The effect of cigarette smoking on the oxidant–antioxidant imbalance in patients with chronic obstructive pulmonary disease

Mahmood A. Al-Azzawi[a], Azal A. Al-Rubaeaee[b], Adel H. Ghoneim[c], Mohamed M.N. AboZaid[c]

[a]College of Dentistry, Al-Ayen University, Dhi Qar, Iraq, [b]Department of Microbiology, College of Medicine, Babylon University, Iraq, [c]Department of Chest Diseases, Zagazig Faculty of Medicine, Zagazig University, Zagazig, Egypt

Correspondence to Dr. Mahmood A. Al-Azzawi, PhD, College of Dentistry, Al-Ayen University, Dhi Qar, Iraq. Tel: +202- 22685071; Mob: 00964-07811244735; e-mail: mmahmood41@yahoo.com

Received 20 November 2018
Accepted 6 February 2019

The Egyptian Journal of Chest Diseases and Tuberculosis 2019, 68:462–470

Background
Predisposing factors for chronic obstructive pulmonary disease (COPD) comprise endogenous genetic factors and exogenous factors such as positive and negative smoking, air pollution, inhalation of dust, and work-related chemicals. Because only a small percentage of smokers develop COPD, it is thought to arise as an outcome of environmental harm and a host's response system, which boosts the tendency of the disease to arise.
Objective
To clarify the effect of smoking on the level of oxidant–antioxidant in patients with COPD.
Patients and methods
Thirty patients with COPD (20 smokers and 10 nonsmokers) and 40 healthy individuals (18 smokers and 22 nonsmokers) participated in this study. The investigations included measurement of malondialdehyde (MDA), superoxide dismutase (SOD) activity, catalase (CAT) activity, glutathione peroxidase (GPx), glutathione reductase (GR), vitamin C, and glutathione (GSH) content.
Results
SOD, CAT, GPx, GSH, GR, and vitamin C were significant lower ($P<0.0001$) among patients with COPD (smokers and nonsmokers) than in the control group, whereas the MDA levels were elevated ($P<0.0001$) in patients with COPD (smokers and nonsmokers) when compared with the control group. Moreover, the values of SOD, GSH, and GPx were significantly lower ($P<0.001$) in smokers with COPD as compared with nonsmokers with COPD. Finally, there was no significant difference in the level of MDA between patients with COPD (smokers and nonsmokers).
Conclusion
There is a significant reduction in the level of some antioxidants (SOD, GSH, and GPx) in smokers with COPD compared with nonsmokers with COPD, but other antioxidants (CAT, GR, and vitamin C) did not change significantly, whereas the oxidant levels (i.e., MDA) were relatively convergent in patients with COPD (smokers and nonsmokers). This, in turn, points to the modest role of smoking in the causation of oxidant–antioxidant imbalance in this disease.

Keywords:
catalase activity, cigarette smoking, chronic obstructive pulmonary disease, glutathione peroxidase, malondialdehyde, oxidant–antioxidant, vitamin C

Egypt J Chest Dis Tuberc 68:462–470
© 2019 The Egyptian Journal of Chest Diseases and Tuberculosis
2090-9950

Introduction

Chronic obstructive pulmonary disease (COPD) is a pulmonary status with large systemic aspects and common morbidity, with adverse effect on characteristic of life [1]. It is differentiated by blockage of airflow and is correlated with lung inflammation and tissue destruction [2]. Cigarette smoking and air pollution were specified as important starting factors and risk for COPD as well as cell destruction and death seen in COPD owing to oxidative stress and carbonyl [3]. Only 20–30% of smokers develop COPD, and 10–15% develop lung cancer [4]. Another study indicated that only 15–20% of smokers develop COPD, and quitting smoking does not inhibit the development of the disease, with a persistent index of inflammatory cell regeneration in the lungs (especially induction of neutrophils) and oxidative stress [1]. This suggests a self-sustained endogenous origin of inflammation in susceptible individuals [5]. Persistent liberation of inflammatory mediators like interleukin 8 and

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

leukotriene B4 [6], in addition, promotes continuous recruitment and stimulation of neutrophils to the lungs. The release of proteases, free radicals, and cytokines of these stimulated cells has been involved in all aspects of COPD, including demolition of surrounding tissues, lack of lung flexibility [7], and hypersecretion of mucus [8], connected with emphysema and chronic bronchitis in COPD. Oxidative stress arises when exposure to free radicals is adequate to antagonize antioxidant defenses. These free radicals, called reactive oxygen species (ROS), are present everywhere, which arise during mitochondrial respiration, signaling, and when contributing to the damage and annihilation of pathogens [9]. Prevalent ROS comprises hydroxyl radical ($^{\bullet}OH^{-}$) and superoxide anion ($O_2^{\bullet\,-}$) containing unpaired electrons, and the unsteady nature of ROS allows the transfer of electrons to other molecules by oxidation, thereby damaging or disrupting or creating more ROS. Prospective objectives for damage by ROS involve proteins, lipids, and DNA [10].

The lung is predominately susceptible to oxidative stress because of the comparatively high oxygen environment, high blood supply, and vulnerability to environmental pathogens and toxins. Cigarettes and biomass smoke appended remarkably to this burden, and one puff of cigarette smoke is evaluated to have more than 1×10^{15} oxidant molecules within it [1]. In this study, we discuss the extent to which cigarette smoking is associated with an oxidant–antioxidant imbalance in patients with COPD and whether cigarette smoking is an important factor in causing this imbalance in these patients; especially some studies have shown that the percentage of smokers in this disease does not exceed 30%.

