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Evaluation of Serum Biomarkers of Oxidative Stress and Airway Inflammation in Chronic Obstructive Pulmonary Disease

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ABSTRACT

A study was undertaken to assess both oxidative stress and inflammation in the lungs of patients with chronic obstructive pulmonary disease (COPD) during mild, chronic, severe and acute exacerbations compared with those with stable COPD, healthy smokers, and non-smokers. Levels of interleukin 8 (IL-8) were measured as markers of airway inflammation and TAS levels were measured as a marker of antioxidant status. MDA levels were measured as a marker of oxidative stress status in the serum of COPD patients. Mean serum MDA levels and levels of IL-8 were significantly high in COPD patients and mean serum antioxidant levels were significantly low in patients as compared to non COPD control. It was further observed that the level of MDA and IL8 elevates while that of total antioxidant level falls with the increase in Gold stage and number of smoking pack years amongst the study subjects. Oxidative stress possibly have role in the pathogenesis of COPD and its complications as indicated by the enhanced levels of lipid peroxidation product malondialdehyde and IL-8 in patients. The lower levels of the antioxidant enzyme status point towards that altered antioxidant defense system in patients. Antioxidant therapeutic use and IL-8 antagonists may have clinical usefulness in the treatment of COPD and in preventing its complication and recurrent exacerbations which may improve disease outcome.

Keywords: Oxidative Stress, Antioxidants, IL8, Gold stage and number of smoking pack years, COPD

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INTRODUCTION

Inflammation is a prominent feature of chronic obstructive pulmonary disease (COPD) as shown by the presence in the airways of activated neutrophils and macrophages and increased numbers of inflammatory mediators.[1, 2]. A current hypothesis in the pathogenesis of COPD is that the increased oxidant burden - both directly as a result of smoking or indirectly by the release of increased amounts of reactive oxygen species from airspace leucocytes-may not be adequately counterbalanced by the lung antioxidant systems, resulting in oxidative stress. An excess of oxidants may then lead to enhanced pro-inflammatory gene expression and protein release, inactivation of anti-proteases, and oxidative tissue injury leading to COPD. The presence of oxidative stress in the airways of smokers and patients with COPD has been shown by increased products of lipid peroxidation and altered antioxidant staus[3, 4, 5].

Exacerbations of COPD are considered to reflect worsening of the underlying chronic inflammation in the airways. The frequency of exacerbations is associated with disease severity [6] and a further increase in airways inflammation[7]. These patients have a reduced quality of life and significantly more hospital admissions and a longer time in hospital, thereby adding to the socioeconomic burden.

The aim of this study was to assess the oxidant/antioxidant imbalance and status of markers of inflammation in patients with mild, chronic, severe and acute exacerbations of COPD with reference to Gold stage and smoking pack years.

MATERIAL AND METHODS

Study was conducted in the Department of General Medicine in collaboration with Department of Biophysics and Department of TB and Respiratory Disease at Institute of Medical Sciences, Banaras Hindu University, Varanasi (India).

Study subjects

A group comprising of 31 cases with moderate COPD (GOLD stage II), diagnosed in accordance with GOLD guidelines[8] with severe COPD were studied during exacerbations with severity assessed by healthcare utilization according to the European Respiratory Society COPD guidelines[9]. Exacerbations were defined as an increase in symptoms beyond normal day-to-day variation requiring a change in medication and hospital admission. According to a recent consensus statement on exacerbations, these patients could be divided into those with severe exacerbations (GOLD stage III) requiring hospital admission, and those with very severe exacerbations (GOLD stage IV) who had respiratory failure and required intervention with

mechanical ventilation. 13 patients (GOLD stage III) with severe or very severe exacerbations of COPD were included in the study. Exclusion criteria were age less than 40 years; pneumonia or other lung diseases; a history suggestive of asthma; and treatment with oral corticosteroids, non-steroidal anti-inflammatory agents, or theophyline preparations during the 2 weeks before admission. Healthy non-smokers and smokers formed control groups for comparison. Smokers had not smoked for 12 hours before bronchoscopy to exclude the acute effects of smoking.

