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NADPH oxidase-driven oxidative stress and the interplay of superoxide dismutase and vitamin C in chronic bronchitis

Anita M. Raut[1], Nilesh V. Kodre[2]

[1]Associate Professor, Department of Biochemistry, DVVPF's Medical College, Ahilyanagar, Maharashtra, India
[2]Associate Professor, Department of Medicine, DVVPF's Medical College, Ahilyanagar, Maharashtra, India.

Abstract

Chronic Obstructive Pulmonary Disease (COPD) is a progressive and debilitating disorder characterized by irreversible airflow limitation and a dysregulated inflammatory response within the lungs parenchyma. Extracellular superoxide dismutase (EC-SOD), a key antioxidant and anti-inflammatory enzyme, is abundantly present in lung tissue and epithelial lining fluids and maintains redox homeostasis. EC-SOD plays a critical protective role by scavenging free radicals and attenuating COPD-associated inflammation. In the present cross-sectional study, oxidative stress biomarkers and antioxidant parameters were assessed in 100 healthy controls and 60 patients with chronic bronchitis. Baseline clinical and biochemical parameters were measured, including serum malondialdehyde as an index of lipid peroxidation, erythrocyte superoxide dismutase (SOD) activity, and plasma vitamin C levels. The results when compared with healthy controls, COPD patients demonstrated a statistically significant elevation in serum MDA levels ($p < 0.001$), accompanied by a significant reduction in both erythrocyte SOD activity and plasma vitamin C levels ($p < 0.001$). Collectively, these findings indicate that diminished antioxidant defenses particularly reduced SOD activity and plasma ascorbate levels contribute to augmented oxidative stress, which may play a pivotal role in the pathogenesis and progression of COPD.

KEYWORDS: Chronic bronchitis; Oxidative stress; Vitamin C; NADPH oxidase; Superoxide dismutase.

Introduction

Chronic obstructive pulmonary disease (COPD) is a progressive, age-related inflammatory airways pathological condition, which is mainly manifested by airflow obstruction and impaired exhalation. Despite of eluded the grasp of researchers, effective disease modifying treatment remain elusive, the current treatment options are largely symptomatic, which provides relief without limiting the pathogenic cascade [1]. The primary pathogenesis of COPD is oxidative stress that is driven by excessive generation of reactive oxygen species (ROS), including both oxygen radicals and non-radical derivatives.

Despite the fact that ROS are physiological byproducts of normal cellular metabolism, their levels are significantly increased by external factors such as cigarette smoke, indoor air pollution, and workplace exposures. Sustained ROS in COPD leads to progressive destruction of cellular proteins, lipids, and DNA, thus causing further inflammation, tissue damage and disease worsening. In addition, ROS also serve as intracellular signaling molecules, regulating apoptosis, cellular senescence and inflammatory cascades. When ROS overproduction exceeds neutralizing efficiency of the innate antioxidant defense system; especially enzymatic antioxidants like superoxide dismutase (SOD) leads to an adverse redox posture [2].

Oxidative stress is now been recognized as a significant cause of both the onset and progression of COPD. The disease is distinguished by

Correspondence: **Dr. Anita M. Raut,** Associate Professor, Department of Biochemistry, DVVPF's Medical College, Ahmednagar, Maharashtra, India. Email: anitaraut009@gmail.com

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impaired or overloaded endogenous antioxidant defenses, which are also undermined in the acute exacerbations. Pulmonary oxidative damage is further enhanced by exogenous oxidant exposure by cigarette smoke, atmospheric pollutants as well as biomass fuels combustion. The two dominant sources of ROS in the inflamed lung are inflammatory cells (mainly neutrophils and macrophages) that produce large amounts of oxidant mainly through the NADPH oxidase system [3]. These ROS not only damage tissues, but also directly compromise endogenous antioxidant systems. Notably, oxidative proteolysis of extracellular superoxide dismutase (EC-SOD) results in its functional inactivation, thereby diminishing antioxidant capacity. This counter-intuitive condition, where persistent free radicals generation of free radicals impairs the antioxidant defense system, only makes the pulmonary damage more severe and promotes the oxidative injury and inflammation [4, 5].

Oxidative stress arises when the production of oxidants exceeds the threshold of neutralization of the antioxidant defenses. These oxidants could be exogenous (cigarette smoke, air pollution) or endogenous (the processes of chronic inflammation). The subsequent oxidative stress facilitates lipid peroxidation causing structural damage to lungs tissues and the eventual deterioration of pulmonary function typical of COPD. The oxygen radical loading to the lung is alleviated by an effective antioxidant defense network.

