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Consumption of GSH with the Increase in Oxidative Stress in Chronic Obstructive Pulmonary Disease (COPD) Patients

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ABSTRACT

Introduction: Oxidant-antioxidant imbalance plays a major role in the pathogenesis of chronic obstructive pulmonary disease (COPD). Recently, most of the studies are concentrated on the evaluation of oxidant-antioxidant balance in exhaled breath condensate and in other body fluids in COPD patients.

Aims and Objectives: In this study, we investigated oxidant-antioxidant balance in systemic circulation of patients with COPD. Serum malondiladehyde (MDA), protein carbonyl (PC) and whole blood reduced glutathione (GSH) levels were determined in 60 patients with stable COPD and it compared with 60 age and sex matched healthy controls.

Materials and Methods: The levels of serum MDA, protein carbonyl and whole blood reduced glutathione were determined according to Buge and Aust method, Levin et al and Beutler et al method.

Results: Serum MDA and PC levels were significantly increase in COPD patients as compared to healthy controls. We found significantly decreased levels of erythrocyte GSH in stable COPD patients as compared to healthy controls.

Conclusion: Tobacco smoking cause increase in oxidative stress and reduction in antioxidant level in COPD patients. From these findings we conclude that there is disturb oxidant – antioxidant balance in COPD patients and this imbalance is related to long term history of tobacco smoking.

Key Words: COPD, Reduced glutathione, Malondialdehyde, Protein carbonyl

INTRODUCTION

COPD is a major public health problem and is projected to rank third leading cause of deaths globally by the year 2030 [1]. Chronic obstructive pulmonary disease is a disease state characterized by progressive and irreversible airflow limitation. This airflow limitation is associated with abnormal inflammatory response of the lungs to the noxious particles and gases [2]. Tobacco smoking is the major risk factor for the development of COPD in India. Tobacco smoke contains 10^{15} free radicals/ oxidants per puff and 4700 toxic chemicals. Tobacco smoke contains gas phase and tar phase. Gas phase of tobacco smoke contains free radicals superoxide and nitric oxide. Long-lived free radical semiquinone present in tar phase of tobacco smoke [3]. Activated neutrophils, macrophages due to smoking produces tremendous amounts of free radicals in alveoli and respiratory tract of COPD patients [4]. Lipid and proteins present on the membrane of airspace epithelium cells are highly susceptible to the free radical mediated damage which cause impair functioning of the cell [5]. Malondialdehyde is end product of lipid peroxidation. With the help of MDA we can indirectly measure free radical activity in body [6]. Protein carbonyl is the product of irreversible non-enzymatic oxidation of proteins. Protein carbonyl formation is a sign of protein dysfunction caused by disease [7,8]

To combat the deleterious effects of oxidants antioxidant system present in the body one of them is GSH. The reduced form of glutathione present in red blood cells. GSH is scavenger of H_2O_2. GSH inhibits lipid peroxidation reactions in lung tissue [9]. The presence of sulfhydryl group in

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GSH makes it function as an antioxidant, it protect the cells against free radicals and oxidants[10]. Intracellular GSH might decreases released of cytokines and chemokines from lung cells by decreasing NF-kB activation [11,12].

Therefore, the present study is undertaken to evaluate serum level of MDA, protein carbonyl and blood GSH in COPD patients and in healthy controls. In addition to this, we determined cut –off level of GSH, MDA and protein carbonyl in COPD patients by using ROC curve analysis.

MATERIAL AND METHODS:

This Case-Control study was carried out in Department of Biochemistry, B.J. Govt. Medical College and Sassoon General Hospital, Pune [Maharashtra]. The study period was in between 2012-2014. The study was approved by Institutional ethical committee [Ref. no. BJMC/IEC/Pharmac/D1210133-35]. Informed consent was obtained from each subject prior to the study. Healthy Controls and COPD cases were selected from Department of Pulmonary Medicine, B.J. Govt. Medical College and Sassoon General Hospital, Pune. Diagnosis of COPD patients were done with the help of spirometry test. The patients and controls were voluntarily participated in the study.

