

STUDY OF OXIDATIVE STRESS IN RELATION WITH ANTIOXIDANT STATUS IN CHRONIC BRONCHITIS

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ABSTRACT

Oxidative stress has been recognized as a central feature of smoke induced chronic bronchitis, where lipid peroxide plays an important role in inflammatory lung disease. Increased lipid peroxide increases epithelial permeability produced by cigarette smoke is likely to be mediated through depletion of the Total Antioxidant Capacity. Imbalance between oxidants and Total Antioxidant Capacity is also an established fact in these patients. 60 patients with chronic bronchitis included in the study. Their base line clinical examination, malondialdehyde (MDA), nitric oxide, alpha tocopherol and Total Antioxidant Capacity were measured. 100 healthy non-smokers were served as controls. The mean malondialdehyde levels and nitric oxide in the patients at base line were higher than Controls ($p < 0.001$). Plasma alpha-tocopherol and total antioxidant capacity were lower ($p < 0.001$) in the patients compared to controls. The present study shows that initially the plasma lipid peroxide (MDA) levels were high and antioxidants (alpha-tocopherol, total antioxidant capacity) were low in patients with chronic bronchitis. Our results suggest the presence of oxidative stress and decrease in total antioxidant capacity in chronic bronchitis.

Keywords: Malondialdehyde, Alpha-tocopherol, Total Antioxidant Capacity, Chronic bronchitis

1. Introduction-

Lung is the organ which is constantly exposed to many atmospheric pollutants such as cigarette smoke, ozone and nitrogen dioxide and is also at risk from oxidant injury by inhalation¹. Inhaled ozone induces toxic processes that impair lung function. Lipid peroxide plays an important role in inflammatory lung diseases. Increased epithelial permeability produced by cigarette smoke is likely to be mediated through depletion of Total Antioxidant Capacity.² Oxidant-antioxidant balance is essential for normal lung function. Both an increased oxidants and or decreased antioxidants may reverse the physiologic oxidant-antioxidants balance in favor of oxidants leading to lung injury.

Chronic bronchitis is defined as presence of persistent cough that it is present in any patient with sputum production for at least two consecutive years, in the absence of any other identifiable cause. In simple chronic bronchitis patients have a productive cough but no physiologic evidence of airflow obstruction where some individual may demonstrate hyperreactive airways with intermittent bronchospasm and wheezing. This condition is called chronic asthmatic bronchitis. Some patients, especially heavy smokers, develop

chronic airflow obstruction usually with evidence of associated emphysema and are classified as obstructive chronic bronchitis.

The earliest feature of chronic bronchitis is hyper secretion of mucus in the large airways associated with hypertrophy of the submucosal glands in the trachea and bronchi. As chronic bronchitis persists there is also marked increase in goblet cells of small airways – small bronchi and bronchioles – leading to excessive mucus production that contributes to airway obstruction³.

Now a days attempt towards oxidative stress status is continuing to prevent oxidative stress. The study of antioxidant capacity in lung disease patients opens a promising field in prevention of oxidative stress related complications in these patients.

2. Aims & objectives

1. To explore the existence of possible peroxidative damage in lung disease patients by estimating the level of serum malondialdehyde as an index of lipid peroxide.
2. To estimate nitric oxide as a marker of oxidative stress.

- To study possible alteration in Total antioxidant Status in lung disease Patients by estimating the total antioxidant capacity.
- To study non enzymatic antioxidant vitamin E.

3. Materials & Methods

The present study was conducted in the department of Biochemistry Dr. Vikhe Patil Medical College and Hospital Ahmednagar. In the present study total 160 subjects both male and females between the age group of 25-75 years were included. Who were diagnosed by physicians on the basis of detailed clinical history, relevant biochemical examinations and clinical condition including spirometry. In the spirometric analysis patients in the clinically stable phase of disease with ratio of FEV1/FVC<700 % were included.

3.1 Study design: Distribution of these subjects was as follows.

Sr. No.	Group	Types	No. of Subjects
1	Control	Healthy subjects	100
2	Chronic bronchitis	Initial stage disease	60

Underlying causes of chronic bronchitis patients were active smoking environmental pollution, heredity, tuberculosis.

The control subjects were completely healthy non smokers and showed no abnormality on clinical examinations and were completely symptom less.

Patients with hypertension, malignancy, overt cardiac failure recent surgery, severe endocrine, hepatic or renal diseases and lung disorders other than COPD were excluded from the present study.

