLD IV. GOLD, Global Initiative for Chronic Obstructive Lung Disease

Discussion

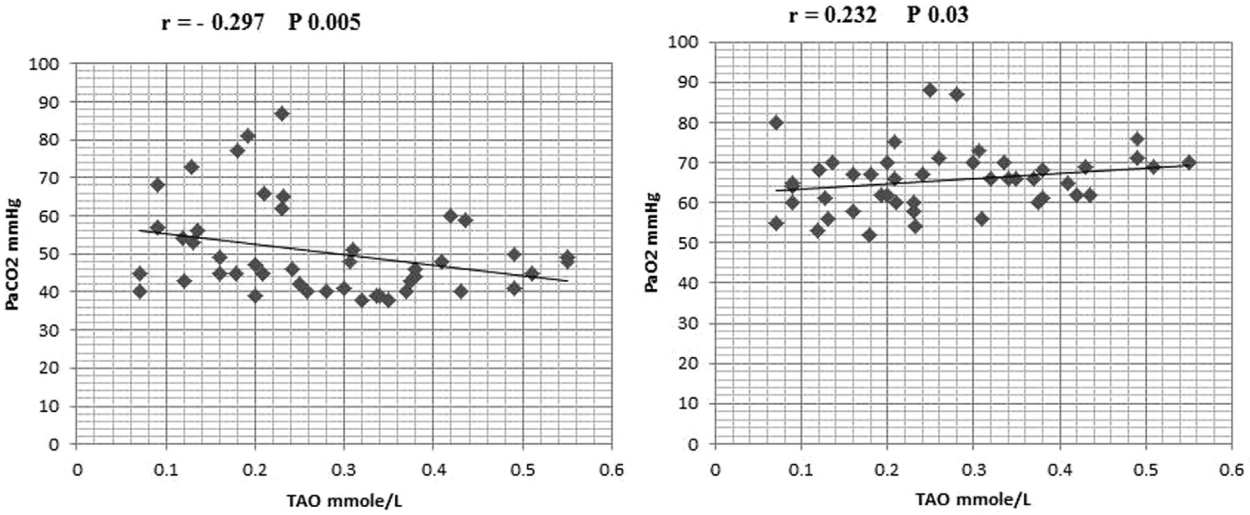
COPD is a common disease associated with progressive deterioration of pulmonary function and quality of life [9,10]. By 2010 it was estimated as the third most common cause of death, and as regards burden of disease it was the fifth worldwide [11]. Reaching a definite treatment for COPD is a much needed outcome, and thus many studies focusing on the pathogenesis of COPD have been conducted. It well

Figure 2



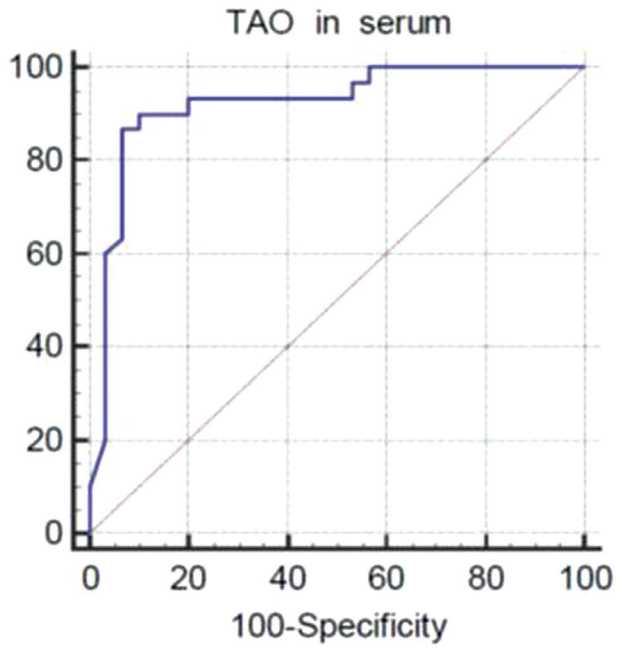
Correlations between serum total antioxidant (TAO) and pulmonary function tests

Figure 3



Correlations between serum total antioxidant (TAO) and arterial blood gases parameters

Figure 4



Receiver operating characteristic curve showing the sensitivity and specificity of serum total antioxidant in chronic obstructive pulmonary disease patients

known that pathogenesis of COPD is multifactorial, depending on the protease–antiprotease imbalance, different inflammatory cytokine and chemokines, and the oxidant–antioxidant imbalance [12]. In this study we were interested in studying the association between reduction of TAO and functional decrement in COPD patients.

The study demonstrated that TAO significantly reduced in COPD patients and healthy smokers compared with healthy nonsmokers; moreover, it was significantly reduced in severe and very severe COPD compared with mild and moderate COPD. In their study, Ambade *et al.* [13] supported our results: they documented the role of oxidative stress in the pathogenesis of COPD and appreciated the role of antioxidants in protecting the lung; they stated that TAO is a measure of antioxidant status. Furthermore, the findings of Mohamed *et al.* [14] were in agreement with our results as they observed that TAO decreased in COPD patients and was correlated with the severity of the disease, but they disagreed with our results in that they observed no significant difference between control smokers and control nonsmokers. Meanwhile, Emin *et al.* [15] supported our results: they proved that TAO reduced in COPD and its level differed according to smoking habits and that it is lower in smokers than in nonsmokers. The findings of Rahman *et al.* [16] and Sahin *et al.* [17] were also in agreement with our results. In their respective studies, Olivieri *et al.* [18] and Salvi *et al.* [19] stated that a reduced TAO level in COPD is the result of reduced antioxidant production or its increased consumption, or there is a role of oxidative stress. We observed significant negative correlations between TAO and each of $PaCO_2$ and HCO_3, whereas it was significantly positively correlated with each of FEV_1/FVC, FEV_1%, and forced expiratory flow 25–75%. Similar results were observed by Mohamed *et al.* [14]. On the other hand, Rahman *et al.* [16] found no correlations between TAO and any of the spirometric parameters; this discrepancy may be attributed to racial and ethnic variability or different smoking habits. Tavilani *et al.* [4] observed that TAO significantly reduced in COPD and smokers than in nonsmokers. They also stated that plasma antioxidant provides protective environment against oxidative stress, and hence a

reduction in plasma antioxidant associated with the development of COPD. They denied any correlation between TAO and the spirometric variables and attributed this to differences in consumption of dietary antioxidants. In this study, TAO in BAL fluid was 0.13 ±0.05 mmol/l. Kontakiotis *et al.* [20] found that TAO in BAL of healthy smokers was 0.12±0.02 mmol/l, and they postulated that it is a form of adaptation of the lung to neutralize oxidant burden of smoking.

By plotting the receiver operating characteristic curve we observed that serum TAO had a sensitivity and specificity of 86.67 and 93.33, respectively, in predicting the severity degree of obstructive dysfunction, with an area under the curve of 0.921. To the best of our knowledge no previous study had examined the sensitivity of TAO in predicting obstructive pulmonary dysfunction. We concluded that deficiency of serum TAO plays a critical role in the pathogenesis of COPD and in determination of severity of airway obstruction.

The strength of this study include recruitment of matched groups, healthy smokers and nonsmokers. We concentrated on both serum and BAL fluid TAO, which give a good idea about the overall antioxidant activity.

The present study had a few limitations. A larger number of COPD patients would have represented different GOLD stages, which should have been involved in the study. In addition, we did not study the sensitivity and specificity of TAO in pulmonary diseases other than COPD. We recommend studying the role antioxidant replacement in modifying the course of COPD.

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Conflicts of interest
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