IJAM

ORIGINAL RESEARCH PAPER ISSN (O) : 2663 - 046X | ISSN (P) : 2663 - 0451

TO STUDY THE PULMONARY FUNCTION TESTS AND ESTIMATE MALONDIALDEHYDE LEVELS, THE LIPID PEROXIDATION BIOMARKER IN COPD PATIENTS.

Dr.Ranjana Assistant Professor ,Dept Of Physiology, Netaji Subhas Medical College& Hospital, Amhara, Bihta, Bihar.

Dr.Mishra Indira Sushil* Former Tutor, Dept Of Physiology, Dr.BSA Medical College & Hospital, Delhi. *Corresponding Author indi mishra@yahoo.com

Dr.Rajiv Ranjan Prasad Assistant Professor, Dept Of Anaesthesia, Netaji Subhas Medical College & Hospital, Amhara, Bihta, Bihar.

ABSTRACT The antioxidants requirement depend on one's exposure to endogenous and exogenous reactive oxygen. Cigarette smoking leads to increased exposure to reactive oxygen species, hence they require more antioxidant nutrients. In this study, we aimed to show the levels of Malondialdehyde (MDA) as a marker of oxidative stress in COPD and smokers.

KEY WORDS : Chronic Obstructive Pulmonary Disease (COPD), Malondialdehyde (MDA)

INTRODUCTION-

Mitchell in the United States of America first mentioned the term "Chronic Obstructive Pulmonary Disease" (COPD) (Mitchell *et al* 1964). COPD is one of the major global health problem and it is the third leading cause of death by 2030.[1] About 210 million people worldwide suffer from COPD, out of this about 80 million people suffer with moderate to severe COPD; which is responsible for about 5% of deaths worldwide. It is going to rank third leading cause of death by 2030 globally[2]. An imbalance between oxidants and antioxidants is responsible in the patho-physiology of COPD, which leads to multisystemic manifestations including weight loss.[3,4]

An oxidative stress leads to peroxidation of membrane lipids which causes cellular damage. Lipid peroxidation leads to a disease COPD[5]. It produces an end-products like MDA (malondialdehyde) which is a ketoaldehyde and a marker for oxidative stress[6]. The important feature of COPD is airflow limitation which is not reversible completely. The small airway compartment changes occur.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.[7] Cigarette smoke leads to direct injury of airway epithelial cells, which causes release of endogenous intracellular molecules. These signals are recognized by receptors like toll receptors 4 and 2 on epithelial cells which initiates inflammatory response.[8] On inflammation cytokines are released, macrophages, neutrophils and dendritic cells are attracted to the site of inflammation which initiates the innate immune response.[9,10] Proteolytic enzymes and reactive oxygen species are produced which if not counterbalanced by antiproteases and antioxidant factors further damage will occur.[11] Reactive oxygen species (ROS) are produced by living organisms during cellular metabolism. At low to moderate concentrations, they have physiological role, but at high concentrations, adverse changes to lipids, proteins and DNA are produced.[12-17] 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.[18] and asthma[19-24] Different biomarkers of oxidative stress are present, including 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.[25] Oxidative stress leads to lipid peroxidation which causes oxidative damage[26]. Many studies have shown the association between these biomarkers levels and different diseases development.[25,27,28] Lipid peroxidation products have gained attention as oxidative stress biomarkers. Lipids are oxidised by enzymes and nonenzymatic oxidants. The mechanisms and lipid peroxidation products have been studied extensively.[29,30] Malondialdehyde (MDA) and thiobarbituric acid reactive substances (TBARS) have been most commonly used indices of oxidative damage.[31,32] Many different studies have demonstrated MDA as potential biomarker to assess oxidative stress status in COPD patients. They applied the method of TBARS wherein 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 in many studies.[33-53] Some studies have reported increase in plasma MDA levels with the progress and severity of the disease.[34,37,54,55] However, few studies did not find any significant change in plasma TBARS MDA levels of COPD patients as compared to healthy controls.[56-61]

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% ($FEF_{25-75\%}$).[62] Cigarette smoking causes decrease in both FEV1/FVC and FEF_{25-75} which resulted into airway obstruction and small airway disease in adult smokers.[63]

A linear relationship between years of smoking and decrease in FEV_1 and FVC was reported.[64] A decrease in FEV_1 was also detected in teenage smokers[65].Smoking cessation led to reduction in smoking-induced decrease in lung function, but also led to reversal to nonsmoking values[66].These findings confirm the deleterious effect of smoking on lung function and prove a beneficial effect of quitting smoking .

