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Care of Adults With Chronic Pulmonary Disorders

Comparison of oxidant/antioxidant balance in COPD and non-COPD smokers

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ABSTRACT

Background/aim: Oxidative stress plays an important role in the pathogenesis of chronic obstructive pulmonary disease (COPD). Smoking is the leading source of oxidants in lungs. However, it is currently unknown why some individuals are more resistant to the detrimental effects of smoking and do not develop COPD. The aim in this study is to measure and compare the oxidant/antioxidant balance between in non-COPD individuals who smoke and COPD patients who smoke.

Materials and methods: Included in the study were 137 patients with COPD and 102 healthy individuals. Participants were divided into groups as COPD patients (former and current smokers), non-COPD individuals who smoke and non-smokers healthy persons. In the following stage, the total antioxidant status (TAS), total oxidant status (TOS) and oxidative stress index (OSI) levels were measured in serum for all participants.

Results: In the current-smoker COPD group, the level of oxidant status were significantly higher than the former-smoker COPD group ($p < 0.001$). Similarly, oxidant levels were significantly high in current-smoker healthy group than never smoker healthy group. According to these results TOS was associated with especially smoking status rather than COPD. Antioxidant status were similar between former-smoker COPD group and current-smoker COPD group. The antioxidant levels were found significantly low in current-smoker COPD patients, compared to the current-smoker non-COPD individuals ($p = 0.007$). Nevertheless, no significant difference was found in OSI levels between two groups. Briefly, high TOS and OSI values were correlated with only smoking, independently from COPD.

Conclusion: It was concluded that there are complex pathogenetic mechanisms, including genetic and individual variations other than oxidant/antioxidant balance, involved in the development of smoking-related COPD. TOS and OSI values are not predictive parameters for the development of COPD, but high level of TAS in non-COPD smokers is promising for future studies.

Introduction

Chronic obstructive pulmonary disease (COPD) is a major source of morbidity and mortality worldwide, resulting in significant healthcare costs and loss of workforce.[1] COPD is currently the fourth leading cause of death, but is expected to rise to third place by 2020.[2]

The precise mechanisms in the pathogenesis of COPD have yet to be defined, although it has been suggested that increased oxidative stress or reduced antioxidant resources and protease-antiprotease imbalance may play a role.[3] A number of studies have shown an increased oxidant burden, and consequently, increased markers of oxidative stress, in the airspaces, breath, blood, sputum and urine in smokers and in patients with COPD.[4–6]

The most prominent risk factor in the clinical manifestation and progression of COPD is smoking tobacco, which is also a major source of oxidants/reactive oxygen species to the lungs.[6] Cigarettes contain a substantial amount of free radicals, which play a significant role in the impairment of oxidative balance and cause cell damage. A single puff of cigarette smoke has been found to contain 10^{17} free radicals and more than 4700 chemicals. More than 90% of patients with COPD are smokers, but for unknown reasons, only 15–20% of cigarette smokers develop the disease.[4]

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The most effective method in the prevention of COPD development is to give up smoking cigarettes; however, this is generally no easy task, and COPD develops in a considerable part of smokers in the long term. It is still impossible to predict in whom COPD will develop, and it is not known why COPD does not develop in some individuals, despite their smoking habit. In these individuals, the oxidant/antioxidant balance may not be impaired due to excessive antioxidant intake in the diet, or due to a genetically increased antioxidant enzyme expression. Evidence that oxidants/free oxygen radicals play an important role in pathogenesis of COPD make it believable that an increasing antioxidant capacity, especially in cigarette smokers, would have a therapeutic benefit.[3] For confirmation of this hypothesis would require demonstrating the increased antioxidant capacity of smoking patients who have not developed COPD. In the present study, the total antioxidant status (TAS), total oxidant status (TOS) were measured in the serum of people who are not COPD whilst smoking, and were compared them with the results of COPD patients and non-smoker healthy individuals.

Material and methods

Participants

The study included 137 ambulatory clinically stable COPD patients and 102 healthy volunteers, and took place between March 2014 and March 2017. The patients had been diagnosed with COPD according to the Global initiative for Chronic Obstructive Lung Disease (GOLD) criteria.[1] Patients with a history of antioxidant (N-acetyl cysteine and carbocisteine, vitamin C and E, ascorbic acid) use and acute exacerbations of COPD in the previous month, accompanying immunosuppression, malignancy, congestive heart failure, insulin-dependent diabetes mellitus, inflammation, infection and neurological dysfunction that could influence the oxidative status were excluded from the study. The participants underwent a COPD Assessment Test (CAT) and a pulmonary function test (PFT), in accordance with the directives of the American Thoracic Society. All pulmonary function tests were carried out by the same technician following the administration of 400 μg salbutamol while the patient was stable. Exacerbation history was noted. A combined COPD assessment scaling was applied according to the GOLD criteria, and a detailed cigarette smoking history was taken from all patients. Patients who had completely given up smoking at least six months ago were considered former-smokers; while individuals who had history of smoking within the last six months were excluded from the study. Of the 137 patients included in the study, 98 had been undergoing corticosteroid treatment for more than one year at the time of the study. All patients were undergoing inhaled bronchodilator therapy in β2 agonists and/or anticholinergic agents.

