

## **Oxidative Stress in Mild and Moderate COPD: Assessment of Oxidant Anti-Oxidant Imbalance.**

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### **Abstract**

Mild COPD is an asymptomatic disease but as oxidant antioxidant imbalance increases the severity of the disease also increases. The aim of the present study was to evaluate the oxidant antioxidant imbalance in mild and moderate COPD groups. The present study included 73 patients with COPD out of which 32 were of Mild impairment and 41 were suffering from Moderate COPD. The results indicated that the mean BMI of Moderate COPD group was lower as compared to Mild COPD group. The lung functions namely FVC, FEV1, FEV1/FVC % and FEV1% Predicted were significantly reduced in Moderate COPD group. The serum levels of antioxidant enzymes were significantly lower in Moderate COPD group as compared to Mild COPD group SOD units/mgm of serum protein( $9.19 \pm 0.09$  Vs  $8.95 \pm 0.08$   $P < 0.01$ ); Catalase units/mgm of serum protein( $8.95 \pm 0.06$  Vs  $8.88 \pm 0.07$   $P < 0.01$ ); GPX nmol NADPH oxidized/min/mgm of serum proteins( $54.32 \pm 0.38$  Vs  $52.95 \pm 0.32$   $P < 0.01$  respectively). The mean Pack Years in Moderate COPD group is more thus indicating smoking induced Oxidant Anti-Oxidant imbalance in disease severity as compared to Mild COPD group in which the Pack Years were less. In present study in COPD patients MDA correlated inversely with SOD ( $r = -0.744, p = 0.01$ ), Catalase( $r = 0.346, p = 0.01$ ), GPX( $r = -0.682, p = 0.01$ ), FEV1( $r = -0.446, p = 0.05$ ) and FEV1% Predicted( $r = -0.567, p = 0.01$ ) The present study indicates that severity of oxidative stress is associated with lung function decline and also decrease in the anti oxidant enzymes levels which in part also contribute to disease severity.

**Keywords:** Oxidant Anti-Oxidant imbalance, Oxidative Stress, Pulmonary Functions, Smoking.

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### **Introduction**

COPD is a major public health problem and is projected to rank third leading cause of deaths globally by the year 2030[1]. An imbalance between oxidant anti-oxidant play an important role in pathophysiology of COPD[2,3,4]. In physiological conditions, antioxidant defense mechanisms maintain a low steady-state concentration of free radicals in the cells and their activities are precisely regulated [5]. Very few studies have compared the Oxidant Anti-Oxidant imbalance in Mild and Moderate COPD. Mild COPD is usually asymptomatic with low levels of oxidative stress but with increase in Oxidant Anti-Oxidant im-

balance the severity of COPD is expected to increase. Membrane lipids are highly susceptible to free radical damage which is found to be highly detrimental to the functioning of the Cell [6]. Malondialdehyde is a product of lipid peroxidation and an indirect measure of free radical activity in body. Oxidative injury resulting from a lack of antioxidants in the body, may at least, in part, be related to reduced FEV; and, thus, they contribute to airflow obstruction [7]. The present study was undertaken to evaluate the oxidant anti oxidant levels in Mild and Moderate COPD patients and also assess the correlation of MDA with Anti-Oxidant enzymes and lung functions in COPD patients.

## Methods

The present study was conducted in Department of Physiology, JNMC, between May to September 2009. All the patients included in the study were taken from TB and Chest Diseases OPD JNMC, AMU, Aligarh. The Study was approved by the Board of Studies, and Institutional Ethical Committee clearance was obtained. The lung functions were done using Mir Spirolab II spirometer and the patients with COPD were selected and grouped into Mild and Moderate severity group according to the guidelines of GOLD[8]. Subjects over 18 years of age with no history of Bronchial Asthma, Diabetes Mellitus, Hypertension, Lung Cancer, Cardiovascular and Renal Diseases, and other diseases in which Oxidative Stress has been documented to be a causative factor, were included in the study. Subjects taking antioxidants were excluded. Estimation of Catalase was done by the method of Aebi [9]. Estimation of Glutathione Peroxidase was done by the method of Paglia and Valentine[10]. Estimation of Superoxide Dismutase was done by method of McCord and Fridovich[11]. Activity of Catalase was estimated as units/mgm of Serum Proteins; Superoxide Dismutase was estimated as units/mgm of Serum Proteins, and Glutathione Peroxidase was estimated as nmol NADPH oxidized/min/mgm of Serum Pro-

teins. MDA was estimated according to the method of Philpot [12]. The levels were estimated as nmol/ml. Protein was estimated using Lowry method[13].