AIMS AND OBJECTIVES: -

1)To study the pulmonary function tests and the lipid peroxidation biomarker, malondialdehyde level in non-smoker controls and COPD patients.
2)To compare the above parameters of smoker COPD patients with non-smoker controls.

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 in collaboration with the

***Corresponding Author Dr.Mishra Indira Sushil**

Former Tutor, Dept Of Physiology, Dr.BSA Medical College & Hospital, Delhi.

Department of T.B. Chest Santosh Hospital, Ghaziabad Uttar Pradesh. Study was approved by the Institutional Ethical Committee.

Group 1, N = 30, control male subjects between the ages of 35-50 years ,who were non-smokers, not exposed to any biomass fuel, with no history of hypertension, lung cancer, bronchial asthma, Diabetes mellitus, cardiovascular 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 with history of exposure to risk factors like smoking, history of chronic cough, dyspnoea or sputum production for at least three months of consecutive two years were selected. Subjects taking antioxidant drugs 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) 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).

PROCEDURE :-

5ml blood was withdrawn from vein with aseptic precautions. Following which the serum MDA was measured using the method of Buege.[67] 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. The malondialdehyde concentration was estimated by plotting the obtained absorbance against the graph. The optical density of the pink colour obtained is proportional to the malondialdehyde 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:

RESULT:

Table 1: DEMOGRAPHIC DATA OF MALE SUBJECTS

DEMOGRAPHIC PARAMETER	GROUP – 1 (CONTROL) N = 30 (Mean ± SD)	GROUP -2 (COPD CASES) N = 3 (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 3 - SPIROMETR

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 (%)	7.900 ± 2.22	5.04 ± 2.87	0.04 (S)	4.29

Table 3- MDA Level

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)

DISCUSSION -

The present study was conducted on two groups. Group 1 is of 30 normal individuals and the other Group 2 is of 30 patients being diagnosed as having chronic obstructive pulmonary disease. The pulmonary function tests were carried out in both the groups. The present study was undertaken to assess the lung functions in COPD patients and compare the result of lung functions with those of normal subject. MDA concentrations in COPD patients were higher than the control group which probably resulted due to smoking induced radical chain reaction leading to lipid peroxidation of membrane phospholipids, altering cellular physiology.[26] It was observed that FEV_1 in first second and PEF rate were markedly decreased in patients with COPD smokers. The ratio FEV_1/FVC was decreased and was found to be sensitive in diagnosing chronic obstructive pulmonary disease.

In the present study, the mean value of FVC of COPD smoker has 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.[68]

A study conducted by Birgul et al, showed that MDA levels are significantly higher in smokers than in non-smoker.[69] 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.[70] Oxidative stress leads to increased lipid peroxidation products i.e MDA and decrease in antioxidants like Vitamin C, vitamin E, superoxide dismutase and catalase.[71] 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[72]

Singh S, Verma SK,et al reported decrease antioxidant levels in SOD, catalase in COPD patients.But MDA levels were increase.[73] 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.

CONCLUSION -

Oxidants such as MDA has a role in oxidative stress and in COPD. The structural changes in respiratory system, as well as the decline in lung functions of COPD smokers demonstrate smoking induced

negative effects which accelerate the onset of respiratory disorders. Smoking cessation as an early intervention may lead to some reversal towards the better health of COPD smokers.

REFERENCES: .

[1]. American Thoracic Society: Standards for the diagnosis and care of patients with chronic obstructive pulmonary disease. Am J Respir Crit Care Med 152: S77-s 121, 1995.

