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A Comparison of Spirometric Parameters and Serum Malondialdehyde Level in Actively Smoking Chronic Obstructive Pulmonary Disease Patients and Non-Smoking Apparently Normals- A Crosssectional Comparative Study

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Authors' contributions

This work was carried out in collaboration among all authors. Data collection and PFT of patients – done by Authors R and RRP. Manuscript preparation and data analysis – done by author MIS. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: The antioxidants requirement depend on one's exposure to endogenous and exogenous reactive oxygen species.Cigarette smoking leads to increased exposure to reactive oxygen species,hence they require more antioxidant nutrients In this study, we aimed to study the serum levels of malondialdehyde (MDA) as a marker of oxidative stress and pulmonary function tests (PFT) and to study if there is any correlation between PFTand MDA levels in, smokers,chronic obstructive pulmonary disease (COPD) patients.
Aim: To compare the pulmonary function tests (PFT) and serum malondialdehyde (MDA) level in smokers, chronic obstructive pulmonary disease (COPD) patients with non-smoker controls.

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Methods and Materials: N=30, 35-50 years age group smokers, COPD patients were enrolled as cases.N=30 age and sex matched were enrolled as control group. Serum MDA and PFT parameters like forced vital capacity (FVC), forced expiratory volume in first second (FEV_1), FEV_1/FVC ratio, Peak expiratory flow rate (PEFR) were measured.
Result: PFT parameters like forced vital capacity (FVC), forced expiratory volume in first second (FEV_1), FEV_1/FVC ratio, peak expiratory flow rate(PEFR) were decreased and found statistically significant in smokers, COPD group.MDA level were increase and found statistically highly significant in smokers, COPD group.
Conclusion: MDA is negatively correlated with FEV_1% predicted, FEV_1/FVC % predicted ratio and FVC in smokers, COPD patients

Keywords: COPD; MDA; PFT.

1. INTRODUCTION

An imbalance between oxidants and antioxidants is responsible in the patho-physiology of chronic obstructive pulmonary disease(COPD), which leads to multisystemic manifestations including weight loss [1]. An oxidative stress leads to peroxidation of membrane lipids which causes cellular damage. It produces an end-products like malondialdehyde(MDA).The important feature of COPD is the loss of elastic recoil by the destruction of parenchyma by the emphysematous changes lead to progressive decline of FEV_1 due to inadequate lung emptying which leads to static and dynamic hyperinflation [2]. Airflow limitation leads to abnormal inflammatory response of lungs to noxious particles and gases [3]. The main risk factor for irreversible air flow limitation is tobacco smoke [4]. On inflammation cytokines are released, macrophages, neutrophils and dendritic cells are attracted to the site of inflammation which initiates the innate immune response [5]. Proteolytic enzymes and reactive oxygen species are produced which if not counterbalanced by antiproteases and antioxidant factors further damage will occur [6]. The shift in balance between oxidants and antioxidants lead to "oxidative stress."Oxidative stress leads to pathological conditions like idiopathic pulmonary fibrosis, chronic obstructive pulmonary disease [7]. Different biomarkers of oxidative stress are present, including reactive oxygen species (ROS). As ROS are very reactive and they have a short half-life, direct measurement in tissues or body fluids is difficult. It is easy to estimate oxidative stress by measuring their oxidation target products like lipid peroxidation end products, oxidized proteins and antioxidants [8]. Oxidative stress leads to lipid peroxidation which causes oxidative damage [9]. MDA and thiobarbituric acid reactive substances (TBARS) have been most commonly used indices of oxidative damage [10]. Many different studies have demonstrated MDA as potential biomarker to assess oxidative stress status in COPD patients.They applied the method of TBARS where in under strong acidic condition and heating, MDA was allowed to react with thiobarbituric acid (TBA)which leads to the formation of a product which is assessed by spectrophotometer. A significant increase in TBARS MDA in COPD patients have been found as compared to healthy controls.Increase in plasma MDA levels with the progress and severity of the disease has been reported [11]. However, few studies did not find any significant change in plasma TBARS MDA levels of COPD patients as compared to healthy controls [12].

There are studies which have reported that smoking decreased the pulmonary function including parameters like forced vital capacity (FVC), forced expiratory volume in one second (FEV_1), FEV_1/FVC, and the forced expiratory flow at 25–75% ($FEF_{25–75\%}$) [13]. Cigarette smoking causes decrease in both FEV1/FVC and $FEF_{25–75}$ which resulted into airway obstruction and small airway disease in adult smokers [14].

A linear relationship between years of smoking and decreasein FEV_1 and FVC was reported [15]. A decrease in FEV_1 was also detected in teenage smokers [16]. Smoking cessation led to reduction in smoking-induced decrease in lung function, but also led to reversal to nonsmoking values [17]. These findings confirm the deleterious effect of smoking on lung function and prove a beneficial effect of quitting smoking.

