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Systemic oxidative stress and antioxidant status in Chronic Bronchitis patients

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Abstract

Background: Chronic Bronchitis is associated with high incidence of morbidity and mortality. The imbalance between oxidants and antioxidants is thought to play an important role in the pathogenesis of Chronic Bronchitis. **Methods & Results:** A total number of 60 subjects comprising of 30 healthy controls and 30 chronic bronchitis cases were studied. In all the subjects, serum levels of malondialdehyde (MDA) as a biomarker of lipid peroxidation and antioxidants like whole blood reduced glutathione (GSH), serum vitamin C, and superoxide dismutase (SOD) activity were estimated. The levels of whole blood reduced glutathione; serum vitamin C and SOD activity were significantly decreased in chronic bronchitis patients when compared to controls. Serum MDA was significantly increased in chronic bronchitis patients when compared to controls. **Conclusion:** The presence of increased systemic oxidative stress in chronic bronchitis patients seems to be associated with current active smoking and systemic inflammation. The decrease in antioxidant levels appears to be mainly a consequence of increased oxidative stress. This suggests that oxidative stress is likely to be involved in pathogenesis of chronic bronchitis.

Keywords: Oxidative stress, Antioxidants, Chronic bronchitis, Reduced glutathione, Vitamin C.

Introduction

Chronic Bronchitis is a chronic inflammation of the bronchi in the lungs. It is generally considered one of the two forms of Chronic Obstructive Pulmonary Disease (COPD). 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] American Thoracic Society defines Chronic Obstructive Pulmonary Disease as "A disease state characterized by the presence of airflow obstruction due to chronic bronchitis or emphysema; the airflow obstruction is generally progressive, may be accompanied by airway hyper-reactivity, and may be partially reversible".[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 in at least 2 consecutive years.[2] 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 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 has correlated inversely with pulmonary function.[4] Antioxidants depletion or deficiency 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 antioxidants such as reduced glutathione functions as an efficient intracellular scavenger of H_2O_2 and plays an important role in the prevention of peroxidative lung damage. Vitamin C is water soluble free radical scavenger, can directly scavenge O_2– and OH– radicals and helps to neutralize physiological oxidant burden created by both exogenous and endogenous sources.[6] The present study is undertaken to evaluate whole blood reduced glutathione, serum vitamin C, superoxide dismutase activity and malondialdehyde in controls and in chronic obstructive pulmonary patients.

Materials and Methods

A cross sectional study of whole blood reduced glutathione, serum vitamin C, superoxide dismutase activity and malondialdehyde in chronic bronchitis patients was carried out from April 2009 to April 2010 in a tertiary care hospital in South India. Controls and chronic bronchitis cases were selected from medicine outpatient department. The patients and controls voluntarily participated in the study. Informed consent was taken and the study was approved by the institutional ethics committee.

Inclusion criteria

i) Cases: 30 clinically and radiologically diagnosed cases of chronic bronchitis were included.

ii) Controls: 30 normal healthy individuals without any history of smoking and chronic lung disease were included.

Exclusion criteria

• Patients with pneumonia, asthma or other chronic respiratory disease

• Patients with history of cardiac failure

• Patients with history of any recent surgical intervention

• Patients with history of diabetes mellitus

• Patients with history of hepatic disease

• Patients with history of renal disease

Collection of blood samples

About 6 ml of blood was collected from a large peripheral vein under aseptic precaution after overnight fasting. Out of which 3ml was taken in an anticoagulant (EDTA) bulb for estimation of whole blood reduced glutathione (GSH), 3 ml in a plain bulb for estimation of serum vitamin C, superoxide dismutase (SOD) and malondialdehyde (MDA).

Estimation of Whole Blood Reduced Glutathione

Whole blood reduced glutathione was estimated by Ernest Beutler et al., Method.[7] It is based on the principle that all of the non-protein sulphydryl groups of red blood cells are in the form of reduced glutathione (GSH). 5,51 - dithiobis-2-nitrobenzoic acid (DTNB) is a disulphide compound, which is readily reduced by sulphydryl compounds, forming a highly colored yellow compound. The optical density of which is measured at 412nm and is directly proportional to the GSH concentration.

