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STUDY OF OXIDATIVE PARAMETERS IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD)

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Abstract

Background: Chronic obstructive pulmonary disease (COPD) proven fact as strong association with oxidative stress.

Methods: Total number of 200 patients was studied, comprising of 100 healthy controls and 100 COPD cases. Among 100 COPD cases diagnosed, 50 were of chronic bronchitis cases and 50 were emphysema cases. Patient's serum was estimated for vitamin- C, reduced glutathione, superoxide dismutase activity and MDA.

Results: In comparison to controls, levels of reduced glutathione, Vit-C and SOD activity are significantly decreased in COPD cases. In emphysema patients also there was significant decrease in erythrocyte reduced glutathione, vitamin C and SOD activity. In comparison to chronic bronchitis cases there was significant increase in MDA in emphysema patients when compared to chronic bronchitis patients.

Conclusion: Imbalance in antioxidants status of erythrocyte reduced glutathione, SOD activity and vit- C in COPD patients is mainly a consequence of increased oxidative stress, which can be corrected by dietary supplements.

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INTRODUCTION

COPD (Chronic obstructive pulmonary disease) is the leading cause of death which stands forth globally. It is prevalent in countries where ever smoking is high. Indian youth has increasing tendency to abuse for tobacco so COPD is emerging to be a major health problem [(1)]. 80% of patients of COPD are due to tobacco abuse, and leftover 20% is due to occupational dusts, air pollution from biomass fuel used, and exposure to environmental tobacco smoke, cooking in poorly ventilated buildings, outdoor air pollution and chemicals. [(2)].

American Thoracic Society defines COPD as a disease characterized by obstruction of air flow due to chronic bronchitis or emphysema [(2)]. Chronic bronchitis is defined as excessive bronchial mucus secretion manifested by daily productive cough for 2 months or more in atleast 3 consecutive years. Emphysema is due to irreversible enlargement of air spaces in distal terminal bronchiole, with destruction of their walls.

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 increase pro-inflammatory gene expression and oxidative tissue injury leading to COPD [(3)]. MDA (Malondialdehyde) a product formed from lipid peroxidation is an indicator of oxidative stress and well correlated indirectly with pulmonary function [(4)]. Imbalance between antioxidants and oxidants may contribute to oxidative stress [(5)].

Erythrocyte antioxidants such as reduced glutathione functions as an efficient intra-cellular scavenger of H2O2 and plays an prime role in the avoiding peroxidative lung damage in patients with COPD. Vitamin C is a water soluble free radical scavenger, can directly scavenge O2* and OH* radicals and helps to neutralize physiological oxidant burden created by both exogenous and endogenous sources [(6)].

Present study is undertaken to evaluate reduced glutathione, vit- C, SOD activity and MDA in COPD in comparison with controls.

METHODOLOGY

It is a study done cross sectionally for a period of one year from February 2014 to January 2015 in Biochemistry department. A total of 200 subjects participated of which 100 were COPD case and 100 were healthy controls. These COPD Patients were clinically diagnosed on basis of detailed clinical history, biochemical examinations and clinical condition including spirometry with FEV1/FVC ratio. Patient Informed consent was obtained and institutional ethical and research committee has approved the study.

Exclusion criteria : Patients with other lung diseases where FEV1 is reduced, history of cardiac problems or history of past surgeries, DM, liver disease, renal disease**,** hypertension, malignancy and other lung disorders.

A detailed proforma was filled up for each patient which included age, sex and of total 100 cases, 50 patients had chronic bronchitis and 50 cases had emphysema.

Past history was noted. 6 ml of blood sample was collected in a test tube from every patient and serum was separated from RBC by centrifugation at 3000 rpm for 10 minutes at room temperature. Following oxidative parameters were carried out in the samples. Samples were collected for estimation of reduced glutathione by Ernest Beutler et al., method [(7)], Vitamin C by 2, 4 –DNPH method [(8)], Serum Superoxide dismutase estimated by Marklund and Marklund method [(9)] and Serum MDA estimated by Kei satoh method [(10)].

Statistical analysis was done by SPSS statistical package version 15.0 and expressed as Mean± SD and range values. P value of < 0.05 is termed as statistical significance.

RESULTS

Of total 100 cases, 50 patients had chronic bronchitis and 50 cases had emphysema.

TABLE- 1: Demographic distribution of subjects

No. of subjects		Control N=100	Cases N=100	P-value
Age's in years	Mean± SD	57.7± 7.4	62.3 ± 7.8	P>0.05
	Range	45-73	50-80	

There is no significance in age groups in both the groups.

