

## OXIDATIVE STRESS AND ANTIOXIDANT STATUS IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE PATIENTS

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### ABSTRACT

Chronic obstructive pulmonary disease (COPD) is associated with high incidence of morbidity and mortality. The imbalance between oxidants and antioxidants is thought to play an important role in the pathogenesis of COPD. A total number of 120 subjects comprising of 60 healthy controls and 60 COPD cases were studied. Among 60 COPD cases, 30 were chronic bronchitis patients and 30 were emphysema patients. In all the subjects, serum levels of Malondialdehyde (MDA) as a biomarker of lipid peroxidation and antioxidants like whole blood reduced glutathione (GSH), serum vitamin C, and superoxide dismutase (SOD) activity were estimated. The levels of whole blood reduced glutathione, serum vitamin C and SOD activity were significantly decreased in COPD cases when compared to controls and they were much lower in emphysema patients when compared to chronic bronchitis patients. Serum MDA was significantly increased in COPD cases when compared to controls and was much higher in emphysema patients when compared to chronic bronchitis patients. The presence of increased systemic oxidative stress seems to be associated with current active smoking and systemic inflammation. The decrease in antioxidants levels among COPD patients appears to be mainly a consequence of increased oxidative stress. This suggests that oxidative stress is likely to be involved in pathogenesis of COPD.

**KEYWORDS:** Oxidative stress; antioxidants; COPD; reduced glutathione; vitamin C; SOD; malondialdehyde.

### 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.<sup>1</sup>

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"<sup>2</sup>. Chronic bronchitis is a clinical diagnosis defined by excessive secretion of bronchial mucus and is

manifested by daily productive cough for 3 months or more in at least 2 consecutive years. Emphysema is a pathologic diagnosis that denotes abnormal permanent enlargement of air spaces distal to the terminal bronchiole, with destruction of their walls without obvious fibrosis.<sup>2</sup>

Cigarette smoking is the most important risk factor for COPD. It is estimated that 80% of COPD patients have significant exposure to tobacco smoke. The remaining 20% have a combination of exposure to environmental tobacco smoke, occupational dusts, chemicals and familial and hereditary factors.<sup>2</sup>

A current hypothesis in the pathogenesis of COPD is that the increased oxidant burden both directly as a result of smoking and indirectly by the release of reactive oxygen species from airspace

leukocytes may not be adequately counter balanced by the lung antioxidant systems, resulting in oxidative stress. An excess of oxidants may then lead to enhanced pro-inflammatory gene expression and oxidative tissue injury leading to COPD.<sup>3</sup> Malondialdehyde (MDA) a lipid peroxidation product is an indicator of oxidative stress has correlated inversely with pulmonary function.<sup>4</sup> Antioxidants depletion or deficiency may contribute to oxidative stress. Antioxidants not only protect against the direct injurious effects of oxidants, but also alter the inflammatory events that play an important role in the pathogenesis of COPD.<sup>5</sup>

Erythrocyte antioxidants such as reduced glutathione functions as an efficient intracellular scavenger of H<sub>2</sub>O<sub>2</sub> and plays an important role in the prevention of peroxidative lung damage in patients with COPD. Vitamin C is water soluble free radical scavenger, can directly scavenge O<sub>2</sub><sup>-</sup> and OH<sup>-</sup> radicals and helps to neutralize physiological oxidant burden created by both exogenous and endogenous sources.<sup>6</sup>

Present study is undertaken to evaluate whole blood reduced glutathione, serum vitamin C, superoxide dismutase activity and malondialdehyde in controls and in chronic obstructive pulmonary disease cases.

#### MATERIALS AND METHODS:

A cross sectional study of whole blood reduced glutathione, serum vitamin C, superoxide dismutase activity and malondialdehyde in chronic obstructive pulmonary disease patients were carried out from April 2009 to April 2010. Controls and COPD cases were selected from Bapuji Hospital and Chigateri General Hospital, Davangere (both these hospitals are attached to teaching institute, J.J.M Medical College, Davangere). Each gave an informed consent and this study was approved by the ethical and research committee of J.J.M Medical College, Davangere, to use human subjects in the research study. The patients and controls voluntarily participated in the study.

