

## A study of oxidative stress, thiol proteins and role of vitamin E supplementation in chronic obstructive pulmonary disease (COPD)

Anita M. Raut<sup>1\*</sup>, Adinath N. Suryakar<sup>2</sup> and Dilip Mhaisekar<sup>3</sup>

<sup>1</sup>Department of Biochemistry, Dr. Vikhe Patil Institute of Medical Sciences, Post: MIDC, Vadgaon Gupta, Ahmednagar-414111, Maharashtra, India, <sup>2</sup>Registrar, Maharashtra University of Health Sciences, Mhasrul, Dindori Road, Nashik-422004 Maharashtra, India and <sup>3</sup>Department of Resp. Medicine, Government Medical College, Vazirabad, Nanded-431601 Maharashtra, India

**Abstract:** *Background:* Lipid peroxide plays an important role in inflammatory lung disease. Increased epithelial permeability produced by cigarette smoke is likely to be mediated through depletion of thiol proteins. Imbalance between oxidants and thiol proteins is also an established fact in these patients. *Materials & methods:* In the present study 30 healthy non-smokers were served as controls and 20 patients with stable COPD were included. Their base line clinical examination, Malondialdehyde (MDA) as an oxidant, alpha tocopherol and erythrocyte superoxide dismutase (SOD) as an antioxidants and thiol proteins levels were measured. All above parameters were repeated after 12 weeks of supplementation with 400 IU of vitamin E daily. *Results:* We observed that the mean malondialdehyde levels in these patients at base line were high ( $p < 0.001$ ) than Control Plasma alpha-tocopherol, SOD and thiol proteins levels were low ( $p < 0.001$ ) in the patients compared to controls. Exogenous vitamin E (400 IU twice daily) Supplementation did not bring about any significant change in plasma Erythrocyte Superoxide Dismutase and vitamin E. But slight increase in the plasma thiol proteins levels was seen. The present study shows that initially the plasma lipid peroxide (MDA) levels were high antioxidant (alpha- tocopherol, SOD) and thiol proteins were low in patients with COPD. Exogenous supplementation with vitamin E increases slightly thiol proteins levels and brings down the levels of MDA showing attenuation of further damage. *Conclusion:* Our study confirmed the existence of oxidative stress and and the augmentation of antioxidant defenses as shown by slight increase in thiol proteins level. The antioxidant therapy is adjunct in lung disease patients and opens a promising field in prevention of oxidative stress related complications in these patients.

**Keywords:** Vitamin E, Malondialdehyde, Superoxide Dismutase, Antioxidants, Chronic Obstructive Pulmonary disease, thiol proteins.

### Introduction

Lung disease is a global health concern and is a major cause of chronic morbidity and mortality. It reduces quality of life causes frequent hospital admission and carries an increase risk of prevalence death for those affected [1]. Lung is an 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 inhalation [2]. Since lung contains the largest endothelial surface area than any other organ, it makes the lung a major target site for circulating oxidants and xenobiotics. Chronic obstructive pulmonary disease (COPD) a chronic slowly progressive disorder is characterized by airflow obstruction. Chronic inflammation by cigarette smoke was associated with a dramatic depletion of thiol proteins. Vitamin E is the most important lipophilic

antioxidant in humans. It contributes to membrane stability and protects critical cell structures against harmful effects from oxygen free radicals and reactive lipoperoxides, which is relevant for several human pathological states, including lung diseases.

The oxidative stress is believed to play a vital role in the pathogenesis of COPD being responsible for a series of events including recruitment of neutrophils and macrophages, increased mucus secretion, vascular permeability, airway inflammation, bronchospasm and inhibition of protease inhibitors [3]. The oxidative stress and protease antiprotease imbalance promote alveolar damage and chronic airway inflammation which are pathophysiologic hallmarks of COPD [4]. While oxidative stress has been well

documented in COPD. There have been only a few studies on the therapeutic role of antioxidants and none using vitamin E. In this study we compared the oxidation product (MDA) and antioxidant superoxide dismutase (SOD) level and total plasma sulphhydryl level in COPD and healthy non- smokers. Further we tested the effect of vitamin E Supplementation at 400 IU per day for 12 weeks on MDA (malondialdehyde), SOD (Superoxide Dismutase) and total plasma sulphhydryl levels.