A total 120 subjects were participated in the study, of which 60 subjects were healthy controls and 60 subjects were of COPD disease. There were no difference in age among cases and controls.

Inclusion Criteria

i) Group 1 (COPD patients): Group 1 consist of 60 clinically diagnosed COPD patients

ii) Group 2 (Healthy Controls): Group 2 consist of 60 normal healthy individuals without any history of smoking and chronic lung disease.

Exclusion Criteria

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

Collection of Blood Samples

Under aseptic condition and with prior written consent of the subject, 5ml of blood was collected from large peripheral vein, after overnight fasting. Out of which 2ml was taken in an anticoagulant (EDTA) bulb for estimation of whole blood reduced glutathione (GSH) and 3ml in plain bulb for the estimation of serum malondialdehyde and protein carbonyl.

Estimation of Whole blood Reduced Glutathione

Whole blood reduced glutathione was estimated by Ernst Beutler et al method [13]. It is based on the principle that all of the non-protein sulphydryl group of red blood cells are in the form of reduced glutathione (GSH). 5,5[1]-dithiobis-2-nitrobenzoic acid (DTNB) is a disulphide compound, which is readily reduced by sulphydryl compounds, forming a highly colored yellow compound. Optical density measured at 412 nm and is directly proportional to GSH concentration[13]. It was expressed as mg/dl.

Estimation of Serum Malondialdehyde

Serum malondialdehyde was determined by Buege and Aust method (1978). It is based on the principle that serum sample is first treated with TCA (Trichloroacetic acid) for protein precipitation and then treated with thiobarbituric acid. The mixture is heated for 10 minutes in boiling water bath. One molecule of MDA (malondialdehyde) reacts with two molecules of thiobarbituric acid. The resulting chromogen is centrifuged and intensity of colour developed in supernatant is measured colourimetrically at 530nm. It was expressed as nmol/ml[14]

Estimation of Serum Protein Carbonyl:

Serum protein carbonyl was determined by Levin et al method (1990). The carbonyl groups in protein reacts with 2, 4- Dinitrophenyl hydrazine (DNPH) to form 2,4–Dinitrophenyl hydrazone which is estimated colorimetrically at 360-390nm. It was expressed as nmol/mg of proteins[15]. Protein concentration in mg/ml was determined by Bradfoed method (1976) [16].

Statistical Analysis

Results are expressed as mean ± SD and range values. Unpaired 't' test is used for comparing different biochemical parameters between cases and controls. P values of <0.05 was consider as statistically significant. In addition to this, a receiver operating characteristic (ROC) curve was used to determine the optimal cut –off point for studied biochemical parameters as a classifier of COPD according to the highest sensitivity and specificity. ROC curve was determined by using MedCalc Software

RESULTS

Table no. 1 shows biochemical characteristics of the study subjects. Serum mean level of malondialdehyde a biomarker

of lipid peroxidation and protein carbonyl a biomarker of protein oxidation were significantly increased in COPD patients when compared to healthy controls. Mean level of whole blood reduced glutathione was significantly decreased in COPD patients than in healthy controls. These results indicate that there is increase in oxidative stress and decrease in antioxidant level in COPD patients as compared to healthy controls.

For evaluation of biochemical marker, we selected blood reduced glutathione cut–off level of 26.7mg/dl for COPD disease (AUC=0.955, SE= 0.02, 95% CI= 0.90 to 0.98, P<0.0001 using the ROC Curve analysis. At this cut-off level of whole blood GSH, we achieved a sensitivity of 93.33% (95%CI=83.8 to 98.2) and specificity of 95.00 % (95%CI=86.1 to 99.0) (Fig. no.1). This result indicates that cut off levels of whole blood GSH decrease with increase in sensitivity and specificity. The values of area under ROC curve for whole blood GSH increase in presence of disease. From our findings we suggest that whole blood GSH may be good classifiers to discriminate the COPD patients from other disease, when patients were unable to perform the spirometry test.

