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ORIGINAL ARTICLE

Role of oxidant–antioxidant imbalance in the pathogenesis of chronic obstructive pulmonary disease

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Abstract *Background:* Chronic obstructive pulmonary disease (COPD) is a common respiratory condition involving the airways and characterized by airflow limitation. Antioxidants are substances that may protect cells from the damage caused by unstable molecules known as free radicals. Antioxidants interact with and stabilize free radicals and may prevent some of the damage free radicals might otherwise cause. Under physiological conditions a balance exists between the amount of reactive oxygen species (ROS) produced in normal cellular metabolism and the endogenous antioxidant defense. An imbalance between the antioxidant capacity and the production of reactive oxygen species leads to oxidative stress, which is associated with the pathogenesis of several human diseases. An oxidant/antioxidant imbalance has been proposed as having a key role in the pathogenesis of COPD. The lung is directly exposed to high levels of oxygen, and therefore has to have efficient antioxidant mechanisms.

Aim of the study: To examine the role of altered levels of oxidant–antioxidants in disease severity of COPD and correlate it with the degree of airflow obstruction in the Egyptian population.

Subjects and methods: Eighty subjects with COPD, 20 healthy smokers, and 20 healthy nonsmokers participated in this study. The investigation included determination of the lung function and the measurements of plasma superoxide dismutase activity (SOD), glutathione content (GSH) reduced form, glutathione peroxidase activity (GSH-Px), catalase activity (CAT), lipid peroxidase (LP), and nitric oxide (NO).

Results: The mean concentration of nitric oxide (NO) was significantly higher in the control subjects (smokers and nonsmokers) compared with the COPD group ($p = 0.001, 0.0001$) respectively.

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Also the mean concentration of nitric oxide (NO) was significantly higher in control nonsmoker group compared to control smoker group ($p = 0.002$). The mean concentration of lipid peroxidase (LP) was significantly higher in COPD patients compared with control subjects (smokers and nonsmokers), ($p = 0.0001, 0.0001$) respectively. The mean concentration of glutathione (GSH) was significantly higher in the control subjects (smokers and nonsmokers) compared with COPD patients ($p = 0.001, 0.001$) respectively. There is no significant difference in the concentration of glutathione-peroxidase (GSH-Px) in all study participants (COPD patients, control smokers, control nonsmokers). The mean concentration of catalase (CAT) was significantly higher in control nonsmokers, compared to COPD patients and control smokers ($p = 0.001, 0.018$) respectively. The mean concentration of superoxide dismutase (SOD) was significantly higher in the control subjects (smokers and nonsmokers) compared with COPD patients ($p = 0.012, 0.001$) respectively. Also the mean concentration of superoxide dismutase (SOD) was significantly higher in control nonsmoker group compared to control smoker group ($p = 0.001$).

Conclusion: These results support the hypothesis that an oxidant–antioxidant imbalance, associated with oxidative stress in COPD patients, plays an important role in the progression of disease severity, also these results revealed the presence of an oxidative presence in smokers and in subjects with COPD and that the imbalance may be important in the pathogenesis of this disease. The use of cigarette increased oxidative stress by causing plasma lipid peroxidation and imbalance in erythrocyte antioxidant. Nitric oxide (NO) metabolism was not increased in patients with chronic obstructive pulmonary disease compared to healthy subjects. It has been reported that GSH plays a major role in pulmonary antioxidant protection.

Introduction

Human ecology requires both oxygen and water with the generation from food of an immediate energy source, ATP, by oxidative phosphorylation. A continuing balance between oxidation and anti-oxidation is necessary for longer less-disabled lives, taking account of oxidative stresses and the critical roles of oxidants in defense against infection, tissue repair and signaling [1]. There is no animal life without oxygen consumption and its conversion to water with the production by leakage from mitochondrial electron transport of free radicals [2], in the course of oxidative phosphorylation and the production of ATP as the ultimate and immediate source of energy [3]. Free radicals may also be formed as nitrogen, carbonyl, chlorine, sulfur and other reactive species [4,5]. During oxidation electrons or hydrogen are transferred from one molecule to another, the latter serving as an antioxidant. Antioxidants, therefore, can stop the formation of free radicals and the chain reactions, which would otherwise result in cell damage or even death.