[2]. WHO site http:// www.who.int world bank / WHO global burden of disease study.

[3]. MacNee W. Accelerated lung ageing :a novel pathogenic mechanism of COPD. Biochem Soc Trans 2009;37:819-823. 8.

[4]. Drost EM, Skwarski KM, Sauleda J et al. Oxidative stress and airway inflammation in severe exacerbations of COPD. Thorax 2005;60:293-300.

[5]. Gutteridge JM. Lipid peroxidation and antioxidants as biomarkers of tissue damage. Clin Chem 1995;41:1819-28

[6]. Dalle-Donne, I., Rossi, R., Colombo, R., Giustarini, D., & Milzani, A.. Biomarkers of oxidative damage in human disease. Clin. Chem 2006; 52, 601–623.

[7]. O'Donnell DE. Hyperinfl ation, dyspnea, and exercise intolerance in chronic obstructive pulmonary disease. Proc Am Thorac Soc 2006; 3: 180–84.

[8]. Opitz B, van Laak V, Eitel J, Suttorp N. Innate immune recognition in infectious and noninfectious diseases of the lung. Am J Respir Crit Care Med 2010; 181: 1294–309.

[9]. Demedts IK, Bracke KR, Van Pottelberge G, et al. Accumulation of dendritic cells and increased CCL20 levels in the airways of patients with chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2007; 175: 998–1005.

[10].Barnes PJ, Shapiro SD, Pauwels RA. Chronic obstructive pulmonary disease: molecular and cellular mechanisms. Eur Respir J 2003; 22: 672–88.

[11].Rahman I, Adcock IM. Oxidative stress and redox regulation of lung inflammation in COPD. Eur Respir J 2006; 28: 219–42.

[12].Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M. Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chem Biol Interact. 2006;160:1–40.

[13].Halliwell B, Gutteridge JMC. Free Radicals in Biology and Medicine. 3rd ed. New York: Oxford University Press; 1999.

[14]Marnett LJ. Lipid peroxidationDNA damage by malondialdehyde. Mutat Res. 1999;424:83–95.

[15].Siems WG, Grune T, Esterbauer H. 4-Hydroxynonenal formation during ischemia and reperfusion of rat small-intestine. Life Sci. 1995;57:785–789.

[16].Stadtman ER. Role of oxidant species in aging. Curr Med Chem. 2004;11:1105–1112.

[17]. Wang MY, Dhingra K, Hittelman WN, Liehr JG, deAndrade M, Li DH. Lipid peroxidation-induced putative malondia ldehyde–DNA adducts in human breast tissues. Cancer Epidemiol Biomarkers Prev. 1996;5:705–710.

[18].Asami S, Manabe H, Miyake J, Tsurudome Y, Hirano T, et al. Cigarette smoking induces an increase in oxidative DNA damage, 8-hydroxydeoxyguanosine, in a central site of the human lung. Carcinogenesis. 1997;18:1763–1766.

[19].Andreadis AA, Hazen SL, Comhair SA, Erzurum SC. Oxidative and nitrosative events in asthma. Free Radic Biol Med. 2003;35:213–225.

[20].Comhair SA, Ricci KS, Arroliga M, Lara AR, Dweik RA, et al. Correlation of systemic superoxide dismutase deficiency to airflow obstruction in asthma. Am J Respir Crit Care Med. 2005;172:306–313.

[21].Comhair SA, Xu W, Ghosh S, Thunnissen FB, Almasan A, et al. Superoxide dismutase inactivation in pathophysiology of asthmatic airway remodeling and reactivity. Am J Pathol. 2005;166:663–674.

[22]Dut R, Dizdar EA, Birben E, Sackesen C, Soyer OU, Besler T, Kalayci O. Oxidative stress and its determinants in the airways of children with asthma. Allergy. 2008;63:1605–1609.

[23].Ercan H, Birben E, Dizdar EA, Keskin O, Karaaslan C, et al. Oxidative stress and genetic and epidemiologic determinants of oxidant injury in childhood asthma. J Allergy Clin Immunol. 2006;118:1097–1104.