#### *Aims and objectives:*

1. The present work was planned to study thiol proteins as well as oxidant/antioxidant balance during inflammation in COPD.
2. Study was also aimed at investigating the effect of vitamin E supplementation on oxidative stress parameters.

#### *Following parameters were studied:*

1. To estimate serum total lipid peroxide.
2. To evaluate the enzymatic antioxidants such as erythrocytic SOD.
3. To evaluate the non- enzymatic antioxidants such as vitamin E in patients with COPD.
4. To study concentration of thiol proteins in the patients with lung disease.

### **Material and Methods**

The present study was conducted in the Department of Biochemistry Dr. Vikhe Patil Medical College and Hospital Ahmednagar and in Dr. V.M. Govt. Medical college Solapur as well as General Hospital Solapur .Patients with Hypertension, Malignancy, overt cardiac failure, recent surgery, severe endocrine hepatic or renal diseases and use of anticoagulant medicine and the lung disorders other than COPD were excluded from the present study. Informed consent was obtained from each participant in the study. The study was cleared by institutional ethics committee. 10 ml blood was collected from

each patient. 5ml of it was collected in EDTA bulb and 5ml was collected in plain bulb. Plasma and serum were separated from respective bulbs by centrifugation at 3000 rpm for 10 minutes at room temperature. All the samples were analyzed on the same day of collection.

Serum MDA levels were measured reacting than with thiobarbituric acid at high temperature to form pink colored complex which was measured at 530 nm [5] Serum vitamin E was measured by reduction of ferric to ferrous ion which forms a red colored complex with alpha-alpha' bipyridyl as in Baker and Frank method [6]. Erythrocyte SOD activity was measured by Kajari Das method [7] which is based on the ability of SOD to inhibit nitrite formation. Plasma thiol protein were measured at 412 nm using DTNB Ellman's reagent by the method of Hu et al [8]. The statistical analysis was performed by using student t test and P values <0.001 were interpreted as statistically significant. The values were expressed as mean  $\pm$  SD.

### **Results**

This study included 20 clinically stable COPD patients in the age group of 35-60 years and 30 healthy controls were also included in the study .The patient were diagnosed by physicians on the basis of detailed clinical history, clinical examination and the relevant biochemical investigations. All patients were given 12 weeks supply of 400 IU twice daily of vitamin E (given as Cap Evion) under medical supervision. Blood samples were collected and processed after 12 weeks and measurement of malondialdehyde, superoxide dismutase, plasma protein sulphhydryl and alpha- tocopherol was repeated.

Parameters	Healthy controls n=20	Stable COPD n= 30	Vitamin E Supplementation for 12 weeks
Serum MDA (umol/L)	1.98 $\pm$ 0.55	3.42 $\pm$ 0.49	1.99 $\pm$ 0.56 *
Serum vitamin E (Mg /dl)	1.25 $\pm$ 0.20	0.89 $\pm$ 0.08	0.90 $\pm$ 0.09 NS
Erythrocyte SOD (U / mg Hb)	1.53 $\pm$ 0.63	0.88 $\pm$ 0.09	0.89 $\pm$ 0.1 NS
Total plasma Thiol proteins (nm / mg protein)	2.0 $\pm$ 0.30	0.48 $\pm$ 0.1	0.53 $\pm$ 0.09

n = number of cases. All values are expressed in mean  $\pm$  SD. Significant when compare with control group (p < 0.001). NS = Non significant

From Table-1, Except for a slight increase in total thiol proteins in plasma ( $p < 0.001$ ) other parameters did not show any significant changes after treatment when compare to the controls. Significantly high levels ( $P < 0.001$ ) of serum lipid peroxide (MDA) were observed in COPD patients as compared to controls mean plasma levels of vitamin E ( $P < 0.001$ ) was lower than controls. Mean SOD level in patients was lower ( $P < 0.001$ ) than controls. Thus at base line patients with COPD had higher MDA levels and lower antioxidant (SOD & Vitamin E) levels than healthy controls.

Twelve weeks of vitamin E supplementation produced reduction in mean MDA level. However no significant change in plasma vitamin E and SOD levels was observed of 12 weeks of vitamin E supplementation.