Oxidant–antioxidant and COPD

The airflow obstruction in COPD is associated with abnormal inflammatory response of the lungs to chronic inhalational exposure from smoke, dust particles and other air pollutants. As a result, the lungs lose their elasticity [6]. Apart from inflammatory reactions, the domination of proteinases over antiproteinases [7] and oxidative stress [8] are also important factors in the pathogenesis of COPD. It has been proven that the incidence of COPD is strictly correlated with the addiction to smoking tobacco [9,10]. It is considered that reactive oxygen species (ROS) is the major cause of cell and tissue damage associated with many chronic inflammatory lung diseases, including COPD [11,12]. Oxidative stress in cells and tissues is induced by the imbalance between the generation and removal of ROS. ROS are derived from inflammation inducing cells (neutrophils, macrophages), large numbers of which migrate to the lungs, also play an important role in the oxidant–antioxidant imbalance observed in the course of COPD [13]. However, the precise mechanism of the etiopathogenesis of COPD is not yet well defined. It is considered that the increased oxidant burden and oxidant–antioxidant imbalance might be the main cause of the disease (COPD) [14]. Increased oxidative burden is generated from airway leucocytes in the blood or in air spaces directly as a result of cigarette smoke and environmental oxidant pollutants, and indirectly by the release of increasing amounts of ROS. The ROS are scavenged by antioxidant compounds and enzymes [11]. Cigarette smoke is the main etiological factor in the pathogenesis of COPD, as it leads to oxidant overload in the lower airways. Cigarette smoke contains more than 1016–1017 oxidant molecules per puff and about 4700 chemicals, including peroxynitrite, superoxide radical and oxides of nitrogen [15]. The adverse contribution of cigarette smoke is considerable; however, the contribution of other risk factors is equally important, as all smokers do not develop COPD. Under normal conditions, the lungs and the blood are adequately protected by various extracellular and intracellular antioxidants against the deleterious effects of oxidants [16]. There is evidence to suggest an imbalance between oxidants and antioxidants in the lungs and the blood in smokers as well as in patients with COPD [17]. One of the results of increased ROS generation is increased lipid peroxidation. It involves free radical chain reactions leading to the decomposition of polyunsaturated fatty acids, constituting, for example, the components of cell membranes [18]. In this process, once a hydrogen atom is detached from a polyunsaturated fatty acid molecule, a reconfiguration of double bonds occurs and leads

to the generation of conjugated dienes (CDs) [19,20]. Among the secondary products of lipid peroxidation, generated as a result of further reactions, (e.g., β-elimination and decomposition of polyunsaturated fatty acid derivatives), are aldehydes, mainlymalondialdehyde (MDA) [21].

Oxidative stress and imbalances in host defense mechanisms may be among the causes of COPD [22]. The increased production of nitric oxide (NO) during inflammatory immune processes involving the respiratory tract is thought to constitute a host defense mechanism, although this comes at a price, because a high level of NO can also cause respiratory tract injury and thus contribute to the pathophysiology of inflammatory airway diseases such as COPD and asthma [23]. Recently, excessive production of NO has been reported in asthmatic airways [24], although its presence is controversial in COPD airways. NO, a small molecule and a strong free radical, influences many aspects of pulmonary function in healthy subjects and patients. It is synthesized from the amino acid L-arginine by nitric oxide synthase (NOS) of which three forms exist. After production, NO can be exhaled, metabolized to nitrite and nitrate, or interact with super oxide to form peroxynitrite [25].

Subjects and methods

Patients and controls

The COPD group consisted of 80 adults, who were diagnosed as COPD at the Chest Department, Zagazig University, Faculty of Medicine, Egypt. The diagnosis was based on the signs and symptoms, as well as on the results of pulmonary function tests including: forced expiratory volume by the end of the first second (FEV1) percentage of predicted value (FEV1/predicted FEV1%) using portable vitalograph copd-6 model 4000 (Vitalograph Ltd., Gort Road Business Park, Ennis, Co. Clare, Ireland). Classification of the disease severity (mild, moderate or severe) was done according to [26,27] as follows depending upon the obtained value of FEV1/predicted FEV1%:

- *Mild:* 80% or above (symptoms should be present to diagnose COPD in people with mild airflow obstruction).
- *Moderate:* 50–79%.
- *Severe:* 30–49%.
- *Very severe:* Below 30% (less than 50% but with respiratory failure).