A total number of 120 subjects were included in the study, of which 60 were chronic obstructive pulmonary disease cases and 60 were healthy controls.

#### Inclusion criteria:

**i) Cases:** Clinically and radiologically diagnosed cases of chronic obstructive pulmonary disease were included. Total 60 cases of COPD patients were divided into 30 cases of emphysema and 30 cases of chronic bronchitis.

**ii) Controls:** 60 normal healthy individuals without any history of smoking and chronic lung disease were included.

#### Exclusion criteria:

- Patients with pneumonia, asthma or other chronic respiratory disease
- Patients with history of cardiac failure
- Patients with history of any recent surgical intervention
- Patients with history of diabetes mellitus
- Patients with history of hepatic disease
- Patients with history of renal disease

#### Collection of blood samples:

About 6ml of blood was collected from large peripheral vein under aseptic precaution after overnight fasting. Out of which 3ml was taken in an anticoagulant (EDTA) bulb for estimation of whole blood reduced glutathione (GSH), 3ml in a plain bulb for estimation of serum vitamin C, superoxide dismutase (SOD) and malondialdehyde (MDA).

#### Estimation of Whole Blood Reduced Glutathione

Whole blood reduced glutathione was estimated by Ernest Beutler et al., Method.<sup>7</sup> It is based on the principle that all of the non-protein sulphhydryl groups of red blood cells are in the form of reduced glutathione (GSH). 5,5<sup>1</sup> - dithiobis-2-nitrobenzoic acid (DTNB) is a disulphide compound, which is readily reduced by sulphhydryl compounds, forming a highly colored yellow compound. Optical density of which is measured at 412nm and is directly proportional to the GSH concentration.

#### Estimation of Serum Vitamin C

Serum vitamin C was estimated by 2, 4 - dinitrophenyl hydrazine method.<sup>8</sup> This method

based on the principle that ascorbic acid is oxidized by copper to form dehydroascorbic acid and diketogulonic acid. These products are treated with 2,4-dinitrophenyl hydrazine (DNPH) to form the derivative bis -2,4-dinitrophenyl hydrazone. This compound, in strong sulfuric acid, undergoes rearrangement to form a colored product which is measured at 520nm. The reaction is run in the presence of thiourea to provide a mildly reducing medium, which helps to prevent interference from non-ascorbic acid chromogen.

#### Estimation of Serum Superoxide Dismutase

Serum superoxide dismutase activity was estimated by Marklund and Marklund method.<sup>9</sup> This method is based on the principle that superoxide anion is involved in auto-oxidation of pyrogallol at alkaline pH (8.5). The superoxide dismutase (SOD) inhibits auto -oxidation of pyrogallol, which can be determined as an increase in absorbance at 420 nm.

#### Estimation of Serum Malondialdehyde

Serum malondialdehyde estimated by Kei Satoh Method.<sup>10</sup> It is based on the principle of auto-

oxidation of unsaturated fatty acids involves the formation of semistable peroxides, which then undergo a series of reactions to form malondialdehyde (MDA). MDA reacts with thiobarbituric acid (TBA) to form pink colored chromogen. The resulting chromogen is extracted with 4.0ml of n-butyl alcohol and the absorbance of which is measured at 530 nm.

#### STATISTICAL ANALYSIS:

Results are expressed as mean  $\pm$ SD and range values. Unpaired 't' test is used for comparing different biochemical parameters between cases and controls. p value of < 0.05 was considered as statistical significance.

#### RESULTS:

Among 60 controls, 35 were male and 25 were female and their mean age was  $57.7 \pm 7.4$  years and among 60 COPD cases, 46 were male and 14 were female and their mean age was  $62.3 \pm 7.8$  years. There were no significant differences in age among cases and controls.

