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EFFECTS OF OXIDANTS AND ANTIOXIDANTS IMBALANCE ON DYNAMIC PULMONARY FUNCTION IN INDIAN COPD PATIENTS

Physiology

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ABSTRACT

An oxidant/antioxidant imbalance is thought to play an important role in the pathogenesis of chronic obstructive pulmonary disease (COPD). We hypothesized that antioxidant capacity reflected by erythrocyte glutathione peroxidase (GPx) and superoxide dismutase (SOD) activities, and serum levels of the lipid peroxidation product malondialdehyde (MDA), may be related to the severity of obstructive lung impairment in patients with COPD. Erythrocyte GPx and SOD activities and serum levels of MDA were measured in 120 consecutive male participants. Erythrocyte GPx activity and erythrocyte SOD activities were significantly lower, and serum MDA levels were significantly higher in patients with COPD compared to non COPD. Findings of the present study suggest that antioxidant capacity reflected by erythrocyte GPx activity and erythrocyte SOD and serum levels of the lipid peroxidation product MDA are linked to the severity of COPD.

KEYWORDS

COPD; oxidant/antioxidant imbalance; glutathione peroxidase (GPx) ; superoxide dismutase (SOD) ; lipid peroxidation; malondialdehyde (MDA)

INTRODUCTION

The lungs are continuously exposed to oxidants generated either endogenously (e.g. released from phagocytes or intracellular oxidants, e.g. from mitochondrial electron transport) or exogenously (e.g. air pollutants or cigarette smoke). The lungs are protected against this oxidative challenge by well-developed enzymatic and nonenzymatic antioxidant systems. Oxidative stress is said to occur when the balance between oxidants and antioxidants shifts in favors of oxidants, from either an excess of oxidants and/or depletion of antioxidants [1].

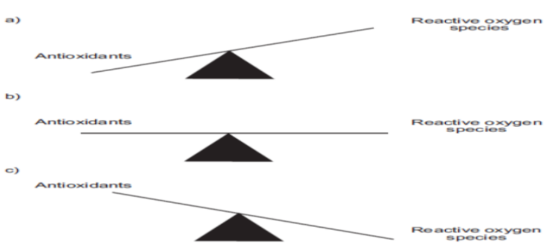

FIGURE 1

Chronic obstructive pulmonary disease (COPD) is one of the major preventable chronic respiratory diseases (CRD). The Global Initiative for Obstructive Lung Disease (GOLD) describes COPD as a common preventable and treatable disease, characterised by persistent airflow limitation that is usually progressive and associated with an enhanced chronic inflammatory response in the airways and the lung to noxious particles or gases [2]. COPD is a major and increasing global health problem and is currently the third leading cause of death in the world [3]. Smoking is the main etiological factor in chronic obstructive pulmonary disease (COPD). Cigarette smoke contains 10^{17} oxidant molecules per puff, and this, together with a large body of evidence demonstrating increased oxidative stress in smokers and in patients with COPD, has led to the proposal that an oxidant/antioxidant imbalance is important in the pathogenesis of this condition [4].

Oxidative stress not only produces direct injurious effects in the lungs, but also activates the molecular mechanisms that initiate lung inflammation [5] and may have a role in many of the processes thought to be involved in the complex pathological events that result in COPD [6].

The aim of the present study was to assess the relationships between the severity of COPD and erythrocyte activities of several key antioxidant enzymes, including GPx, and superoxide dismutase (SOD). In addition, we characterized the degree of oxidative stress, reflected by MDA levels, in relation to the severity of obstructive lung impairment.

RELATION OF AGE WITH PULMONARY FUNCTIONS

Airflow obstruction, usually measured using spirometry, is a useful marker of disease since testing is reasonably reproducible and widely available. GOLD has proposed spirometric criteria for diagnosis and severity assessment of COPD using the forced expiratory volume in one second (FEV_1) and the ratio of the FEV_1 over the forced vital capacity (FVC) after bronchodilator.[7,8]

Interestingly, the peak of lung function appears to occur after maximum height has been achieved, so that the improvements in spirometry are not just due to increased lung size (and thus airway caliber).Lung function remains stable at this plateau (with men having a higher plateau value than women) until approximately age 25 to 30 years, after which FEV_1 slowly declines. Although there is some controversy as to whether the decline in FEV_1 is linear with age or accelerates with aging,[9] some of the most robust data suggest that lung function decreases approximately 20 mL per year during middle age, and then decreases more rapidly by about 38 mL per year after age 65.[10,11]