Very severe cases were excluded from this study.

Classification of the severity of cigarette smokers was done according to the number of pack-years (P-Y): number of cigarettes smoked per day multiplied by the duration in years (Smoking Index) and divided by 20 as follows [28]:

- *Mild smokers:* Less than 20 P-Y.
- *Moderate smokers:* 20–49 P-Y.
- *Heavy smokers:* More than 49 P-Y.

Note: (1 pack has 20 cigarettes).

Healthy controls (smokers and nonsmokers) collected from non-chesty patients referred from Outpatient Clinics and inpatients of different Departments of Zagazig University Hospitals to Chest Outpatient Clinic for clinical and functional assessment, e.g., before abdominal, eye operations, etc., and also from voluntary people who work in this hospital. All control cases had no historical, clinical or radiological data suggestive of chest problems and they had normal spirometric data even the smoker ones and their pulmonary function tests showed a FEV1/FVC ratio $>70\%$.

Collection of blood samples

5 mL venous blood was drawn into heparinized vials from each subject. The collected blood in heparinized tubes was divided into 2 parts, the first used for the determination of reduced glutathione content (GSH), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), the other part of the sample was centrifuged and separated, plasma was used for the determination of nitric oxide (NO), lipid peroxidation (LP), catalase activity (CAT).

Methods

Determination of superoxide dismutase activity (SOD) in blood

Superoxide dismutase activity is measured according to the method of [29].

Principle

Superoxide dismutase (SOD) catalyzes the dismutation of the superoxide radical (O_2-) into hydrogen peroxide (H_2O_2) and elemental oxygen (O_2).

$$O_2^- + 2H \xrightarrow{SOD} H_2O_2 + O_2$$

Superoxide ions, generated from auto-oxidation of pyrogallol, convert the nitro blue tetrazolium chloride (NBT) to NBT-diformazan which absorbs light at 550 nm. Superoxide dismutase reduces the superoxide ion concentration thereby lowering the rate of NBT-diformazan formation. The extent of reduction in the appearance of NBT-diformazan is a measure of SOD activity present in samples.

Determination of glutathione content (GSH) reduced form in blood

Glutathione was measured according to the colorimetric method of [30].

Principle

This method is based on spectrophotometric measurement of the yellow color of 2-nitro-5-thiobenzoic acid which was produced as one product of this reaction:

$$\text{Glutathione} + 5,5'\text{-dithiobis(2-nitrobenzoic acid)(DTNB)} \rightarrow \text{2-nitro-5-thiobenzoic acid} + \text{glutathione disulfide(GSSG)}.$$

Determination of glutathione peroxidase activity (GSH-Px) in blood

The activity of GSH-Px is determined by using the method of [31,32].

Principle

This method is a linked enzyme reaction in which the oxidized glutathione (GSSG) formed by the action of H_2O_2 and GSH-Px, is converted back to its reduced form in the presence of glutathione reductase (GSSG-R) and NADPH. GSH is thus maintained at a constant concentration and the reaction is followed by measuring the stoichiometric oxidation of NADPH. In this method the amount of residual GSH left after exposure to enzyme activity for a fixed time is measured colorimetrically.

Determination of catalase activity (CAT) in plasma

The assay is based roughly on the method of [33].

Principle

The dichromate/acetic acid reagent can be thought of as a stop bath for catalase activity. As soon as enzyme reaction mixture hits the acetic acid, its activity was destroyed; any hydrogen peroxide which hasnot been split by the catalase will react with the dichromate to give a blue precipitate of perchromic acid. This unstable precipitate was then decomposed by heating to give the green solution. This green color was measured by a spectrophotometer at 570 nm.

Determination of lipid peroxidation level in plasma

Lipid peroxidation is measured colorimetrically as described by [34].

Principle

This method is based on measurement of malondialdehyde (MDA) as one of the main end products of lipid peroxidation by thiobarbituric acid test.

Determination of nitric oxide in plasma

Nitric oxide was determined according to the method described by [35].

