Research Article

Evaluation of antioxidants in patients with chronic obstructive pulmonary disease (COPD)

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Abstract

Background: Chronic obstructive pulmonary disease (COPD) is associated with high morbidity and mortality. Several studies showed evidence that there is a strong association between oxidative stress and COPD. Decrease in antioxidants also contributes to oxidative stress, because antioxidants not only protect against the direct injurious effects of oxidants, but also alter the inflammatory events that play an important role in the pathogenesis of COPD.

Objective: To evaluate the erythrocyte reduced glutathione (GSH), superoxide dismutase (SOD) activity, and malondialdehyde (MDA) as a biomarker of lipid peroxidation in patients with COPD and in controls.

Materials and Methods: A total number of 120 subjects were studied, comprising 60 healthy controls and 60 COPD cases. Among 60 COPD cases, 30 were patients with chronic bronchitis and 30 were patients with emphysema. Erythrocyte GSH was estimated by Ernest Beutler et al method, serum SOD activity by Marklund and Marklund method, and MDA by Kei Satoh method.

Result: The levels of erythrocyte GSH and SOD activity were significantly decreased in COPD cases when compared with controls. They were significantly decreased in patients with emphysema when compared to controls and were significantly increased in patients with emphysema when compared with patients with chronic bronchitis.

Conclusion: This study suggests the increased MDA levels in patients with COPD because of increased lipid peroxidation mediated by toxic free radicals such as superoxide ion, hydrogen peroxide, and hydroxyl radical. The decrease in antioxidant levels of erythrocyte GSH and SOD activity among patients with COPD appear to be mainly a consequence of increased oxidative stress.

KEY WORDS: COPD, superoxide dismutase, malondialdehyde, antioxidants, erythrocyte reduced glutathione

Introduction

Chronic obstructive pulmonary disease (COPD) is the fourth leading cause of deaths globally. The prevalence of COPD is higher in countries where smoking is highly prevalent. In India, there is an increasing tendency to abuse tobacco and COPD is emerging to be a major public health problem.[1]

Cigarette smoking is the most important risk factor for COPD. It is estimated that 80% of the patients with COPD have significant exposure to tobacco smoke. The remaining 20% have a combination of exposure to environmental tobacco smoke, occupational dusts and chemicals, indoor air pollution from biomass fuel used for cooking in poorly ventilated buildings, outdoor air pollution, and airway infection; and familial and hereditary factors have been implicated in the development of COPD.[2]

Chronic bronchitis is a clinical diagnosis defined by excessive secretion of bronchial mucus and is manifested by daily productive cough for 3 months or more for at least 2 consecutive years. Emphysema is a pathologic diagnosis that denotes

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abnormal permanent enlargement of air spaces distal to the terminal bronchiole with destruction of their walls without obvious fibrosis.[2]

A current hypothesis in the pathogenesis of COPD is that because of 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 counterbalanced by the lung antioxidant systems, resulting in oxidative stress. An excess of oxidants may then lead to enhanced pro-inflammatory gene expression and oxidative tissue injury leading to COPD.[3]

Malondialdehyde (MDA), a lipid peroxidation product, is an indicator of oxidative stress that is correlated inversely with pulmonary function.[4]

Antioxidant depletion may contribute to oxidative stress. Antioxidants not only protect against the direct injurious effects of oxidants, but also alter the inflammatory events that play an important role in the pathogenesis of COPD.[5]

Erythrocyte antioxidant such as reduced glutathione (GSH) functions as an efficient intracellular scavenger of H_2O_2 and plays an important role in the prevention of peroxidative lung damage in patients with COPD.[6]

This study is undertaken to evaluate erythrocyte GSH, superoxide dismutase (SOD) activity, and MDA in controls and in COPD cases.

Materials and Methods

A cross-sectional study of erythrocyte GSH, SOD activity, and MDA in patients with COPD were carried out from July 2013 to April 2015.

Controls and COPD cases were selected from MNR Medical College and Hospital, Sangareddy, Telangana, India. Each gave an informed consent and this study was approved by the ethical and research committee of MNR Medical college and Hospital to use human subjects in the research study. The patients and controls voluntarily participated in the study.

