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Study of Correlation of Pulmonary Function Test with the Markers of Oxidative Stress and Non-enzymatic Antioxidants in Chronic Obstructive Pulmonary Disease (COPD) Patients

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Authors' contributions

This work was carried out in collaboration between all authors. Author SAA Concept, designed and supervised the study. Author RSP conducted the study, review of literature, and wrote the manuscript. Author PB performed all the statistical analysis. Author RL did the diagnosis of COPD patients and managed the sample collection. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: Chronic Obstructive Pulmonary Disease (COPD) represents a major health problem. Its prevalence is increasing worldwide. The aim of our study was to assess the relationship between markers of oxidative stress (malondialdehyde (MDA) and protein carbonyl) and the non-enzymatic antioxidants (vitamin E, vitamin C and reduced glutathione (GSH) with the marker of airflow obstruction (FEV_1% predicted) in COPD patients.
Study Design: Case-control study.

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Place and Duration of Study: Department of Biochemistry, B.J. Govt. Medical College and Sassoon General Hospital, Pune. [Maharashtra]. The study period was in between Feb 2012 to Aug 2013.

Methodology: Study comprised of 120 stable COPD patients of different stages were selected as per (GOLD) guidelines, each group consisting 30 patients, of age 40-75 yrs and 30 healthy controls. Pulmonary function test was done by using spirometer. Serum levels of MDA, protein carbonyl, vitamin E, vitamin C and GSH were estimated by spectrophotometric method. Statistical analysis was performed by using SPSS 17 software.

Results: Lung function tests namely FEV_1/FVC% ratio and FEV_1 % predicted showed significant reduction in stage I: (65.28±2.78; 90.23±11.36), stage II: (59.76±6.56; 63.13±7.85), stage III: (49.16±6.17; 39.76±6.34) and stage IV: (37.44±4.78; 22.43±5.55) COPD patients as compared to healthy controls (100.33±7.471;105.03±13.08 $P<0.001$) respectively. The level of serum MDA and protein carbonyl was increased significantly in [stage I: (6.23±0.81nmol/ml, 5.64±2.94nmol/mg) stage II: (7.94±1.26nmol/ml, 8.1±2.33 nmol/mg), stage III: (9.42±1.51nmol/ml, 9.66±3.12nmol/mgs) and stage IV: (11.53±1.23nmol/ml, 11.13±2.17nmol/mg] COPD patients as compared to controls (4.19±1.79nmol/ml, 3.50±1.87nmol /mg) respectively. Where as a significant concomitant decreased was observed in vitamin E, vitamin C and reduced glutathione in [stage I: (1.09±0.37mg/dl; 0.98±0.34mg/dl; 28.24±6.12mg/dl), stage II: (0.806±0.27mg/dl, 0.69±0.28mg/dl, 22.42±4.50mg/dl), stage III: (0.608±0.15mg/dl, 0.53±0.09mg/dl, 17.67±4.45mg/dl) and stage IV: (0.48±0.11mg/dl, 0.43± 0.10mg/dl, 13.73±2.76mg/dl) COPD patients as compared to controls (1.51±0.40mg/dl, 1.41±0.59 mg/dl, 34.26±4.96mg/dl) respectively. We found a significant negative correlation between the MDA and protein carbonyl with the FEV_1% predicted and positive correlation between the vitamin C, vitamin E and GSH with the marker of airflow obstruction (FEV_1% predicted) in COPD patients.

Conclusion: From this study we conclude that as the severity of disease increases FEV_1% predicted decreases. These changes are associated with an increase in oxidative stress and a concomitant decrease in non-enzymatic antioxidants studied.

Keywords: GOLD - global Initiatives for obstructive lung disease; FEV1 - force expiratory volume in one second; FVC- force vital capacity; MDA- malondialdehyde; GSH - reduced glutathione; ROS - reactive oxygen species.

1. INTRODUCTION

Chronic Obstructive Pulmonary Disease (COPD) is a slow, progressive condition characterized by airflow limitation, which is largely irreversible [1]. The most common risk factor for COPD is tobacco smoking [2]. The prevalence of COPD is higher in countries where smoking is highly prevalent. Tobacco smoke contains oxidants such as carbon monoxide, nicotine, semiquinone, acroline which produces oxidative stress. The coexistence of airway inflammation and parenchymal inflammation occurs in most COPD patients [3]. The increase of oxidative stress in patients with COPD results from the action of exogenous oxidants (such as air pollutants and tobacco components) as well as endogenous oxidants produced during the inflammatory process [4-6].