Principle

Nitric oxide is relatively unstable in the presence of molecular oxygen, with an apparent half life of approximately 3–5 s and is rapidly oxidized to nitrate and nitrite totally designated as NO_x. A high correlation between endogenous nitric oxide production and nitrite/nitrate (NO_x) levels has been established. The measurement of these levels provides a reliable and quantitative estimate of nitric oxide output in vivo. The assay determines total nitrite/nitrate level based on the reduction of any nitrate to nitrite by vanadium followed by the detection of total nitrite by Griess reagent. The Griess reaction entails formation of a chromophore from the diazotization of sulfanilamide by acidic nitrite followed by coupling with bicyclic amines such as N-(1-naphthyl) ethylenediamine. The chromophoric azo derivative can be measured colorimetrically at 540 nm.

Statistical analyses

All statistical analyses were carried out using the SPSS (statistical package for the social science software) statistical package version 20.0 (SPSS Inc., Chicago, IL, USA) for Windows, and the software Microsoft Excel V.5 (2003). USA Quantitative data were expressed as mean and standard deviation (X + SD) and analyzed by applying Student's *t*-test for comparison of two groups of normally distributed variables. The results of the "*t*"-value are then checked on Student's "*t*"-table to find out the significance level (*p*-value) according to the degree of freedom. All these tests were used as tests of significance at $p < 0.05$ [36].

Results

A total of 120 subjects, including 80 COPD patients, 20 healthy smokers and 20 nonsmokers as control subjects were studied. The mean age of the COPD patients was 54.9 ± 9, male/female ratio was 27/7, number of pack-years was 46.4 ± 1.7 and the FEV1/FVC ratio was 59 ± 3.7. The clinical characteristics of the COPD patients are shown in Table 1.

Among whole patients, 40 of them were classified as moderate, 24 as severe and 16 as a mild. In healthy smoker and nonsmoker control groups the mean age was 52.2 ± 2.1 and 55.7 ± 2.3) respectively, male/female ratio was 20/0 and 11/9, respectively. Number of pack-years was 33.7 ± 1.2 in healthy smoker controls. The clinical characteristics of the healthy smoker and nonsmoker control groups are shown in Tables 2 and 3.

Nitric oxide level

The mean concentration of nitric oxide (NO) was significantly higher in the control subjects (smokers and nonsmokers) (18.2 ± 0.8), (21.4 ± 1.1) μmol/L, respectively compared with 7.1 ± 0.4 μmol/L, in the COPD group ($p = 0.001$, 0.0001) respectively. Also the mean concentration of nitric oxide (NO) was significantly higher in control nonsmoker group (21.4 ± 1.1), compared to control smoker group (18.2 ± 0.8) ($p = 0.002$). As shown in Table 4 and Fig. 1.

Lipid peroxidation (LP) level

The mean concentration of lipid peroxidase (LP) was significantly higher in COPD patients (31.3 ± 1) mM/L, compared with control subjects (smokers and nonsmokers) (14.1 ± 0.6), (13.6 ± 0.7) mM/L, respectively with $p = 0.0001$, 0.0001 respectively, as shown in Table 4 and Fig. 1.

Glutathione content (GSH) reduced form level

The mean concentration of glutathione (GSH) was significantly higher in the control subjects (smokers and nonsmokers) (22.3 ± 0.6), (24.2 ± 0.5) mg/mol, respectively compared with 8.9 ± 1.1 mg/mol, in COPD patients ($p = 0.001$, 0.001) respectively, as shown in Table 4 and Fig. 2.

Table 1 Clinical characteristics of the COPD patients.

Character	Mean ± SD
Age	54.9 ± 9
Sex (M/F)	27/7
Cigarette (pack-years)	46.4 ± 1.7
FEV1 (%)	59 ± 3.7

Table 2 Clinical characteristics of the smoker control.

Character	Mean ± SD
Age	52.2 ± 2.1
Sex (M/F)	20/0
Cigarette (pack-years)	33.7 ± 1.2

Table 3 Clinical characteristics of the nonsmoker control.

Character	Mean ± SD
Age	55.7 ± 2.3
Sex (M/F)	11/9

Glutathione peroxidase activity (GSH-Px) level

There is no significant difference in the concentration of GSH-peroxidase (GSH-Px) in all study participants (COPD patients, control smoker, control nonsmokers), (12.2 ± 0.6, 12.1 ± 0.8, 12 ± 0.9) min/mL respectively, as shown in Table 4 and Fig. 2.