Patients and methods

Patients

Thirty stable (no exacerbation since at least 6 months) patients with COPD (mean age, 64.7±6.32 years; BMI, 28.241±3.410 kg/m^2) were included in this study. Of them, 20 were smokers and 10 were nonsmokers, with no exposure to negative smoking or occupational. Forty healthy participants (mean age, 45.30±11.41 years; 12 females and 28 males; 18 smokers and 22 nonsmokers) were studied as a control group. None of the control personnel had any historical, radiological, or spirometry evidences indicating chest troubles, even smokers, and lung function tests (>80%). The study was performed in accordance with the corresponding corporate ethics committees, and all included patients provided written informed consents. In this study, all participants underwent the following: detection of the full history, full clinical examination (general and local), plain chest radiography (posteroanterior view), spirometric pulmonary function tests, full blood picture, random blood sugar, liver function, and kidney function tests. Patients with COPD were diagnosed clinically by symptoms and signs (coughing, wheezing, dyspnea, and exercise intolerance), chest radiography, and ensured by spirometry. Spirometry was performed using Masterscreen Pneumo (Erich Jaeger, Wurzburg, Germany), with a calculation of the forced expiratory volume at the end of the first second (FEV1) (% of predicted) and the forced vital capacity (FVC). The proportion of FEV1/FVC is a criterion of airflow obstruction if it is less than 80%, depending on age. These calculations were conducted as stated by the standards of the European Respiratory Society and the American Thoracic Society [11]. The greatest values of FEV1 of three forced expiratory trials were used. The best FEV1, FVC, and FEV1/FVC rates were adopted for the analysis (FEV1/predicted FEV1%), namely, mild more than 80%; moderate, 50–79%; severe, 30–49%; and very severe, less than 30% (<50% but with respiratory failure). Very severe cases were not engaged in this study [12–14]. The exclusion criteria were as follows: (a) patients with chronic or acute diseases (liver diseases, renal diseases, heart diseases, thyroid disorders, coagulation disorders, hematologic problems, patients with diabetes mellitus, and patients with acute respiratory distress syndrome); (b) patients younger than 18 years; and (c) pregnant women.

Methods

Sampling

In a heparinized tube, 5 ml of venous blood was taken from all participants in the early morning, and the sample was centrifuged (2500 g for 10 min at 4°C). The plasma collected is used for the determination of vitamin C, and the red blood cells (RBCs) were washed with sodium chloride (0.9%) three times and were kept at −80°C until the performance of other biochemical tests. The samples were treated instantly after obtaining to reduce the potential oxidation of vitamin C.

Biochemical procedures

Catalase

The base of the method relies on the measurement of an absorbance, which decreases in the test sample by the induced dissolution of hydrogen peroxide (H_2O_2) in the existence of an analyte enzyme. This average is set down by estimating the decrease in absorbance every 30 s for 3 min at 240 nm in 1.5 ml of the reaction mixture comprising 13.2 mmol/l H_2O_2 in

50 mmol/l phosphate buffer (pH 7.0) and 0.1 ml of the RBC. The control is a combination of 50 mmol/l phosphate buffer (pH 7.0) and 0.1 ml of the cell homogenate [15].

Superoxide dismutase

The test is performed in two sets. The first tube (assay mixture) contains 1.0 ml of sodium pyrophosphate buffer, 0.2 ml of phenazine methosulfate, 0.3 ml of nitroblue tetrazolium, 1.0 ml distilled water, and 0.2 ml RBC (enzyme source). The second tube (blank) contains all the aforementioned reactants, excluding for enzyme source. The reaction was initiated by the addition of 0.1 ml NADH. After incubation at 30°C for 90 S, 1.0 ml glacial acetic acid was added to each tube to check the reaction. The absorbance has been read at 560 nm versus blank [16].

Glutathione peroxidase

The test mixture contained 0.1 ml H_2O_2 (2.5 mmol/l), 0.2 ml EDTA (0.8 mmol/l), 0.2 ml of reduced glutathione reductase (GR), 0.1 ml sodium azide (NaN_3) (10 mmol/l), 0.4 ml phosphate buffer (0.4 M) pH 7.0, and 0.2 ml of RBC. The mixture is incubated for 10 min at 37.8°C. The reaction was turned off by the addition of 0.5 ml of 10% trichloroacetic acid (TCA), and the tubes are centrifuged at 2000 rpm. Then, 3.0-ml disodium hydrogen phosphate (0.3 mmol/l) and 1.0 ml DTNB (0.04%) are added to the supernatant. The developed color was read photometrically at 420 nm immediately. The blank contains all the aforementioned reactants, except the enzyme source (RBC) [17].

Glutathione reductase

The test mixture includes 0.1 ml of RBCs to a total volume of 3.0 ml, including 0.2 ml oxidized glutathione (GSSG), 0.2 ml EDTA, 2.5 ml phosphate buffer, and 0.1 ml NADPH. The blank also runs at the same time with the test mixture to rectify the net differences in OD after NADPH auto-oxidation and contains all the aforementioned reactants, except the RBC. The addition of NADPH at the beginning of the reaction was followed at 340 nm. The decrease in absorbance at 340 nm is followed at 30 s period. The content of protein in the sample was also stated. Enzyme unit was described as nmole of NADPH oxidized per minute per mg protein [18].

Reduced glutathione

Overall, 0.5 ml of RBCs or plasma was precipitated with 2.0 ml (5%) trichloroacetic acid (TCA) and centrifuged at 6000g for 5 min. 2.0 ml of supernatant was mixed with 1.0 ml of Ellman's reagent (DTNB) and 4.0 ml (0.3 M) disodium hydrogen phosphate. The yellow color that appeared was read at 412 nm. A set of standards (20–100 μg) was processed in a similar way with a blank consisting 1.0 ml of buffer [19].

Lipid peroxide levels

Lipid peroxidation values were evaluated by the reaction of thiobarbituric acid (TBA) by the method of Ohkawa *et al.* [20]. This method was applied to calculate the color developed by the reaction of TBA with malondialdehyde (MDA) at 532 nm. Overall, 50 μl of erythrocyte supernatant and 2 μl of butylated hydroxytoluene in methanol were added to test tubes. Then, 50 μl of TBA solution and 50 μl of acid reagent (1 M phosphoric acid) were added. The tubes were stirred repeatedly and incubated for 60 min at 60°C. Then, the mixture was centrifuged at 10 000*g* for 3 min. The absorbance of the supernatant was measured at 532 nm. Standard solution of MDA (10 nmol) was turned on at the same time. Lipid peroxide content in the sample expressed as nmol of MDA/dl.

