CLINICO-BIOCHEMIAL STUDY OF OXIDATIVE STRESS IN COPD PATIENTS IN A TERTIARY CARE HOSPITAL

J K Mishra[1], Ashvendra Singh Chawra[1], Girish Singh[2], H D Khanna[3]

1. Department of T.B. & Respiratory Diseases, Institute of Medical Sciences, BHU, Varanasi
2. Division of Biostatistics, Institute of Medical Sciences, BHU, Varanasi
3. Department of Biophysics, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India

Corresponding Author:
Prof. J. K. Mishra, Professor, Department of T.B. & Respiratory Diseases, Room No.1352,
Institute of Medical Sciences, Banaras Hindu University, Varanasi, 221005 UP, India,
e-mail: jkmishra.imsbhu@gmail.com

ABSTRACT

Background: Chronic obstructive pulmonary disease (COPD) characterized by airflow limitation that is usually progressive and associated with an enhanced chronic inflammatory response in the airways is a major worldwide health problem that has an increasing prevalence and mortality. **Aim:** The aim of study was to estimate serum malondialdehyde (MDA) and Antioxidant level from blood samples of COPD patients and to compare with age and sex matched healthy controls and further to compare these levels between severity grades and smoking pack years. **Methods:** The present study was conducted in the Department of TB and Respiratory disease, Institute of Medical Sciences, Banaras Hindu University, Varanasi during June 2012 to June 2013. A total of 44 COPD patients who met the prescribed inclusion and exclusion criteria were registered for the study and 44 age and sex matched healthy non COPD individuals were selected as the controls. MDA level was estimated by thiobarbiburic acid assay by Philpot Method and assay of TNF-α done by TNF-α (Human) ELISA KIT Protocol. **Results:** The percentage of male cases was 72.7 and 47.1% of cases were over 60 years of age. 50% cases belonged to poor socioeconomic status. Cough, sputum production and dyspnoea were the predominant symptoms. 40.9% cases had history of previous hospitalization. A statistically significant difference in mean MDA as well as total antioxidant level was found between cases and controls. It was observed that the level of MDA increases and total antioxidant level decreases as severity of COPD increases. The level of MDA increased and level of total antioxidant decreased with increase in the smoking pack years. **Conclusion** The inflammatory mediators involved in COPD have been clearly defined, and an early approach on smoking cessation and controlling oxidative stress may retard disease progression.

Key words: Malondialdehyde, MDA, Antioxidant

INTRODUCTION

The chronic obstructive pulmonary disease (COPD) is a type of obstructive lung disease. COPD is associated with a chronic inflammatory response, predominantly in small airways characterized by increased numbers of macrophages, neutrophils, & T lymphocytes. The inflammatory mediators involved in COPD have not been clearly defined, but it is now apparent that many lipid mediators, inflammatory peptides, reactive oxygen and nitrogen species, chemokines, cytokines, and growth factors lead to complex inflammatory process.

COPD is a major and increasing global health problem that is now a leading cause of morbidity and mortality. Total deaths from COPD are projected to increase by more than 30% in the next 10 years unless urgent action is taken to reduce the underlying risk factors, especially tobacco use. Estimates show that COPD in 2030 will become the third leading cause of death worldwide. The primary cause of COPD is tobacco smoke (including second-hand or passive exposure). WHO estimates that in 2005 5.4 million people died due to tobacco use. Tobacco-related deaths are projected to increase to 8.3 million deaths per year by 2030.[1]

The causes for COPD have opposite patterns according to the geographic areas.[2] In high- and middle-income countries tobacco smoke is the biggest risk factor, meanwhile in low-income countries exposure to indoor air pollution, such as the use of biomass fuels for cooking and heating, causes the COPD burden. Other risk factors for COPD include occupational dusts and chemicals (such as vapours, irritants, and fumes) and frequent lower respiratory infections during childhood.

It is not fully understood how tobacco smoke and other inhaled particles damage the lungs to cause COPD however, the most important processes causing lung damage are: (a) oxidative stress produced by the high concentrations of free

radicals in tobacco smoke, (b) cytokine release due to inflammation as the body responds to irritant particles such as tobacco smoke in the airway and (c) tobacco smoke and free radicals impair the activity of antiprotease enzymes such as alpha 1-antitrypsin, allowing protease enzymes to damage the lung.