44 age and sex matched healthy non COPD individuals were selected as the controls, whose blood samples were drawn with their consent for comparison with the blood samples of the cases. Ethical approval for the study was obtained from the Ethical Committee of the Institute of Medical Sciences, Banaras Hindu University. Detailed history and clinical examination was done in all selected cases and controls. Informed consent from all study subjects was taken prior to collection of blood samples. Venous blood sample, about 10 ml, was collected from each individual in a clean and dry plain vial without any anticoagulant. The blood was allowed to clot at room temperature. The sera was removed through centrifugation and stored at -20^0C in a sterile plain glass vial. All the study subjects underwent following investigations -Complete Blood Count, Renal Function Test, Liver Function Test, Random Blood Sugar, Urine- Routine and Microscopy, Electrocardiogram, Chest X-ray, Pulmonary Function test(FEV1/FVC and % of FEV1) and CT Scan of Chest (wherever feasible).

Evaluation of Oxidative Stress and Antioxidant Status

Oxidative status of the serum of patients and controls was assessed by measuring serum lipid peroxidation levels by the thiobarbituric acid (TBARS) method [10]. Total antioxidant status in serum was determined by a spectrophotometric method using the Randox assay kit [11].

Estimation of IL8

IL-8 concentrations in serum samples were assessed by a human enzyme-linked immuno-sorbent sandwich assay (ELISA) using specific monoclonal biotinylated antibodies (Orgenium Laboratories, FINLAND). Sulphuric acid was added to terminate the reaction and the optical density was determined at 450 nm. The levels were expressed as pg/ml.

Statistical Analysis

The analysis of data was done by using SPSS16 software. All the data were expressed as the mean ± standard error of mean. Data were analyzed statistically by independent Student's t-test for comparison of parametric variables.

RESULTS AND DISCUSSION

Forty four patients with COPD were studied- thirty one with Gold stage 2 and 13 with Gold stage 3. Forty four healthy individuals with no respiratory symptoms and normal spirometric data were studied for comparison. The characteristics of the subjects at the time of enrolment into the study are shown in table 1. All healthy subjects had normal lung function. Signs and symptoms of study subjects are depicted in table 2. Non-smokers and healthy smokers were younger than those with stable COPD and those with severe exacerbations. Patients with very severe exacerbations of COPD had a significantly greater number of pack years of smoking than healthy smokers although all smokers had at least a 22 pack year history.

Table 1 Characteristics of patients

1.	**Age group (years)**	**No. of cases (44)**
	a. 40-50	09
	b. 51-60	14
	c. >60	21
2.	M/F	32/12
3.	Socioeconomic Status	
	a. Upper	06
	b. Middle	16
	c. Lower	22
4.	Smoking Pack Year	
	a. < 10	10
	b. 11-20	13
	c. >20	21
5.	Gold stage	
	a. 2	31
	b. 3	13

The mean level of MDA was significantly higher in COPD cases as compared to control cases. The elevated levels of MDA in patients with higher Gold stage as well as with increasing smoking pack year are a marker of increased lipid peroxidation or oxidative stress (Table 3).

The levels of serum total antioxidant status (TAS) in COPD cases were lower than in the control group. The depleted levels of TAS in patients with higher Gold stage as well as with increasing smoking pack year are a marker of decreased antioxidant capacity with the increased oxidative stress (Table 3).

Table 2 Sign and symptoms in study subjects

Sign and symptoms	No. of cases (44)
Smoking	44
Cough	44
Dyspnoea	29
Sputum production	44
Pallor	09

Clubbing	0
Cyanosis	17
Pedal edema	16
Icterus	0
Chest x-ray	11
Previous hospitalization	18
Exacerbation	30
Associated Comorbidities	07

Table 3- Levels of MDA, TAS and IL8 with reference to Gold grade, Smoking pack year in study subjects

Parameter (mean±SD)	**Case (44)**	**Control (44)**	**t-value**	**p-value**
MDA level (mmol/l)	1.35±0.52	0.33±0.05	12.823	<0.001
a. Gold stage 2	0.306±0.043		18.640	<0.001
b. Gold stage 3	0.399±0.039			
Smoking Pack Year				
a. < 10	0.297±0.034			
b. 11-20	0.338±0.0620.			
c. >20	0.371±0.063		4.882	0.003
Total antioxidant (mmol/l)	0.041±0.022	0.130±0.047	11.192	<0.001
a. Gold stage 2	0.147±0.045		8.606	<0.001
b. Gold stage 3	0.088±0.012			
Smoking Pack Year				
a. < 10	0.165±0.063			
b. 11-20	0.132±0.042		3.415	0.021
c. >20	0.109±0.035			
IL8 (pg/ml)	1.294±0.426	0.138±0.064	17.79	<0.001
a. Gold stage 2	1.104±0.331		336.19	<0.001
b. Gold stage 3	1.747±0.247			
Smoking Pack Year				
a. < 10	1.121±0.141			
b. 11-20	1.321±0.254			
c. >20	1.497±0.388		228.67	<0.001