Vitamin C

Vitamin C levels were measured by the method described by Omaye *et al.* [21]. The assay mixture includes 0.5 ml of RBC, 1.0 ml of (10%) TCA, and 0.5 ml of distilled water, mixed completely, and then centrifuged for 20 min. In 1.0 ml of the supernatant, 0.2 ml of DTC reagent is added. The mixture is incubated at 37°C for 3 h. Then, 1.5 ml of 65% sulfuric acid is added. Mixed well and allowed to stand 30 min at room temperature. The color that appeared was read at 520 nm. Graduated quantities of standards were also processed in the same manner. The vitamin C level was described as μg/ml.

Statistical analyses

The results were described as the mean±SD. Statistical analysis of the results was carried out employing the statistical package software (SPSS), version 20.0 (SPSS Inc., Chicago, IL, USA). Student's *t* test was used to compare two groups of normally distributed variables. The results of the *t* value is then examined on Student's *t* table to detect the significance level (*P* value) in accordance with the degree of freedom. All the above tests applied as tests of significance at *P* value less than 0.05 [22].

Results

Table 1 shows the clinical and physiological features of patients with COPD. Table 2 and Figure 1 show a comparison for the oxidant–antioxidant activity

Table 1 Clinical and physiological characteristics of chronic obstructive pulmonary disease group

Clinical and physiological parameters	Values
Age (year) (mean±SD)	64.7±6.32
Male/female (*n*)	21/9
BMI (kg/m^2) (mean±SD)	28.71±1.36
Smoking status: smokers/nonsmokers (*n*)	20/10
Pack years in smokers (mean±SD)	56.6±1.8
FEV1/FVC (%) (mean±SD)	60±3.8

FEV1, forced expiratory volume at the end of the first second; FVC, forced vital capacity.

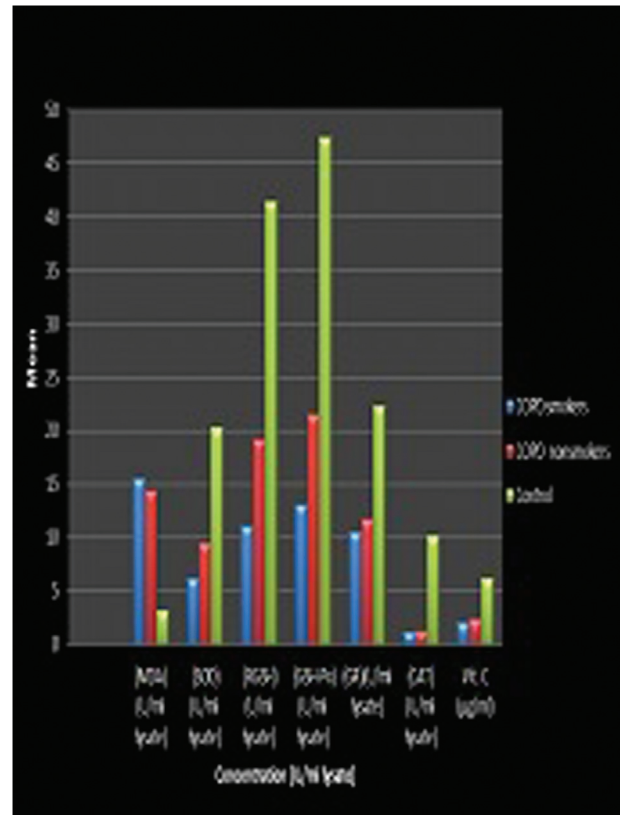
Table 2 Oxidant levels and antioxidant status of the study groups

	COPD smokers (*n*=20) (mean ±SD)	COPD nonsmokers (*n*=10) (mean±SD)	Control (*n*=40) (mean ±SD)	*P* value
Malondialdehyde (U/ml lysate)	15.51 ±1.95	14.4±1.67	3.23 ±0.29	0.0001[a] 0.0001[b] –
Superoxide dismutase (U/ml lysate)	6.23 ±0.71	9.5±0.94	20.32 +1.93	0.0001[a] 0.0001[b] 0.001[c]
Reduced glutathione (U/ml lysate)	11.12 ±1.2	19.2±1.36	41.53 ±1.54	0.0001[a] 0.0001[b] 0.001[c]
Glutathione peroxidase (U/ml lysate)	13.1 ±0.91	21.5±1.7	47.5 ±1.82	0.0001[a] 0.0001[b] 0.001[c]
Glutathione reductase (U/ml lysate)	10.5 ±0.82	11.8±0.92	22.3 +1.62	0.0001[a] 0.0001[b] –
Catalase (U/ml lysate)	1.2±0.12	1.33±0.34	10.2 ±0.45	0.0001[a] 0.0001[b] –
Vitamin C (μg/ml)	2.110 ±0.509	2.412±0.880	6.130 ±0.124	0.0001[a] 0.0001[b] –

COPD, chronic obstructive pulmonary disease. [a]Significance between smoker COPD and control. [b]Significance between nonsmoker COPD and control. [c]Significance between smoker COPD and nonsmoker COPD.

between patients with COPD (smokers and nonsmokers) and controls on the one hand and between smoker patients with COPD and nonsmoker patients with COPD on the other hand. The concentrations of MDA, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione (GSH), GR, and vitamin C of patients with COPD (smokers and nonsmoker) and controls are shown in (Table 2). The evaluated levels of SOD, CAT, GSH, GR, GPx, and vitamin C were significantly lower ($P<0.0001$) in the patients with COPD (smoker and nonsmoker) compared with controls. The indicator of oxidative stress MDA was significantly elevated ($P<0.0001$) in patients with COPD (smoker and nonsmoker) in comparison with controls. The results of this study revealed that the level

Figure 1



Oxidant and antioxidant levels of the study groups.

of MDA in smoker patients with COPD compared with nonsmoker patients with COPD did not vary significantly, but there is a relative elevation in the oxidative stress marker (MDA) in the smoker patients with COPD. We did observe that only some of the antioxidants (SOD, GSH, and GPx) were significantly lower ($P<0.001$) in smoker patients with COPD in comparison with nonsmoker patients with COPD, but there is no significant variance for the rest antioxidants (CAT, GR, and vitamin C). Despite that, we detected a relative decrease in the markers of all antioxidants in the smoker patients with COPD.

