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STUDY OF LIPID PEROXIDATION AND SERUM ASCORBIC ACID LEVELS AS INDICES OF OXIDATIVE STRESS IN VARIOUS LUNG DISORDERS

Ivvala Anand Shaker[1*], Suresh Babu Kondaveeti[1], Hemraz Palwan[2], Dabburu Kumaraswamy[3], Raja G[1], S Saleem Basha[1]
[1]Dept of Biochemistry, Melmaruvathur Adhiparasakthi Institute of Medical Science & Research, Melmaruvathur, Tamil Nadu, India
[2]Ottawa Fertility Centre, 66 – 890, Cahill Dr (W), Ottawa, ON K1V 9A4, Ottawa, Canada
[3]Departments of Pharmacology, SRM Medical College, Kattankalathur-602239, Tamilnadu

***Corresponding Author**: **Dr. Ivvala Anand Shaker**, Dept of Biochemistry, Melmaruvathur Adhiparasakthi Institute of Medical Science & Research, Melmaruvathur, Tamil Nadu, India

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Abstract: The present study was planned to assess the levels of oxidant and antioxidant in various lung disorders and to evaluate the existence of correlation between oxidant-antioxidant levels. Asthma, Bronchitis, COPD and Lung fibrosis well know chronic inflammatory disorders where disturbances in the oxidative system have been observed. To investigate the lipidperoxidation in terms of plasma MDA and antioxidant capacity in terms of ascorbic acid (Vitamin C) were measured. Results revealed statistically significantly Increase serum MDA ($P<0.001$) and decrease vitamin C ($P<0.001$) in patients suggestive of oxidative imbalance in various lung disorders was revealed. However there is negative correlation between lipidperoxidation and ascorbic acid levels correlation. The study thus supported the emerging concept of free radical injury in various lung disorders and therefore a thought can be given to whether antioxidant supply could have a beneficial impact on the free radical induced injury and improvement of respiratory reserve in various lung disorders.

Keywords: Asthma, Bronchitis, COPD, Lung fibrosis, Oxidants, Antioxidants, MDA, Ascorbic acid.

INTRODUCTION

Large segments of population in both rural and urban area are affected with lung disorders. In all forms of terminal diseases the lungs are secondarily involved. Some degrees of pulmonary disorders are found at every autopsy. Our study mainly focused primarily on diseases affecting important organs at cellular level.

Among respiratory disorders the majority of the diseases were Acute and Chronic respiratory diseases, Bronchial asthma, chronic obstructive pulmonary disease (COPD) and tumors of the bronchus and lungs. In addition to the traditional aspects of diagnosis such as imaging and other techniques, the study of cellular and molecular changes in the organ is mainstay of recent work.

Asthma is an airway disease characterized by increase responsiveness of the trachea-broncheal tree to a multiplicity of stimuli. It is an episodic disease, acute exacerbation being inter spread with symptom free periods. It is manifested physiologically by narrowing the air passages which may be relieved spontaneously or as a result of therapy and clinically by paronysms of dyspnoea, cough and wheezing [1].

Chronic obstructive pulmonary disease (COPD) includes chronic bronchitis, obstructive bronchitis and pulmonary emphysema. There is no longer any doubt that cigarette smoking is a major risk factor for chronic bronchitis, emphysema, COPD, cancer and cardiovascular disease [2]. One of the ways in which cigarette smoke damages the body is by profoundly raising the extent of oxidative stress in the lungs. A single puff of cigarette smoke contains billions of free radicals and can literally "burn up" antioxidants [3]. A still greater source of oxidative stress results from the lung inflammation resulting from smoking. The total oxidative damage caused by smoke corresponds directly to the degree of lung injury, respiratory compromise, morbidity and mortality found in individual patients [4].

The disorder is associated with hyperplasia and hypertrophy of the mucus producing glands found in sub mucosa of large cartilaginous airways [5].

Bronchitis is a condition associated with excessive tracheobronchial mucus production sufficient to cause cough with expectoration [6]. Lung fibrosis is an inherited multisystem disorder characterized by an abnormality in exocrine gland function. Nearly all patients develop chronic progressive disease of the respiratory system. Pulmonary disease is the common cause of the death and morbidity in patients with cystic fibrosis [7].