All study parameters were expressed as mean±standard deviation. Student's t test was used to compare the means between two groups. Pearson's correlation test was used to find the correlation of MDA with Anti Oxidant enzymes and lung functions. P value of < 0.05 was taken as statistically significant.

## Results

73 Subjects (48 male and 25 females) with COPD were selected and grouped into Mild (32, M/F 24/8) and Moderate (41, M/F 24/17) groups. The mean age of mild COPD group was 39.78±9.70 while that of Moderate COPD group was 42.19±15 years. All the subjects who participated in the study were smokers and the mean pack years in Moderate COPD group was higher as compared to Mild COPD group (17.07±8.53 Vs 11.62 ±4.22). BMI was significantly reduced in Moderate COPD group as compared to Mild COPD group (p=0.001)[table 1 and 2]. The spirometric values namely FVC, FEV1, FEV1/FVC % and FEV1 % Predicted were significantly lower in Moderate COPD group in comparison with those having Mild COPD (P<0.001 for all)[table 3].

*Table 1. Anthropometry*

Study group	Age(years)	Sex		Height(cm)	Weight(kg)	BMI(Kg/m <sup>2</sup> )*
		male	female			
Mild COPD(n=32)	39.78±9.70	24(75%)	8(25%)	164.15±5.62	60±6.27	22.39±2.68
Moderate COPD(n=41)	42.19± 15	24(58.53%)	17(41.47%)	162.73±7.49	53.04±7.50	20.07±2.90

\* BMI is significantly reduced in Moderate COPD group as compared to Mild COPD group (p=0.001)

*Table 2. Pack years*

Pack Years	Mild COPD	Moderate COPD
Number of sticks/20 x years	11.62± 4.22	17.07± 8.53

*Table 3. Pulmonary Functions*

Study group	Mild COPD	Moderate COPD	P
FVC(liters)	3.97 ±0.86	3.05 ±0.73	<0.001
FEV1(liters)	2.47± 0.47	1.88± 0.46	<0.001
FEV1/FVC(%)	63.20 ±6.21	61.27± 4.96	<0.001
FEV1 % Predicted	81.06 ±5.29	62.48 ±6.57	<0.001

**Table 4.** MDA and Anti Oxidant Enzymes

Parameter	Mild COPD	Moderate COPD	P
SOD(units/mgm of serum proteins)	9.19±0.09	8.95± 0.08	<0.001
Catalase(units/mgm of serum proteins)	8.95 ±0.06	8.88± 0.07	<0.001
GPX(nmol NADPH oxidized/min/mgm of serum proteins)	54.32±0.38	52.95± 0.32	<0.001
MDA(nmol/ml)	1.18 ±0.05	1.32± 0.06	<0.001

**Table 5.** Correlation of MDA with Anti oxidant enzymes and lung functions in COPD cases

Parameter	r	P Value
SOD	-0.744	0.01
Catalase	-0.346	0.01
GPX	-0.682	0.01
FEV1	-0.446	0.05
FEV1 % Predicted	-0.567	0.01

The estimated values of GPX, SOD and Catalase were significantly lower (p<0.001 for all) in Moderate COPD group but the levels of MDA were higher(p<0.001) in the same as compared to Mild COPD group(table 4). In COPD patients MDA correlated inversely with SOD (r=-0.744,p=0.01),Catalase(r=0.346,p=0.01),GPX(r=-0.682, p=0.01), FEV1 (r=-0.446,p=0.05)and FEV1% Predicted (r=-0.567,p=0.01)[Table 5].