Discussion

Of proven facts, that increased oxidants and/or decreased in antioxidant levels may act to reverse the physiological balance of oxidant-antioxidant in favor of oxidants, thus oxidative stress may contribute in the causing of COPD [1]. The present study detected that patients with COPD have remarkable heightened oxidative stress, as described in the greater values of lipid peroxidation (LPO) products. This is associated with variations in activity of some enzymatic antioxidant in the blood and plasma, comprising SOD, CAT, GSH, GR, GPx, and vitamin C. The results of this study observed that oxidative stress is a

serious pathophysiologic variation in patients with COPD. The excess in lipid peroxide products in patients with COPD enhances the importance of oxidative stress and its association in the pathogenesis of this disease [23]. Based on our findings, we observed a slight increase in oxidants in smoker patients with COPD compared with nonsmoker patients, and we found that only some antioxidants (not the all) were significantly decreased in smoker patients with COPD when compared with nonsmoker patients. Our results showed a significant decrease ($P<0.0001$) in antioxidant values of CAT, GPx, SOD, GSH, GR, and vitamin C in patients with COPD (smoker and nonsmoker) when compared with the control group, whereas MDA values were greater in patients with COPD (smokers and nonsmokers) ($P<0.0001$) in comparison with controls. Moreover, the results showed that only some of the antioxidants (SOD, GSH, GPx) were significantly lower ($P<0.001$) in smoker patients with COPD in comparison with nonsmoker patients with COPD, but we did not observe a significant decrease for the rest of antioxidants (CAT, GR, and vitamin C). Regardless, we found a relative decrease in the markers of all antioxidants in the smoker patients with COPD. Several studies have also indicated an elevated level of plasma MDA in healthy smokers and patients with COPD [24]. COPD is one of the most common pulmonary diseases resulting from cigarette smoking and in itself indicates the prevalence of smoking in any country [25]. A previous study noted that in healthy smokers and smokers with COPD, a final product of lipid peroxidation is established in airway endothelial cells, epithelial cells, neutrophils, and macrophages [26]. They assumed that the oxidative stress caused by cigarette smoking looks to be more evident in those who have experienced COPD. Moreover, another study exhibited that there is no relation between the marker of oxidative stress (MDA) and levels of H_2O_2 in COPD related to cigarette smoking [27]. Despite our results should be considered as preliminary, we have observed an inclination in MDA levels in individuals with COPD related to smoking. Oxidative stress is chemically linked to an increase in the generation of oxidants or a significant reduction in the activity of antioxidant protection, such like GSH [28]. Generation of ROS is the mirror that reflects the damaging side of oxidative stress and includes peroxides and free radical species. Several of these species are less interactive (e.g. superoxide) but can be changed through oxidoreduction reactions with transition metals or other redox compounds (e.g. Quinones) to greater damaging species that can cause long-term cellular deterioration [29]. Certainly, DNA damage causes serious and long-term changes [30]. The most common enzyme related to H_2O_2 damage and expressed in the intracellular alveolar macrophages is the CAT enzyme. A comparison of the effects of oxidative stress and antioxidant activity between patients and healthy participants has been identified by the neutrophil study. Several studies have demonstrated the presence of oxidative stress in the systemic circulation and airways of patients with COPD [24,31]. The activity of antioxidant defense mechanisms against oxidative stress in COPD and systemic changes in them is not fully revealed [32]. Previous studies have shown that there is an elevation of oxidative stress in patients with COPD, which appear in the form of an excessive increase in the production of LPO compounds and diminished total activity of antioxidants [33]. Moreover, antioxidants are indicators of oxidative stress. An antioxidant has been expressed as any substance that when exist in a depressed concentrations compared to that of an oxidizable substrate significantly delays or prevents the oxidation of that substrate. Plasma includes a different set of antioxidants. The lack of antioxidants in the plasma may result in permanent oxidative stress. A state of oxidative stress arises when antioxidants decrease and oxidative substances increase, which cause severe molecular and tissue damage [34]. Apart from some classifications, vitamin C has been considered as a standard for the biochemical estimation of vitamin C state [35]. Vitamin C is particularly influential because it inhibits or reduces plasma lipid peroxidation *in vitro,* which is induced by the gas phase of cigarette smoke [36]. Some studies have shown that dietary antioxidants (e.g. vitamins A, C, and E) may have a protective role in smokers [37]. Several studies have indicated lower concentrations of antioxidants in patients with COPD compared with healthy people [33,38,39]. However, other studies have failed to come up with that antioxidants have a protective effect on lung functions [40]. Molecular mechanisms related to the interpretation of the protective side of antioxidants in COPD still lack detection and clarity [41,42]. Regarding our results, we indicated previously that only some of the antioxidants (SOD, GSH, and GPx) were significantly lower ($P<0.001$) in smoker patients with COPD as compared with nonsmoker patients with COPD, but we did not observe a significant difference for the rest of antioxidants (CAT, GR, and vitamin C). However, we observed a relative decrease in all of antioxidant markers associated with the smoker patients. Numerous