The changes in lung parenchyma (decreased elastic recoil), chest wall (increased stiffness), and the respiratory muscles (decreased force generation) explain the observed changes in lung volume with aging. Total lung capacity remains relatively preserved since the increased distensibility of the lung is offset by the stiffer chest wall. Residual volume (RV) and functional residual capacity (FRC) both increase, but expiratory reserve volume (ERV) decreases.[12,13]

OXIDATIVE STRESS AND ANTIOXIDANTS

Chronic obstructive pulmonary disease (COPD) is a disease characterized by irreversible and progressive airflow obstruction that is associated with lung inflammation, according to American Thoracic Society guidelines[14]. The most common causes of COPD are cigarette smoking, environmental exposure to dust particles and air pollutants such as allergens, bacterial and viral spores [15]. The lungs are continuously exposed to oxidants, either generated from inside the body or from outside the body due to exposure to air pollution or cigarette smoke. These particles increase the levels of oxidants in the lungs, and have the potential to interact with biological systems to produce oxidative stress, which results in the destruction of alveoli, the tiny air sacs in the lungs where the exchange of oxygen and carbon dioxide takes place, and also results in narrowing of airway lumen diameters. Oxidative stress promotes inflammation of the airways of the lungs as seen in COPD patients. This causes an irreversible decrease in reduction of forced expiratory volume in 1s (FEV_1) as seen in COPD cases [16]. COPD is a major, ever increasing global health problem due to increase in smoking rates and lifestyle changes and is projected to be the fourth leading cause of death worldwide by 2030 [17]. Many markers of oxidative stress have been shown to have direct correlation among themselves and severity of airway obstruction represented by FEV_1. [18] Simultaneously, other studies have highlighted the deficiency of native antioxidant defense mechanisms of lungs in COPD. The important naturally occurring antioxidants in body include glutathione system, catalase, and superoxide dismutase (SOD) system[19]

SOD detoxifies the superoxide anion and converts into H_2O_2 and O_2, a less toxic product. Catalase (CAT) enzyme completes the detoxification process initiated by SOD via decomposition of H_2O_2 to water and oxygen [20].

Glutathione peroxidase (GPx) is a family of selenium dependent and independent antioxidant enzymes [21]. There are six known isoforms of GPx and the most abundant isoforms, GPx-1, is ubiquitously expressed in the cytoplasm of all mammalian cells [22, 23]. The main function of GPx is to detoxify hydrogen peroxide to H_2O_2 and O_2. However, it also detoxifies the lipid peroxides to release water and alcohol. There is evidence that erythrocyte GPx activity is lower in smokers compared to non-smokers [24].

OXIDATIVE STRESS AS A COMPONENT OF COPD PATHOGENESIS

Lungs are exposed to high levels of free radicals. Production of reactive oxygen species has been found directly linked to oxidation of proteins, DNA, and lipids, which may cause direct lung injury or may induce a variety of cellular responses through the generation of secondary metabolic reactive species. Membrane lipids are highly susceptible to free radical damage which is found to be highly detrimental to the functioning of the cell. MDA is a product of lipid peroxidation and an indirect measure of free radical activity in body. As free radical injury increases lung function decreases. Oxidative stress is reported to play an important role in the pathophysiology of COPD.

Oxidative stress occurs in response to the production of cellular ROS and RNS during endogenous metabolic reactions. Inhaled oxidants in the ambient air, including ozone, nitrogen dioxide, diesel exhaust and cigarette smoke, are also well-established causes of oxidative stress. Under normal conditions, the production of endogenous ROS is tightly regulated, and endogenous antioxidants protect tissues against the exposure to free radicals [25]. Although low-normal conditions, the production of endogenous ROS is tightly regulated, and endogenous antioxidants protect tissues against the exposure to free radicals [25]. Although low-to-moderate concentrations of ROS and RNS are necessary for physiological functions such as defense against infectious agents and cellular signaling pathways[25], uncontrolled activation of ROS production leading to an imbalance between oxidants and antioxidants such as GSH can have detrimental consequences . The most common ROS, i.e. the superoxide anion (O_2^-) and the hydroxyl radical (OH), are directly associated with oxidative modifi cations of biochemical systems such as proteins, lipids and carbohydrates. Oxidative stress is involved many of the pathogenic processes underlying COPD, such as direct tissue damage, inactivation of antiproteases, mucus hypersecretion, vascular barrier dysfunction leading to edema of the bronchial wall, bronchoconstriction and enhanced lung inflammation through activation of redox-sensitive transcription factors in leukocytes [26,27,28]