**Discussion**

In present study, the severity of COPD has been found increasing with increase in the mean Pack Years. It identifies smoking as a risk factor. Airway obstruction in patients with COPD is thought to occur in susceptible smokers as a result of years of accelerated decline in FEV1. In non-smokers, the FEV1 declines at a rate of 20-30 ml/year [14]. Smokers have an accelerated decline in FEV1 and the rate increases with increasing numbers of cigarettes smoked. Reported changes in patients with COPD, range between 48-91ml/year[15]. In the present study BMI was significantly reduced in Moderate COPD group as compared to Mild COPD group (p=0.001). Low BMI may be attributed to skeletal muscle atrophy and weight loss [16].Malnutrition and unexplained weight loss are also known with more advanced COPD. Although we have not included inflammatory markers or correlated BMI with lung function decline,MDA or Anti Oxidant enzymes levels but possible explanation could be that the Pack Years is more in the Moderate COPD group which may result in the present findings. We have found that the Moderate COPD Patients have higher Oxidant Anti-Oxidant imbalance as compared to patients having Mild COPD. Cigarette smoke contains more than 6000 chemical compounds, and both free radicals and oxidants are present in abundance [17]. Smoking induces inflamma-

tion and alters repair mechanisms. The explanation for reduced SOD activity is a possible direct inactivation of

the enzyme by hydrogen peroxide or by the superoxide anion itself [18,19].Various studies have reported that the plasma levels of MDA are increased in healthy smokers and in patients with COPD[20,21]. A study conducted by Birgul I et al, 2007, showed that MDA levels are significantly higher in smokers than in non- smokers[22]. ZA Solak et al, 2005, reported an increase in MDA levels and decrease in GPX activity in smokers, as compared to healthy non-smokers[23]. Yessica D et al, 2009, reported an increase in MDA levels in all stages of COPD severity, as compared to healthy controls[24]. Premanand R et al, 1994, reported in elevated levels of MDA with concomitant reduction in GPX activity in respiratory diseases[25]. Nadeem et al, 2005, reported an increase in MDA levels in COPD patients, as compared to healthy non-smoking controls. They found an increase in levels of SOD, Catalase and GPX in severe COPD patients, as compared to Moderate COPD patients[26].A study conducted by Kluchova Z et al, 2007, showed a decrease in GPX activity and higher MDA levels in patients with Severe COPD, compared to Moderate COPD[27]. We have studied the Oxidant Anti-Oxidant imbalance in Mild and Moderate COPD and in accordance with the above studies we have found that oxidative stress increases as disease severity increases. Sethi JM et al[28] , reported that higher the smoking intensity, greater will be the decline in FEV1. We have also found decline in lung functions in Moderate COPD group as compared to Mild COPD group which is explained on the basis of interplay of inflammation, remodeling, bronchospasm, mucus hypersecretion, loss of elastic recoil and increased airway resistance, resulting in progressive reduction in the expiratory airflow. Movahed M et al in 2007 reported increase in pulmonary symptoms once ten pack years’ history is reached[29].Our study shows inverse correlation of MDA with lung func-

tions and antioxidant enzymes in COPD patients. Inverse correlation between lung functions and MDA have been demonstrated [30, 31]. As oxidative stress increases, MDA would be expected to increase [32]. In present study smokers with COPD have higher MDA levels which probably resulted due to smoking induced radical chain reaction leading to lipid peroxidation of membrane phospholipids, altering cellular physiology. Similarly increase in MDA and decrease anti oxidant enzymes have been reported in COPD patients compared to healthy non smokers/controls[33,23]. Liu Ling Yun et al [34], reported an increase in MDA and decreased levels of SOD and GPX in stable and acute exacerbation in COPD group.

In conclusion on the basis of study the increasing pack years contribute to disease severity. Oxidant Anti - Oxidant imbalance is more in Moderate COPD as compared to Mild COPD. The levels of lipid peroxidation biomarker MDA correlate negatively with lung functions and anti oxidant defenses in body.

Limitations and drawbacks: The study is not free from selection bias and sample size is small. The study needs comparison of results with healthy non smokers and smokers without COPD. Socioeconomic status, contributory/ risk factors resulting in COPD need to be evaluated.

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