previous studies have detected variations in various endogenous antioxidants in patients with COPD, and this may be stimulated or inhibited relying on the defense response. Research has shown that the SOD which is an enzymatic antioxidant is able to restrain superoxide anion and provide protection for aerobic cells against the risk of oxidative stress. CAT has been indicated to be more effective with high levels of H_2O_2. Therefore, the amounts of H_2O_2 produced can be scavenged by the presence of normal levels of antioxidants such as CAT and GSH, which form a defensive line against H_2O_2-mediated toxicity [43]. A study [44] showed a marked decrease in erythrocyte CAT and SOD function in patients with COPD compared with controls and could be a form of compensatory reaction to elevated oxidative stress. Moreover, another study [45] revealed reduced SOD function in patients with COPD. It is scientifically proven that GPx provides an important role in the mechanisms of pyroxyl scavenging and in keeping the functional integrity of the cell membrane. It has been perceived that there is considerable reduction in GPx function in patients with COPD [44]; comparable results also have formerly been observed [40]. This can be because of the activity of superoxide anion and hydroxyl, which acts as a critical part in the stimulation of GPx in the erythrocyte [46]. Regarding the main topic in this study, which is that smoking has an important role in the imbalance of antioxidant oxidation in patients with COPD, although this study is preliminary, it showed that smoking does not play an important role in causing the mentioned imbalance. We certainly need more studies in this regard with a larger number of patients and healthy people, taking into account many other factors that are relevant to the participants in this study [e.g. accurate diagnosis of the disease, make sure that the participants were smokers or nonsmokers, cigarette smoking severity in pack years, stages of the disease (mild, moderate, severe), probability of other diseases, and exacerbation incidence] to prove or disprove the findings of this study and pave the way for more specialized studies. The emergence of oxidative stress from sources other than cigarette smoking has been supported by many studies as it was proved beyond doubt that there are other sources of exposure that can increase the risk of developing COPD. Mostly, all of these exposures are related to particles [47,48]. These exposures could involve biomass burning and air pollution particles. Most of these particulate exposures, ecological and vocational, can lead to oxidative stress risks. ROS are produced either immediately from the particles that support inappropriate electron transmits or from the direct interaction of cell proteins with particles (such as, electronically transmit compounds in the mitochondria). Oxidative stress occurs after exposure to the particles as a primary phase in biological action [49]. Exposure of cells to particles in vitro results in the production of a large-scale oxidative stress in different types of cells. It also confirmed the generation of oxidizing substances after exposure to the particles *in vivo* [50]. The role of genetic and epigenetic effect on oxidative stress status in COPD also received special attention in such studies. Apoptosis, senility of respiratory epithelial cell, and damage of DNA occur as a result of the activation of lipid peroxidation and carbonyl stress by cigarette smoke [51]; such crucial pathways participate in emphysema and reshaping of the airway. Nevertheless, just a small percentage of cigarette smokers can develop COPD, confirming the significance of environmental harm and a host's response in governing cigarette smoke-provoked oxidative stress and COPD. To investigate the genetic factors inducing COPD, a number of scientific strategies have been taken into account. It is believed that the oxidative stress caused by cigarette smoking contributes effectively to causing many diseases and physiological disorders, including COPD [52]. However, a number of studies have shown that a small percentage of smokers may have COPD, which leads us to think that factors other than cigarette smoking may play an important role in the development of oxidative stress or may in some way regulate the oxidative stress caused by cigarette smoke. There is clearly a hidden hand working in the dark to promote the state of imbalance between oxidants and antioxidants, contributing to the development of COPD and other diseases [53]. To uncover these factors, scientists took it upon themselves to study the participants through a set of trends. Some of them concentrated on the study of transcription and regulation of the epigenetic of certain molecular and cellular pathways that interfere with certain respiratory disorders, whereas a group of researchers studied large groups of patients at the genetic level to detect candid genome-extensive combinations and find out single nucleotide polymorphisms (SNPs) that may be consistent with the disease [54]. The genes that regulate oxidants such as P450, CYP2C18, and aryl hydrocarbon receptor nuclear translocator like 2, which encodes a basic helix-loop-helix transcription factor (active at low atmospheric and cellular oxygen levels), have been more frequently expressed in patients with COPD [55]. By adopting the principle of the candidate gene, it is concluded that there is a relationship between many mutations that occur in the genes of antioxidants and the intensity of