2. AIMS AND OBJECTIVES

To study the pulmonary function test(PFT) parameters and serum malondialdehyde(MDA) level in smoker,chronic obstructive pulmonary disease(COPD) patients and non-smoker control

group and to study if there is any correlation between PFTand MDA levels in smokers, chronic obstructive pulmonary disease (COPD) patients.

3. MATERIALS AND METHODS

It is a cross sectional comparative study conducted from May 2012 to January 2014 in the Department of Physiology & Biochemistry, Santosh Medical College, Ghaziabad,India in collaboration with the Department of T.B. Chest Santosh Hospital, Ghaziabad Uttar Pradesh,India . Study was approved by the Institutional Ethical Committee. Analysis was done using student unpaired t test.Sample size was calculated using AI - Therapy Statistics BETA large effect size (0.7). Alpha value was 0.05, power of study was at 80% for one tailed hypothesis. So total number of people required were 52 (26 in each group).

Group 1, N = 30, control male subjects between the ages of 35-50 years ,who were non-smokers, not exposed to any smokers in family or friends routinely,with no history of hypertension, lung cancer, bronchial asthma, Diabetes mellitus,cardiovascular biomass and renal diseases in which oxidative stress has been documented to be a causative factor were selected from the institution. Radiographic findings were normal. The position of the diaphragm was normal and lungs were found to be normal.

Group 2, N = 30, COPD patients who were active smokers for more than 10 years,history of chronic cough, dyspnoea or sputum production for atleast three months of consecutive two years were selected. Subjects taking antioxidant drugs, Diabetes mellitus, hypertension were excluded from the study. Body height (Ht) in centimeters was measured without shoes by asking the subjects to stand with their heels, head and buttocks against a stadiometer.Body weight was measured in kilograms (kg) without shoes and minimal clothing. BMI (Kg/m^2) [18] was calculated by dividing body weight in kilogram by height in meters square.

PFT was done on volume based PK Morgan RS 232 Dry Rolling Spirometer.Spirometric analysis was performed with the help of computerized spirometer with patient in sitting posture wearing the nose clip and breathing through mouth piece (Recommendation of American thoracic society was followed while performing spirometric analysis). After recording age (years), following parameters were assessed like forced vital capacity (FVC),forced expiratory volume in first second (FEV_1), FEV_1/FVC ratio,peak expiratory flow rate (PEFR) [19].

Procedure: 5ml blood was withdrawn from vein with aseptic precautions.Following which the serum MDA was measured using the method of Buege and Aust [20]. Serum-100µL was diluted to 500µL distilled water. To this diluted sample about 1ml of Trichloroacetic acid (TCA) - thiobarbutric acid (TBA) -hydrochloric acid HCl reagent was added.The samples were kept in boiling water bath for 15 minutes. The reaction mixture was cooled and centrifuged. The supernatant was removed and optical density of the pink colour formed was read at 535nm. A blank was also maintained simultaneously by taking 500µL of water instead of sample in the reaction mixture. Themalondialdehyde concentration was estimated by plotting the obtained absorbance against the graph. The optical density of the pink colour obtained is proportional to the MDA concentration in the given sample.

Calculation: The optical density of the test samples is proportional to MDA concentration in the sample and calculated by the plotting against the standard graph and multiplied by the respective dilution factors the final concentration is expressed as µm/L.

Concentration of MDA nmol/ml = O.D. of Test X O.D. of Std Reference value

Group 1 - 3.025nmol/ml Group 2 – 4.444nmol/ml

4. RESULT

The above study assessed the lung functions in COPD patients and correlate the result of PFT with MDA using student unpaired t test. MDA concentrations in COPD patients was 4.444nmol/ml and was found to be statistically significant than the control group. It was observed that FEV1 in first second was 1.90 ± 0.91 and PEFR rate was 5.04 ± 2.8 and statistically significant in patients with COPD smokers. The ratio FEV1/FVC was 72.04 ± 7.66 found to be statistically significant in COPD group.

Table 1. Demographic data of male subjects

Demographic Parameter	Group – 1 (Control) N = 30 (Mean ± SD)	Group -2 (COPD Cases) N = 30 (Mean ± SD)
Age (years)	43.7 ±0.95	45.2 ±1.09
Weight (kg)	55.13 ± 7.61	50.17 ± 7.86
Height (cm)	157.87 ± 4.89	156.72 ± 5.23
BMI (kg/m^2)	21.4 ± 3.03	20.36 ± 2.13

Table 2. Spirometric parameters of COPD, smokers and non-smoker control group analysis by student unpaired t test