Table-2: Comparison of oxidative parameters in controls and COPD

Groups		Vitamin-C (mg/dL)	MDA(nmol/mL)	GSH (mg/dL)	SOD(U/ml)
Controls	**Range**	0.79 -1.4	1.43-3.68	26-37	5.10- 13.01
	Mean±S.D	1.10±0.16	2.62±0.52	35.14±2.12	10.01±1.69
Cases	**Range**	0.38-0.81	3.48-6.87	23.2 -30.92	2.29-6.95
	Mean±S.D	0.56±0.12	5.36±0.74	26.93±1.99	4.65 ±1.32
Controls V/S Cases	**t-value**	20.91	22.84	14.93	19.12
	p-value	<0.001	<0.001	<0.001	<0.001

All the parameters are significant in cases of COPD when compared with controls.

Table-3: Comparison of Oxidative parameters in controls and chronic bronchitis

Groups		Vitamin-C(mg/dl)	MDA(nmol/ml)	GSH (mg/dl)	SOD (U/ml)
Controls	Range	0.79 -1.4	1.43-3.68	26-37	5.10- 13.01
	Mean±S.D	1.10±0.16	2.62±0.52	35.14±2.12	10.01±1.69
Chronic bronchitis cases	Range	0.4-0.79	3.38-6.97	26.96-30.82	4.12-6.95
	Mean±S.D	0.58±0.14	5.36±0.77	29.17±0.99	5.76±0.79
Controls V/S Chronic Bronchitis Cases	t-value	18.21	19.42	12.91	16.01
	p-value	<0.001	<0.001	<0.001	<0.001

p value < 0.001(highly significant)

Erythrocyte reduced glutathione, serum vitamin-C; SOD activity and MDA in controls and chronic bronchitis are significantly changes

Table-4: Comparison of erythrocyte reduced glutathione, serum vitamin C, SOD activity and MDA emphysema cases in comparison with controls

Groups		Vitamin-C (mg/dl)	MDA(nmol/ml)	GSH (mg/dl)	SOD (U/ml)
Controls	Range	0.79 -1.4	1.43-3.68	26-37	5.10- 13.01
	Mean±S.D	1.10±0.16	2.62±0.52	35.14±2.12	10.01±1.69
Emphysema cases	Range	0.38-0.81	3.48-6.87	23.97-31.12	2.29 -7.01
	Mean±S.D	0.66±0.12	6.36±0.74	29.2±1.69	4.89±0.98
Controls V/S Emphysema Cases	t-value	25.44	27.91	17.27	12.10
	p-value	<0.001	<0.001	<0.001	<0.001

p value < 0.001, highly significant

All parameters are significantly changed in controls and emphysema cases

TABLE- 5: Relationship between various antioxidants in COPD cases

Correlation between	Person correlation coefficient 'r' value	p- value
GSH Vs Vit. C	+ 0.59	< 0.001
GSH Vs SOD	+ 0.62	< 0.001
Vit. C Vs SOD	+ 0.75	< 0.001
GSH Vs MDA	- 0.61	< 0.001
Vit. C Vs MDA	- 0.62	< 0.001
SOD Vs MDA	- 0.69	< 0.001

It is observed that positive significant correlation between reduced GSHVs Vit. C, GSH Vs SOD and Vit. C Vs SOD in COPD cases is significant. There is a negative significant correlation between GSH VsMDA, Vit C Vs MDA and SOD Vs MDA in COPD cases it is highly significant.

DISCUSSION

COPD prevalence is high in areas where smoking is high. In our country there is increasing tendency to abuse tobacco so COPD is major public health problem [(1)]. Oxidative stress explains its role in COPD pathogenesis as it is due to imbalance between the production of oxidants and the presence of antioxidants [(11)].

In comparison with controls COPD cases have significant decreased (p value< 0.001) level of reduced glutathione. This study is in correlation with the study done by Mercken EM et al., [(12)] and Mukaddercalikoglu et al., [(13)] There is significant decrease in GSH (P-value < 0.001) in emphysema cases in comparison with chronic bronchitis cases, well correlates with the study of done by J. Gea et al., [(14)]. Smoking tobacco is most important cause of COPD, due to the airways of smokers are exposed to highly reactive components, the lung airways of smokers are at risk of oxidative injury. In normal conditions most of the glutathione stored intracellularly in the reduced form (GSH) in COPD, there is increased oxidative stress so free sulfhydryl(-SH) groups become oxidized resulting in loss of GSH. Reason for this could be smoke due to cigarette, which irreversibly reacts with GSH to form GSH derivatives ,

there by depleting the total pool of GSH [15, 16]. The activities of redox system enzymes such as gamma-glutamyl cysteine synthatase, glutathione peroxidase, and glucose-6-phosphate dehydrogenase and glutathione synthesis were decreased in epithelial cells of alveoli after exposure to cigarette smoke. Thus there is independent depletion of soluble GSH intracellularly, concomitant with the formation of GSH conjugates [17].