Estimation of Serum Vitamin C

Serum vitamin C was estimated by 2, 4 – dinitrophenyl hydrazine method.[8] This method based on the principle that ascorbic acid is oxidized by copper to form dehydroascorbic acid and diketogulonic acid. These products are treated with 2,4-dinitrophenyl hydrazine (DNPH) to form the derivative bis -2,4-dinitrophenyl hydrazone. This compound, in strong sulfuric acid, undergoes rearrangement to form a colored product which is measured at 520nm. The reaction is run in the presence of thiourea to provide a mildly reducing medium, which helps to prevent interference from non-ascorbic acid chromogen.

Estimation of Serum Superoxide Dismutase

Serum superoxide dismutase activity was estimated by Marklund and Marklund method. [9] This method is based on the principle that superoxide anion is involved in an auto-oxidation of pyrogallol at alkaline pH (8.5). The superoxide dismutase (SOD) inhibits auto -oxidation of pyrogallol, which can be determined as an increase in absorbance at 420 nm.

Estimation of Serum Malondialdehyde

Serum malondialdehyde estimated by Kei Satoh Method.[10] It is based on the principle of auto-oxidation of unsaturated fatty acids involves the formation of semistable peroxides, which then undergo a series of reactions to form malondialdehyde (MDA). MDA reacts with thiobarbituric acid (TBA) to form pink colored chromogen. The resulting chromogen is extracted with 4.0ml of n-butyl alcohol and the absorbance of which is measured at 530 nm.

Statistical Analysis

Results are expressed as mean ±SD and range values. The unpaired 't' test is used for comparing different biochemical parameters between cases and controls. The P value of < 0.05 was considered as statistical significance.

Results

A total number of 60 subjects were included in the study, of which 30 were chronic bronchitis cases and 30 were healthy controls. Among 30 controls, 20 were male and 10 were female and their mean age was 57.7 ± 7.4 years and among 30 chronic bronchitis cases, 23 were male and 7 were female and their mean age was 62.3 ± 7.8 years. There were no significant differences in age between cases and controls.

Table 1: Comparison of Whole Blood Reduced Glutathione, Serum Vitamin C, SOD activity and Malondialdehyde in Chronic Bronchitis patients and healthy controls

Groups		**GSH (mg/dl)**	**Vit. C (mg/dl)**	**SOD activity (U/ml)**	**MDA (nmol/ml)**
Controls	Mean ± SD	33.55 ± 2.23	1.10 ±0.16	9.93 ± 1.73	2.62 ± 0.52
	Range	25.0038.02	0.82-1.40	5.00-12.90	1.53-3.58
Chronic bronchitis	Mean ± SD	28.97 ±1.11	0.65 ±0.09	5.67 ± 0.81	4.83 ± 0.51
	Range	26.76 31.42	0.47 – 0.81	4.00 – 7.05	3.58 – 5.89
Controls Vs Chronic bronchitis	Mean difference	4.58	0.45	4.26	2.21
	t*	13.01	17.67	15.86	19.20
	P	< 0.001	< 0.001	< 0.001	< 0.001

* Unpaired t- test, P value < 0.001, highly significant

Table 1 shows biochemical characteristics of the study subjects. The mean level of whole blood reduced glutathione (28.97 ±1.11 mg/dl), serum vitamin C (0.65±0.09 mg/dl) and SOD activity (5.67±0.81 U/ml) were significantly decreased in chronic bronchitis patients than in controls (33.55 ±2.23 mg/dl, 1.10 ±0.16 mg / dl, 9.93±2.23 respectively) and were statistically significant (P<0.001). Serum mean level of Malondialdehyde (4.83±0.51 nmol/ml) a biomarker of lipid peroxidation was significantly increased in chronic bronchitis patients when compared to controls (2.62 ±0.52 nmol/ml) and was statistically significant (P<0.001). These results indicate that an increase in oxidative stress and decrease in antioxidant levels in chronic bronchitis patients when compared to controls.