Spirometricparameter	Group – 1 (Control N=30 (Mean ± SD)	Group -2 (COPD Cases) N= 30 (Mean ± SD)	P Value	T Value
VC	3.621± 0.692	2.662 ±0.962	0.001(S)	4.431
FVC (Litres)	4.32 ± 0.54	3.84 ± 0.6	0.001 (S)	4.42
FEV_1 (Litres)	3.10 ± 0.63	1.90 ± 0.91	0.00001 (S)	5.87
FEV_1/FVC (%)	82.64 ±9.55	72.04 ± 7.66	0.0001 (S)	4.61
PEFR	7.9±2.2	5.04±2.8	0.001(S)	4.29

Statistics - P<0.05 Significant

Table 3. MDA Level of COPD, Smokers and non smokers control group analysis by student unpaired t test

Biochemical Parameter	GROUP – 1 (Control) N=30 (Mean ±SD)	GROUP -2 (COPD Cases) N=30 (Mean ± SD)	P value
MDA Level(nmol/ml)	3.025 ±0.807	4.444 ±0.335	0.00001(HS)

Statistics - P<0.05 Significant

Table 4. Correlation of Malondialdehyde (MDA) with pulmonary function test (PFT)markers in smoker,COPD patients with non-smoker healthy controls

Correlations	**GROUP - 1 (Control) N=30** r	**GROUP - 1 (Control) N=30** P	**GROUP -2 (COPD Cases) N=30 r**	**GROUP -2 (COPD Cases) N=30 p**
MDA-FEV1% Predicted	+0.04	0.703	-0.70	<0.001
MDA-FEV1/FVC % Predicted	-0.14	0.310	-0.71	<0.001
MDA-FVC	0.03	0.72	-0.61	<0.001

Statistics - P<0.05 Significant

5. DISCUSSION

The present study was undertaken to assess the lung functions in COPD patients and correlate the result of PFT with MDA. MDA concentrations in COPD patients were higher and statistically significant than the control group which probably resulted due to airflow limitation. Airflow limitation is progressive and associated with abnormal inflammatory response of lungs to noxious particles and gases [3]. The major factor for irreversible air flow limitation is tobacco smoking [4]. Smoking induced radical chain reaction leads to lipid peroxidation of membrane phospholipids, alters cellular physiology [9]. It was observed that FEV_1 in first second and PEFR rate were markedly decreased in patients with COPD smokers. The ratio FEV_1/FVC was decreased and was found to be sensitive in diagnosing COPD. MDA was negatively correlated with FEV_1% predicted, FEV_1/FVC % predicted ratio and FVC in smokers, COPD patients (Table 4).

In the present study, the mean value of FVC of COPD smoker was reduced significantly ($P<0.001$) as compared to control subjects. Inflammation induced by cigarette smoke is capable of both stimulating acute production of airway secretions and inducing persistent anatomic changes in the airway. For example, goblet cell metaplasia may predispose to a hypersecretory state peribronchial fibrosis may result in airflow obstruction [21].

A study conducted by Birgulet al, showed that MDA levels are significantly higher in smokers than in non-smoker [22]. Long-term exposure to smoke results into systemic oxidants-antioxidants imbalance which leads to increased lipid peroxidation products and decreased levels of antioxidants like vitamins A and C in the plasma [23]. Oxidative stress leads to increased lipid peroxidation products i.e MDA and decrease in antioxidants like Vitamin C, vitamin E, superoxide dismutase and catalase [24]. Oxidative damage seen in COPD is due to exposure to the oxidants from cigarette smoke, tobacco and endogenously produced oxidants due to activated inflammatory cells. Measuring these oxidants and antioxidants in the blood,the magnitude of oxidative stress in COPD can be determined [25].

Singh S, Verma S K,et al reported decrease antioxidant levels in superoxide dismutase activity (SOD), catalase in COPD patients. But MDA levels were increase [26]. Although in our study the smokers with COPD have not been subdivided on the basis of years of smoking and severity but we have found that MDA levels are higher as compared to control group which can be attribute to the fact that free radicals generation and irreversible airflow limitation in COPD is due to smoking. Hence airflow limitation secondary to smoking is a major risk factor of COPD.

6. CONCLUSION

The structural changes in respiratory system, as well as the decline in lung functions of COPD smokers demonstrate smoking induced deleterious effects which accelerate lipid peroxidation. Increase levels of MDA in serum is directly related with the degree of injury and impairment of lung function in COPD patients. MDA can be a good marker of severity in COPD patients. Smoking cessation as an early intervention may lead to some reversal towards the better health of COPD smokers

7. LIMITATION AND STRENGTH OF STUDY

Although in our study the smokers with COPD have not been subdivided on the basis of years of smoking and severity but we have found that MDA levels are higher as compared to control group which can be attribute to the fact that free radicals generation is more in smokers and smoking is a major risk factor of COPD. Air pollution too can affect both the groups of study and it is a limiting factor.

CONSENT

As per international standard or university standard, patients' written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

Study was approved by the Institutional Ethical Committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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