COPD. Polymorphisms in SOD3, glutathione S-transferase M1 (GSTM1), and glutathione S-transferase P (GSTP1) were attendant with reduced lung function and higher frequency of COPD hospitalization, adding to the increased potential incidence of exacerbations [56]. Moreover, it has been specified that SNPs in microsomal epoxide hydrolase (EPHX1), surfactant protein B (SFTPB), transforming growth factor beta 1 (TGFB1), and latent transforming growth factor beta binding protein 4 (LTBP4) were associated with airflow obstruction, dyspnea symptoms, and intolerance of exercise capacity [57]. Oxidative stress also stimulates some epigenetic regulative processes. Through various proper pathways, muscle-related miRNAs control muscle progression and rehabilitation of damage. In COPD patients, the inspiratory burdens to which the respiratory muscle is continuously exposed may be a major player accounting for this specified pattern of miRNA expression. In different stages (mild, moderate, and severe) of patients with COPD, expression of several muscle-related miRNAs miR-1, miR-133, and miR-206 in inspiratory muscles has been downregulated [58]. These epigenetic events are potentially act as biological adaptive mechanisms to better conquer the persistent respiratory burden enforced to the respiratory system in COPD. It has become known that cigarette smoking is correlated with the epigenetic modifications in the airway epithelium [59,60] and that these epigenetic modifications play a role in the regulation of respiratory inflammation [61,62]. Thus, focusing on epigenetic mechanisms may provide a broader ground for research into the therapeutic field of COPD. Methylation of DNA is considered one of these regulative mechanisms. In human and other mammal cells, methylation of DNA mostly places in the cytosine residues in the cytosine-phosphate-guanine (CpG) dinucleotides [63]. The large variation in the smokers' tendency to develop COPD may be partly interpreted by changes in DNA modification. Screening of more than 27 000 genes at the genome level around the DNA methylation has shown that 349 CpG sites are closely related to the severity of COPD and reduced lung function [64]. It was also observed that NAD-dependent deacetylase sirtuin-1 levels have been decreased in patients with COPD, indicating that the expression of class I histone deacetylase (HDAC) and oxidative stress are correlated with COPD [65]. Several polymorphisms related to COPD have been recognized by whole genome association study (WGAS). However, the incidence of these SNPs in the people does not clarify the greater frequency of the disease, making attention to support the notion that environmental oxidants and/or epigenetic modifications have a profound effect on the phenotype [66]. Standards for the severity of COPD have been determined by studying thousands of participants' phenotypes by GOLD, where smokers with obstruction airflow were compared with smokers who did not have obstruction airflow. The studies revealed weighty levels of SNP expression on chromosomes (chr) 15 covering some genes comprising α-neuronal nicotinic acetylcholine receptor subunit (CHRNA3/5), and iron-responsive element-binding proteins 2, also known as IRE-BP2 [67,68]. A significant regulator for oxidative stress and apoptosis in the lung cells of patients with COPD, namely, family with sequence similarity 13 member A (FAM13A) on chromosome (chr) 4, had been specified.[69]. In a COPD gene study that included approximately 7000 patients with moderate to severe COPD and approximately 6000 healthy control from different ethnic origin, three recognized COPD GWAS gene candidates were determined: CHRNA3, FAM13A, and Hedgehog interacting protein (HHIP). Furthermore, three recent combinations were determined: Ras and Rab interactor 3 (RIN3), matrix metalloproteinase-12 (MMP12), and transforming growth factor beta 2 (TGFB2) [70]. After defining and comparing the phenotypes, new groups at the genome level of COPD have become apparent. WGAS was performed for a phenotype of emphysema by a chest computed tomography scan in more than 2000 patients with COPD, and indicated that there is a near-important relationship through the genomic area including protein bicaudal D homolog 1 (BICD1) and severe emphysema [71]. There are a number of target genes that enhance the predisposition of COPD through oxidative-mediated pathways. In addition, oxidation signaling pathways play a key role in chronic bronchitis-related airway remodeling. Future insights needed to understand how genetic factors play a major role in COPD require early study of the disease in children and even infants taking into account all hereditary epigenetic factors. If the factors that caused reduced lung growth or the antioxidant capacity are detected, this will lead us to new ways of preventing or treating the disease.

Conclusion

This study evidenced that the high level of MDA, the final product of oxidant stress, seems to be more significant in Patients with COPD. Simultaneously, a considerable decrease in the responses of defensive

antioxidant was found to be related to both smoker and nonsmoker patients with COPD. Oxidant–antioxidant imbalance appears to be a major incident associated with the onset of COPD. This study focused on the extent of cigarette smoking association with oxidative stress. In this regard, the study found that the Patients with COPD (smokers and nonsmokers) have neither significant difference in the marker of oxidative stress (MDA) nor in all the antioxidants (but in the some of them). This indicates the humble role of cigarette smoking in causing the oxidant–antioxidant imbalance of patients with COPD, and thus leads us to search for other causes of this imbalance, especially the genetic factor. As we have mentioned earlier that this study is a preliminary study and we need to repeat it, taking accurately into consideration all the aspects and condition associated with patients and methods, to prove the results or refute them and to start a serious work, which can lead us to an explanation for the oxidant–antioxidant imbalance that occurred in patients with COPD.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

References

1 McGuinness A, Sapey E. Oxidative stress in COPD sources, markers, and potential mechanisms. J Clin Med 2017; 6:21.

2 Huang G, Xu XC, Zhou JS, Li ZY, Chen HP, Wang Y, *et al.* Neutrophilic inflammation in the immune responses of chronic obstructive pulmonary disease: lessons from animal models. J Immunol Res 2017; 2017:7915975.

3 Barnes PJ. Inflammatory mechanisms in patients with chronic obstructive pulmonary disease. J Allergy Clin Immunol 2016; 138:16–27.

4 Green CE, Turner AM. Role of chronic obstructive pulmonary disease in lung cancer pathogenesis. World J Respirol 2013; 3:67–76.

5 Alvarado A. Autoimmunity in chronic obstructive pulmonary disease: un update. Clin Res Trials 2018; 4:1–9.

6 Stone H, McNab G, Wood AM, Stockley RA, Sapey E. Variability of sputum inflammatory mediators in COPD and α1-antitrypsin deficiency. Eur Respir J 2012; 40:561–569.

7 Russel RE, Thorley A, Culpitt SV, Dodd S, Donnelly LE, Demattos C, *et al.* Alveolar macrophage-mediated elastolysis: roles of matrix metalloproteinases, cysteine, and serine proteases. Am J Physiol Lung Cell Mol Physiol 2002; 283:867–873.

8 Voynow JA, Young LR, Wang Y, Horger T, Rose MC, Fischer BM, *et al.* Neutrophil elastase increases muc5ac mRNA and protein expression in respiratory epithelial cells. Am J Physiol 1999; 276:835–843.

9 Al-Dalaen SM. Review article: oxidative stress versus antioxidants. Am J Biosci Bioeng 2014; 2:60.

10 Birben E, Sahiner UM, Sackesen C, Erzurum S, Kalayci O. Oxidative stress and antioxidant defense. World Allergy Organ J 2012; 5:9–19.

11 Quanjer PH, Tammeling GJ, Cotes JE, Pedersen OF, Peslin R, Yernault JC. Lung volumes and forced ventilatory flows. Report of the working party for standardization of lung function tests, European community for steel and coal. Official statement of the European Respiratory Society. Eur Respir J 1993; 16:5–40.