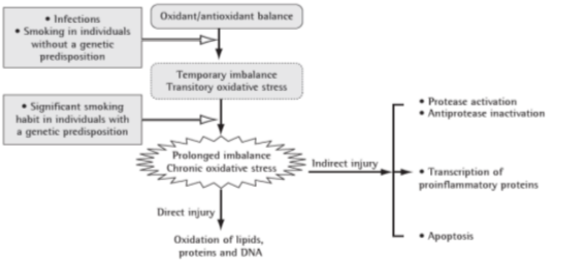

Figure 2 - The oxidative stress in COPD causes direct injury to lung components and triggers and exacerbates the other etiopathogenic mechanisms.

MATERIAL AND METHODS

Patients of COPD presenting to outpatient, indoor, and intensive care unit in the Department of Respiratory Medicine and general medicine were studied. Total 120 (60 cases and 60 controls) subjects were included in the study and the study was conducted in middle to late elderly male (age group of 40-70 years) individuals in the people's college of medical sciences and research centre Bhanpur Bhopal. The study was approved by the review and ethics committee and due consent of the subjects was taken. Height and weight was taken and BMI cut off point calculated for Indian population by Indian government.[29](Table -1)

Table – 1 BMI cut off point for Indian population

CATEGORIES	BMI (in kg/m2)
Underweight	<18.5
Normal weight	18.5-22.9
Overweight	23-24.9
Obese	≥25

SELECTION OF SUBJECTS

Included patients those who having only COPD (with single diagnosis) and who met the diagnostic criteria of COPD [COPD was defined according to the GOLD criteria] [1], none of these were suffer from diabetes, renal disease, rheumatoid, cardiac and other chronic diseases where oxidizing agents load are already more. These patients are compared with age matched healthy nonsmoker subjects with normal pulmonary function test. Here none of the any patients and control subjects was taking dietary supplements such as antioxidant vitamins or minerals.

Pulmonary Function Tests (FEV1, FVC, FEV1/ FVC %) recorded in one sitting on the same day by PC based spirometry COSMED (software version 9.1b). Three satisfactory efforts were recorded according to the norms given by American thoracic society.

STATISTICAL ANALYSIS

All data were analyzed by SPSS (Statistical package of social sciences version 20). The pulmonary function tests were compared in both the case (diseased) and control (normal) groups by the 'unpaired t' test. Data were expressed as Mean ± SD. Statistical significance was indicated by 'P' value <0.05. Simple Regression was used to determine the relation between the variables body fat % and WHR with Pulmonary Function Tests (FEV1, FVC, FEV1/ FVC %). Coefficient of correlation expressed as 'r'.

Table – 2 Baseline parameters of study subjects

	Case (patients) (n=60)	Control (n=60)
Age		
40-50	14 (23%)	15 (25%)
50-60	24 (40%)	22 (37%)
60-70	22 (37%)	23 (38%)
Smokers	54 (90%)	NA
Pack year (mean±SD)	15.83±11.92	NA
Smoking index		
<100	10 (17%)	NA
100-500	24 (40%)	NA
500-1000	14 (23%)	NA
>1000	12 (20%)	NA
COPD GOLD stages		
Stage 1	10 (17%)	NA
Stage 2	18 (30%)	NA
Stage 3	20 (33%)	NA
Stage 4	12 (20%)	NA

For GPX and SOD estimation 5ml of whole blood was centrifuged for 10 minutes at 3000 rpm, plasma was separated and erythrocytes were washed four times with 3ml of 0.9% NaCl solution, and centrifuged for 10 minutes at 3000 rpm after each wash. Washed and centrifuged erythrocytes were made up to 2.0 ml with cold redistilled water and mixed and left to stand at 4° C for 15 minutes. The haemolysate was diluted with 0.01mmol/liter phosphate buffer pH 7.0, so that the 5 fold inhibition was between 30% and 60%. SOD estimation was based on the method of Suttle et al where as glutathione peroxidase was estimated by the method of Paglia and Valentine. Both the enzymes SOD & GPX were determined by Ransel anti-oxidant enzyme kit provided by Randox laboratories Ltd, Crumlin, UK based on the methods described by Andersen et. Malondialdehyde (MDA) was estimated according to the method of Stocks and Dormandy.