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 [7]. The product of lipid peroxidation is malondialdehyde [MDA], which serve as an indicator of oxidative damage in vivo [8-10]. Another important biomolecule targeted by free radicals is protein. Its irreversible non-enzymatic oxidation or carbonylation causes loss of protein function resulting in accumulation of protein carbonyl products in the cell or in biological fluids [11,12].

The harmful effects of these free radicals are balanced in the cell by scavenging action of enzymatic and non-enzymatic antioxidants [13,14]. Vitamin E is lipid soluble antioxidant, represents body's principle defense against oxidant-induced membrane injury in human tissue via its role in breaking the lipid peroxidation chain reaction [15]. Vitamin E is present in lipid membranes and in extracellular fluids [16]. Vitamin C is the water soluble antioxidant, present abundantly in epithelial lining fluid of the lungs [17,18]. It scavenges the superoxide, hydroxyl and peroxyl radicals [17]. It also contributes in regeneration of vitamin E. Vitamin C functions as a chain breaking antioxidant [19]. In addition to this, vitamin C plays a role in immune function and is transported into neutrophils and lymphocytes [20].

GSH is the most abundant intracellular molecule, capable of protecting cells against oxidants and toxic xenobiotics [21,22]. It plays a role in synthesis of leukotrienes, proteins, nucleic acids as well as the activation of enzymes and regulation of immune response [22]. GSH in the epithelial lining fluids provides sensor system for the production of lung surfactant proteins [23-26]. A low level of GSH in the cells increases its risk for the oxidative damage.

Increase in oxidative stress in the circulation causes a fall in the serum non-enzymatic antioxidants (vitamins E, vitamin C and reduced glutathione) in smoking people was observed in previous reports [27,28]. Study of non-enzymatic antioxidants in patients suffering from lung disease opens a promising field in prevention of oxidative stress related complications.

There are many studies available on oxidants and antioxidants levels in COPD patients but only few has compared the levels of oxidants and antioxidants with lung function parameters in COPD patients, which may be important in deciding the severity or recurrence of the disease.

Keeping this fact in mind we have decided to find out correlation between lung function test and marker of oxidative stress and non-enzymatic antioxidants in COPD patients.

2. MATERIALS AND METHODS

2.1 Selection of Cases and Control

a) Control Group: Consist of 30 normal, age and sex matched healthy volunteers. Healthy volunteers for this group serving as control were selected from the Institution.
b) COPD patients Group: The study population was selected from among 120 consecutive patients of COPD, who attended TB and Chest Department of B. J. Govt. Medical College and Sassoon General Hospital, Pune [Maharashtra]. The subjects who were enrolled in the study were ≥40 years of age with symptoms of COPD (dyspnea, chronic cough or sputum production, forced expiratory volume in 1st second/forced vital capacity (FEV_1/FVC) <0.7 on spirometry and with post-

bronchodilator FEV_1 reversibility, that is, less than 12%). One hundred and twenty COPD patients were classified into four stages according to GOLD (Global Initiative for Obstructive Lung Disease) guidelines based on the values of FEV1% predicted with FEV1/FVC % ratio <0.7 after performing lung function test, these are as follows:

a) Stage I COPD: – (n=30, post-bronchodilator FEV1≥ 80%),
b) Stage II COPD:- (n=30,post-bronchodilator FEV1 ≥50% and <80%),
c) Stage III COPD:- (n=30,post-bronchodilator FEV1≥30% and <50%),
d) And Stage IV COPD :- (n=30, post-bronchodilator FEV1<30%).

Informed consent form was obtained from each subject prior to the study.

2.2.1 Exclusion criteria for control and COPD patients group

Patients who were suffering from or who were known to have tuberculosis, pneumonia, asthma, bronchiectesis, lung cancer, interstitial lung diseases, respiratory failure, cardiac failure, diabetes mellitus, hepatic disease, renal disease and who had any recent surgical intervention and who are unable to performed lung function test were excluded from COPD patients group. Healthy individual with any past history of lung/respiratory disease or with abnormal lung function test were excluded from control group.