IL-8 levels, inflammatory marker, were significantly higher in all study subjects. The elevated levels of IL8 in patients with higher Gold stage as well as with increasing smoking pack year are a marker of increased inflammation pointing towards a link between decreased antioxidant levels and increased cellular alterations due to oxidative damage or persistence of oxidative stress in COPD patients (Table 3).

Chronic obstructive pulmonary disease (COPD) is a major worldwide health problem that has an increasing prevalence and mortality [12, 13]. COPD predominantly occurs in those over 40 years of age. An oxidant is a substance capable of causing oxidation (an interaction between oxygen

molecules and other substances). The lungs are continuously exposed to oxidants, generated from either inside the body (released from the cells), or outside the body (*e.g.* cigarette smoke or air pollution). Oxidative stress, an increased exposure to oxidants and/or decreased antioxidant capacities, is widely recognized as a central feature of many diseases [14, 15]. Considerable evidence now links COPD with increased oxidative stress [16,17]. COPD is characterized by obstruction of the airways and limitation of airflow both into and out of the lungs. The airflow limitation is usually progressive (gets worse with time) and associated with an abnormal inflammatory response of the lungs to noxious particles or gases such as tobacco smoke, second hand smoke, air pollution or harsh chemicals in the workplace.

Pathogenesis of COPD is not completely understood. Chronic inflammation of the cells which line the bronchial tree play a major role. Smoking, and other airway irritants, perpetuates an ongoing inflammatory response which leads to hyperactivity of the airways, whereby the smooth muscle of the airways constricts and narrows excessively. This causes the airways to become swollen, excess mucus to be produced and the cilia to function poorly. As the disease progresses, COPD patients find increasing difficulty to clear their secretions, developing a chronic, productive cough, wheezing and dyspnoea, the hallmark symptoms of COPD. To further complicate matters, as excess mucus is produced, it begins to pool in the airways, providing a perfect breeding ground for bacteria to multiply. This leads to further inflammation, the formation of diverticuli (pouch-like sacs) in the bronchial tree and bacterial infections which occur frequently in COPD patients.

Increased neutrophils were reported in the distal airspaces of patients with COPD, which increased further during exacerbations of the disease. This increase in neutrophil numbers is concomitant with an increase in IL-8 levels in the proximal airway secretions in patients with stable and exacerbated COPD compared with smokers and non-smokers, and an increase in IL-8 levels in the distal airspaces in exacerbations of COPD. A new finding in this study is the depletion of GSH in the airspaces in exacerbations of COPD, indicating increased oxidative stress[18.]

Although the pathological events that lead to the development of airways obstruction and exacerbations in COPD are still not completely understood, inflammation of the airways appears to be a critical factor. Indeed, by examining lung tissue sections, Hogg and colleagues[19] found an increase in airways inflammation—evidenced by the enhanced presence of mucous exudates and inflammatory leucocytes—to be associated with COPD disease severity[19]. Oxidative stress is thought to be an important component of inflammation through the activation of oxidant sensitive transcription factors leading to increased transcription of pro-inflammatory genes. Critical to the effects of oxidative stress is the protective counterbalance of antioxidant systems. A shift in this

oxidant/antioxidant balance could result in an increase in oxidative stress which may cause cellular damage. In this regard, GSH appears to be an important antioxidant in the lungs and is present in high concentrations in epithelial lining fluid[20].

CONCLUSION

The present study points there may be a link between decreased antioxidant levels and increased cellular alterations due to oxidative damage, supporting the possibility of the persistence of oxidative stress in COPD. More studies are necessary to confirm whether these alterations are the cause or the consequence of disease. Antioxidant therapeutic use and IL-8 antagonists may have clinical usefulness in the treatment of COPD and in preventing its complication and recurrent exacerbations which may improve disease outcome.

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CONFLICT OF INTEREST

None of the authors has any financial relationship with organizations that could be perceived as influencing the described research. All authors have read the journal's policy on disclosure of potential conflict of interest.

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