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Original Research Article

Study of Pulmonary Function Test, Oxidative Stress Marker and Non-enzymatic Antioxidants in Chronic Obstructive Pulmonary Disease

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Abstract: Chronic Obstructive Pulmonary Disease (COPD) is a health problem with increasing severity, as exposure to risk factors such as cigarette smoke, pollution is inevitable. Oxidant antioxidant imbalance cause oxidative burden leading to lung tissue damage. The aim of present study was to evaluate oxidative stress marker (MDA) and non enzymatic antioxidant in COPD patients. MDA level were estimated by thiobarbituric acid method, Vitamin-E estimated by the method of Baker et al and Vitamin-C level were estimated by Lowry method. In Results Serum mean level of MDA was significantly higher in COPD patients as compared to normal healthy individual while mean of level of vitamin E & vitamin C decreased in COPD patients as compared to control. In Conclusion increased MDA level and lower Ascorbic acid & Tocopherol levels indicate excess of oxidative stress in COPD patients. Dietary supplementation of antioxidant vitamins to COPD patients is strongly recommended.
Keywords: MDA, Oxidative Stress, COPD, Non-Enzymatic Antioxidant

INTRODUCTION:

Chronic obstructive pulmonary disease (COPD) is the fourth leading cause of death globally. The prevalence of COPD is higher in countries where smoking is highly prevalent. In India, there is an increasing tendency to abuse tobacco and COPD is emerging to be a major public health problem [1]. American Thoracic Society defines Chronic Obstructive Pulmonary Disease as "A disease state characterized by the presence of air flow obstruction due to chronic bronchitis or emphysema; the airflow obstruction is generally progressive, may be accompanied by airway hyper-reactivity, and may be partially reversible [2]**.**

COPD has been defined by global initiative of COPD (GOLD), as a state characterized by airflow limitation that is not fully reversible. There is considerable evidence that an increased oxidative burden occurs in the lungs of patients with COPD which plays a role in its pathogenesis. The lungs are continuously exposed to oxidants generated either endogenously or exogenously. Cigarette smoke, consisting of the gas phase and particulate phase (tar phase) is thought to contain 1017 oxidants molecules per puff [3].

Oxidant/antioxidant imbalance is thought to play a part in the pathogenesis of COPD. Oxidative stress leads to increase in concentration of free radicals which can cause damage to the biomolecules (protein, lipids, DNA) present in the cells. One of the targets of oxidants is polyunsaturated fatty acids (PUFA) present in the cell membrane [4].

Vitamin E is lipid soluble antioxidant, represents body's principle defence against oxidant-induced membrane injury in human tissue via its role in breaking the lipid peroxidation chain reaction [4]. Vitamin E is present in lipid membranes and in extracellular fluids [6]. Vitamin C is the water soluble antioxidant, present abundantly in epithelial lining fluid of the lungs. It scavenges the superoxide, hydroxyl and peroxyl radicals [7]. It also contributes in regeneration of vitamin E. Vitamin C functions as a chain breaking antioxidant [8]. In addition to this, vitamin C plays a role in immune function and is transported into neutrophils and lymphocytes [9].

Among the main biological targets of oxidative stress, membrane lipids are the most commonly involved class of bio molecules. Lipid peroxidation forms a number of secondary products able to boost oxidative damage. In addition to their cytotoxic properties, lipid peroxides are increasingly recognized as being important in signal transduction for a number of events in the inflammatory response. Malondialdehyde (MDA) has been widely studied as a product of polyunsaturated fatty acid peroxidation. High MDA levels have been observed in several biological fluids from patients with different airways diseases including asthma COPD and bronchitis [10].

MATERIALS AND METHODS:

The study was carried out in Department of Biochemistry, Santosh Medical College and Hospital, Ghaziabad from June 2012 to July 2013. This study was conducted on 30 healthy individuals and 30 COPD patients. These COPD Patients 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. The control subjects were completely healthy non smokers and showed no abnormality on Clinical examinations.

Exclusion Criteria:

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.

Methods:

6 ml of 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.

1. The level of serum Malondiadehyde (MDA) was determined by Kei Satoh method [11].
3. Serum Vitamin-E (α – Tocopherol) was estimated by the method of Baker and Frank [12].
4. Serum Vitamin-C was estimated by the method of Lowry *et al.;* [13].

Statistical Analysis:

Results were statistically analyzed by 'GraphPad Quick Cals t-test calculator'. Student's t-test was used to assess the significance of difference between the groups. All results are presented as mean ± S.D. A 'p' value of less than 0.05 was considered significant.