2.2 Collection of Blood Samples

Under aseptic condition and with prior consent of the subject, 7ml of blood was collected from large peripheral vein, after overnight fasting. Out of which 2ml was taken in an EDTA bulb for the estimation of whole blood reduced glutathione (GSH) and remaining 4ml blood was collected in a plain bulb, allowed to clot for 1 hr. Serum was separated by centrifugation at 3000 rpm for 10 minutes at room temperature, separated serum was aliquot and stored at -80ºC until the analysis and was used for the estimations of serum MDA, protein carbonyl, vitamin E and vitamin C.

2.3 Estimation of Serum Malondialdehyde [MDA]

Serum malondialdehyde was determined by Buege and Aust method (1978). It was expressed as nmol/ml [29].

2.4 Estimation of Serum Protein Carbonyl

Serum Protein Carbonyl was determined by Levin et al method (1990). It was expressed as nmol/mg of proteins [30]. Protein concentration in mg/ml was determined by Bradfoed method (1976) [31].

2.5 Estimation of Whole Blood Reduced Glutathione

Total blood reduced glutathione (GSH) was determined by Ernest Beutler et al. Method [32]. GSH was determined by use of standard curve and was expressed as mg/dl.

2.6 Estimation of Serum Vitamin C

Serum vitamin C was determined by Ayekyaw method [33]. It was expressed as mg/dl.

2.7 Estimation of Serum Vitamin E

Serum Vitamin E was measured by Braker and Frank method [34]. It was expressed as mg/dl.

2.8 Pulmonary Function Test

Pulmonary Function test was done by using Spirometer. Measurement of Forced Vital Capacity and Forced Expiratory Volume was done in First seconds. The FEV1/FVC is calculated using the maximum FEV1 and FVC from the techanically acceptable, though not from the same curves. The Data was obtained from the printer, attached to spirometer.

2.9 Statistical Analysis

Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS version 17). The data for biochemical analysis was expressed as mean ± SD. The statistical significance of the results was analyzed by using unpaired "t" test. *P* value of <0.05 was considered as statistically significant. Pearson correlation was used to analyze the relationship between biochemical markers and lung function parameters.

3. RESULTS

(Table no.1) shows that irrespective of the sex in healthy volunteers the normal FEV_1% predicted and FEV_1/FVC% ratio was observed, which decreases with the advancement of the stage.

(Table no.2) shows the levels of serum malondialdehyde, protein carbonyl, whole blood reduced glutathione, serum vitamin C and vitamin E levels in healthy controls and in different stages of COPD patients. In present study we used malondialdehyde as a marker of lipid peroxidation for to assess oxidative stress in COPD patients. In our study, we found a significant increase in the level of serum malondialdehyde in stage I COPD patients versus healthy controls (P<0.0001), stage II versus stage I (P<0.0001), stage III versus stage II (P<0.001) and stage IV versus stage III COPD patients (P<0.0001). Higher serum MDA level was observed in stage IV COPD patients compared to other stages (Table no.2).

Protein carbonyl is a marker of protein oxidation. In our study, we observed a significant increase in the level of serum protein carbonyl in stage I COPD patients as compared to healthy controls (P<0.01). In addition to this when we compared the levels of protein carbonyl in stage II versus stage I (P<0.001), stage III versus stage II (P<0.05) and stage IV versus stage III (P<0.05), a remarkable increase was observed in COPD patients (Table no.2). There was significant increase in values of protein carbonyl like MDA. But this increase is lesser than that of MDA in stages of COPD patients (Table no.2).

Table 1. Demographic data and pulmonary function tests for patients with different COPD severities and healthy controls

Parameters	Healthy controls (n=30)	COPD patients			
		Stage I (n=30)	Stage II (n=30)	Stage III (n=30)	Stage IV (n=30)
Sex (M/F)	20/10	24/6	25/5	28/2	27/3
Age (yrs)	52.93±9.08	58.86±10.05	62.03±9.12	60.43±7.39	63.4±8.27
Smoking history (Pack years)	----------	52.1±4.74	52.36±7.25	53.56±8.26	55.16±8.97
FEV1% Predicted	105.03±13.08	90.23±11.36[a]	63.13±7.85[b]	39.76±6.34[c]	22.43 ±5.55[d]
FEV1/FVC% ratio	100.33±7.471	65.28±2.78[a]	59.76±6.56[b]	49.16±6.17[c]	37.44±4.78[d]