For many years, the science of free radicals was the preserve of physical and inorganic chemists. However, when it came to the impact of free radicals in biology, many at first did not foresee the impact it could have on disease pathology. It is only relatively recently, in the last 20 years, that free radicals in the form of reactive oxygen species (ROS) have become increasingly recognized as playing a major role in many disease processes. ROS such as superoxide anion ($O_2^{\cdot}$) and the hydroxyl radical ($OH^{\cdot}$) are unstable molecules with unpaired electrons, capable of initiating oxidation. This can result in the oxidation of proteins, DNA and lipids that may cause direct tissue injury or induce a variety of cellular responses, through the generation of secondary metabolic Reactive species. The lung exists in a high-oxygen environment and together with its large surface area and blood supply is highly susceptible to injury mediated by oxidative stress. Consequently, the lung contains many antioxidant defenses in order to protect itself from oxidant-induced tissue damage. ROS can be generated either endogenously by metabolic reactions, such as from mitochondrial electron transport during respiration or during activation of circulating inflammatory cells or phagocytes, and exogenously from air pollutants or cigarette smoke. As a result, increased levels of ROS have been shown to affect the extracellular environment impacting on a variety of physiological processes [8].

In addition, ROS can initiate inflammatory responses in the lungs through the activation of redox-sensitive transcription factors [9]. It is proposed that ROS produced by phagocytes that have been recruited to sites of inflammation is a major cause of the cell and tissue damage associated with many chronic inflammatory lung diseases including asthma and chronic obstructive pulmonary disease (COPD) [10].

However, the composition of inflammatory cell types varies widely in asthma and COPD and this could account for the differences in ROS production as well as the patho-physiology between these diseases [11].

This paper discusses the impact of ROS and its role in the pathogenesis of various lung disorders. Moreover, it also highlights the antioxidant mechanisms in place to protect against the damaging effects of ROS.

MATERIALS AND METHODS

Selection of Study Participants:

A cross sectional study of serum vitamin C and Malondialdehyde as one of the marker for lipid peroxidation in various lung disorder

patients and Controls were carried out in Department of Biochemistry, B.J. Medical College & Sassoon General Hospitals, Pune. Informed consent was obtained and this study was approved by the ethical and research committee. A total number of 110 subjects were included in the study, of which 20 cases of disorders i.e Asthma bronchitis, COPD and lung fibrosis were taken including 30 healthy controls.

Inclusion criteria:

i) **Cases:** Clinically and radiologically diagnosed cases were included. Total 80 cases of lung disorder patients were divided into 20 cases of Asthma, 20 cases of bronchitis, 20 cases of COPD and 20 cases of lung fibrosis.

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, history of cardiac failure, recent surgical intervention, diabetes mellitus, hepatic disease and renal diseases were excluded from the study.

Collection of blood samples:

About 5ml of blood was collected from large peripheral vein under aseptic precaution after overnight fasting in a plain bulb for estimation of serum vitamin C and Malondialdehyde (MDA).

Estimation of Serum Vitamin C:

Serum vitamin C was estimated by 2, 4-dinitrophenyl hydrazine method [12]. This method based on the principle that ascorbic acid is oxidized by copper to form dehydro ascorbic 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 Malondialdehyde:

Malondialdehyde levels were estimated by the double-heating method of Wasowitz [13]. MDA, an end product of fatty acid peroxidation, reacts with thiobarbituric acid (TBA) to form a colored complex. The principle of the method is the spectrophotometric measurement of the color generated by the reaction of TBA with MDA. The concentration of MDA was calculated by the absorbance coefficient of the MDA–TBA complex (absorbance coefficient e = 1.56 × 105 L/mol per cm)

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 value of < 0.05 was considered as statistical significance.

RESULTS

Among 30 controls, 15 were male and 15 were female whose mean age was 52.7 ± 4.4 years and among 80 lung disorder cases, 46 were male and 14 were female and their mean age was 60.3 ± 5.8 years. There were no significant differences in age among cases and controls

Table 1: Comparison of Serum Vitamin C, and Malondialdehyde in Controls and lung disorder Cases at the time of admission

Group	No of cases	MDA µmoles/ml (Mean ± SD)	Vitamin C mg% (Mean ± SD)
Control	30	3.36±.0.42	1.39±0.34
Asthmatic	20	10.11±0.73*	0.54±.0.21*
Bronchitis	20	7.14±0.81*	0.58±0.33*
COPD	20	9.38±0.43*	0.69±0.18*
Lung fibrosis	20	6.67±0.26*	0.41±0.27*

* P < 0.001 highly significant when compared with control subjects

Table no.1 shows comparative analysis of serum vitamin C and MDA levels between controls and various lung disorder cases. Statistical analysis by unpaired t-test shows that mean levels of serum vitamin C levels were significantly decreased (p < 0.001) and mean level of serum MDA was significantly increased in various lung disorder cases when compared to healthy controls and are statistically highly significant (p < 0.001).

The mean values of serum vitamin C, and MDA in controls were in the range of 1.39±0.34 mg/dl and 3.36±0.42 µmol/ml respectively. In Asthma, bronchitis, COPD and lung fibrosis cases they were in the range of 0.56±0.12 mg/dl, and 5.36±0.74 µmol/ml respectively. These results indicate that increase in oxidative stress and decrease in antioxidant levels in various lung disorder cases when compared to controls.

