Increased Oxidative Stress and Altered Levels of Antioxidants in Chronic Obstructive Pulmonary Disease

Ahmed Nadeem,1,2 Hanumanthrao Guru Raj,1,2 and Sunil Kumar Chhabra1,2,3

Abstract—An imbalance between oxidative stress and antioxidative capacity has been proposed to play an important role in the development and progression of chronic obstructive pulmonary disease. We carried out a study to assess the systemic oxidant-antioxidant status in patients with chronic obstructive pulmonary disease (COPD) and relate it to the severity of disease. We measured a wide range of parameters of oxidant-antioxidant balance in leukocytes, plasma and red cells of 82 patients with COPD and 22 healthy non-smoking controls (HNC). Lung function was measured by spirometry. Staging of COPD was done as per the recommended guidelines. Red cell antioxidative enzyme activities were altered, with glutathione peroxidase (GSH-Px) having lower, superoxide dismutase (SOD) having greater and catalase having similar activity in patients as compared to HNC. In plasma, ferric reducing antioxidant power (FRAP) and total protein sulfhydryls were lower and GSH-Px, lipid peroxides measured as MDA-TBA products, and protein carbonyls were higher in the patients as compared to HNC. Plasma total nitrates and nitrites (NOx) were similar in the two groups. Superoxide anion (O2•−) release from leukocytes upon stimulation with N-formyl-L-methionyl-L-leucyl-L-phenylalanine (fMLP) and total blood glutathione were also higher in patients as compared to HNC. Plasma FRAP had a positive whereas total blood glutathione had a significant negative correlation with the severity of airways obstruction (FEV1% predicted). Further, comparisons between clinical stages of severity of COPD revealed significant differences in plasma FRAP and total blood glutathione. Our observations suggest there is a systemic oxidant-antioxidant imbalance in the patients with COPD.

INTRODUCTION

Chronic Obstructive Pulmonary Disease (COPD) is a condition characterized by progressive and largely irreversible airways obstruction and influx of inflammatory cells into the lungs (1–4). While the precise mechanisms of the pathogenesis of COPD have not been defined, increased oxidant burden and protease-antiprotease imbalance have been proposed. The increased oxidant burden derives from the fact that cigarette smoke, the main etiological factor in the pathogenesis of COPD, contains...
an estimated $10^{14}$ radicals/puff and about 4700 chemicals that include peroxynitrite, superoxide radical and oxides of nitrogen. The tar phase also contains hydroxyl radical and hydrogen peroxide In addition, nitric oxide ($\text{NO}^\bullet$) is present in cigarette smoke in concentrations of 500–1000 ppm (5, 6). The oxidant burden in the lungs is further enhanced in smokers by the release of reactive oxygen species (ROS) from alveolar macrophages and sequestered neutrophils in the lung (4, 7, 8). Cigarette smoking also exposes various components of the blood in the pulmonary microvasculature i.e. red cells, plasma and leukocytes to an increased oxidant burden of ROS, either directly by diffusion into the blood (9, 10) or indirectly from the ROS generated from activated inflammatory cells in the lung and/or peripheral leukocytes (4, 7, 8, 11–14).

Oxidants generated in the biological fluids are efficiently scavenged by antioxidants. Under normal conditions, the lung and blood are adequately protected by various antioxidant components (15–18). There are several lines of evidence that suggest an imbalance between oxidants and antioxidants in the lung and blood in smokers and the patients with COPD (4, 19, 20). The reported abnormalities include, decreased plasma protein sulfhydryl groups, decreased total antioxidant capacity, increased lipid peroxidation products (4), increased superoxide generation from peripheral neutrophils (4, 13, 14) and increased $\text{F}_2$-isoprostanes (21, 22).

Most investigations have centered on a few parameters of the oxidant-antioxidant balance. A comprehensive study with a wide range of parameters has not been carried out in patients with COPD. Further, the relationship with disease severity has not been explored in most studies. The present study was carried out to address these lacunae in literature.

**MATERIALS AND METHODS**

**Study Patients**

Eighty two patients of COPD, belonging to the age group 45–70 years, were included in the study from the outpatient department of the Institute. Twenty two age-matched healthy non-smoking controls were included as controls. The diagnosis was based on the GOLD criteria of COPD (23). All the patients had a smoking history of greater than 10 packs/year, cough, expectoration and exertional breathlessness, and a change in FEV1 of less than 200 mL and 12% following inhalation of 200 µg salbutamol. In India, moderate or heavy smoking is uncommon in females (24) and most of the female patients with COPD arise out of exposure to biomass fuels. Although, we intended to include both males and females in the study, there were very few females who met the inclusion criteria. Therefore, only males were included. The patients were classified according to severity as suggested in the GOLD criteria (23). There were 46 subjects with Stage II disease and 23 with Stage III disease. There was no other concurrent pulmonary or systemic disease or evidence of any upper or lower respiratory tract infection. None of them had had an acute exacerbation of COPD within the past four weeks. No drug was allowed on the day of test. Informed consent was obtained. The study was approved by the Institutional Ethics Committee.

**Spirometry**

Spirometry was carried out after withdrawal of bronchodilators—inhaled beta-2 agonists for at least 12 h and oral bronchodilators (salbutamol/theophylline derivatives) for 24 h. Corticosteroid therapy (oral/inhaled), if any, was allowed unchanged. The test was performed on a dry, rolling-seal spirometer of the Transfer Test C model lung function machine (P.K. Morgan, Kent, UK). Maximal Expiratory Flow Volume curves were obtained. Three acceptable and at least two reproducible curves (the two highest FVC and FEV1 being within 200 ml of each other) were obtained in each subject. The highest values of Forced expiratory volume in the 1st second (FEV1) was selected for analysis.

Unless otherwise stated all the chemicals were purchased from Sigma Chemical Co. (Bangalore, India)

**Leukocyte Harvesting**

Twenty milliliter of blood was withdrawn and mixed with dextran (509,000 mw) and allowed to stand for 45 min at room temperature. The resulting leukocyte-rich plasma was centrifuged at 250 × g (4°C) for 12 min to obtain leukocytes. Contaminating red cells were lysed by 0.2% w/v NaCl for 30 s followed by restoration of molarity by addition of 1.6 w/v NaCl. After centrifugation, the leukocytes were washed twice in Krebs-Ringer Phosphate buffer, pH-7.35 containing 0.2% w/v dextrose and finally suspended in it at a concentration of 5 million cells/mL. The viability of leukocytes harvested with this technique was greater than 95% as determined by means of trypan blue exclusion.

**Superoxide Anion ($\text{O}_2^{\bullet-}$) Assay**

Superoxide anion generation by leukocytes (2.5 million cells) was measured as the superoxide