Vitamin C (ascorbic acid) a water soluble antioxidant and it is highly found in the lung epithelial lining fluid plays a significant electron donor, capable of scavenging and neutralizing ROS. As a potent electron donor, it is necessary to maintain good levels of vitamin C to sustain lung volume and counter oxidative damage in COPD [6, 7]. Therapeutic strategies aimed to decrease oxidative stress represents promising value in prevention and management of oxidative damage in COPD. In this context, the current research was aimed at examining NADPH oxidase-mediated oxidant/antioxidant balance in pulmonary inflammation.

Materials and Methods

Study Setting: Participants were recruited from both inpatient and outpatient units. Diagnostic assessments were done by Pulmonologists certified on the basis of thorough clinical histories, physical examinations, relevant biochemical and spirometric tests.

Study Population: There were 160 subjects (100 healthy controls and 60 with recent chronic obstructive pulmonary disease (COPD) of the earliest clinically recognized stage. COPD cohort involved both men and women aged between 25 to 75 years. Key etiological agents that had been found to cause pulmonary disease among this group of people were active tobacco use, exposure to ambient pollution, being predisposed genetically, atopic reactions, and the history of previous pulmonary tuberculosis.

Inclusion Criteria: The patients with conformed chronic bronchitis (COPD) at the latest stage of the disease, determined by the pulmonologists and airflow limitation as evidenced by spirometry, which is operationalized as a ratio of FEV1/FVC less than 70%. Patient's clinically stable state, with no acute exacerbation at time of enrollment was included.

Exclusion Criteria: Patients having other respiratory disorders than chronic bronchitis, patients with such important comorbidities as HIV infection, diabetes mellitus, systemic hypertension, malignancy, congestive heart failure, recent surgical operations, liver, kidney, or endocrine illnesses will be excluded. Participants that are taking antioxidant supplements or any medication that is known to affect the oxidant-antioxidant balance at the same time

Blood Sample Collection

Following written informed consent, all participants underwent clinical screening and spirometric evaluation under the supervision of a respiratory physician. A total of 5 mL of venous blood was collected from each subject via puncture of the antecubital vein under aseptic conditions. Blood was collected into a plain tube for serum separation; serum was obtained by centrifugation at 3,000 rpm for 10–15 minutes at room temperature. Hemolyzed samples were excluded from analysis. All biochemical assays were performed on the same day of collection to eliminate storage-related analytical variability.

Biochemical Parameters: The following biochemical parameters were measured in both healthy controls and COPD patients:

Serum Malondialdehyde (MDA): As an index of lipid peroxidation, measured by the Kei Satoh method (1989) [8].

Erythrocyte Superoxide Dismutase (ESOD) Activity: Modified spectrophotometric method of Kajari Das et al. (1991), based on nitrite formation from superoxide radicals [9].

Plasma Vitamin C: Quantified using Caraway's method (1955) [10].

Results

Table 1. Biochemical parameters in healthy controls and COPD patients.

Group	Serum MDA (µmol/L)	SOD (U/g Hb)	Plasma Vitamin C (mg/dL)
Healthy Controls (n = 100)	1.66 ± 0.277	1.37 ± 0.128	0.926 ± 0.127
COPD Patients (n = 60)	4.51 ± 2.80	0.38 ± 0.11	0.35 ± 0.067
$p < 0.001$ for all three parameters (COPD patients vs. healthy controls)			

Values expressed as mean ± SD. MDA = malondialdehyde; SOD = superoxide dismutase; Hb = hemoglobin.

Discussion

Malondialdehyde (MDA) as a Marker of Oxidative Stress: Table 1 demonstrates significantly elevated serum MDA levels in COPD patients compared with healthy controls ($p < 0.001$). This finding is consistent with a substantial body of published evidence reporting increased serum lipid peroxide and thiobarbituric acid reactive substances (TBARS) levels in patients with lung disease [11].

The overproduction of reactive oxygen species - the primary constituent of which is the superoxide anions generated by the activity of NADPH oxidase in activated phagocytes located at inflammatory sites - is a determinant of cellular and tissue damage found in chronic inflammatory pulmonary disorders. Of the variety of pulmonary cell phenotypes, alveolar type II epithelial cells have been shown to be especially vulnerable to various oxidative insults and this has been partly ascribed to their relentless and direct contact with any inhaled xenobiotic. Oxidative perturbations that affect pulmonary tissues are chronically presented by the inherent metabolic fluxes as well as external environmental exposures. Inhaled oxidants, primarily ozone, which is a potent oxidant in the atmosphere, exaggerate inflammatory reactions in the lungs, triggering lipid peroxidation, biomolecule remodeling, and an increased influx of neutrophils, airway hyper-responsiveness, and subsequent respiratory dysfunction, even in people who appear healthy [12].