Serum Vitamin C:

The mean value of serum vitamin C is 1.39 ±0.34 mg/dl in controls and 0.56±0.12 mg/dl various lung disorder cases. When compared to controls various lung disorder patients have significantly (p value < 0.001) decreased level of vitamin C. The mean value of serum vitamin C is 0.54±_0.21mg / dl in Asthma patients, 0.58±0.33 mg/dl in bronchitis patients, 0.69±0.18 in COPD patients and 0.41±0.27 in lung fibrosis patients. Vitamin C is much decreased (p value < 0.001) in lung fibrosis patients when compared to asthma, bronchitis and COPD patients.

Serum Malondialdehyde:

The mean value of serum MDA is 3.36±_0.42µmol/ml in controls 10.11±0.73µmol/ml in Asthma patient's 7.14±0.81 µmol/ml in bronchitis patients, 9.38±0.43 µmol/ml in COPD patients and 6.67±0.26µmol/ml in lung fibrosis patients.

MDA level is much elevated (p value < 0.001) in Asthmatic patients when compared to bronchitis, COPD and lung fibrosis patients.

DISCUSSION

Respiratory disorders are multi factorial in aetiology and origin. Reactive oxygen species (ROS) have been closely associated with a number of pathological diseases [14]. The aim of the present study was to measure serum MDA levels, which is a marker of lipid peroxidation eventually sequelae of the injury caused by ROS. Vitamin C levels are also measured to study the antioxidant status and both are correlated to establish the relationship between them, if any.

The potential danger of the oxygen free radical is an increased lipid peroxidation of lung tissue causing alveolo capillary destruction and extension exudation with derangement of lung function [15].

In the present study, lipid peroxide levels were found to be very significantly increased in the patients with asthma (10.11±0.73µmoles/ml) bronchitis (7.14±0.81 µmoles/ml) COPD (9.38±0.43µmoles/ml) and lung fibrosis (6.67±0.26µmoles/ml). Plasma level of ascorbic acid decreased in lung disorders [16]. In the present study the levels of ascorbic acid in plasma were found to be significantly decreased in the patients with Asthma (0.54±0.21mg %), bronchitis (0.58±0.33mg %), COPD (0.69±0.18mg %) and lung fibrosis (0.41±0.27mg %).

Lung injuries induced by inhalation of mineral dust eg. Asbestos, quartz, silica, at least partly be mediated by free radicals. These agents can affect epithelial, mesothelial and fibroblastic cells. Occupational exposure to asbestos has been associated with increased risk of pulmonary fibrosis (asbestosis) mesthelioma and bronchogenic carcinoma [17]. Inhaled silica particles and asbestos fibres can be phagocytosed by pulmonary macrophages. These cells can then rupture releasing proteolytic enzymes and chemotactic mediators causing infiltration of other cells such as neutrophils, thus initiating an inflammatory process, leading to an increased generation of ROS [18]. The condition leading to fibrotic lung

disorders these disorders characterized by injury to the alveolar region with accumulation of alveolar macrophages and neutrophils, parenchymal cell injury and fibrosis of the alveolar walls. This is a chronic process generally involving the entire respiratory part of lung. Experimental data suggest that exaggerated release of oxidants by alveolar macrophages and neutrophils play a major role in injury to the epithelium and endothelium [18].

When neutrophils or monocytes are exposed to the phagocytic or chemotactic stimulus they experience a respiratory burst characterized by an increase in oxygen concentration and activation of the hexose monophosphate shunt [19, 20, 21].

It is postulated that most of the lung disorders are characterized by injury, mainly to the alveolar region with the accumulation of macrophages and neutrophils parenchymal cell injury and fibrosis of the alveolar walls.

Ascorbic acid is an aqueous phase antioxidant. It quenches the superoxide peroxyl and hydroxyl radicals by electron transfer reaction ascorbic acid function as an electron sink as it donates its electron to the free radical species thereby converting it to the less harmful forms thus prevents the chain reactions of lipid peroxidation Ascorbic acid as a scavenger of oxidants derived from polymorphonuclear leukocytes may have clinical significance in antioxidant prevention of lung tissue injury

In the present study we found a highly significant correlation between MDA concentration and ascorbic acid level initiating increased lipid peroxidation and decreased ascorbic acid levels which will severely alter cell membrane and intracellular organelles function in the lung with the degree of oxidant injury being dependent on the degree of oxidant activity and the status of endogenous antioxidant defenses.

CONCLUSION

A statistically significant negative correlation between serum MDA and serum ascorbic acid levels in the various lung disorders has also been established in this study.

Therefore it can be concluded that in various lung disorders there is a significant increase in lipid peroxidation accompanied by a significant decrease in antioxidant mechanism and that inverse relationship between them has been found in this study.

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