### Discussion

In lung disease oxidative stress oxidize thiols of various proteins this irreversible modification under biological relevant condition associated with oxidative injury thus lowering thiol levels [9]. Oxidized species arises leads to the formation of protein thiolates can be readily oxidized to a sulphenic acid that quickly form a disulphide with nearby thiol, strong oxidants will oxidize sulphenic or sulphonic acid derivatives [10]. As thiol groups maintain the overall redox balance this may prevent the inactivation of alpha-1AT by protecting the active site from oxidants this mechanism helps to restore its activity [11]. The increase seen in these endogenous antioxidants in vitamin E supplemented groups may therefore have beneficial effects. Oxidative stress has been shown to be increased in patients with COPD [12] increased oxidative stress has been suggested to play an important role in the pathogenesis of the disease, present study showed that the supplementation of vitamin E (400 IU) for 12 weeks reduces the oxidant load. The mechanism for this may involve activation of macrophages, neutrophils and eosinophils generates superoxide anion, which is rapidly converted to hydrogen peroxide by superoxide dismutase (SOD), and hydroxyl radicals, formed nonenzymatically in the presence of  $Fe^{++}$  as a secondary reaction. In neutrophils, myeloperoxidase also catalyses the formation of the oxidant hypochlorous acid from hydrogen peroxide in the presence of chloride ions. ROS which may also be released by lung

epithelial cells may also stimulates cells directly, thereby amplifying lung inflammatory and oxidant events.

In this study vitamin E supplementation for 12 weeks produced significant decreased in lipid peroxidation level (MDA) In a similar study vitamin E supplementation by Hoshino & Coworkers [13] showed similar results. Our results are also consist with those of Brown K M & Coworkers [14]. Our study demonstrates that vitamin E under our protocol is an effective antioxidants as it reduced the level of plasma MDA by inhibiting lipid peroxidation caused by free radicals. This is in response as vitamin E exerts its antioxidant property by preventing chain propagation as a result of its ability to transfer phenolic hydrogen to a peroxy free radical of a peroxidized polyunsaturated fatty acid that terminates chain reaction, prevents neutrophil chemotaxis, inhibits monocyte adhesion to endothelium and inhibits platelet aggregation thus minimizing the lipid peroxidation that improves the lung function. The mean plasma alpha-tocopherol levels were lower in COPD than controls. The fact that COPD patients have lower tocopherol levels and thus are more prone to free radical injury. It may also be used to prevent decrease in lung function and further damage to the lung tissue. We found no significant increase in plasma alpha tocopherol level in COPD patients after 12 weeks of vitamin E Supplementation. However another study showed increase in alpha tocopherol levels in smokers after vitamin E supplementation at 1000 mg /day for 14 days [15]. Patch & Coworkers demonstrated increase in alpha tocopherol concentration in bronchoalveolar lavage at 2400 IU / day. This difference in results can be explained on the basis of using different doses of vitamin E [9]. Mean SOD levels were low in stable COPD patients. This may point towards the fact increased production of free radicals in COPD patients leads to increased consumption of SOD (superoxide dismutase) an antioxidant enzymes [8,10].

However vitamin E Supplementation in our study caused no significant increase in SOD levels. Our study had a few limitations firstly the sample size was small. A small difference

between groups would not be detected as significant with small samples. Secondly the clinical outcome parameters were limited. The focus in the present study was mainly on biochemical parameters. Thirdly the nutritional intake was not standardized keeping patients on a standardized diet was not feasible in a study of this duration carried out in an outpatient setting.

### Conclusion

In conclusion our study shows that initially the plasma lipid peroxidation products (MDA levels) were high & antioxidants (alpha tocopherol, SOD and thiol proteins) were low in patients with

COPD. Vitamin E supplementation brings down the level of MDA suggesting attenuation of oxidative damage. The lowering of lipid peroxides may prove beneficial by preventing further damage in pulmonary epithelium and improves the lung function test. Before supplementation FEV1/FVC was < 70% it improves slightly after supplementation. Further studies with different doses for longer periods may throw more light on the role of free radical injury and protective effects of antioxidants in COPD.

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\*All correspondences to: Dr. Anita M. Raut, Department of Biochemistry, Dr. Vikhe Patil Institute of Medical Sciences, Post: MIDC, Vadgaon Gupta, Ahmednagar-414111, Maharashtra, India. E-mail: anitara09@gmail.com