Oxidative stress has an important role on several events of lung physiology and for the pathogenesis of COPD.[3-4] These include oxidative inactivation of Antiproteases and surfactant mucus hypersecretion, membrane lipid per oxidation, mitochondrial respiration, alveolar epithelial injury, remodeling of extracellular matrix, and apoptosis. An increased level of REACTIVE OXYGEN SPECIES (ROS) produced in the airways is reflected by increase markers of oxidative stress in the airspaces, sputum, breath, lungs and blood in patients with COPD.

The biomarkers of oxidative stress such as H_2O_2, F_2-isoprostanes, malondialdehyde (MDA), and 4-Hydroxy-2-nonenal has been successfully measured in breath condensate. ROS and aldehydes play a key role in enhancing the inflammation through the activation mitogen-activated protein kinases and redox sensitive transcription factors such as nuclear factor kappa beta (NFκβ) and activator protein-1. Oxidative stress also alters nuclear histone acetylation and deacetylation leading to increased gene expression of pro-inflammatory mediator in the lung.

The aim of study was -

1. To estimate serum **MDA** and total antioxidant level from blood samples of COPD patients.
2. To compare the levels of **MDA**, total antioxidant in COPD with age and sex matched healthy controls.
3. To compare the levels of **MDA** and total antioxidant between different severity grade, smoking pack years and age group of COPD patients.

MATERIALS AND METHODS

The present study was conducted in the Department of TB and Respiratory disease, Institute of Medical Sciences, Banaras Hindu University, Varanasi in collaboration with Department of Biophysics during the period of June 2012 to June 2013.

The patients were screened on following criteria:

(a) **Inclusion criteria:** COPD patients in any stage (mild chronic, moderate chronic, severe chronic and acute exacerbation) of age group of 40 to70 years

(b) **Exclusion Criteria:** (1) COPD patients having co-morbid conditions like Infectious disease, Septicemia, Sickle cell disease, Hypertension, Diabetes, Alzheimer's disease, Parkinson's disease etc. (2) COPD patients taking some drug which can have antioxidants action like Multivitamins, Antioxidants, Lycopene, Beta carotene, Astaxanthine, Selenium, Green tea etc.

A total of 44 COPD patients who met above criteria were registered for the study and 44 age and sex matched healthy non COPD individuals were selected as the controls, whose blood samples were drawn with their consent for comparison with the blood samples of the cases.

Patients and controls were subjected to (1) Informed consent, (2) Detailed history and clinical examination like, Complete Blood Count, Renal Function Test, Liver Function Test, Random Blood Sugar, Urine- Routine and Microscopy, Electocardiogram (ECG), Chest X-ray, Pulmonary Function test (FEV1/FVC and % of FEV1) and CT Scan of Chest (wherever feasible).

The estimation of following parameters was done:

Assay of oxidative stress:- Malondialdehyde level by thiobarbiburic acid assay by Philpot Method.[5]

Assay of TNF-α: By TNF-α (Human) ELISA KIT Protocol.

Criteria for Severity of COPD

Gold Grade	Severity	Symptoms	Spirometry
0	At Risk	Chronic cough, sputum production	Normal
I	Mild	With or Without chronic cough or sputum production	FEV1/FVC<0.7 And FEV1 ≥80% predicted
II	Moderate	With or Without chronic cough or sputum production	FEV1/FVC<0.7 And 50%≤FEV1<80% predicted
III	Severe	With or Without chronic cough or sputum production	FEV1/FVC<0.7 And 30%≤FEV1<50%, predicted
IV	Very Severe	With or Without chronic cough or sputum production	FEV1/FVC<0.7 and FEV1<30% Predicted Or FEV1<50% Predicted with respiratory failure or signs of right heart failure

OBSERVATIONS AND RESULT

The present study was based on 44 cases of COPD and 30 Non-COPD subjects as controls. The percentage of male and female cases was 72.7 and 27.3 respectively. 47.1% cases were of age more than 60 years. 50% cases belonged to poor socioeconomic status. The percentage of cases belonging to middle class socioeconomic status was 36.4 whereas, 13.6% belonged to good socioeconomic status.

The predominant symptoms observed in cases were cough (100%), sputum production (100%), dyspnoea (65.9%) and exacerbations (68.2%). 40.9% cases had history of previous hospitalization. The percentage of cases with associated co-morbidities was 15.9 (Table-1).

It was observed that all the cases were smokers where, 29.6% were under 11-20 pack year code 47.7% smoked greater than 20 pack year and 22.7% smoked less than 10 pack year. Further it was found that 70.5% cases belonged to GOLD Grade-II and 29.5% cases GOLD Grade-III.