[24].Fitzpatrick AM, Teague WG, Holguin F, Yeh M, Brown LA. Severe Asthma Research Program. Airway glutathione homeostasis is altered in children with severe asthma: evidence for oxidant stress. J Allergy Clin Immunol. 2009;123:146–152.

[25].Dalle-Donne I, Rossi R, Colombo R, Giustarini D, Milzani A. Biomarkers of oxidative damage in human disease. Clin Chem. 2006;52:601–23.

[26].Niki E. Lipid peroxidation: physiological levels and dual biological effects. Free Radic Biol Med. 2009;47:469–84.

[27].Niki E. Lipid peroxidation products as oxidative stress biomarkers. Biofactors. 2008;34:171–80.

[28].Giustarini D, Dalle-Donne I, Tsikas D, Rossi R. Oxidative stress and human diseases: origin, link, measurement, mechanisms, and biomarker. Crit Rev Clin Lab Sci. 2009;46:241–81.

[29].Yin H, Xu L, Porter NA. Free radical lipid peroxidation: mechanisms and analysis. Chem Rev. 2011;111:5944–72.

[30].Niki E, Yoshida Y, Saito Y, Noguchi N. Lipid peroxidation: Mechanisms, inhibition, and biological effects. Biochem Biophys Res Commun. 2005;338:668–76.

[31].Lykkesfeldt J. Malondialdehyde as biomarker of oxidative damage to lipids caused by smoking. Clin Chim Acta. 2007;380:50–8.

[32].Del Rio D, Stewart AJ, Pellegrini N. A review of recent studies on malondialdehyde as toxic molecule and biological marker of oxidative stress. Nutr Metab Cardiovasc Dis. 2005;15:316–28.

[33].Dhakal N, Lamsal M, Baral N, Shrestha S, Dhakal SS, Bhatta N, Dubey RK. Oxidative stress and nutritional status in chronic obstructive pulmonary disease. J Clin Diagn Res. 2015;9:BC01–4.

[34].Arja C, Surapaneni KM, Raya P, Adimoolam C, Balisetty B, Kanala KR. Oxidative stress and antioxidant enzyme activity in South Indian male smokers with chronic obstructive pulmonary disease. Respirology. 2013;18:1069–75.

[35].Woźniak A, Górecki D, Szpinda M, Mila-Kierzenkowska C, Woźniak B. Oxidant-antioxidant balance in the blood of patients with chronic obstructive pulmonary disease after smoking cessation. Oxid Med Cell Longev. 2013;2013:897075.

[36].Cristóvão C, Cristóvão L, Nogueira F, Bicho M. Evaluation of the oxidant and antioxidant balance in the pathogenesis of chronic obstructive pulmonary disease. Rev Port Pneumol. 2013;19:70–5.

[37].Ahmad A, Shameem M, Husain Q. Altered oxidant-antioxidant levels in the disease prognosis of chronic obstructive pulmonary disease. Int J Tuberc Lung Dis. 2013;17:1104–9.

[38].Raut AM, Suryakar AN, Mhaisekar D. A study of oxidative stress, thiol proteins and role of vitamin E supplementation in chronic obstructive pulmonary disease (COPD). Al Ameen J Med Sci. 2013;6:134–7.

[39].Gencer M, Aksoy N, Dagli EC, Uzer E, Aksoy S, Selek S, Celik H, Cakir H. Prolidase activity dysregulation and its correlation with oxidativeantioxidative status in chronic obstructive pulmonary disease. J Clin Lab Anal. 2011;25:8–13.

[40].Stanojkovic I, Kotur-Stevuljevic J, Milenkovic B, Spasic S, Vujic T, Stefanovic A, Llic A, Ivanisevic J. Pulmonary function, oxidative stress and inflammatory markers in severe COPD exacerbation. Respir Med. 2011;105:S31–7.

[41].Joppa P, Petrásová D, Stancák B, Dorková Z, Tkácová R. Oxidative stress in patients with COPD and pulmonary hypertension. Wien Klin Wochenschr. 2007;119:428–34.