RESULTS:

Table 1 shows biochemical characteristics of the Cases and Control subjects. The mean level of FEV1 significantly decrease in COPD patients as compared to control (p =0.009). The mean level of FVC was increased significantly in COPD as compared to Healthy Individual. The levels of Vitamin-C and Vitamin-E were found to be lowered as control which was statistically significant. Serum mean Malondialdehyde level was significantly increased in COPD patients when compared to controls (p <0.0001).These results indicate that an increase in oxidative stress and decrease in antioxidant levels in chronic bronchitis patients when compared to controls.

Table 1: Showing Demographic Data, Pulmonary Function, Antioxidant vitamin and Malondialdehyde levels in COPD and normal Healthy Individuals

Variables	Control	Cases	p-Value
No. of Participants	30	30	
FEV1(L)	2.46±0.45	2.15±0.45	p =0.009 **S**
FVC (L)	3.11±0.52	3.70±0.77	p =0.009 **S**
FEV1/FVC %	80.06±12.21	60.08±16.42	p <0.0001 **S**
MDA (nmol/ml)	1.78±0.52	2.40±0.20	p <0.0001 **S**
Vitamin-E (mg/dl)	1.53±0.25	0.92±0.06	p <0.0001 **S**
Vitamin-C (mg/dl)	1.47±0.11	0.48±0.05	p <0.0001 **S**

S = statistically significant

DISCUSSION:

Oxidative stress plays an important role in the pathogenesis of chronic bronchitis. Our results indicate that there is an increase in oxidative stress marker and decrease in antioxidant levels in COPD Patients.

In our study, we found significantly decreased level of Vitamin-C in COPD patients as compared to control (p <0.0001). Our results are in accordance with the studies performed by Nagraj *et al.;* [14], Raghunath *et al.;* [15] Sargeant *et al.;* [16] and Mukadder *et al.;* [17]. Vitamin C functions as an important free radical scavenger. The mechanism involved in the reduction of vitamin C level in COPD is due to rapid oxidation of ascorbic acid by free radicals. The negative relationship between vitamin C and MDA may be due to the depletion of vitamin C when the oxidant burden is increased [17]. Vitamin C functions as an antioxidant

by donating its electrons it prevents other compounds from being oxidized, however by the very nature of this reaction vitamin C itself is oxidized in the process. The species formed after the loss of one electron is a free radical i.e., ascorbyl radical. As compared to other free radicals ascorbyl radical is relatively stable with half life of 10^{-5} seconds and is fairly unreactive which explains the antioxidant nature of vitamin C and its preference. Reduction of a reactive free radical with formation of a less reactive compound is sometimes called free radical scavenging or quenching [18].

We also found significantly decreased level of Vitamin-E ($p < 0.0001$) in COPD patients as compared to control. Tug *et al.;* [19], Daga *et al.;* [20] and Raut *et al.;* [21] obtain the same results. 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.

We also evaluate MDA (marker of lipid peroxidation) as measurement of cellular damage in COPD patients. We found significantly increased level of MDA in COPD patients as compared to healthy individuals ($p < 0.0001$). Our results are in accordance with Nagraj *et al.;* [14] , Daga *et al.;* [20] and Raut *et al.;* [21] Kirkil *et al.;* [22]. Lipid peroxidation, resulting from the reaction of free radicals with polyunsaturated fatty acid side chains in membrane lipoproteins, is a further reaction that can results in cell damage, and is a self perpetuating process that continues as chain reactions.

Oxidative stress has been implicated in the pathogenesis of tobacco smoke induced chronic obstructive pulmonary disease. Reactive oxygen species present in the tobacco smoke may cause damage to human alveolar epithelial cells by lipid peroxidation of cell membranes. Increased MDA concentration in patients with COPD is due to increased production of reactive oxygen species and hence more lipo oxidation products [20]. Increased MDA level in emphysema patients indicates more oxidative stress compared to chronic bronchitis patients. This may be due to patients with emphysema having more severe lung function impairment, lower body mass index, poor quality of life and more serious systemic dysfunction [23].

CONCLUSION:

Present study demonstrates that there is increased oxidative stress in patients with COPD when compared to controls. This study also emphasizes the decreased antioxidants namely serum vitamin C and vitamin-E in COPD patients when compared to controls. This study demonstrates the role of oxidative stress and antioxidant imbalance in pathogenesis of COPD. Hence by advising diet rich in antioxidants or supplementation of antioxidants may prevent the further oxidative damage in COPD patients. 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 antioxidant 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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