Table-1: Presence of various signs and symptoms in COPD cases

Symptoms	No.	%
Smoking	44	100.0
Cough	44	100.0
Dyspnoea	29	65.9
Sputum production	44	100.0
Pallor	9	20.5
Clubbing	0	0.0
Cyanosis	17	38.6
Pedal edema	16	36.4
Icterus	0	0.0
Chest X-ray	11	25.0
Previous hospitalization	18	40.9
Exacerbation	30	68.2
Associated co-morbidities	7	15.9

The mean and standard deviation (SD) of MDA level among cases was 1.35±0.52 (μmol/l) and in control was 0.33±0.05 (μmol/l). The difference between means was found statistically highly significant (t=12.82 and p<0.001) (Table-2). The mean and SD of total antioxidant level in cases was 0.041±0.022 (μmol/l), and in control was 0.130±0.047 (μmol/l). The difference between means was found statistically highly significant (t=11.19 and p<0.001) (Table-2).

Table 2: MDA and Total antioxidant level between cases and controls

Group	**MDA level (μmol/l) Mean±SD**	**Total antioxidant level (μmol/l) Mean±SD**
Case	1.35±0.52	0.041±0.022
Control	0.33±0.05	0.130±0.047
Intergroup comparison (Independent sample t-test)	t = 12.82 p < 0.001	t = 11.192 p < 0.001

The mean and SD of MDA level in GOLD Grade-II and GOLD Grade-III cases were 0.306±0.043 and 0.399±0.039 (μmol/l) respectively. The inter GOLD Grade groups comparison of mean MDA level was found statistically highly significant (t= 6.70, p<0.001) (Table-3). Similarly, the mean and SD of total antioxidant level in GOLD Grade-II and GOLD Grade-III cases were 0.147±0.045 and 0.088±0.012 (μmol/l) respectively. The comparison of mean total antioxidant level between the two GOLD Grades was found statistically highly significant (t= 4.55, p<0.001) (Table-3).

Table 3: MDA and Total antioxidant level according to GOLD Grade of cases

Parameters	**GOLD Grade-II**	**GOLD Grade-III**	**Inter GOLD Grade comparison**
MDA (μmol/l)	0.306±0.043	0.399±0.039	t=6.70, p < 0.001
Total antioxidant (μmol/l)	0.147±0.045	0.088±0.012	t=4.55, p < 0.001

The mean and SD of MDA and total antioxidant level of cases having different smoking number of pack year were determined and statistically significant difference was found between different numbers of pack year. Mean MDA was higher and mean total antioxidant was lower for cases belonging to 11 and above number of pack year as compared to those with less than 10 number of pack per year (Table-4).

Table- 4: MDA and Total antioxidant level according to smoking number of pack year

Parameters	**Smoking (pack year)**			**p-value**
	< 10 yrs	**11-20 yrs**	**> 20 yrs**	
MDA (μmol/l)	0.297±0.034	0.338±0.062	0.371±0.063	0.004
Total antioxidant (μmol/l)	0.165±0.063	0.132±0.042	0.109±0.035	0.021

The mean and SD of MDA and total antioxidant level of cases according to different age groups were also determined however, the differences between different age groups were found not statistically significant (Table-5).

Table- 5: MDA and Total antioxidant level according to age of cases

Parameters	**Age Group (yrs)**	**p-value**		
	40 - 50	**51 - 60**	**> 60**	
MDA (μmol/l)	0.331±0.067	0.319±0.060	0.343±0.057	0.526
Total antioxidant (μmol/l)	0.130±0.042	0.145±0.061	0.120±0.037	0.320

DISCUSSION

In the present study it was observed that more numbers of cases of COPD were over 60 years of age and it was found more common in males. It was observed that COPD is more common in lower socio-economic status. Similar result was also reported Eva Prescott and Jørgen Vestbo in a study between socio-economic status and chronic obstructive pulmonary disease.[6]

The study shows that most cases have smoking pack year >20. The exact threshold for the duration/intensity of cigarette smoking that will result in COPD varies from one individual to another. In the absence of genetic/ environmental/ occupational predisposition, smoking less than 10 to 15 pack years of cigarettes is unlikely to result in COPD. On the other hand, the single best variable for predicting which adults will have airflow obstruction on spirometry is a history of more than 40 pack years of smoking (positive likelihood ratio [LR], 12 [95% CI, 2.7-50]).[7-8]