In comparison to controls, cases of COPD have significantly (p value< 0.001) decreased vit-C levels. This correlates with study done by Raghunath R, Rai et al. , [6] L.A.Sargeant et al., [18] and Mukaddercalikoglu et al [19]. Vitamin C is significantly decreased (p value < 0.001) in emphysema patients when compared to chronic bronchitis patients. This is similar to study of Papaioannou et al [20].

Vit- C functions as free radical scavenger, so mechanism involved is reduction of vit- C level in COPD, and this is due to rapid oxidation of ascorbic acid by its free radicals mechanism. Vit- C and MDA are inversely related,and this may be due to the depletion of vit- C when the oxidant burden is increased [19]. Vitamin C functions as an antioxidant by donating its electrons, and it prevents other compounds from being oxidized, however by the very nature of this reaction vit- C itself is oxidized in the process. The species formed after the loss of one electron is a free radical i.e., ascorbyl radical. Reduction of a reactive free radical with formation of a less reactive compound is sometimes called free radical scavenging or quenching [21].

Decreased levels of SOD are found in COPD cases when compared to controls. COPD patients have significant p value <0.001.This is similar to study done by Raghunath Rai R et al, [6] Gamzekirkil et al. [22] SOD is significantly decreased (P-value < 0.001) in emphysema patients when compared to chronic bronchitis patients. This correlates with the study done by AI. Papaioannou et al [20]. The imbalance in antioxidant enzymes SOD activity emphasizes the redox alteration in COPD patients. Mechanism involved in decreased serum SOD activity is due to increased production of free radicals in COPD patients leading to increased depletion of antioxidant enzymes [22].When compared to controls, COPD patients have significantly (p value < 0.05) with increased level of MDA. This is in coincidence with the study of M.K. Daga et al, [23] BirgulIsik et al, [24] and Gamzekirkil et al [22]. MDA concentration is significantly elevated (p value < 0.001) in emphysema patients when compared to chronic bronchitis patients, this is similar to the study done by J. Gea et al [14].

Results implicate that there is increase in oxidative stress and decrease in antioxidants in COPD cases when compared to controls. Erythrocyte reduced glutathione functions as an efficient intracellular scavenger of H2O2 and plays an important role in the prevention of peroxidative lung damage in patients with COPD. In oxidative stress, levels of erythrocyte reduced glutathione is decreased, which plays specific role in COPD pathogenesis .Oxidative stress has role in the pathogenesis of tobacco smoke induced COPD. Human epithelial cells of alveoli have damaged cell membranes because of reactive oxygen species present in the smoke of tobacco. Increased MDA, a lipoxidation product in COPD cases is due to increased production of reactive oxygen species [23].Increased MDA level in emphysema patients indicates more oxidative stress in comparison with chronic bronchitis cases. Reason for this could be that, the patients with emphysema have more severe lung dysfunction, lower body mass index, poor quality of life and more serious systemic dysfunction [20, 25].

CONCLUSION

From present study it is known that there is increased oxidative stress in cases of COPD and it is higher in emphysema patients when compared to chronic bronchitis patients. It is observed that tobacco smoke causes oxidative stress in smokers which results in COPD (chronic obstructive pulmonary disease). Further oxidative damage can be avoided by dietary supplements which are rich in antioxidants in COPD patients.

Conflict of interest- None

Acknowledgement-None

REFERENCES

1. Prem anand R, Santhosh kumar PH, Mohan A. Study of thiobarbituric reactive substances and total reduced glutathione as indices of oxidative stress in chronicsmokers with and without chronic obstructive pulmonary disease. J Chest Dis Allied Sci 2007;49:9-12

2. Mephie SJ, Papadakis MA, Lawrence MT. Current medical diagnosis andtreatment. 47th edition, McGraw Hill Medical Publisher, New Delhi, 2008; 216 -221.

3. Drost EM, Skwarski KM, Sauleda J, Soler N, Roca J, Agusti A et al. Oxidativestress and airway inflammation in severe exacerbations of COPD. Thorax2005;60:293-300.

4. Kluchova Z, Petrasova D, Joppa P, Dorkova Z, Tkacova R. The associationbetween oxidative stress and obstructive lung impairment in

patients with COPD.Physiol Res. 2007;56:51-56.