**Table 1: Comparison of Whole Blood Reduced Glutathione, Serum Vitamin C, SOD activity and Malondialdehyde in Controls and COPD Cases**

No.		GSH (mg/dl)		Vit. C (mg/dl)		SOD activity (U/ml)		MDA (n mol/ml)	
		Range	Mean $\pm$ SD	Range	Mean $\pm$ SD	Range	Mean $\pm$ SD	Range	Mean $\pm$ SD
Controls	60	25.00– 38.02	33.55 $\pm$ 2.23	0.82-1.40	1.10 $\pm$ 0.16	5.00-12.90	9.93 $\pm$ 1.73	1.53 – 3.58	2.62 $\pm$ 0.52
COPD Cases	60	24.17 – 31.42	27.63 $\pm$ 1.96	0.35 – 0.81	0.56 $\pm$ 0.12	2.30 – 7.05	4.78 $\pm$ 1.27	3.58– 6.71	5.36 $\pm$ 0.74
Mean Diff.		5.92		0.54		5.15		2.74	
t-value *		15.45		21.10		18.54		23.44	
p-value		< 0.001, HS		< 0.001, HS		< 0.001, HS		< 0.001, HS	

\* Unpaired t- test

**Table no.1** shows comparative analysis of whole blood reduced glutathione, serum vitamin C, SOD activity and MDA levels between controls and COPD cases. Statistical analysis by unpaired t-test shows that mean levels of whole blood reduced

glutathione, serum vitamin C, SOD activity were significantly decreased ( $p < 0.001$ ) and mean level of serum MDA was significantly increased in COPD cases when compared to healthy controls and are statistically highly significant ( $p < 0.001$ ).

**Table 2: Comparison of Whole Blood Reduced Glutathione, Serum Vitamin C, SOD activity and Malondialdehyde in Different Phenotypes of COPD**

Groups		GSH (mg/dl)	Vit.C (mg/dl)	SODactivity (U/ml)	MDA (nmol/ml)
Controls	Mean ± SD	33.55 ± 2.23	1.10 ± 0.16	9.93 ± 1.73	2.62 ± 0.52
	Range	25.00-38.02	0.82-1.40	5.00-12.90	1.53-3.58
Chronic bronchitis	Mean ±SD	28.97 ±1.11	0.65 ±0.09	5.67 ± 0.81	4.83 ± 0.51
	Range	26.76 - 31.42	0.47 – 0.81	4.00 – 7.05	3.58 – 5.89
Emphysema	Mean ±SD	26.29 ±1.70	0.47 ±0.08	3.90 ±0.99	5.89 ±0.52
	Range	24.17-30.98	0.35-0.75	2.30-7.00	4.42-6.71
Controls Chronic bronchitis	Mean difference	4.58	0.45	4.26	2.21
	t*	13.01	17.67	15.86	19.20
	p	< 0.001	< 0.001	< 0.001	< 0.001
Controls emphysema	Mean difference	7.26	0.63	5.03	3.27
	t*	17.17	25.34	20.91	28.11
	p	< 0.001	< 0.001	< 0.001	< 0.001

\* Unpaired t- test

p value < 0.001, highly significant

**Table 3: Comparison of Whole Blood Reduced Glutathione, Serum Vitamin C, SOD activity and MDA in Chronic Bronchitis and Emphysema Cases**

COPD Groups	No.	GSH (mg/dl)	Vit.C (mg/dl)	SOD activity (U/ml)	MDA (nmol/ml)
Chronic Bronchitis	30	28.97 ±1.11	0.65 ± 0.09	5.67 ± 0.81	4.83± 0.51
Emphysema	30	26.29 ±1.70	0.47 ± 0.08	3.90 ± 0.99	5.89 ± 0.52
Mean difference		2.68	0.18	1.77	1.06
t*-value		7.23	8.44	7.57	7.98
p-value		< 0.001	< 0.001	< 0.001	< 0.001

\* Unpaired t- test

p value < 0.001, highly significant

The mean values of whole blood reduced glutathione, serum vitamin C, SOD activity and MDA in controls were in the range of 33.55±2.23 mg/dl, 1.10±0.16 mg/dl, 9.93 ±1.73 U/ml and 2.62±0.52 nmol/ml respectively. In COPD cases they were in the range of 27.63 ±1.96 mg/dl, 0.56±0.12 mg/dl, 4.78 ±1.27 U/ml and 5.36 ±0.74 nmol/ml respectively. These results indicate that increase in oxidative stress and decrease in antioxidant levels in COPD cases when compared to controls.