The participants were divided into four subgroups according to the following characteristics:

1. Former-smoker COPD group: Comprised 75 stable COPD patients who had stopped smoking at least 6 months ago.
2. Current-smoker COPD group: Comprising 62 stable COPD patients who were current smokers.
3. Current-smoker non – COPD group: (healthy smokers): Comprising 50 adult patients aged above 50 who had smoked at least 20 packs per year without developing any health problems and who had normal pulmonary function test and physical examination results.
4. Non-smoker non-COPD group: (healthy non-smokers) Comprising 52 adult patients who had no history of smoking or any health problems, and who had normal pulmonary function test results.

All patients were informed about the aim of the study, and written consent was obtained. Ethical committee approval was obtained from the University Medical Faculty Ethics Committee.

Assays

Venous blood samples were taken from the participants after a minimum two hour absence of smoking. Serum was separated from the cells by centrifugation at 1500 g for 10 min, after which the serum samples were immediately frozen and stored at − 80 °C until the analyses.

Total antioxidant status (TAS)

TAS levels were measured using commercially available kits (Relassay, Turkey). The novel automated method is based on the bleaching of the characteristic color of a more stable ABTS (2.2′-Azino-bis [3-ethylbenzothiazoline-6-sulfonic acid]) radical cation by antioxidants. The results were expressed as mmol Trolox equivalent/L.[7]

Total oxidant status (TOS)

TOS levels were measured using commercially available kits (Relassay, Turkey). In the new method, oxidants present in the sample oxidized the ferrous ion-o-dianisidine complex to ferric ion. The oxidation reaction was enhanced with the glycerol molecules that are abundant in the reaction medium. The ferric ion produced a colored complex of xylenol orange in an acidic medium. The color intensity, which could be measured spectrophotometrically, indicates the total amount of oxidant molecules present in the sample. The assay was calibrated using hydrogen peroxide, and the results were expressed in terms of micromolar hydrogen peroxide equivalent/L (μmol H_2O_2 equivalent/L).[8]

Oxidative stress index (OSI)

The ratio of TOS to TAS was accepted as the oxidative stress index (OSI). For calculation, the resulting unit of TAS was converted to μmol/L, and the OSI value was calculated according to the following Formula: OSI (arbitrary unit) = TOS (μmol H_2O_2 equivalent/L)/TAC (μmol Trolox equivalent/L).

Statistical analysis

Statistical analyses were carried out using the SPSS/PC software (version 21.0. IBM). Evaluation of normality was performed with the Shapiro–Wilk test. Normally distributed continuous variables were compared with Student's *t*-test for independent groups, not-normally distributed variables were compared with nonparametric (Mann–Whitney U) test, and categorical variables were compared by Chi square test. Associations between variables were identified using Pearson's correlation analysis. A p value ≤ 0.05 was considered significant.

Results

A total of 239 individuals were included in the study, of which 137 had COPD and 102 were healthy controls. After being classified into the patient and the control groups, no difference was identified between the two groups of patients in terms of sex, age and BMI. When the participants were categorized as former-smoker COPD, current-smoker COPD, current-smoker non-COPD control and never-smoker control groups, no difference was apparent between the groups in terms of age, sex and BMI. Furthermore, no difference was identified between former-smoker and current smoker subgroups of COPD in terms of PFT, CAT, exacerbations and GOLD Class. The demographical and clinical features of groups are presented in Table 1.

While antioxidant levels equal between the smoker and former-smoker COPD patients; a significant difference was found among oxidant levels. Higher TOS values was found in the current-smoker COPD group. Hence OSI values was higher in current-smoker COPD patients (Table 2).

Likewise; in control group, TOS and OSI values were significantly higher in smokers, than never-smoker healthy individuals (Table 3). Also TAS values was similar between in the control groups.