12 Zhu B, Xiao C, Zheng Z. Vitamin D deficiency is associated with the severity of COPD: a systematic review and meta-analysis. Int J Chron Obstruct Pulmon Dis 2015; 10:1907–1916.

13 Mahlin C, von Sydow H, Osmancevic A, Emtner M, Gronberg AM, Larsson S, Slinde F. Vitamin D status and dietary intake in a Swedish COPD population. Clin Respir J 2014; 8:24–32.

14 Janssens W, Bouillon R, Claes B, Carremans C, Lehouck A, Buysschaert I, *et al.* Vitamin D deficiency is highly prevalent in COPD and correlates with variants in the vitamin D-binding gene. Thorax 2010; 65:215–220.

15 Aebi H. Catalase in vitro. Meth Enzymol 1984; 105:121–126.

16 McCord JM, Fridovich I. Superoxide dismutase. An enzymic function for erythrocuprein (hemocuprein). J Biol Chem 1969; 244:6049–6055.

17 Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med 1967; 70:158–169.

18 Hazelton GA, Lang CA. Glutathione contents of tissues in the aging mouse. Biochem J 1980; 188:25–30.

19 Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys 1959; 82:70–77.

20 Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979; 95:351–358.

21 Omaye ST, Turnbull JD, Sauberlich HE. Selected methods for the determination of ascorbic acid in animal cells, tissues, and fluids. Meth Enzymol 1979; 62:3–11.

22 Morton RF. Medical statistics. In Morton RF, Hebel JR, McCarter RJ, eds. A study guide to epidemiology and biostatistics. 5th ed. Maryland: Aspen Publication, Gaithersburg; 2001. 71–74

23 Cristóvão C, Cristóvão L, Nogueira F, Bicho M. Evaluation of the oxidant and antioxidant balance in the pathogenesis of chronic obstructive pulmonary disease. Rev Port Pneumol 2013; 19:70–75.

24 Barnes PJ, Shapiro SD, Pauwels RA. Chronic obstructive pulmonary disease: molecular and cellular mechanisms. Eur Respir J 2003; 22:672–688.

25 Gan W, Man S, Senthilselvan A, Sin D. Association between chronic obstructive pulmonary disease and systemic inflammation: a systematic review and a meta-analysis. Thorax 2004; 59:574–680.

26 Rahman I, van Schadewijk AA, Crowther AJ, Hiemstra PS, Stolk J, MacNee W, *et al.* 4-Hydroxy-2-nonenal, a specific lipid peroxidation product, is elevated in lungs of patients with chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2002; 166:490–495.

27 Nowak D, Kasielski M, Antczak A, Pietras T, Bialasiewicz P. Increased content of thiobarbituric acid-reactive substances and hydrogen peroxide in the expired breath condensate of patients with stable chronic obstructive pulmonary disease: no significant effect of cigarette smoking. Respir Med 1999; 93:389–396.

28 Schafer FQ, Buettner GR. Redox environment of the cell as viewed through the redox state of the glutathione disulfide/glutathione couple. Free Radic Biol Med 2001; 30:1191–1212.

29 Valko M, Morris H, Cronin MT. Metals, toxicity and oxidative stress. Curr Med Chem 2005; 12:1161–1208.

30 Evans MD, Cooke MS. Factors contributing to the outcome of oxidative damage to nucleic acids. BioEssays 2004; 26:533–542.

31 van der Vaart H, Postma DS, Timens W, ten Hacken NH. Acute effects of cigarette smoke on inflammation and oxidative stress: a review. Thorax 2004; 59:713–721.

32 Tavilani H, Nadi E, Karimi J, Goodarzi MT. Oxidative stress in COPD patients, smokers, and non-smokers. Respir Care 2012; 57:2090–2094.

33 Rahman I, Morrison D, Donaldson K, MacNee W. Systemic oxidative stress in asthma, COPD, and smokers. Am J Respir Crit Care Med 1996; 154:1055–1060.

34 Gutteridge JM. Lipid peroxidation and antioxidants as biomarkers of tissue damage. Clin Chem 1995; 41:1819–1828.

35 Omaye ST, Turnbull JD, Sauberlich HE. Selected methods for determination of ascorbic acid in animal cells, tissues and fluids. In McCormick DB, Wright LD eds. Methods of enzymology, vitamins and coenzymes. New York: Academic Press 1979. 3–11

36 Cross CE, O'Neill CA, Reznick AZ, Hu ML, Marcocci L, Packer L, *et al.* Cigarette smoke oxidation of human plasma constituents. Ann N Y Acad Sci 1993; 686:72–89.

37 Vestbo J. Epidemiology. In Voelkel NF, MacNee W, eds. Chronic obstructive lung diseases. Canada: BC Decker Inc. 2002. 41–55

38 Agacdiken A, Basyigit I, Özden M, Yildiz F, Ural D, Maral H, *et al.* The effects of antioxidants on exercise-induced lipid peroxidation in patients with COPD. Respirology 2004; 9:38–42.

39 Nadeem A, Raj HG, Chhabra SK. Increased oxidative stress and altered levels of antioxidants in chronic obstructive pulmonary disease. Inflammation 2005; 29:23–32.

40 Kluchova Z, Petrasova D, Joppa P, Dorkova Z, Tkacova R. The association between oxidative stress and obstructive lung impairment in patients with COPD. Physiol Res 2007; 56:51–56.

41 Foronjy R, Wallace A, d'Armiento J. The pharmokinetic limitations of antioxidant treatment for COPD. Pulm Pharmacol Ther 2008; 21:370–379.

42 Rahman I. Antioxidant therapeutic advances in COPD. Ther Adv Respir Dis 2008; 2:351–374.

43 van Asbeck BS, Hoidal J, Vercellotti GM, Schwartz BA, Moldow CF, Jacob HS. Protection against lethal hyperoxia by tracheal insufflation of erythrocytes: role of red cell glutathione. Science 1985; 227:756–759.

