

COPD-An Imbalance between Oxidants and Antioxidants

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Abstract: Chronic obstructive pulmonary disease (COPD) is a progressive condition characterized by airflow limitation associated with an abnormal inflammatory response of the lungs to noxious particles and gases, caused primarily by cigarette smoking. Increased oxidative burden plays an important role in the pathogenesis of COPD. An imbalance between oxidants and antioxidants is considered to play a role in the pathogenesis of chronic obstructive pulmonary disease (COPD). There is a delicate balance between the toxicity of oxidants and the protective function of the intracellular and extracellular antioxidant defense systems, which is critically important for the maintenance of normal pulmonary functions. Several biomarkers of oxidative stress are available and have been evaluated in COPD.

Keywords: COPD; oxidant/antioxidant imbalance; oxidative stress; smoking.

INTRODUCTION

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 [1]. COPD is a major and increasing global health problem and is currently the third leading cause of death in the world [2].

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 favour of oxidants, from either an excess of oxidants and/or depletion of antioxidants [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. Increasingly, COPD is recognised not only to affect the lungs, but to have significant systemic consequences, such as muscle dysfunction and weight loss [6]. Oxidative stress is also thought to play an important role in this aspect of the disease [7].

ANTIOXIDANTS IN THE LUNGS

Antioxidants are usually classified as either enzymatic or nonenzymatic and are the primary defences against reactive oxygen/reactive nitrogen species.

- Antioxidant enzymes include the superoxide dismutase (SOD) family, catalase, glutathione (GSH) peroxidase, GSH S-transferase and thioredoxin [8].
- The nonenzymatic antioxidants include low molecular weight compounds, such as GSH, ascorbate, urate, alpha-tocopherol, bilirubin, lipoic acid and trace elements.

Concentrations of these nonenzymatic antioxidants vary in the lungs. Some, for example GSH, are more concentrated in epithelial lining

fluid compared with plasma [9] and others, such as albumin, are found in high concentration in serum, but at much lower concentrations in the epithelial lining fluid [10].

OXIDATIVE STRESS AS A COMPONENT OF COPD PATHOGENESIS

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 [11]. Although low-to-moderate concentrations of ROS and RNS are

necessary for physiological functions such as defense against infectious agents and cellular signaling pathways^[11], uncontrolled activation of ROS production leading to an imbalance between oxidants and antioxidants such as GSH can have detrimental consequences (Figure 1). The most common ROS, i.e. the superoxide anion (O₂⁻) and the hydroxyl radical (OH•), are directly associated with oxidative modifications 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 [12-14] (Figure 2 & 3).

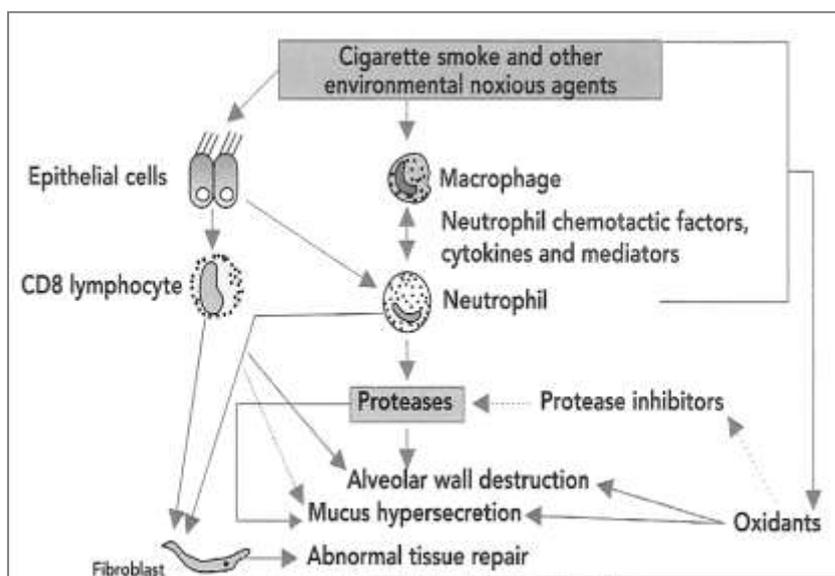


Fig-1: An excess in pro-oxidants (e.g. cigarette smoke) may overwhelm the antioxidant defense system of the body, causing an oxidant/antioxidant imbalance and therefore oxidative stress. Oxidative stress causes direct tissue damage, inactivation of antiproteases, mucus hypersecretion, vascular barrier dysfunction leading to edema of the bronchial wall, bronchoconstriction (both via direct action and through the production of isoprostanes from lipid peroxidation) and enhanced lung inflammation through activation of redox-sensitive transcription factors in leukocytes