RESULT

120 subjects, 60 case and 60 control, were enrolled in this study. They were generally middle-aged (mean age 54.7±1.8 years), with the average smoking history of 38.9±5.9 packyears. Mild COPD (Stage 1) was present in 10 (17%) patients, moderate COPD (Stage 2) was present in 18 (30%) patients, severe COPD (Stage 3) was present in

20 (33%) patients, and very severe COPD (Stage 4) was present in 12 (20%) patients. FVC, FEV1, and the ratio of FEV1/FVC were all significantly lower in patients with severe COPD compared to patients with moderate COPD (p<0.001 for all spirometric variables).

Linear regression analysis revealed a significant direct relationship between FEV1 and erythrocyte GPx activity (r = 0.216, p<0.05) erythrocyte SOD activities (r = 0.202, p<0.05), and a significant inverse relationship between FEV1 and serum MDA levels (r = -0.241, p<0.05).

Table – 3 Values of respiratory status, oxidative stress status and inflammatory markers in COPD patients vs. the control group

Parameter	Case	Control	p Value
% predicted FEV1	40.6±19.8	91.6±10.2	< 0.001*
FEV, L	2.04±0.47	3.29±0.58	< 0.001*
% predicted FVC	68.12±21.6	89.4±8.1	< 0.001*
FVC, L2.	83±0.61	4.07±0.65	< 0.001*
FEV1/FVC ratio	59.3±10.8	93.4±3.9	< 0.001*
MDA, mmol/L	2.59±1.38	1.06±0.32	< 0.001*
SOD, IU/L	136.24±41.53	186±52.67	< 0.001*
GPx U/gm Hb	22.4±2.31	43.16±3.60	< 0.001*

FEV1: Forced Expiratory Volume in the first second; FVC: Forced Vital Capacity, MDA: malondialdehyde; SOD: superoxide dismutase, GPX: glutathione peroxidase

Table – 4 Effect of oxidative stress on pulmonaryfunction test andassessmentofseverity

Parameters	Stage 1	Stage 2	Stage 3	Stage 4	p
FVC (L)	3.08±0.36	2.86±.093	1.93±0.36	1.39±0.29	< 0.001
FEV1(L)	3.19±0.73	2.64±0.33	1.97±0.67	1.81±0.29	< 0.001
FEV1/FVC(%)	0.74±0.23	0.67±0.19	0.59±0.18	0.48±0.13	< 0.001
MDA, mmol/L	1.88±0.79	2.01±.0.72	2.81±0.24	3.18±0.21	< 0.001
SOD, IU/L	146.28±21.52	139.14±.18.31	128.81±6.24	113.16±8.28	< 0.001
Gpx U/gm Hb	30.88±3.82	25.73±.4.32	20.19±1.46	17.18±0.20	< 0.001

The mean levels of serum biomarker of oxidative stress (MDA) was significantly higher in cases compared to controls (P = 0.0001), consequently, the antioxidant levels (GPx and SOD) were significantly lower in cases (P = 0.0001). These all measured data corroborate with a greater oxidative and anti-oxidative pathway imbalance in smokers. However, nonsmoking COPD patients still have significantly higher values of oxidative markers compared to their non-COPD counterparts. The serum levels of oxidative stress marker MDA were found to be significantly elevated in higher GOLD Classes (III, IV) when compared to lower GOLD Classes (I, II). The levels of serum antioxidants (GPx and SOD) diminished significantly in higher GOLD grades when compared with lower grades.

DISCUSSION

The present study, we have demonstrated that the activity of GPx and SOD in erythrocytes and plasma MDA levels correlate with disease severity as assessed by FEV1. Patients with severe COPD have the lowest activity of GPx, and SOD but the highest MDA levels. Considerable evidence now links COPD with increased oxidative stress and, therefore, the status of antioxidant defense mechanisms assumes paramount importance.

In addition, an increased burden of free radicals originates from activated neutrophils, the main inflammatory cells in COPD. In addition, the oxidant/antioxidant balance is deteriorated further by the depletion of antioxidant mechanisms. In COPD both enzymatic and nonenzymatic antioxidative systems are deficient. Indeed, several but not all studies documented that certain markers of oxidative stress may be related to smoking and to the severity of obstructive lung impairment in patients with COPD. In our study out of 60 cases (patients), 6 patients were non smokers and 54 were smokers, it shows that other than smoking environmental factors (pollution and radiation) also play an important role for COPD etiopathogenesis.

CONCLUSION

In the present study it is concluded that there is imbalance between oxidant and antioxidant plays a major role in pathogenesis of COPD and its progression as well as complications (co-morbid conditions like pulmonary artery hypertension, obstructive sleep apnea, obesity etc.).

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