44 Ahmad A, Shameem M, Husain Q. Relation of oxidant-antioxidant imbalance with disease progression in patients with asthma. Ann Thorac Med 2012; 7:226–232.

45 Rahman I, Biswas SK, Jimenez LA, Torres M, Forman HJ. Glutathione, stress responses, and redox signaling in lung inflammation. Antioxid Redox Signal 2005; 7:42–59.

46 Tsukamoto M, Tampo Y, Sawada M, Yonaha M. Paraquat-induced oxidative stress and dysfunction of the glutathione redox cycle in pulmonary microvascular endothelial cells. Toxicol Appl Pharmacol 2002; 178:82–92.

47 Balmes J, Becklake M, Blanc P, Henneberger P, Kreiss K, Mapp C, *et al.* Environmental and Occupational Health Assembly, American Thoracic Society. American Thoracic Society Statement: occupational contribution to the burden of airway disease. Am J Respir Crit Care Med 2003; 167:787–797.

48 Sangani RG, Ghio AJ. Lung injury after cigarette smoking is particle related. Int J Chron Obstruct Pulmon Dis 2011; 6:191–198.

49 Tao F, Gonzalez-Flecha B, Kobzik L. Reactive oxygen species in pulmonary inflammation by ambient particulates. Free Radic Biol Med 2003; 35:327–340.

50 Kadiiska MB, Mason RP, Dreher KL, Costa DL, Ghio AJ. In vivo evidence of free radical formation in the rat lung after exposure to an emission source air pollution particle. Chem Res Toxicol 1997; 10:1104–1108.

51 Kirkham PA, Barnes PJ. Oxidative stress in COPD. Chest 2013; 144:266–273.

52 Boukhenouna S, Wilson MA, Bahmed K, Kosmider B. Reactive oxygen species in chronic obstructive pulmonary disease. Oxid Med Cell Longev 2018; 2018:5730395.

53 Louhelainen N, Rytilä P, Haahtela T, Kinnula VL, Djukanović R. Persistence of oxidant and protease burden in the airways after smoking cessation. BMC Pulm Med 2009; 9:25.

54 Kan M, Shumyatcher M, Himes B. Using omics approaches to understand pulmonary diseases. Respir Res 2017; 18:149.

55 Steiling K, Lenburg ME, Spira A. Personalized management of chronic obstructive pulmonary disease via transcriptomic profiling of the airway and lung. Ann Am Thorac Soc 2013; 10:190–196.

56 Postma DS, Kerkhof M, Boezen HM, Koppelman GH. Asthma and chronic obstructive pulmonary disease: common genes, common environments? Am J Respir Crit Care Med 2011; 183:1588–1594.

57 Hersh CP. Pharmacogenetics of chronic obstructive pulmonary disease: challenges and opportunities, Pharmacogenomics 2010; 11:237–247.

58 Puig-Vilanova E, Aguiló R, Rodríguez-Fuster A, Martínez-Llorens J, Gea J, Barreiro E. Epigenetic mechanisms in respiratory muscle dysfunction of patients with chronic obstructive pulmonary disease. PLoS One 2014; 9: e111514.

59 Belinsky SA, Palmisano WA, Gilliland FD, Crooks LA, Divine KK, Winters SA, *et al.* Aberrant promoter methylation in bronchial epithelium and sputum from current and former smokers. Cancer Res 2002; 62:2370–2377.

60 Barnes PJ. Reduced histone deacetylasein COPD: clinical implications. Chest 2006; 129:151–155.

61 Adcock IM, Tsaprouni L, Bhavsar P, Ito K. Epigenetic regulation of airway inflammation. Curr Opin Immunol 2007; 19:694–700.

62 Barnes PJ, Adcock IM, Ito K. Histone acetylation and deacetylation: importance in inflammatory lung diseases. Eur Respir J 2005; 25:552–563.

63 Li E. Chromatin modification and epigenetic reprogramming in mammalian development. Nat Rev Genet 2002; 3:662–673.

64 Qiu W, Baccarelli A, Carey VJ, Boutaoui N, Bacherman H, Klanderman B, *et al.* Variable DNA methylation is associated with chronic obstructive pulmonary disease and lung function. Am J Respir Crit Care Med 2012; 185:373–381.

65 Lawless MW, O'Byrne KJ, Gray SG. Oxidative stress induced lung cancer and COPD: opportunities for epigenetic therapy. J Cell Mol Med 2009; 13:2800–2821.

66 Weiss ST. What genes tell us about the pathogenesis of asthma and chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2010; 181:1170–1173.

67 Pillai SG, Ge D, Zhu G, Kong X, Shianna KV, Need AC, *et al.* ICGN Investigators. A genomewide association study in chronic obstructive pulmonary disease (COPD): identifcation of two major susceptibility loci. PLoS Genet 2009; 5:e1000421.

68 Cho MH, Boutaoui N, Klanderman BJ, Sylvia JS, Ziniti JP, Hersh CP, *et al.* Variants in FAM13A are associated with chronic obstructive pulmonary disease. Nat Genet 2010; 42:200–202.

69 Repapi E, Sayers I, Wain LV, Burton PR, Johnson T, Obeidat M, *et al.* Genome-wide association study identifies five loci associated with lung function. Nat Genet 2010; 42:36–44.

70 Cho MH, McDonald ML, Zhou X, Mattheisen M, Castaldi PJ, Hersh CP, *et al.* NETT Genetics, ICGN, ECLIPSE and COPD Gene Investigators. Risk loci for chronic obstructive pulmonary disease: a genome-wide association study and metaanalysis. Lancet Respir Med 2014; 2:214–225.

71 Kong X, Cho MH, Anderson W, Coxson HO, Muller N, Washko G, *et al.* ECLIPSE Study NETT Investigators. Genome-wide association study identifies BICD1 as a susceptibility gene for emphysema. Am J Respir Crit Care Med 2011; 183:43–49.