FEV1% Predicted: Forced Expiratory volume in one second % Predicted, FEV1/FVC% ratio: Forced Expiratory volume in one second/forced vital capacity % ratio. Values are expressed as mean± SD [a]P <0.001 - significant when compared to healthy controls [b]P <0.001 - significant when compared to stage I COPD patients. [c]P<0.001 - significant when compared to stage II COPD patients. [d]P < 0.001 - significant when compared to stage III COPD patients

Table 2. Comparisons of serum malondialdehyde, protein carbonyl level, whole blood reduced glutathione, serum vitamin C and vitamin E levels in healthy controls and patients with different COPD severities

Sr. No.	Parameters	Healthy controls (n=30)	COPD patients			
			Stage I (n=30)	Stage II (n=30)	Stage III (n=30)	Stage IV (n=30)
1	Serum Malondialdehyde (nmol/ml)	4.19±1.79	6.23±0.81[a]	7.94±1.26[d]	9.42±1.51[g]	11.53±1.23[j]
2	Serum Protein Carbonyl (nmol/mgs of proteins)	3.50±1.87	5.64±2.94[b]	8.1±2.33[e]	9.66±3.12[h]	11.13±2.17[k]
3	Whole Blood Reduced Glutathione (mg/dl)	34.26±4.96	28.24±6.12[c]	22.42±4.50[e]	17.67±4.45[g]	13.73±2.76[l]
4	Serum Vitamin C (mg/dl)	1.41±0.59	0.98±0.34[b]	0.69±0.28[e]	0.53±0.09[i]	0.43±0.10[l]
5	Serum Vitamin E (mg/dl)	1.51±0.40	1.09±0.37[c]	0.806±0.27[f]	0.608±0.15[i]	0.48±0.11[l]

Values are expressed as mean± SD. [a]P<0.0001: statistically significant when compared to healthy controls [b]P<0.01: statistically significant when compared to healthy controls [c]P<0.001: statistically significant when compared to healthy controls [d]P<0.0001: statistically significant when compared to stage I COPD patients [e]P<0.001: statistically significant when compared to stage I COPD patients [f]P<0.01: statistically significant when compared to stage I COPD patients [g]P<0.001: statistically significant when compared to stage II COPD patients [h]P<0.05: statistically significant when compared to stage II COPD patients [i]P<0.01: statistically significant when compared to stage II COPD patients [j]P<0.0001: statistically significant when compared to stage III COPD patients [k]P<0.05: statistically significant when compared to stage III COPD patients [l]P<0.001: statistically significant when compared to stage III COPD patients

In our study, we observed the non-enzymatic antioxidant reduced glutathione (GSH) level was significantly decreased in stage I COPD patients as compared to healthy controls (P<0.001). In addition to this when we compared blood level of reduced glutathione (GSH) in stage II versus stage I, stage III versus stage II and stage IV versus stage III (P<0.001,Table no.2) a stepwise decrease was observed in COPD patients.

Serum levels of vitamin C and vitamin E were significantly decreased in stage I COPD patients as compared to healthy controls (P<0.01 and P<0.0001 respectively). Vitamin E and vitamin C were significantly decreased in stage II COPD as compared to stage I COPD (P<0.01 and P<0.001 respectively). Vitamin C and vitamin E were significantly decreased in stage III as compared to stage II (P<0.01) COPD patients. Vitamin E and vitamin C were much decreased in stage IV COPD as compared to stage III COPD patients, this decreased was statistically significant. (P<0.001) (Table no. 2).

As shown in (Table no.3), we found significant negative correlation between the markers of oxidative stress (MDA and protein carbonyl) with pulmonary function parameters FEV_1 % predicted (r=-0.825 and r =-0.582, P<0.0001) and FEV_1/FVC% predicted ratio (r =-0.732 and r =-0.480, P<0.0001) in COPD patients respectively. In contrast to that, we found significant positive correlation between non-enzymatic antioxidants (reduced glutathione, vitamin C and vitamin E) with pulmonary function parameters FEV1 % predicted(r=+0.745, r =+0.563 and r =+0.630, P<0.0001) and FEV_1/FVC % predicted ratio (r=+0.650, r=+0.538 and r =+0.522, P<0.0001,Table no.3) in COPD patients respectively. We found no significant correlation between markers of oxidative stress (MDA and protein carbonyl) and non-enzymatic antioxidants (vitamin E, vitamin C and GSH) with FEV1% predicted and FEV1/FVC% predicted ratio in control group (Table no 3).