#### Whole Blood Reduced Glutathione

The mean value of whole blood reduced glutathione is 33.55 ±2.23 mg/dl in controls and 27.63 ±1.96 mg/dl in COPD cases. When compared to controls COPD patients have significantly decreased (p value < 0.001) level of GSH. The mean value of whole blood reduced glutathione is 28.97 ±1.11 mg / dl in chronic bronchitis patients and 26.29 ±1.70 mg/dl in emphysema patients. GSH is much decreased (p value < 0.001) in emphysema patients when compared to chronic bronchitis patients.

#### Serum Vitamin C

The mean value of serum vitamin C is 1.10 ±0.16 mg / dl in controls and 0.56±0.12 mg/dl in COPD cases. When compared to controls COPD patients

have significantly (p value < 0.001) decreased level of vitamin C. The mean value of serum vitamin C is 0.65 ±0.09 mg / dl in chronic bronchitis patients and 0.47 ±0.08 mg/dl in emphysema patients. Vitamin C is much decreased (p value < 0.001) in emphysema patients when compared to chronic bronchitis patients.

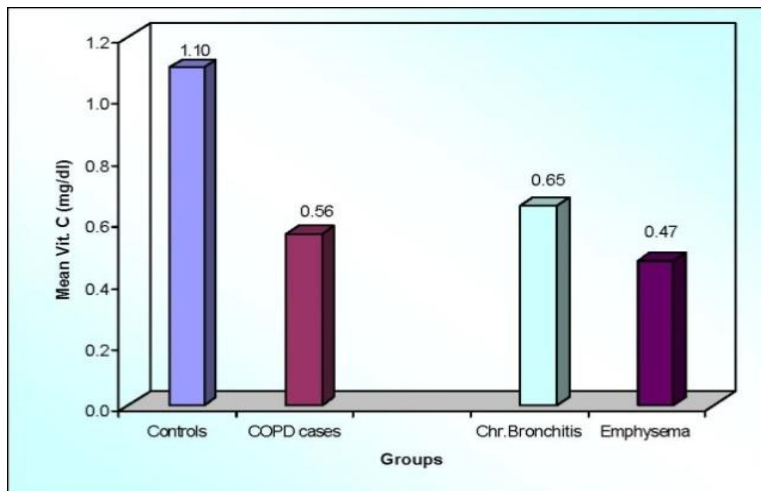
#### Serum superoxide dismutase activity

The mean value of SOD is 9.93 ±1.73 U/ml in controls and 4.78±1.27 U/ml in COPD cases. When compared to controls COPD patients have significantly (p value < 0.001) decreased level of SOD. The mean value of SOD is 5.67 ±0.81 U/ml in chronic bronchitis patients and 3.90 ±0.99 U/ml in emphysema patients. SOD activity much decreased (p value < 0.001) in emphysema patients when compared to chronic bronchitis patients.