[42].Vibhuti A, Arif E, Deepak D, Singh B, Qadar Pasha MA. Correlation of oxidative status with BMI and lung function in COPD. Clin Biochem. 2007;40:958–63.

[43]. Ceylan E, Kocyigit A, Gencer M, Aksoy N, Selek S. Increased DNA damage in patients with chronic obstructive pulmonary disease who had once smoked or been exposed to biomass. Respir Med. 2006;100:1270–6.

[44].Nadeem A, Raj HG, Chhabra SK. Increased oxidative stress and altered levels of antioxidants in chronic obstructive pulmonary disease. Inflammation. 2005;29:23–32.

[45].Calikoğlu M, Unlü A, Tamer L, Ercan B, Buğdayci R, Atik U. The

levels of serum vitamin C, malonyldialdehyde and erythrocyte reduced glutathione in chronic obstructive pulmonary disease and in healthy smokers. Clin Chem Lab Med. 2002;40:1028–31.
[46].Hanta I, Kocabas A, Canacankatan N, Kuleci S, Seydaoglu G. Oxidantantioxidant balance in patients with COPD. Lung. 2006;184:51–5.
[47].Rahman I, Morrison D, Donaldson K, MacNee W. Systemic oxidative stress in asthma, COPD, and smokers. Am J Respir Crit Care Med. 1996;154:1055–60.
[48].ben Anes A, Fetoui H, Bchir S, ben Nasr H, Chahdoura H, Chabchoub E, Yacoub S, Garrouch A, Benzarti M, Tabka Z, Chahed K. Increased oxidativestress and altered levels of nitric oxide and peroxynitrite in Tunisian patients with chronic obstructive pulmonary disease: correlation with disease severity and airflow obstruction. Biol Trace Elem Res. 2014;161:20–31.
[49].Zeng M, Li Y, Jiang Y, Lu G, Huang X, Guan K. Local and systemic oxidative stress status in chronic obstructive pulmonary disease patients. Can Respir J. 2013;20:35–41.
[50].Tsukagoshi H, Shimizu Y, Iwamae S, Hisada T, Ishizuka T, Iizuka K, Dobashi K, Mori M. Evidence of oxidative stress in asthma and COPD: potential inhibitory effect of theophylline. Respir Med. 2000;94:584–8.
[51].Vibhuti A, Arif E, Mishra A, Deepak D, Singh B, Rahman I, Mohammad G, Pasha MA. CYP1A1, CYP1A2 and CYBA gene polymorphisms associated with oxidative stress in COPD. Clin Chim Acta. 2010;411:474–80.
[52].Isik B, Isik RS, Ceylan A, Calik O. Trace elements and oxidative stress in chronic obstructive pulmonary disease. Saudi Med J. 2005;26:1882–5.
[53].Premanand R, Kumar S, Mohan A. Study of thiobarbituric reactive substances and total reduced glutathione as indices of oxidative stress in chronic smokers with and without chronic obstructive pulmonary disease. Indian J Chest Dis Allied Sci. 2007;49:9–12.
[54].Kluchová Z, Petrásová D, Joppa P, Dorková Z, Tkácová R. The association between oxidative stress and obstructive lung impairment in patients with COPD. Physiol Res. 2007;56:51–6.
[55].Folchini F, Nonato NL, Feofiloff E, D'Almeida V, Nascimento O, Jardim JR. Association of oxidative stress markers and C-reactive protein with multidimensional indexes in COPD. Chron Respir Dis. 2011;8:101–8.
[56].Jammes Y, Steinberg JG, Ba A, Delliaux S, Brégeon F. Enhanced exerciseinduced plasma cytokine response and oxidative stress in COPD patients depend on blood oxygenation. Clin Physiol Funct Imaging. 2008;28:182–8.
[57].Koechlin C, Couillard A, Cristol JP, Chanez P, Hayot M, Le Gallais D, Préfaut C. Does systemic inflammation trigger local exercise-induced oxidative stress in COPD? Eur Respir J. 2004;23:538–44.