Table 1
Demographic and clinical data of subjects

	Group 1 Former-smoker COPD $n = 75$	Group 2 Current-smoker COPD $n = 62$	Group 3 Current-smoker Controls $n = 50$	Group 4 Never-smoker Controls $n = 52$	p value
Age (years)	67.7 ± 8.8	60.2 ± 9.1	61.3 ± 8.1	65.3 ± 5.9	0.264
Sex (m/f)	69/6	55/7	43/7	45/7	0.08
BMI (kg/m^2)	25.3 ± 4.8	25.1 ± 4.8	26.3 ± 5.2	26.2 ± 3.1	0.264
Pack years	42.9 ± 21.2	48.6 ± 18.8	40.3 ± 10.8	na	0.036
FVC, predicted%	68.65 ± 16.1	66.23 ± 15.2	86.52 ± 11.9	86.38 ± 16.8	0.372*
FEV_1, predicted%	48.28 ± 15.4	49.37 ± 14.1	82.06 ± 12.4	80.50 ± 15.5	0.670*
FEV_1/FVC	54.28 ± 9.8	58.06 ± 8.3	76.18 ± 5.6	80.02 ± 8.3	0.017*
CAT	16.49 ± 9.5	15.29 ± 8.9	na	na	0.452*
Exacerbation 0/1/>2	25/27/23	28/20/14	na	na	0.335*
GOLD Class A/B/C/D	14/19/9/33	18/9/8/27	na	na	0.317*

COPD: Chronic Obstructive Lung Disease; CAT: COPD Assessment Test; BMI: Body mass index; na: not available, FVC: Forced vital capacity; FEV_1: Forced expiratory volume in 1 s; GOLD: Global Initiative for Chronic Obstructive Lung Disease.
* Between group 1 and 2. Values are presented as the mean ± standard deviation.

TOS levels in healthy smokers was significantly higher than the Current-smoker COPD group. At the same time, TAS levels was significantly higher in healthy smokers when compared to the COPD group.

Interestingly OSI values was similar in the smoker COPD and the smoker healthy control groups ($p = 0.093$) (Table 4).

OSI was significantly high in the current-smoker COPD group when compared with the former-smoker COPD group ($p < 0.001$), and OSI was found to be significantly high in current-smoker control group than never-smoker control group ($p < 0.001$) (Table 2 and 3). The results were similar in both the current-smoker COPD group and smoker control groups ($p = 0.093$) (Table 4).

TOS and OSI were found high in smokers, regardless of COPD status.

Spirometric values, particularly% predicted FEV_1, did not show any significant correlation with the TAS, TOS or OSI levels.

Discussion

In this study, plasma oxidant levels were higher in current-smokers COPD patients than former-smoker COPD patients. Similarly, TOS levels were higher in current-smokers healthy peoples than never-smoker healthy individuals. Oxidant levels were higher both current-smoker healthy individuals and current-smokers COPD patients. TOS was associated with especially smoking status rather than COPD.

The results of the current study demonstrate that oxidant/antioxidant balance is impaired in smokers, whether with or without COPD. In the study population, while TOS was found high in smokers, TAS was found to be similar among the smoker and non-smokers. Thus OSI value was lower in smokers, and OSI was correlated with cigarette smoking rather than COPD. Nevertheless, TAS was significantly lower in patients with COPD. The TAS levels of the group who did not develop COPD - despite smoking - were high when compared with COPD group. According to these results, lower TAS levels may be associated with the development of COPD.

It is currently unknown why some individuals are more resistant to the detrimental effects of smoking and do not develop COPD. As known, oxidant/antioxidant balance plays important role in the development of COPD. According to the hypothesis of the current study, the reason for this may be, “due to an excessive antioxidant intake in the diet or a genetically increased expression of antioxidant enzymes, oxidant/antioxidant balance might be unimpaired”. If this hypothesis is confirmed, there would be strong evidence that smokers should be recommended to take excessive antioxidants so as not to develop COPD in the future. Furthermore, an increased susceptibility to COPD in early smokers with impaired oxidant/antioxidant balance could be identified.

Antioxidant defenses in the lung are provided by endogenous enzyme systems and non-enzymatic antioxidant compounds. The main enzymatic antioxidants are superoxide dismutase, which degrades superoxide anion, and the glutathione redox system, which inactivates hydrogen peroxide. Nonenzymatic antioxidants such as vitamin E, vitamin C and β-carotene, ubiquinone, flavonoids, and selenium are present in foods, and are considered dietary antioxidants.[9] The current study was based the hypothesis that these antioxidant defense mechanisms are stronger in individuals who smokers without COPD. Also it seems rational to expect high antioxidant levels in cigarette-resistant persons, as expected genuinely, antioxidant levels were found to be significant high in smoker healthy (non-COPD) individuals than smoker COPD groups. The increased TAS values may be protecting them from COPD. In our study, this distinctive feature of the TAS did not have in terms of TOS. So, TOS was only related to smoking. TOS levels were found to be high in both smokers control group and smokers COPD group. Even was higher in the smokers control group. Because of high levels of TOS, found no difference between the patient and control groups in terms of OSI.