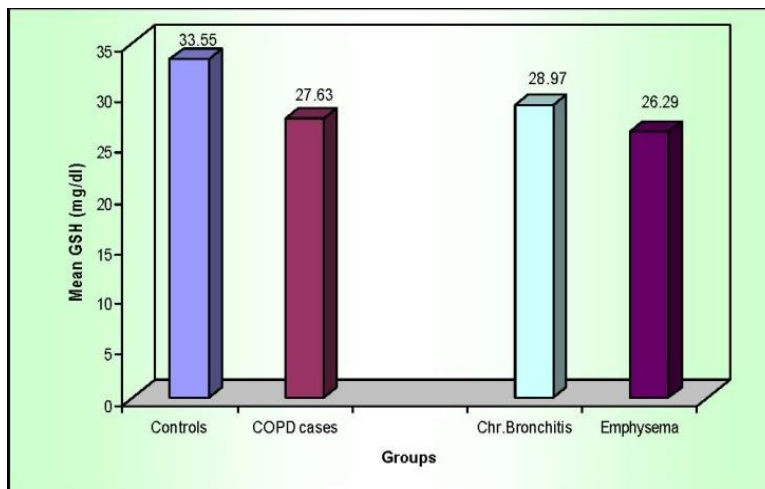
#### Serum Malondialdehyde

The mean value of serum MDA is 2.62 ±0.52 nmol/ml in controls and 5.36±0.74 nmol/ml in COPD cases. When compared to controls COPD patients have significantly (p value < 0.001) increased level of MDA. The mean value of serum MDA is 4.83 ±0.51 nmol/ml in chronic bronchitis and 5.89 ±0.52 nmol/ml in emphysema patients. MDA level is much elevated (p value < 0.001) in

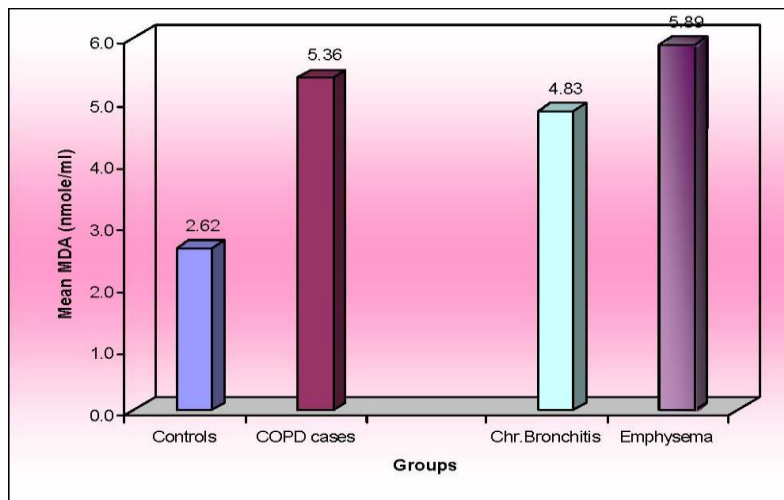
emphysema patients when compared to chronic bronchitis patients.



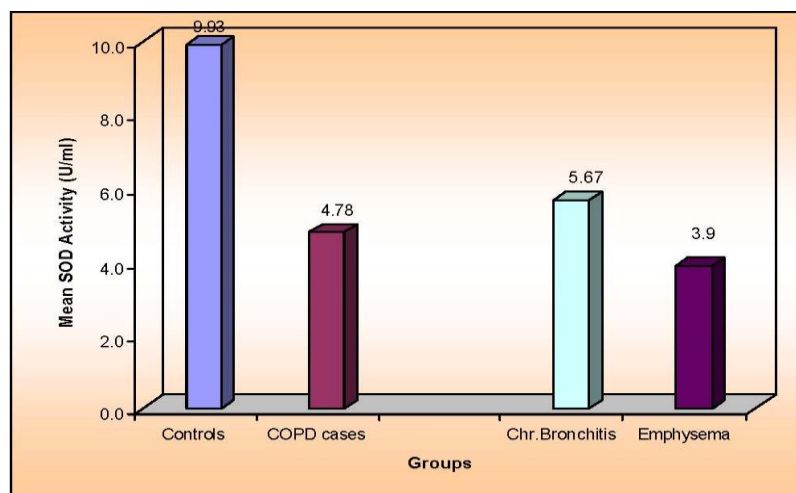
Graph - 1: Comparison of serum vitamin C in controls and in COPD cases



Graph-2: Comparison of whole blood reduced glutathione in controls and in COPD cases