Catalase activity (CAT) level

The mean concentration of catalase (CAT) was significantly higher in control nonsmokers (19.6 ± 0.6) μmol/mL, compared to COPD patients and control smokers (6.8 ± 0.3, 7.7 ± 0.6) μmol/mL, respectively, (p = 0.001, 0.018) respectively, as shown in Table 4 and Fig. 2.

Superoxide dismutase activity (SOD) level

The mean concentration of superoxide dismutase (SOD) was significantly higher in the control subjects (smokers and nonsmokers) (8.9 ± 0.6), (14.9 ± 1.1) μmol/L, respectively compared with 5.4 ± 0.7 μmol/L, in the COPD patients (p = 0.012, 0.001) respectively. Also the mean concentration of superoxide dismutase (SOD) was significantly higher in

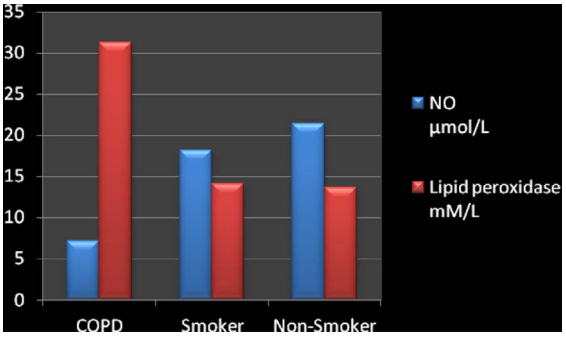

Figure 1 Mean concentration of nitric oxide, lipid peroxidase in COPD patients and control groups (smokers and nonsmokers).

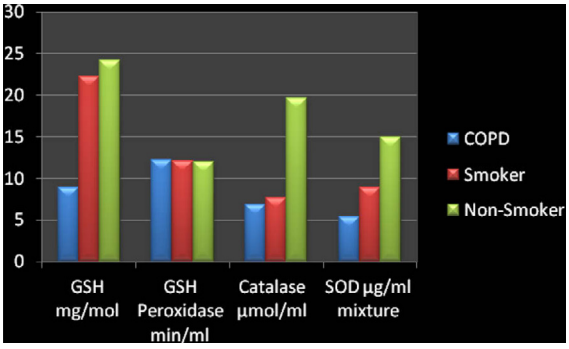

Figure 2 Mean concentration of glutathione, GSH-peroxidase, catalase and superoxide dismutase in COPD patients and control groups (smokers and nonsmokers).

Table 4 Mean concentration of nitric oxide, lipid peroxidase, glutathione, GSH-peroxidase, catalase and superoxide dismutase in COPD patients and control groups (smokers and nonsmokers).

Groups parameter	COPD patients	Smoker control	Nonsmoker control	p
NO (μmol/L)	7.1 ± 0.4 0.001[b] 0.002[c]	18.2 ± 0.8	21.4 ± 1.1	0.0001[a]
Lipid peroxidase (mM/L)	31.3 ± 1 0.0001[b]	14.1 ± 0.6	13.6 ± 0.7	0.0001[a]
GSH (mg/mol)	8.9 ± 1.1 0.001[b]	22.3 ± 0.6	24.2 ± 0.5	0.001[a]
GSH-peroxidase (min/mL)	12.2 ± 0.6	12.1 ± 0.8	12 ± 0.9	NS
Catalase (μmol/mL)	6.8 ± 0.3 0.018[c]	7.7 ± 0	19.6 ± 0.6	0.001[a]
SOD (μg/mL mixture)	5.4 ± 0.7 0.012[b] 0.001[c]	8.9 ± 0.6	14.9 ± 1.1	0.001[a]

[a] Significant COPD with nonsmoker.
[b] Significant COPD with smokers.
[c] Significant smoker with nonsmoker.

control nonsmoker group (14.9 ± 1.1), compared to control smoker group (8.9 ± 0.6) (p = 0.001), as shown in Table 4 and Fig. 2.

Discussion

It has been observed that patients with COPD manifested increased oxidative stress, as shown by the higher levels of LPO products and plasma protein carbonyls, higher concentration of total blood glutathione and decreased protein sulfhydryls in plasma. This is accompanied by alterations in several endogenous enzymatic antioxidants in the blood, including SOD, catalase and GPx activity. Significant changes were also observed in the total antioxidant status of plasma. Various studies have reported increased plasma MDA levels in healthy smokers and in patients with COPD [37].