The cut–off level of malondiladehyde 7.654 ng/ml for COPD disease (AUC=0.999, SE= 0.00, 95% CI= 0.96 to 1.00, P<0.0001) were decided by using ROC Curve analysis. At this cut-off level of serum malondiladehyde, we achieved a sensitivity of 98.33% (95%CI=91.1 to 100.0) and specificity of 100.00 % (95%CI=94.0 to 100) (Fig. no.2). Similarly, for protein carbonyl we obtained cut–off level of 7.51ng/mg of proteins for COPD disease (AUC=0.980, SE= 0.01, 95% CI= 0.93 to 0.99, P<0.0001) by using ROC Curve analysis. At this cut-off level of serum protein carbonyl, we achieved a sensitivity of 90.00% (95%CI=79.5 to 96.2) and specificity of 100.00 % (95%CI=94.0 to 100) (Fig. no.3). These results showed that as cut-off levels of serum malondilaldehyde and protein carbonyl increases with increase in sensitivity and specificity. The values of area under ROC curve, for serum MDA and protein carbonyl increase in presence of the disease. From our findings we suggest that serum levels of MDA and protein carbonyl can be used as marker to diagnose the COPD patients, when patients were unable to perform the spirometry test.

DISCUSSION

Oxidative stress plays important role in pathogenesis of COPD. In our study we selected smoker COPD patients. In our body strong antioxidant system exists to combat the deleterious effects of oxidants. In our study we found significantly decreased levels of reduced glutathione in COPD patients as compared to healthy controls. This is in accordance with study of Pawar RS et al [17], Parija M et al[18] and Calikoglu M et al [19]. We observed decreased levels of reduced glutathione in COPD patients this might be due to smokers lungs have higher content of oxidants. In normal individual glutathione stored in the cell in reduced form (GSH). In condition of increased oxidative stress this reduced form of glutathione is oxidized. Cigarette smoke contains oxidants that irreversibly binds to reduced form of glutathione to form GSH adduct that cannot be converted back to oxidized form of glutathione (Table no.1) [20]. This is the reason behind this decreased level of GSH in blood in COPD patients.

Lipids are present abundantly on the cell membrane. These lipids are more vulnerable to attack by free radicals. In this process of lipid peroxidation malondialdehydes are form. MDA is the end product of lipid peroxidation[21]. In our study we observed increased levels of serum malondiadehyde in COPD patients as compared to healthy controls (Table no.1). Our results are in accordance with Yesica DT et al[22], Daga MK et al [23] and Kirkil G et al [24]. Tobacco smoke contains tremendous amount of free radicals it may cause damage of alveolar epithelial cell membrane of lung tissues by stimulating process of lipid peroxidation. Increased MDA levels in patients with COPD may be due to increased production of free radicals and hence more lipid peroxidation products[25]. Similar to lipids, proteins are also oxidized by free radicals. In our study we observed increased levels of protein carbonyl in COPD patients as compared to healthy controls(Table no.1). This increase in PC might be due to increase 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 [26]. Increase oxidative stress leading to increase protein carbonyl formation in COPD patients (Table 1) [27]. The increased level of protein carbonyl in COPD patients was also reported in the study of Yessica DT et al. [22]. In contrast to our study, Mesia- Vela S et al. has fails to find significant increase in the level of protein carbonyl in COPD patients [28].

We did ROC curve analysis of MDA, PC and GSH by using Medcalc software. ROC curve analysis of oxidative stress markers showed cut –off values for MDA 7.654 ng/ml and for PC 7.51ng/mg of proteins. From these results it is clear that the area under ROC curve values for MDA and PC increases with highest sensitivity and specificity in presence of disease. Similarly for reduced glutathione we obtained cut-off level 26.7mg/dl by using ROC curve analysis. This result showed that area under ROC curve values for GSH increases with highest sensitivity and specificity in presence of disease. Hence, ROC curve analysis revealed the fact that all these markers are very sensitive and specific to diagnose the disease. However, the dietary supplementation of vita-

mins may reduce severity of the disease by reducing oxidative stress. Further study with the dietary supplementation of vitamins is needed to prove this.