A total number of 120 subjects participated in the study, of which 60 were COPD cases and 60 were healthy controls. Total 60 cases of patients with COPD were divided into 30 cases of emphysema and 30 cases of chronic bronchitis.

Inclusion Criteria

Clinically and radiologically diagnosed cases of COPD were included. Healthy normal individuals without any history of smoking and chronic lung disease were included.

Exclusion Criteria

Patients with pneumonia, asthma, or other chronic respiratory disease, history of cardiac failure, any recent surgical intervention, diabetes mellitus, hepatic disease, and renal disease were excluded.

Estimation of Erythrocyte Reduced Glutathione

Method

Ernest Beutler et al. method.[7]

Principle

All of the nonprotein sulfhydryl groups of red blood cells are in the form of GSH. 5,5'-Dithiobis-2-nitrobenzoic acid (DTNB) is a disulfide compound, which is readily reduced by sulfhydryl compounds, forming a deep yellow-colored compound. Optical density (OD) that is measured at 412 nm is directly proportional to the GSH concentration.

Reagents

Phosphate solution, sodium citrate, precipitating solution, DTNB reagent, and GSH standard are the reagents used.

Calculation

Concentration of erythrocyte GSH = OD of test/OD of test control × 100 mg/dl

Estimation of Serum Superoxide Dismutase

Method

Marklund and Marklund method.[8]

Principle

Superoxide anion is involved in auto-oxidation of pyrogallol at alkaline pH (8.5). The SOD inhibits auto-oxidation of pyrogallol, which can be determined as an increase in absorbance of 420 nm.

Reagents

1. Tris-buffer, 0.05 M, pH 8.5 containing 1 mM EDTA
2. Pyrogallol (20 mM): 25 mg pyrogallol dissolved in 10 mL distilled water

Calculation

Serum SOD activity = $Control - Test / Control \times 50 \times 1000$ U/mL

Estimation of Serum Malondialdehyde

Method

Kei Satoh method.[9]

Principle

Auto-oxidation of unsaturated fatty acids involves the formation of semistable peroxides, which then undergo a series of reactions to form MDA. MDA reacts with thiobarbituric acid (TBA) to form pink-colored chromogen. The resulting chromogen is extracted with 4.0 mL of n-butyl alcohol and the absorbance of which is measured at 530 nm.

Reagents

A total of 20 g/dL trichloroacetic acid (TCA) in distilled water (20% TCA), sulfuric acid 0.05 M, sodium sulfate solution, TBA reagent (0.67%) were the reagents.

Calculation

Concentration of serum MDA (nmol/mL) = Absorbance of test (*A*) × 51.28 nmol/mL

Result

A total number of 120 subjects were included in this study. Among them, 60 were controls who were normal healthy individuals and 60 were COPD cases. Of the 60 controls, 35 were men and 25 were women and their mean age was 57.7 ± 7.4 years. Among the 60 COPD cases, 46 were men and 14 were women and their mean age was 62.3 ± 7.8 years. Based on clinical and radiological findings, CPOD cases were divided into two groups as chronic bronchitis and emphysema. Of the 60 cases, 30 cases had chronic bronchitis and 30 cases had emphysema.

Result shows that mean levels of erythrocyte GSH and SOD activity were significantly decreased ($p < 0.001$) and mean levels of serum MDA were significantly increased in COPD cases when compared with the healthy controls and were statistically highly significant ($p < 0.001$) [Table 1].

Statistical analysis by unpaired *t*-test shows that the patients with chronic bronchitis had significantly lower levels of erythrocyte GSH and SOD activity than the controls ($p < 0.001$) and significantly higher level of serum MDA than those of controls ($p < 0.001$) [Table 2].

Statistical analysis of unpaired *t*-test shows that patients with emphysema had significantly lower levels of erythrocyte GSH and SOD activity than the controls ($p < 0.001$) and significantly higher level of serum MDA than the controls ($p < 0.001$) [Table 3].

Statistical analysis of unpaired *t*-test shows that patients with emphysema had significantly lower levels of erythrocyte GSH and SOD activity ($p < 0.001$) and significantly higher level of serum MDA than the chronic bronchitis cases ($p < 0.001$) [Table 4].