Also it is reported that increased oxidative stress in COPD patients included the presence of higher LPO products, decreased sulfhydryls and total antioxidant status [38]. An earlier study reported an increase in MDA levels in all stages of COPD severity. The mean MDA values were also found to be significantly increased in all COPD groups according to severity [39].

Our study found that the mean concentration of lipid peroxidase (LP) was significantly higher in COPD patients (31.3 ± 1) mM/L, compared with control subjects (smokers and nonsmokers) (14.1 ± 0.6), (13.6 ± 0.7) mM/L, respectively with p = 0.0001, 0.0001 respectively. This result agrees with a group of researchers who reported that plasma levels of lipid peroxidation products, measured by MDA derivatives, are higher in patients with COPD and are highest in COPD patients presenting with exacerbations [40–42]. Furthermore, the plasma levels of these substances have been reported to be higher in healthy smokers than in nonsmokers. Also Wofniak et al. support our result as proved that the level of lipid peroxidation products both in blood plasma and in erythrocytes was significantly higher in smoker controls than in the healthy nonsmokers [43]. Contrary to LPO products, we found that the mean concentration of nitric oxide (NO) was significantly higher in the control subjects (smokers and nonsmokers) (18.2 ± 0.8), (21.4 ± 1.1) μmol/L, respectively compared with (7.1 ± 0.4) μmol/L, in the COPD group (p = 0.001, 0.0001) respectively. Also the mean concentration of nitric oxide (NO), was significantly higher in control nonsmoker group (21.4 ± 1.1), compared to control smoker group (18.2 ± 0.8) with a p value of (p = 0.002). This result was supported by Çalıkoğlu et al. who found that mean levels of serum nitrite/nitrate in patients with chronic obstructive pulmonary disease were significantly lower than in the healthy control group and nitrite/nitrate levels were significantly lower in healthy smokers than in healthy nonsmokers [42]. Increased exhaled NO levels have been demonstrated by other researchers in patients with COPD, and a positive correlation has been suggested between exhaled NO and FEV1 [44,45].

Ichinose's study has shown that exhaled NO levels were significantly increased in asthma but not in COPD patients as compared with healthy subjects, although iNOS-immunopositive cells were observed to be almost of the same degree in asthma and COPD patients [46]. In accordance with these results, Ichinose suggests that NO produced in the airways appears to be consumed by its reaction with superoxide anion and/or by peroxidase-dependent nitrite oxidation [46].

Low NO levels in COPD patients may be explained by the reaction of NO with increased free oxygen radicals to form peroxynitrite which eventually leads to relatively low serum levels of NO [42]. In healthy controls, NO_2/NO_3 levels were significantly lower than in smokers than in nonsmokers. These results confirm the observations of Rutgers et al. and Corradi et al. demonstrated that exhaled NO levels were lower in smokers than in nonsmokers in both healthy subjects and COPD patients, and they suggested that smoking can cause chronic damage in cells which produce NO in airways [25,44]. Pryor and Stone have reported that gas-phase cigarette smoking contains 1015 radicals per puff, primarily alkyl and peroxyl types. In addition, nitric oxide is present in cigarette smoking in concentrations of 500–1000 ppm per puff [47]. Nitric oxide ($NO^{\bullet}$) reacts quickly with the super oxide anion ($O^{2\bullet-}$) to form peroxynitrite ($ONOO^-$). This compound is very harmful to cells and tissues [47]. Increased NO inhalation in smokers may increase the release of both NO and superoxide anion. These two compounds react rapidly to form peroxynitrite, thereby lowering serum NO levels. This situation may be a way to reveal the decrease of serum NO_2/NO_3 levels in healthy smokers. The other view is that increased inhalation of NO in cigarette smoke may have a down regulatory effect on nitric oxide synthase [25].

Our study showed that the mean concentrations of each of glutathione (GSH) was significantly higher in the control subjects (smokers and nonsmokers) (22.3 ± 0.6), (24.2 ± 0.5) mg/mol, respectively compared with 8.9 ± 1.1 mg/mol, in COPD patients, with p = 0.001, 0.001 respectively, the mean concentration of superoxide dismutase (SOD) was significantly higher in the control subjects (smokers and nonsmokers) (8.9 ± 0.6), (14.9 ± 1.1) μmol/L, respectively compared with 5.4 ± 0.7 μmol/L, in the COPD patients (p = 0.012, 0.001) respectively.