CONCLUSION

Tobacco smoking cause increase in oxidative stress and reduction in antioxidant level in COPD patients. From these findings we conclude that there is disturb oxidant – antioxidant balance in COPD patients and this imbalance is related to long term history of tobacco smoking.

Strength and limitations of the study:

Strength:

1. We are the first to report the level of protein carbonyl in serum in smoker COPD patients.

LIMITATIONS

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

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Table 1: Comparison of serum MDA, Protein Carbonyl and Whole blood reduced Glutathione in healthy controls and COPD patients.

Groups	Serum MDA ng/ml)	Serum Protein Carbonyl (ng/mg of proteins)	Reduced glutathione (mg/dl)
Healthy Controls (n=60)	4.4 ± 1.7	3.55 ± 1.86	33.49 ± 5.3
COPD patients (n=60)	9.87 ± 0.98	9.61 ± 3.43	19.36 ± 5.8
Mean difference	5.47	6.18	14.13
t*	-20.38	-12.62	14.68
P	P<0.001	P<0.001	P<0.001

* Unpaired t-test

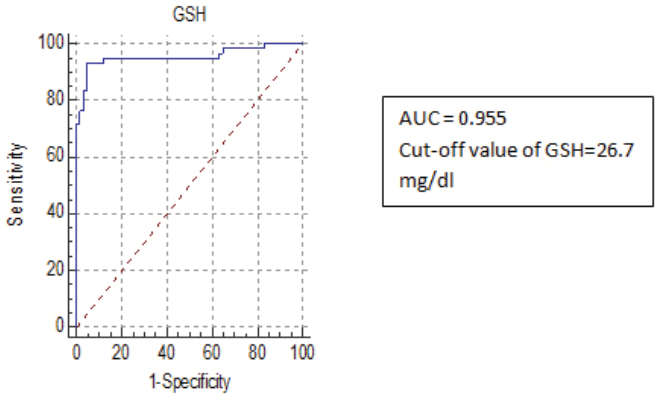

Figure 1: ROC curve analysis of GSH in COPD patients.

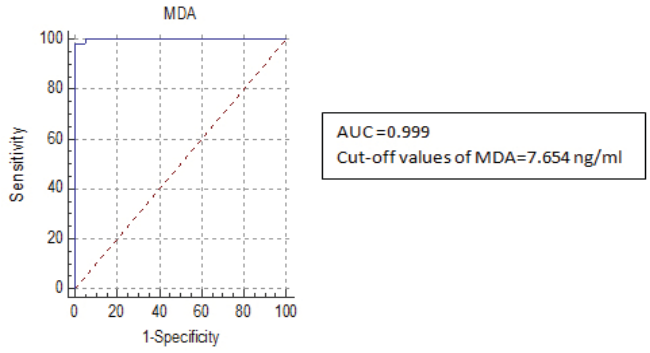

Figure 2: ROC curve analysis of MDA in COPD patients.

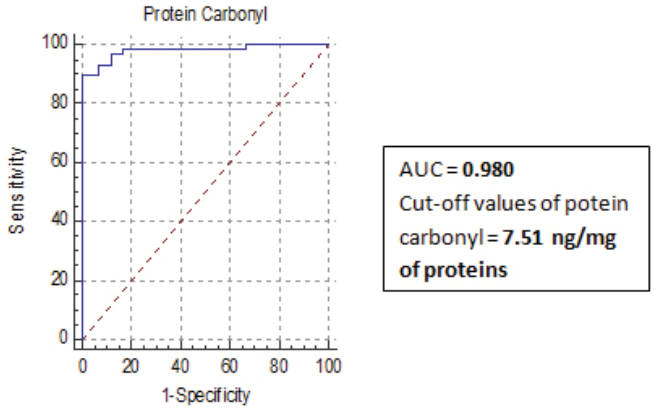

Figure 3: ROC curve analysis of protein carbonyl in COPD patients.