Informed consent was obtained from each patient in the study. The study was cleared by institutional ethics committee.

3.2 Study procedure- 10 ml blood was collected from each patient. Serum was separated by centrifugation at 3000 rpm for 10 minutes at room temperature. Following parameters were carried out on the samples on the same day of collection.

- The level of serum total lipid peroxide in terms of Malondiadehyde (MDA) was determined by Kei Satoh method.⁴
- Serum Nitric oxide (NO[•]) as nitrite was estimated by Najwa Cortas and Nabil Wakid method⁵

- Serum Vitamin 'E' (α – Tocopherol) was estimated by the method of Baker and Frank.⁶
- Total antioxidant capacity in plasma (TAC) was assayed by FRAP analysis.⁷

4. Results and Observations:

Table No. 1 Illustrate the levels of MDA, NO[•], Vitamin E and TAC in the healthy controls and chronic bronchitis patients

Test	Healthy Controls n=100	Chronic Bronchitis patients n=60
Sr. MDA (umol/L)	1.66±0.289	4.61±2.7 *
Sr. No [•] (umol/L)	33.15±6.13	137.58±12.19*
Sr. Vit E (mg/dl)	0.927±0.12	0.32±0.09 *
Sr.TAC (umol/L)	1253.12±170.22	354.43±88. *

n = number of cases

All values are expressed in mean \pm SD

* = Significant when compared with control group

4.1 Statistical analysis: was carried out using students unpaired' test. Probability values 0.05 were considered as significant. Also data were expressed in mean \pm SD form.

5. Discussion:

5.1 Table No.1 – Results of the present study showed significant high levels ($p < 0.001$) of oxidants serum lipid peroxide (MDA) and serum nitric oxide (NO[•]) in cases with chronic bronchitis as compared to healthy controls. The mean plasma levels of Vit. E ($P < 0.001$) and total antioxidant capacity (TAC) were lower in patients with chronic bronchitis than controls. Lung cells, in particular alveolar epithelial type II cells, are susceptible to the injurious effects of oxidants. Lungs are continuously exposed to oxidants, either generated endogenously by metabolic reactions or exogenously, such as air pollutants or cigarette smoke and since Cigarette smoking, another environmental hazard, also delivers oxidants and free radicals to the lungs. Cigarette smoke contains many oxidants and free radicals, both in the gas and the tar phase and causes sequestration of neutrophils into the pulmonary microcirculation and accumulation of macrophages in respiratory bronchioles. Once recruited, these cells become activated and generate ROS. ROS, which may also be released by lung epithelial cells, may also stimulate inflammatory cells directly thereby amplifying

lung inflammatory and oxidant events, there by increases the MDA significantly in chronic bronchitis patients⁸.

In the respiratory tract, NO[•] is generated enzymatically by all three distinct isoforms of NO[•] synthase i.e. NOS-1, NOS-2 and NOS-3. Of these three forms NOS-2 activity is primarily regulated transcriptionally and is commonly induced by bacterial products and pro-inflammatory cytokines. Inflammatory diseases of the respiratory tract such as chronic bronchitis are commonly characterized by an increased expression of NOS-2 within respiratory epithelial and inflammatory immune cells. This increases the local production of NO[•] in the patients with lung diseases⁹

Vitamin E is the most important lipophilic antioxidant in humans in this study we observed the reduced vitamin E level in lung disease patients could be due to its overconsumption as an antioxidant subsequent to increased production of free radicals by cigarette smoke and inflammatory reaction.

The total antioxidative potential of the plasma reflects the ability of an individual to resist the oxidative stress. Ferric reducing ability of plasma (FRAP) evaluates plasma total antioxidant capacity due to known and unknown antioxidants in the plasma¹⁰ Therefore in the present study significant reduction observed in total ferric reducing ability of plasma may be due to increased free radical activity either because of inflammation or complications that results in imbalance between antioxidant capacity and prooxidant affecting lung function. Extensively amplified oxidant burden and declined individual antioxidant levels might be responsible for the observed significant fall in total antioxidant capacity of patients with lung disease.

Conclusion

Thus evaluating oxidative stress in lung disease patients by measuring lipid peroxidation and antioxidant status can lead to better understanding of free radical mediated damage in chronic bronchitis patients. An inequity between oxidative stress and antioxidative capacity has been proposed to play an important role in the development and progression of chronic bronchitis and it is related to the severity of disease.

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