Table 3. Correlation of pulmonary functions test parameters with markers of oxidative stress and non-enzymatic antioxidants in controls and COPD patients

Correlation	Control group (n=30)		COPD group (n=120)	
	r	p	r	P
MDA-FEV1 % Pred.	0.047	0.803	-0.825	<0.0001
MDA-FEV1/FVC % Pred.	-0.156	0.410	-0.732	<0.0001
PC-FEV1 % Pred.	0.152	0.422	-0.582	<0.0001
PC-FEV1/FVC % Pred.	0.150	0.428	-0.480	<0.0001
GSH-FEV1% Pred.	0.060	0.751	0.745	<0.0001
GSH-FEV1/FVC % Pred.	-0.278	0.136	0.650	<0.0001
VITC-FEV1 % Pred.	-0.156	0.410	0.563	<0.0001
VITC-FEV1/FVC % Pred.	-0.150	0.428	0.538	<0.0001
VIT E-FEV1 % Pred.	-0.056	0.770	0.630	<0.0001
VIT E-FEV1/FVC % Pred.	-0.328	0.076	0.522	<0.0001

4. DISCUSSION

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 during metabolic reactions or exogenously, such as air pollutant or cigarette smoke. Cigarette smoke contains many oxidants and free radicals, both in the gas and the tar phase and cause sequestration of neutrophils into the pulmonary microcirculation and accumulation of macrophages in respiratory bronchioles [35]. All these factors tend to decrease lung

function so we did pulmonary function test in COPD patients. In present study ,we observed lung function parameters namely FEV1% predicted and FEV1/FVC % ratio were significantly decreased in all stages of the COPD according to the severity of the disease (P<0.001,Table no.1). These findings were supported by the study of Pierachille S et al. who reported a reduction of FEV1 and FEV1% in COPD patients [36]. In the study conducted by Daphne CR et al he reported FEV1 % decline in COPD groups as compared to healthy controls [37]. In COPD, there appears interplay of inflammation, remodeling, bronchospasm, mucus hypersecretion, loss of elastic recoil and increased airway resistance, resulting in progressive reduction in the expiratory airflow. This reduction in airflow obstruction causes generation of reactive oxygen species (ROS) [2].

ROS causes lipid peroxidation as well as protein oxidation, which may cause direct lung injury or may induce a variety of cellular responses through the generation of secondary metabolic reactive species. So in the present study we estimated MDA as a marker of lipid peroxidation and protein carbonyl as a marker protein oxidation. In our study we observed a significant stepwise increase in serum MDA level from stage I to stage IV COPD patients (Table no. 2). This increase in MDA level observed was increased with the severity of disease or stage. This might be due to the stage IV COPD patients having more severe lung function impairment, poor quality of life and more serious systemic dysfunction [38,39]. This finding is in accordance with the study of Daga MK et al. [40], Kirkil G et al. [41], Isik B et al. [42] and Lee SI [43]. Yessica D et al. and Menon B et al. reported an increase in MDA level in all stages of COPD severity as compared to controls. Our finding is in agreement with report of Yessica D. et al. and Menon B et al. [44,45]

In contrast, to our findings Henneke JH et al. reported that the malondialdehyde level do not significantly increased in serum of COPD patients [46].

In our study we observed an increase in serum protein carbonyl level from stage I to stage IV COPD patients (Table no. 2).We observed a significant increase in protein carbonyl with the advancement of stage but the increase observed was less as compared to MDA. This increase might be due to oxidation of proteins when exposed to reactive oxygen species. Oxidative damage results in altered structure and function of circulating proteins, leading to altered antigenecity and immune response, contraction of smooth muscle, impairment of β adrenoreceptor function, stimulation of airway secretion, activation of mast cells and activation of proteases. Increased oxidative stress leading to increased protein carbonyl formation in COPD patients [47]. The increased level of protein carbonyl in COPD patients was also reported in the study of Yessica DT et al. [44]. However, In contrast to our finding, no significant change in the level of protein carbonyl in COPD patients have also been reported in the study of Vala SM et al. [48].