Lung epithelial cells function not only as targets but also as active sources of ROS, and their oxidant production can further activate inflammatory cells, establishing a self-perpetuating cycle of oxidative stress and inflammation. Elevated MDA levels in COPD patients serve as biomarkers of increased ROS activity and lipid peroxidation. Oxidative stress also amplifies the release of pro-inflammatory cytokines and chemokines—including TNF-α, IL-1β, and IL-8—which recruit neutrophils and activate transcription factors such as NF-κB and AP-1. This inflammatory cascade intensifies tissue damage through ROS-mediated inactivation of antiproteases, epithelial cell apoptosis, mitochondrial dysfunction, impaired extracellular matrix repair, and sustained chronic inflammation; processes central to the pathogenesis of COPD and acute respiratory distress syndrome (ARDS) [13, 14].

Superoxide Dismutase (SOD) Activity

Table 1 illustrates significantly reduced erythrocyte SOD activity in COPD patients relative to healthy controls ($p < 0.001$). Although prior studies on the relationship between SOD activity and oxidative stress in lung disease have yielded variable results, our findings are concordant with earlier reports documenting decreased SOD activity in patients with lung disease [15].

In COPD, excessive production of superoxide anions by polymorphonuclear leukocytes, macrophages, and monocytes disrupts the oxidant/antioxidant equilibrium. The resultant increased utilization of SOD to neutralize these harmful radicals leads progressively to enzyme depletion.

Copper-zinc SOD (Cu-Zn SOD), the predominant cytosolic isoform, is a homodimer comprising two identical subunits of approximately 32 kDa, each bearing an active site formed by copper and zinc ions coordinated through a shared histidine ligand. This enzyme is susceptible to irreversible inactivation by hydrogen peroxide (H_2O_2), one of its own reaction products. As mature erythrocytes lack protein synthetic capacity, any inactivation of erythrocyte SOD directly compromises their antioxidant reserve.

Under physiological conditions, limited SOD inactivation may be inconsequential. However, in states of heightened oxidative stress—as prevails in COPD—substantial inactivation occurs, critically compromising erythrocyte antioxidant defenses. The inactivation mechanism involves oxidative modification of histidine residues essential for copper coordination at the active site; the resulting structural alterations render the enzyme susceptible to proteolysis, with damaged SOD fragments rapidly degraded by intracellular peptidases. The consequent decline in SOD activity further amplifies plasma oxidative burden and contributes to the self-perpetuating oxidative injury characteristic of COPD [15].

Plasma Vitamin C (Ascorbic Acid) Levels: In addition to reduced SOD activity, the present study demonstrated significantly decreased plasma vitamin C levels in COPD patients ($p < 0.001$). Vitamin C (ascorbate) is an important water-soluble antioxidant in plasma and epithelial lining fluid. In the context of oxidative stress, the NADPH oxidase system generates reactive oxygen species including nitric oxide (NO•) and superoxide anions; these interact to form peroxynitrite, a highly cytotoxic oxidant. Peroxynitrite rapidly oxidizes ascorbic acid, thereby depleting the plasma antioxidant reserve [16].

Ascorbate is considered a key aqueous-phase antioxidant, and its deficiency may substantially contribute to oxidative stress in COPD. An additional mechanism underlying vitamin C depletion is the increased conversion of vitamin E to the vitamin E radical under conditions of elevated oxidative stress. Since vitamin C

is required for the regeneration of vitamin E from its radical form, augmented vitamin E recycling demand leads to accelerated ascorbate consumption and consequent depletion [17].

Together, the decline in both SOD activity and plasma vitamin C levels in COPD patients reflects a profoundly compromised antioxidant defense system, which contributes to the oxidative pathology of the disease.

Conclusion

The present study demonstrates that there are strong oxidative disturbances in the patients with chronic bronchitis as indicated by significantly higher levels of serum malondialdehyde in comity with significantly reduced erythrocytic superoxide dismutase (SOD) activity and plasma vitamin C levels when compared to healthy controls ($p < 0.001$ in all parameters). The increased oxidative load in COPD leads to increased vitamin C consumption, as indicated by much lower levels of plasma ascorbate levels in all COPD individuals compared to healthy ones in this study. The overall results indicate the overall weakening of antioxidant defense systems in COPD, to which both enzyme (SOD) and non-enzyme (vitamin C) antioxidants contribute to the development of the disease. This research forms the foundation of future research studies, on antioxidant based therapeutic interventions.

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Ethical Approval: The study protocol was reviewed and approved by the Institutional Ethics Committee of DVVPF's Medical College, Ahilyanagar. Written informed consent was obtained from all participants prior to enrolment.

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