It is evident from Table 5 that there is a positive correlation between GSH and SOD cases and it is highly significant. It is also evident from the table that there is a negative correlation between GSH VS sod, GHS Vs MDA, SOD Vs MDA in COPD cases and it is highly significant.

Discussion

Oxidative stress plays an important role in the pathogenesis of COPD. Oxidative stress is caused by an imbalance between the production of oxidants and the presence of antioxidants.[10] The aim of this study was to know the alterations in erythrocyte GSH, SOD activity, and MDA in patients with COPD and healthy controls.

Erythrocyte GSH functions as an efficient intracellular scavenger of H_2O_2 and plays an important role in the prevention of peroxidative lung damage in patients with COPD. In this study, when compared with controls, patients with COPD had significantly decreased ($p < 0.001$) level of GSH. This is in accordance with the study by Parija et al.,[11] Mercken et al.,[12] and Calikoglu et al.[13] GSH is significantly decreased ($p < 0.001$) in patients with emphysema when compared with patients with chronic bronchitis. This is in accordance with the study by Gea et al.[14]

Cigarette smoking is an important factor for the development of COPD. The airways of those who smoke are exposed to highly reactive components and the lung is always at the risk of oxidative injury. Under nonstress conditions, most of the intracellular glutathione is stored in the reduced form (GSH). During increased oxidative stress, the free sulfhydryl (-SH) groups become oxidized resulting in the loss of GSH. The gaseous phase of cigarette smoke may also irreversibly react with GSH to form GSH derivatives that cannot be reduced back, thereby depleting the total available GH pool.[15,16]

The activities of glutathione synthesis and redox system enzymes such as glutathione peroxidase, gamma glutamyl cysteine synthetase, and glucose-6-phosphate dehydrogenase were transiently decreased in alveolar epithelial cells after exposure to cigarette smoke condensate, possibly as a result of the action of highly electrophilic free radicals on the active site of enzymes. Thus, there is a time-dependent depletion of intracellular soluble GSH concomitant with the formation of GSH conjugates.[17]

SOD functions as a scavenger of superoxide radical in the body. The level of SOD is decreased in oxidative stress, which plays an important role in the pathogenesis of various diseases.[6]

In this study when compared with controls, patients with COPD have significantly ($p < 0.001$) decreased levels of SOD. This is in accordance with the studies by Rai and Phadke[6] and Kirkil et al.[18] SOD is significantly decreased ($p < 0.001$) in patients with emphysema when compared with patients with chronic bronchitis. This is in accordance with the study by Papaioannou et al.[19]

The alterations in antioxidant enzymes such as SOD emphasize the redox imbalance in patients with COPD. Mechanism involved in decreased serum SOD activity is due to increased production of free radicals in patients with COPD leading to increased consumption of antioxidant enzymes.

MDA is a lipid peroxidation product that is formed during oxidative process of PUFA (poly unsaturated fatty acid) by

Table 1: Comparison of erythrocyte GSH, SOD activity, and MDA in controls and in patients with COPD

		GSH (mg/dL)		SOD activity (U/mL)		MDA (nmol/mL)	
		Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD
Controls	60	25.00–38.02	33.55 ± 2.23	5.00–12.90	9.93 ± 1.73	1.53–3.58	2.62 ± 0.52
Cases	60	24.17–31.42	27.63 ± 1.96	2.30–7.05	4.78 ± 1.27	3.58–6.71	5.36 ± 0.74
Mean difference		5.92		5.15		2.74	
t-value		15.45		18.54		23.44	
p-value		<0.001, HS		<0.001, HS		<0.001, HS	

COPD, chronic obstructive pulmonary disease; GSH, reduced glutathione; MDA, malondialdehyde; SD, standard deviation; SOD, superoxide dismutase.

Table 2: Comparison of erythrocyte GSH, SOD activity, and MDA in controls and in patients with chronic bronchitis

Groups		GSH (mg/dL)	SOD activity (U/mL)	MDA (nmol/mL)
Controls	Mean ± SD	33.55 ± 2.23	9.93 ± 1.73	2.62 ± 0.52
	Range	25.00–38.02	5.00–12.90	1.53–3.58
Chronic bronchitis	Mean ± SD	28.97 ± 1.11	5.67 ± 0.81	4.83 ± 0.51
	Range	26.76–31.42	4.00–7.05	3.58–5.89
Controls vs. chronic bronchitis	Mean difference	4.58	4.26	2.21
	t	13.01	15.86	19.20
	p	<0.001	<0.001	<0.001

GSH, reduced glutathione; MDA, malondialdehyde; SD, standard deviation; SOD, superoxide dismutase.