Malondialdehyde (MDA) is a highly toxic byproduct of the lipid per-oxidation of unsaturated fatty acids by free radicals. Since it is the stable product that is why it is used as the marker of oxidative damage of unsaturated fatty acids. Oxidative stress play an important role in the pathogenesis of COPD and may also be involved in the pathogenesis of lung cancer by causing DNA damage.[9] There is some evidence that diet rich in antioxidants is associated with higher lung function and lower prevalence of COPD especially in male smokers.[10] It was also reported that short term improvement in adult asthma might be achieved through a food-based intervention to modify dietary anti oxidant intake.[11-12] The antioxidant level in the present study was found significantly low in cases as

compared to healthy controls and a similar trend was also reported by Pirabbasi E et al.[13] The MDA was found to be significantly higher in cases as compared to healthy control. A similar finding has also been reported in by Bartoli ML et al.[14]

When the level of MDA and total antioxidant of cases were further examined according to the severity of disease based on Gold Grade, it was observed that the level of MDA increases as Gold Grade increases but total antioxidant level decreases as Gold Grade increases. A similar trend was seen as regards smoking pack year status that is, level of MDA increases as pack year increases and level of total antioxidant decreases as pack year increases. However, the study shows that the levels of MDA and total antioxidant were independent of different chosen age groups.

In this study majority of patients were in acute exacerbation of COPD and so future study in patient without exacerbation needs to be done. Further, the study was more stressed on cigarette exposure though, there might be many other risk factor for COPD future studies on individual risk factor are required.

CONCLUSION

COPD has a major impact on patient health status and associated morbidity and mortality. Smoking is most important factor in causing COPD. Early approaches to smoking cessation, proper medication and controlling oxidative stress would certainly alter disease progression; improve symptoms and functional status as well as quality of life in patients of COPD.

REFERENCES

1. World Health Organization. The World Health Report 2002: reducing risks, promoting healthy life. Geneva (Switzerland): WHO, 2002.
2. Soriano JB, Miravitlles M, Borderı´as L, et al. Geographical variations in the prevalence of COPD in Spain: relationship to smoking, death rates and other determining factors. Arch Bronconeumol 2010; 46: 522–30.
3. Ciencewicki J, Trivedi S, Kleeberger SR. Oxidants and the pathogenesis of lung diseases. J Allergy Clin Immunol 2008; 122: 456-68.
4. Waseem SMA, Mobarak Hussain M, Islam N. Oxidative Stress in Mild and Moderate COPD: Assessment of Oxidant Anti-Oxidant Imbalance. Biomedical Research 2014; 25 (1): 115-119.
5. Philpot J. Assay for MDA levels. Rad Res 1963; 3: 55- 80.
6. Eva Prescott, Peter Lange, Jørgen Vestbo. Socioeconomic status, lung function and admission to hospital for COPD: results from the Copenhagen City Heart Study. The European Respiratory Journal 1999; 13(5):1109-14.
7. Qaseem A, Wilt TJ, Weinberger SE, Hanania NA, Criner G, van der Molen T, et al. Diagnosis and Management of Stable Chronic Obstructive Pulmonary Disease: A Clinical Practice Guideline Update from the American College of Physicians, Ann Intern Med. 2011;155:179-191.
8. Simel DL, Rennie D, Bossuyt PMM. The STARD Statement for reporting studies of diagnostic accuracy: application to the history and clinical examination. J Gen Intern Med. 2008:23:768-74.

9. Filaire E, Dupuis C, Galvaing G, et al. Lung cancer: what are the links with oxidative stress, physical activity and nutrition. Lung Cancer 2013; 82:383-9.

10. Shaheen SO, Jameson KA, Syddall HE, et al. The relationship of dietary patterns with adult lung function and COPD. Eur Respir J 2010; 36:277-84.

11. Wood LG, Garg ML, Smart JM, et al. Manipulating antioxidant intake in asthma: a randomized controlled trial. Am J Clin Nutr 2012; 96:534-43.

12. Shaheen SO. Antioxidants and respiratory disease: the uric acid paradox. Thorax 2014; 69:978-979.

13. Pirabbasi E, Najafiyan M, Cheraghi M, Shahar S, Abdul Manaf Z, Rajab N, et al. Predictors' factors of nutritional status of male chronic obstructive pulmonary disease patients. ISRN nursing. 2012 Dec;2012 :782626.

14. Bartoli ML, Novelli F, Costa F, et al. Malondialdehyde in Exhaled Breath Condensate as a Marker of Oxidative Stress in Different Pulmonary Diseases. Mediators of Inflammation 2011; Article ID 891752, 7 pages, 2011. doi:10.1155/2011/891752.