Discussion

Oxidative stress plays an important role in the pathogenesis of chronic bronchitis.[11] These results indicate that there is an increase in oxidative stress and decrease in antioxidant levels in chronic bronchitis patients when compared to controls. When compared to controls, chronic bronchitis patients have significantly decreased (P value < 0.001) level of GSH. This is in accordance with the study of Madhuri Parija *et al*[12] Mercken EM *et al*[13] and Mukadder calikoglu *et al*[14].

Cigarette smoking is the most important factor for the development of chronic bronchitis. Under non stress 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 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 GSH pool.[15]

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 (CSC), 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.[16]

When compared to controls chronic bronchitis patients have significantly (P value <0.001) decreased levels of vitamin C. This is in accordance with studies of Raghunath R.Rai *et al*[6], L.A. Sargeant *et al*[17] and Mukadder calikoglu *et al*[14]. Vitamin C functions as an important free radical scavenger. The mechanism involved in the reduction of vitamin C level in chronic bronchitis is due to rapid oxidation of ascorbic acid by free radicals. The negative relationship between vitamin C and MDA may be due to the depletion of vitamin C when the oxidant burden is increased.[18]

Vitamin C functions as an antioxidant by donating its electron, it prevents other compounds from being oxidized, however, by the very nature of this reaction vitamin C itself is oxidized in the process. The species formed after the loss of one electron is a free radical is ascorbyl radical. As compared to other free radicals ascorbyl radical is relatively stable with half life of 10-5 seconds and is fairly unreactive which explains the antioxidant nature of vitamin C and its preference. Reduction of a reactive free radical with the formation of a less reactive compound is sometimes called free radical scavenging or quenching.[19]

Superoxide dismutase functions as a scavenger of super oxide radical in the body. The level of SOD decreases in oxidative stress, which plays an important role in the pathogenesis of various diseases.[6] When compared to controls, chronic bronchitis patients have significantly (P value < 0.001) decreased level of SOD. This is in accordance with studies of Raghunath R Rai *et al*[6] and Gamze kirkil *et al*[20]. The alterations in antioxidant enzymes such as SOD emphasize the redox imbalance in chronic bronchitis patients. The mechanism involved in a decreased serum SOD activity is due to increased production of free radicals in chronic bronchitis patients, leading to increased consumption of antioxidant enzymes.[20]

MDA is a lipid peroxidation product which is formed during the oxidative process of PUFA by reactive oxygen species. MDA is the sensitive marker of lipid peroxidation. Chronic bronchitis patients are subjected to enhanced oxidative stress and increased level of MDA. When compared to controls, chronic bronchitis patients have significantly (P value < 0.001) increased level of MDA. This is in accordance with the study of M.K. Daga *et al*[21], and Gamze kirkil *et al*[20].

Oxidative stress has been implicated in the pathogenesis of tobacco smoke induced chronic obstructive pulmonary disease. Reactive oxygen species present in the tobacco smoke may cause damage to human alveolar epithelial cells by lipid peroxidation of cell membranes. Increased MDA concentration in patients with Chronic bronchitis is due to increased production of reactive oxygen species and hence more lipoxidation products.[21]

Conclusion

The present study demonstrates that there is increased oxidative stress in patients with chronic bronchitis when compared to controls and also decreased levels of antioxidants namely whole blood reduced glutathione, serum vitamin C and SOD activity in chronic bronchitis patients when compared to controls. This study demonstrates the role of oxidative stress and antioxidant imbalance in pathogenesis of chronic bronchitis. By advising diet rich in antioxidants or supplementation of antioxidants may prevent the further oxidative damage in chronic bronchitis patients.

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Conflict of interest

None declared.

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