Table 3: Comparison of erythrocyte GSH, SOD activity, and MDA in controls and in patients with emphysema

Groups		GSH (mg/dL)	SOD activity (U/mL)	MDA (nmol/mL)
Controls	Mean ± SD	33.55 ± 2.23	9.93 ± 1.73	2.62 ± 0.52
	Range	25.00–38.02	5.00–12.90	1.53–3.58
Emphysema	Mean ± SD	26.29 ± 1.70	3.90 ± 0.99	5.89 ± 0.52
	Range	24.17–30.98	2.30–7.00	4.42–6.71
Controls vs. emphysema	Mean difference	7.26	5.03	3.27
	t	17.17	20.91	28.11
	p	<0.001	<0.001	<0.001

GSH, reduced glutathione; MDA, malondialdehyde; SD, standard deviation; SOD, superoxide dismutase.

Table 4: Comparison of erythrocyte GSH, SOD activity, and MDA in chronic bronchitis and emphysema cases

COPD groups	No.	GSH (mg/dL)	SOD activity (U/mL)	MDA (nmol/mL)
		Mean ± SD	Mean ± SD	Mean ± SD
Chronic bronchitis	30	28.97 ± 1.11	5.67 ± 0.81	4.83 ± 0.51
Emphysema	30	26.29 ± 1.70	3.90 ± 0.99	5.89 ± 0.52
Mean difference		2.68 ↓	01.77 ↓	1.06 ↓
t-value		7.23	7.57	7.98
p-value		<0.001	<0.001	<0.001

COPD, chronic obstructive pulmonary disease; GSH, reduced glutathione; MDA, malondialdehyde; SD, standard deviation; SOD, superoxide dismutase.

Table 5: Relationship between various antioxidants in COPD cases

Correlation between	Pearson's correlation coefficient "*r*" value	Significant *p*-value
GSH and SOD	+0.66	<0.001
GSH and MDA	–0.68	<0.001
SOD and MDA	–0.68	<0.001

COPD, chronic obstructive pulmonary disease; GSH, reduced glutathione; MDA, malondialdehyde; SOD, superoxide dismutase.

reactive oxygen species. MDA is the sensitive marker of lipid peroxidation. Patients with COPD are subjected to enhanced oxidative stress and increased level of MDA. Lipid peroxidation products measured as MDA content correlated inversely with the time elapsed from the last exposure of cigarette smoke to the degree of small airway obstruction reflected by low maximal expiratory flow rates in those who smoke.[20]

In this study when compared with controls, patients with COPD have significantly ($p < 0.001$) increased level of MDA. Our findings suggest that high plasma MDA may be associated in patients with severe COPD.

Increased MDA concentrations in patients with COPD is due to the increased production of reactive oxygen species and hence, more lipoxidation products.[21] Increased MDA level in patients with emphysema indicates more oxidative stress when compared with patients with chronic bronchitis. This may be due to the fact that patients with emphysema have more severe lung function impairment, lower body mass index, poor quality of life, and more serious systemic dysfunctions.[22,23]

Rahman et al.[24] underlined that because of the variability of oxidative stress in those who smoke, it is unlikely that measurements of antioxidant capacity in plasma would correlate closely with the measurements of airway obstruction. However, there is generally less intraindividual variability in the activities of anti-oxidative enzymes in erythrocytes.[25]

Conclusion

This study demonstrates that there is an increased oxidative stress in patients with COPD when compared with controls and it is higher in patients with emphysema when compared with patients with chronic bronchitis. This study also emphasizes the decreased antioxidants namely erythrocyte GSH, and SOD activity in patients with COPD when compared with controls. Antioxidants are particularly decreased in patients with emphysema when compared with patients with chronic bronchitis. This study demonstrates that tobacco smoke induces oxidative stress in those who smoke, which results in COPD. Hence, by advising diet rich in antioxidants or supplementation of antioxidants may prevent the further oxidative damage in patients with COPD.

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