We found significant inverse correlation between serum malondialdehyde and protein carbonyl with the marker of airflow obstruction i.e. forced expiratory volume in one second % predicted (FEV1% predicted) in COPD patients (r=-0.825 and r =-0.582, *P*<0.0001 respectively) (Table no. 3). These results show that the levels of malondialdehyde and protein carbonyl increases with the severity of the disease and related to decrease in lung function test. This findings is in agreement with the study conducted by Schunemann HJ et al. [49], Arpana V et al. [50] who showed that FEV1% predicted decreases as the mean value of MDA increases.

Under normal condition, oxidative stress is counterbalanced by efficient antioxidant system in the body. Antioxidant defense in the lungs are provided by endogenous enzyme system

and non-enzymatic antioxidant compounds. In the present study we studied few non-enzymatic antioxidants such as reduced glutathione, vitamin C and vitamin E.

We observed a significant decrease in the level of GSH from stage I to stage IV (P<0.001, Table no.2) in COPD patients. This observed decrease may be due to the utilization of GSH in order to overcome oxidative stress thereby depleting total available GSH pool [51]. The decreased level of GSH was also observed in COPD patients in the study of Nagraj et al. [52].

The other antioxidants vitamin C and E levels was also observed to be decreased. Vitamin E is a lipid soluble chain breaking antioxidant. It converts superoxide, hydroxyl and lipid peroxyl radicals to less reactive form [53,54]. In present study vitamin E significantly lowered in all stages of COPD compared to controls (Table no. 2). Rout A et al. observed a significant decreased level of vitamin E in serum in COPD patients [55].

The stage wise decrease was observed in vitamin C levels as the stage advances (Table no.2). This decrease observed might be due to two reasons first is the rapid oxidation of ascorbic acid by free radicals and second is vitamin C is also required to regenerate vitamin E, once the vitamin C is converted to tocopheroxyl radical. This is in accordance with studies of Sargeant LA et al. [56], Rai RR et al. [57] and Calikoglu M et al. [58].

In our study we observed significant positive correlation of vitamin C, vitamin E and reduced glutathione (GSH) with forced expiratory volume in one seconds % predicted (FEV1 % predicted) (r=+0.563, r=+0.630 and r=+0.745, P<0.0001) in COPD patients respectively (Table. no 3). We found no significant correlation between markers of oxidative stress (MDA and protein carbonyl) and non-enzymatic antioxidants (vitamin E, vitamin C and GSH) with pulmonary function test parameters namely: FEV1% predicted and FEV1/FVC% predicted ratio in control group (Table no.3).

5. CONCLUSION

We conclude that in COPD patients oxidative stress is increased with the decrease in pulmonary function test values this indicates that respiratory capacity of the lung decreases with the increase in oxidative stress. In the present study we observed that with the increase in oxidative stress there is concomitant decrease in non-enzymatic antioxidants studied: vitamin E, vitamin C and reduced glutathione (GSH).

This led us to think that the additional supplementation of dietary antioxidants as well as GSH might be useful in the alleviation of the disease.

So our next aim is to study the supplementation of dietary antioxidants to the COPD patients and studied the effects.

6. STRENGTH AND LIMITATIONS OF THE STUDY

Strength:

1) We are the first to report the levels of oxidative stress markers (MDA and protein carbonyl) and non-enzymatic antioxidant (vitamin E, vitamin C and reduced glutathione) in different stages of COPD patients.

2) We correlated pulmonary function test markers namely: FEV1% predicted and FEV1/FVC % ratio with the markers of oxidative stress (MDA and protein carbonyl) and non-enzymatic antioxidants (vitamin E, vitamin C and reduced glutathione) in COPD patients.

Limitations: Considering the prevalence of the COPD patients this work has to be done with the larger sample size for to confirm the results.

CONSENT

Declare that written informed consent was obtained from the patient.

ETHICAL APPROVAL

The study protocol was examined and authorized by the Medical Research and Ethics Committee of the B. J. Govt. Medical College and Sassoon General Hospital in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki on biomedical research on human subjects. Obtained necessary Institutional ethical approval [Ref. No. BJMC/ IEC/ Pharmac/ D1210133-35].

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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