[58].Couillard A, Koechlin C, Cristol JP, Varray A, Prefaut C. Evidence of local exercise-induced systemic oxidative stress in chronic obstructive pulmonary disease patients. Eur Respir J. 2002;20:1123–9.
[59].Erden ES, Motor S, Ustun I, Demirkose M, Yuksel R, Okur R, Oktar S, Yakar Y, Sungur S, Gokce C. Investigation of Bisphenol A as an endocrine disruptor, total thiol, malondialdehyde, and C-reactive protein levels in chronic obstructive pulmonary disease. Eur Rev Med Pharmacol Sci. 2014;18:3477–83.
[60].Torres-Ramos YD, García-Guillen ML, Olivares-Corichi IM, Hicks JJ. Correlation of plasma protein carbonyls and C-reactive protein with GOLD stage progression in COPD patients. Open Respir Med J. 2009;3:61–6.
[61].Ben Moussa S, Sfaxi I, Tabka Z, Ben Saad H, Rouatbi S. Oxidative stress and lung function profiles of male smokers free from COPD compared to those with COPD: a case–control study. Libyan J Med. 2014;9:23873.
[62].Kuperman AS, Riker JB: The variable effect of smoking on pulmonary function. Chest, 1973, 63: 655–660 [PubMed] [Google Scholar]
[63].Zamel N, Altose MD, Speir WA: Statement on spirometry: a report of the section of respiratory pathophysiology of the American College of Chest Physicians. J Asthma, 1983, 20: 307–311 [PubMed] [Google Scholar]
[64].Xu X, Li B, Wang L. Gender difference in smoking effects on adult pulmonary function. Eur Respir J. 1994;7:477–83.
[65].Lee SK, Park JW, Kim KH, Jung JH. An Analysis of the Thickness of Abdominal Muscles during Forceful Expiration and Pulmonary Function in Teenage Smokers and Nonsmokers. J Phys Ther Sci. 2013;25:789–91.
[66].Nemery B, Moavero NE, Brasseur L, Stanescu DC. Changes in lung function after smoking cessation: an assessment from a cross-sectional survey. Am Rev Respir Dis. 1982;125:122–4
[67].Kumari, Suchetha K., Jayaprakash V., Sharada. (2011). Status Of Lipid Peroxidation And Total Antioxidant Capacity In Regular Voluntary Blood Donors. Journal of Health and Allied Sciences NU. 01. 03-06. 10.1055/s-0040-1703530.
[68].Curran DR, Cohn L. Advances in mucous cell metaplasia: a plug for mucus as a therapeutic focus in chronic airway disease. Am J Respir Cell Mol Biol. 2010 Mar;42(3):268-75. doi: 10.1165/rcmb.2009-0151TR. Epub 2009 Jun 11. PMID: 19520914; PMCID: PMC2830403.
[69].Birgul Isik, Ali Ceylan & Recep Isik (2007) Oxidative Stress in Smokers and Non-smokers, Inhalation Toxicology, 19:9, 767-769, DOI: 10.1080/08958370701401418
[70}Yanbaeva DG, Dentener MA, Creutzberg EC, Wesseling G, Wouters EF. Systemic effects of smoking. Chest. 2007 May;131(5):1557-66. doi: 10.1378/chest.06-2179. PMID: 17494805.
[71] Cavalcante AG, de Bruin PF. The role of oxidative stress in COPD: Current concepts and perspectives. J Bras Pneumol. 2009;35:1227–37. [PubMed] [Google Scholar]
[72]MacNee W. Oxidants/antioxidants and COPD. Chest. 2000;117:303S–17S. [PubMed] [Google Scholar]
[73] Singh S, Verma SK, Kumar S, Ahmad MK, Nischal A, Singh SK, Dixit RK. Evaluation of Oxidative Stress and Antioxidant Status in Chronic Obstructive Pulmonary Disease. Scand J Immunol. 2017 Feb;85(2):130-137. doi: 10.1111/sji.12498. PMID: 28256060.