The results of the present study showed that oxidant levels were higher in smokers, whether or not they were COPD.

In the COPD group, antioxidant levels were found to be similar among the smoker and non-smokers, but oxidant levels were significantly higher in patients with current-smoker COPD. Likewise in the healthy individuals, although similar levels of TAS, TOS levels were higher in the smokers. Thus increased oxidant levels were attributed

Table 2
Oxidant/antioxidant status for COPD groups

	Group 1 Former-smoker COPD $n = 75$	Group 2 Current-smoker COPD $n = 62$	p value
TAS (μmol Trolox equivalent/L)	1.78 ± 0.20	1.77 ± 0.26	0.625
TOS (μmol H_2O_2 equivalent/L)	8.41 ± 3.88	13.44 ± 8.98	<**0.001**
OSI (arbitrary unit)	4.79 ± 2.34	7.45 ± 4.20	<**0.001**

TAS: Total antioxidant status; TOS: Total oxidant status; OSI: Oxidative stress index.

Table 3
Oxidant/antioxidant status for non-COPD (control) groups

	Group 3 Current-smoker Controls $n = 50$	Group 4 Never-smoker Controls $n = 52$	p value
TAS (μmol Trolox equivalent/L)	1.85 ± 0.19	1.82 ± 0.21	0.560
TOS (μmol H_2O_2 equivalent/L)	14.51 ± 6.29	9.63 ± 2.78	<**0.001**
OSI (arbitrary unit)	7.74 ± 2.93	5.36 ± 1.75	<**0.001**

Table 4
Comparison of oxidant/antioxidant status between healthy smokers and current-smoker COPD group

	Group 2 Current-smoker COPD $n = 62$	Group 3 Current-smoker Controls $n = 50$	p value
TAS (μmol Trolox equivalent/L)	1.77 ± 0.26	1.85 ± 0.19	***0.007***
TOS (μmol H_2O_2 equivalent/L)	13.44 ± 8.98	14.51 ± 6.29	***0.019***
OSI (arbitrary unit)	7.45 ± 4.20	7.74 ± 2.93	0.093

to the effect of smoking. Parallel to this, both COPD group and control group, OSI values were significantly higher in smokers. OSI values were correlated with cigarette smoking status rather than COPD.

In this regard, these results do not fully confirm the hypothesis that antioxidant capacity is high and oxidant/antioxidant balance is maintained in smoker individuals without COPD. Furthermore, these results demonstrate that OSI values are not predictors of COPD development. On the other hand a significantly high level of TAS, partially confirm our hypothesis and it is promising for future studies.

In previous studies, it has also been demonstrated that cigarette smoking increases oxidative stress. In the current study, TOS and OSI were found to be high in the smoker group, similar to the study of Stankovic et al.[10] As in the current study, TAS values have also been found to be low in patients with COPD in previous studies.[11–14]

In the light of these results, obtaining definitive proof that oxidants contribute to COPD and/or that antioxidant therapy is beneficial in COPD remains problematic.[9,15,16] Exogenous antioxidants, such as vitamins, do not seem to prevent cigarette smoke-related lung injury.[17,18] This situation reveals that oxidant/antioxidant balance alone does not play a role in COPD pathogenesis, but rather that a complex mechanism is involved that includes also genetic factors and other mechanisms.

In the current situation, as there is no evidence that antioxidants will prevent the development of COPD in smokers in the future, it would seem that there is no option aside from quitting smoking.

Our correlation studies have shown that there is no significant correlation between spirometric data and the status of the antioxidant system in healthy non-smokers, smokers or subjects with COPD, and this finding is compatible with previous studies.[13,14,19]

To the best of our knowledge, there has been no study to date involving these four groups, in particular, a comparison of smoker COPD and healthy smoker groups. This could be considered as strength of the current study.

There are several limitations of this study; for instance, enzymatic stress markers were not investigated due to high cost. The second limitation in our study was not measured systemic oxidative stress in sputum, BAL or exhaled breath.

In the future, in order to be able to predict COPD development in smokers, and thus to be able to develop treatment modalities for protection from COPD, there is a need to carry out studies to reveal the genetic and individual variations that affect the oxidative injury mechanisms and to understand mechanisms other than oxidant/antioxidant imbalance that are effective on COPD pathogenesis.

Contributions

Conceptualization, Funding acquisition, Methodology, Project administration, Writing-original draft, Writing-review & editing: Y Aydemir; Formal analysis: Ö Aydemir; Data curation: AC Güngen, H Çoban, C Taşdemir, H Düzenli, A Sengul, A Şehitoğulları.

Ethical Statement

The study was approved by the Ethics Committee of Sakarya University,

Declaration of Competing Interest

The authors have no conflicts of interest to declare.

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