Graph - 3: Comparison of serum MDA in controls and COPD cases



Graph - 4: Comparison of serum SOD activity in controls and COPD cases

**DISCUSSION:**

Oxidative stress plays an important role in the pathogenesis of COPD.<sup>11</sup> these results indicate that there is increase in oxidative stress and decrease in antioxidant levels in COPD cases when compared to controls. When compared to controls COPD patients have significantly decreased (p value < 0.001) level of GSH. This is in accordance with the

study of Madhuri Parija et al,<sup>12</sup> Mercken EM et al,<sup>13</sup> and Mukadder calikoglu et al<sup>14</sup> and among COPD phenotypes GSH level is much lower (p value < 0.001) in emphysema patients when compared to chronic bronchitis patients.

Cigarette smoking is the most important factor for the development of COPD. Under non stress conditions, most of the intracellular glutathione is

stored in the reduced form (GSH). During increased oxidative stress, the free sulfhydryl (-SH) groups become oxidized resulting in loss of GSH. The gaseous phase of cigarette smoke may also irreversibly react with GSH to form GSH derivatives that cannot be reduced back, thereby depleting the total available GSH pool.<sup>15</sup>

The activities of glutathione synthesis and redox system enzymes such as glutathione peroxidase, gamma-glutamyl cysteine synthetase and glucose-6-phosphate dehydrogenase were transiently decreased in alveolar epithelial cells after exposure to cigarette smoke condensate (CSC), possibly as a result of the action of highly electrophilic free radicals on the active site of enzymes. Thus there is a time dependent depletion of intracellular soluble GSH, concomitant with the formation of GSH conjugates.<sup>16</sup>

When compared to controls COPD patients have significantly ( $p$  value  $< 0.001$ ) decreased level of vitamin C. This is in accordance with studies of Raghunath R. Rai et al,<sup>6</sup> L.A. Sargeant et al<sup>17</sup> and Mukadder calikoglu et al.<sup>14</sup> In our study vitamin C is much decreased ( $p$  value  $< 0.001$ ) in emphysema patients when compared to chronic bronchitis patients. 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.<sup>18</sup> 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.<sup>19</sup>

Superoxide dismutase functions as a scavenger of super oxide radical in the body. The level of SOD is

decreased in oxidative stress, which plays an important role in the pathogenesis of various diseases.<sup>6</sup> When compared to controls COPD patients have significantly ( $p$  value  $< 0.001$ ) decreased level of SOD. This is in accordance with studies of Raghunath R Rai et al,<sup>6</sup> Gamze kirkil et al<sup>20</sup> and in our study SOD is much decreased ( $p$  value  $< 0.001$ ) in emphysema patients when compared to chronic bronchitis patients.

The alterations in antioxidant enzymes such as SOD emphasize the redox imbalance in COPD patients. Mechanism involved in decreased serum SOD activity is due to increased production of free radicals in COPD patients leading to increased consumption of antioxidant enzymes.<sup>20</sup>

MDA is a lipid peroxidation product which is formed during oxidative process of PUFA by reactive oxygen species. MDA is the sensitive marker of lipid peroxidation. COPD patients are subjected to enhanced oxidative stress and increased level of MDA. When compared to controls COPD patients have significantly ( $p$  value  $< 0.001$ ) increased level of MDA. This is in accordance with the study of M.K. Daga et al<sup>21</sup>, Birgul Isik et al<sup>22</sup>, and Gamze kirkil et al<sup>20</sup>. In our study MDA level is much higher ( $p$  value  $< 0.001$ ) in emphysema patients when compared to chronic bronchitis patients.

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 lipoxidation products.<sup>21</sup> 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.<sup>23, 24</sup>



## CONCLUSION

Present study demonstrates that there is increased oxidative stress in patients with COPD when compared to controls and oxidative stress is much higher in emphysema patients when compared to chronic bronchitis patients. This study also emphasizes the decreased antioxidants namely whole blood reduced glutathione, serum vitamin C and SOD activity in COPD patients when compared to controls. Antioxidant levels are particularly much decreased in emphysema patients when compared to chronic bronchitis patients. 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.

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