5. MacNee W. Oxidants / Antioxidants and COPD. Chest 2000;117:303S-317S.

6. Rai RR, Phadke MS. Plasma oxidant-antioxidant status in different respiratorydisorders. Indian J ClinBiochem 2006;21(2):161-164

7. Beutler E, Duron O, Kelly BM. Improved method for the determination of blood glutathione. J Lab Clin Med 1963;61(5):882-888.

8. Kaplan LA, Pesce AJ, Methods of Analysis Ascorbid Acid (Vitamin C). In:Clinical Chemistry. 3rd ed., Chapter 39, p.786-787.

9. Nischal HK, Sharma MP, Goyal RK, Kaushik GG. Serum superoxide dismutaselevels in diabetes mellitus with or without microangiopathic complications. JAPI1998;46:853-55.

10. Satoh K. Serum Lipid peroxide in cerebrovascular disorders determined by a newcolorimetric method. ClinicaChimicaActa 1978;90:37-43.

11. Gerritsen WBM, Asin J, Zanen P, van den Bosch JMM, Haas FJLM. Markers ofinflammation and oxidative stress in exacerbated chronic obstructive pulmonarydisease patients. Respir Med 2005;99:84-90.

12. Mercken EM, Gosker HR, Rutten EP, Wouters EF, Bast A, Hageman GJ et al.Systemic and pulmonary oxidative stress after single -leg exercise in COPD.CHEST 2009;136:1291-1300.

13. Calikoglu M, U A, Tamer L, Ercan B, Bugdayci R, Atik U. The levels of serum vitamin C, Malondialdehyde and erythrocyte reduced glutathione in chronicobstructive pulmonary disease and in healthy smokers. ClinChem Lab Med2002;40(10):1028-1031.

14. Gea J, Mas S, Balcells E, Sauleda J, Gomez F, Galdiz JB et al. Systemic oxidativestress and COPD phenotypes. Am J RespirCrit Care Med 2009;179:A4329.

15. Van der Toorn M, Vries MPS, Slebos DJ, de bruin HG, Abello N, van OosterhoutAJM, et al. Cigarette smoke irreversibly modifies glutathione in airway epithelialcells. Am J Physiol Lung Cell MolPhysiol 2007;293:L1156-L1162.

16. Vina J, Servera E, Asensi M, Sastre J, Pallardo FV, Ferrero JA et al. Exercisecauses blood glutathione oxidation in chronic obstructive pulmonary disease :prevention by O2 therapy. J ApplPhysiol 1996;81(5):2199-2202.

17. Rahman I, MacNee W. Oxidant/antioxidant imbalance in smokers and chronic obstructive

pulmonary disease. Thorax 1996;51:348-350.

18. Sargeant LA, Jaeckel A, . Interaction of vitamin C with the relationbetween smoking and obstructive airways disease in EPIC Norfolk. EurRespir J2000;16:397-403.

19. Calikoglu M, Tamer L, Calikoglu I, Atis S, Ulubas B, Ercan B. Oxidative stressand products of nitric oxide metabolism in chronic obstructive pulmonary diseaseand in healthy smokers. Turkish Respir J 2002;3(1):24-27.

20. Papaioannou AI, Mazioti A, Kiropoulos T, Tsilioni I, Koutsokera A, Tanou K e al. Systemic and airway inflammation and the presence of emphysema in patientswith COPD. Respir Med 2010;104:275-282.

21. Padayatty SJ, Katz A, Wang Y, Eck P, Kwon O et al. Vitamin C asan Antioxidant : Evaluation of its role in disease prevention. J Am CollNutr2003;22(1):18-35.

22. Kirkil G, Muz MH, Seckin D, Sahin K, Kucuk O. Antioxidant effect of zincpicolinate in patients with chronic obstructive pulmonary disease. Respir Med2008;102:840-844.

23. Daga MK, Chhabra R, Sharma B, Mishra TK. Effects of exogenous vitamin Esupplementation on the levels of oxidants and antioxidants in chronic obstructivepulmonary disease. J Biosci 2003;28(1):7 -11.

24. Isik B, Isik SR, Yolacan H, Isik MR. Serum malondialdehyde and paraoxonaselevels in chronic obstructive pulmonary disease. Turkish Respir J 2005;6(1):19 -21

25. Boschetto P, Quintavalle S, Zeni E, Leprotti S, Potena A, Ballerin L et al.Association between markers of emphysema and more severe chronic obstructivepulmonary disease. Thorax 2006;61:1037-1042.