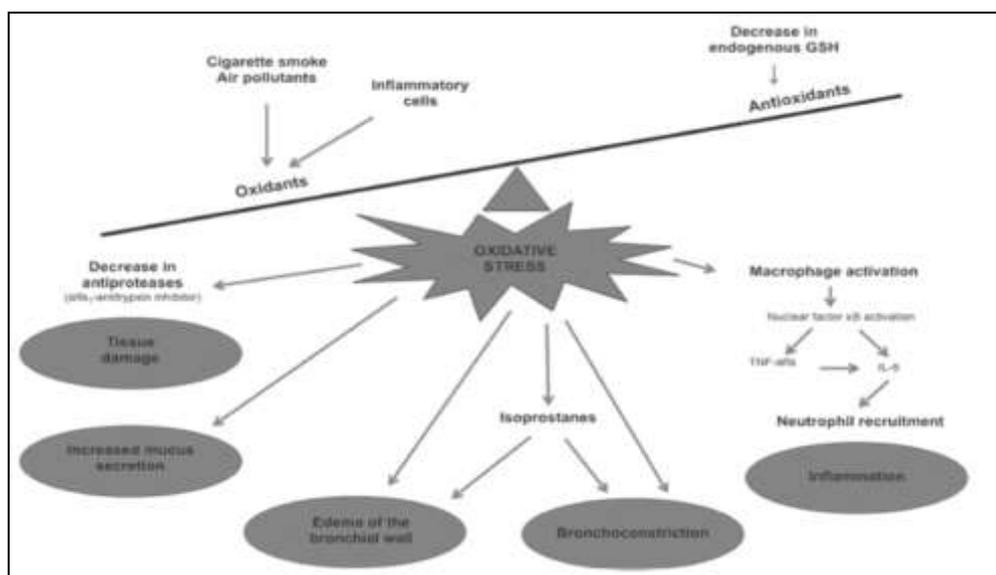
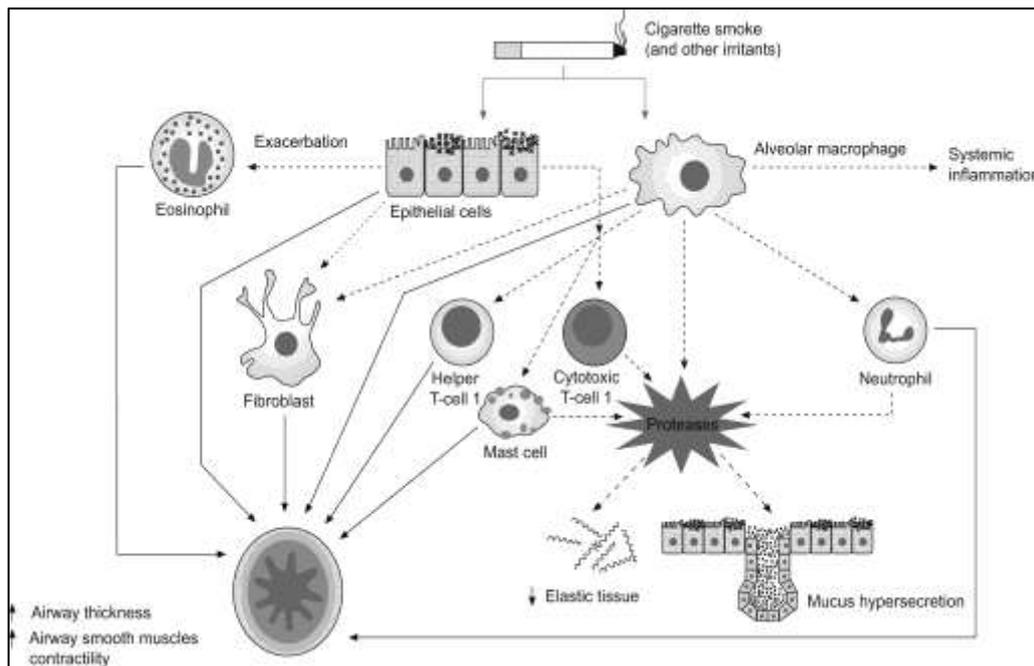


Fig-2 & 3: The pathogenic triad of COPD consists of oxidative stress, protease-antiprotease imbalance, and inflammation, of which oxidative stress forms prime component. Oxidative stress affects airways by myriad mechanisms including mucus hypersecretion, damage to airway epithelium, neutrophil influx, airway inflammation, and increased apoptosis. Several processes lead to oxidative stress-related tissue damage, primary among them is lipid peroxidation which leads to the formation of various lipid hydroperoxides and aldehydic products

MATERIALS AND METHODS

After clinical examination and confirmed diagnosis, 60 male participants (30 patients and 30 control group) were included in this study. Included patients those who having only COPD (with single diagnosis) and who met the diagnostic criteria of COPD [COPD was defined according to the Global initiative for Chronic Obstructive Lung Disease (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 no pulmonary, cardiovascular, or oncological disease, inflammation, infection, and neurological dysfunction that could influence the oxidative status were enrolled as controls with normal pulmonary function test. Here none of the any patients and control subjects was taking dietary supplements such as antioxidant vitamins or minerals.