Also the mean concentration of superoxide dismutase (SOD) was significantly higher in control nonsmoker group (14.9 ± 1.1), compared to control smoker group (8.9 ± 0.6) with a p value of p = 0.001 and the mean concentration of catalase (CAT) was significantly higher in control nonsmokers (19.6 ± 0.6) μmol/mL, compared to COPD patients and control smokers (6.8 ± 0.3, 7.7 ± 0.6) μmol/mL, respectively, (p = 0.001, 0.018) respectively. Except the concentration of GSH-peroxidase (GSH-Px) was not significant difference in all study participants (COPD patients, control smoker, control nonsmokers), (12.2 ± 0.6, 12.1 ± 0.8, 12 ± 0.9) min/mL respectively. Several earlier studies have reported alterations in different endogenous antioxidants in COPD patients, which may increase or decrease depending on the defense response. SOD is an intracellular antioxidant enzyme that inhibits superoxide anion and protects aerobic cells against oxidative stress. Catalase is more effective in the presence of high H_2O_2; produced H_2O_2 may thus be scavenged by normal levels of catalase and glutathione, which is the primary defense against H_2O_2-mediated toxicity [48].

Tavilani et al. showed that SOD activity is significantly lower in smokers and COPD subjects than in non-smoker subjects. This enzyme is the only enzyme family with activity against superoxide radicals, and is mainly a cytosolic enzyme that expresses in the bronchial epithelium of human lung. This decrease can possibly be due to the response to increased reactive oxygen species production, which with severe or chronic oxidant exposure conditions may be inadequate to

detoxify high levels of reactive oxygen species, this is compatible with our results [17]. Additional studies are required to show the exact mechanisms that decrease catalase and SOD activities.

In the study of Tavilani et al. it is observed that there was no significant difference in plasma glutathione peroxidase and glutathione reductase activities in nonsmokers, smokers, and subjects with COPD [17], and this finding is compatible with the work of Montãno et al. [49], also compatible with our results regarding glutathione peroxidase, but not with reduced glutathione. Glutathione peroxidase is synthesized in bronchial epithelial cells, alveolar macrophages, and other lung cell lines, and is induced by hypoxia and decreased by exposure to ozone [50].

Ahmed et al. found a notable decrease in erythrocyte SOD and catalase activity in COPD patients than controls that might be a compensatory response to increased oxidative stress [12]. Rai et al. also reported decreased SOD activity in COPD patients [51]. It is well known that GPx plays a significant role in peroxyl scavenging mechanisms and in maintaining the functional integration of cell membranes. Ahmed et al. observed a significant decrease in GPx activity in COPD patients [12], similar findings have previously been reported by Kluchova et al. [52].

This might be due to hydroxyl and superoxide anion, which plays a key role in the activation of red cell GPx [53], these results are not consistent with our results; we observed no significant difference in all study participants (COPD patients, control smokers, control nonsmokers).

Some studies have reported alterations in the glutathione system in lung inflammatory conditions such as COPD. These alterations may be aggravated by the upregulation of gamma-glutamyl cysteine synthetase by cigarette smoke [38].

Ahmed et al. demonstrate that COPD patients have significantly higher levels of total blood glutathione [12], these results also not agree with our results, we observed that the glutathione (GSH) was significantly higher in the control subjects (smokers and nonsmokers) than COPD patients. Altuntaş et al. observed that there was no significance in each of the three parameters (LPO, GSH, and catalase) between smokers and patients with COPD, but a significant difference was observed in each of the three parameters between nonsmokers and patients with COPD (MDA: $p = 0.001$, GSH: $p = 0.028$ and catalase: $p < 0.001$) and between smokers and nonsmokers (LPO: $p = 0.035$, GSH: $p = 0.016$ and catalase: $p = 0.005$) [54].

Conclusions

The results of this study reinforce the evidence that oxidant/antioxidant imbalance is associated with airways obstruction. This study provides clear evidence for altered oxidant–antioxidant balance in COPD patients that increases in parallel with the severity of the disease. Further studies analyzing the pathophysiological mechanisms involved in lung injury related to oxidant/antioxidant imbalance are required.

Conflict of interest

There is no conflict of interest.

Acknowledgment

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