Table-1: Baseline parameters of study subjects

	Case (patients) (n=30)	Control (n=30)
Age		
40-50	8 (27%)	10 (33%)
50-60	13 (43%)	12(40%)
60-70	9 (30%)	8 (27%)
Smokers	26 (87%)	NA
Pack year (mean±SD)	7.56±2.82	NA
Smoking index		
<100	6 (23%)	NA
100-500	16 (61%)	NA
>500	4 (16%)	NA

Collection of sample

Use a serum separator tube and allow samples to clot for 2 hours at room temperature or overnight at 2-8°C. Centrifuge at approximately 1000 × g (or 3000 rpm) for 15 minutes. Remove serum and assay immediately or aliquot and store samples at -20°C or -80°C.

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 whereas 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. Malondialdehyde (MDA) was estimated according to the method of Stocks and Dormandy. Zinc level was measured by using Centronic GmbH-Germany Kit by colorimetric estimation. Ascorbic acid

level was measured by the method of Ayekyaw. Uric acid was measured by Biosystem Kits and by uricase / peroxidase method.

STATISTICAL ANALYSIS

The collected data was analyzed using SPSS windows (version 20). Results were expressed as mean ± SD. Student's t-test was employed to determine statistical significance. P value less than 0.05 were considered statistically significant.

RESULT

Distribution of biomarkers among chronic obstructive pulmonary disease cases and controls,

- superoxide dismutase levels (in U/ml) were significantly decreased in cases ($P = 0.001$),
- serum Malondialdehyde levels (in nmol MDA/ml) were significantly higher in cases ($P = 0.001$),
- glutathione peroxidase levels (U/gm Hb) were significantly diminished in cases ($P = 0.001$),
- serum zinc levels ($\mu\text{g/dl}$), were diminished in cases ($P = 0.001$),
- serum ascorbic acid levels (mg/dl) were significantly lower in cases ($P = 0.001$)
- serum uric acid levels (mg/dl) were significantly lower in cases ($P = 0.001$)

Table -2: Comparison of biomarkers between cases and controls

Parameters	Units	Patient (n=30)	Control (n=30)	P value
Ascorbic acid	mg/dl	0.38± 0.31	0.91± 0.40	< 0.001*
GPX	U/gm Hb	22.4±2.31	43.16±3.60	< 0.001*
MDA	nmol/ml	2.59±1.38	1.06±0.32	< 0.001*
SOD	U/ml	136.24±41.53	186±52.67	< 0.001*
Uric acid	mg/dl	2.48±0.68	3.18± 0.93	< 0.001*
Zinc	$\mu\text{g/dl}$	43.67±21.86	92.89±16.73	< 0.001*

Unpaired t test, *significant, SOD= superoxide dismutase, GPX= glutathione peroxidase, MDA= malondialdehyde

DISCUSSION

The study results support the oxidant and antioxidant imbalance theory of COPD. 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. In our study out of 30 cases (patients), 4 patients were nonsmokers and 26 were smokers, it shows that other than smoking environmental factors (pollution and radiation) also play an important role for COPD etiopathogenesis.

An important observation was that uric acid may function as an antioxidant, which is of great importance in plasma and plays the protective role during the formation of free radicals. The plasma concentration of uric acid is almost 10-fold higher than other antioxidants, such as vitamin C and E [15]. Moreover; uric acid has much higher antioxidant capacity.

Urate (the soluble form of uric acid in the blood) can scavenge super oxide, hydroxyl radical, and singlet oxygen and can chelate transition metals. Peroxynitrite is a particularly toxic product formed by the reaction of super oxide anion with nitric oxide that can injure cells by nitrosylating the tyrosine residues (nitro tyrosine formation) of proteins. Uric acid can also block this reaction. Significantly decreased levels of uric acid have been found by means of an enzymatic method using a colorimetric assay in plasma of COPD subjects compared to healthy controls [16] whereas a significant decrease was found only in very-severe COPD by HPLC with electrochemical detection [17]. No difference has been found using an automated analyzer [18].

Zinc, an essential dietary metal, plays essential roles in protein structure, it is the intrinsic metal component or activating cofactor for more than 70 important enzyme systems, including carbonic anhydrase, the alkaline phosphatases, dehydrogenases, and carboxypeptidases [19], participates in cellular and humoral immunity, and has anti-inflammatory and antioxidant function [20]. When zinc deficiency occurs in conjunction with acute lung injury or asthma, inflammation is more intense, it results in enhanced oxidative damage in the airways [20]. Trace element (Se, Mn, and Zn) status is altered in critically ill patients with COPD [21].

CONCLUSION

The present study provides a strong evidence for the oxidant-antioxidant imbalance in the pathogenesis of COPD. It is likely that antioxidants may have in future combination therapies for COPD patients. A biomarker-based (preferably MDA) study can be utilized to assess the efficacy of novel antioxidant or other agents in modifying the course of this disease. This study may have useful clinical implications in view of increased understanding of COPD risk parameters. It has also been recognized that

additional burden of comorbidities has negative impact on economies and also patient related outcomes.

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