PLASMA OXIDANT-ANTIOXIDANT STATUS IN DIFFERENT RESPIRATORY DISORDERS

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ABSTRACT

This study confirms the fact that in different respiratory disorders, the status of plasma oxidants and antioxidants shifts from normal. The status of oxidants in plasma as represented by malondialdehyde (MDA) levels increased significantly in the conditions of chronic obstructive pulmonary disease (COPD), emphysema, bronchiectasis and bronchial asthma. The two vitamin antioxidants vitamin C and vitamin E showed decreased levels than in controls. In patients with COPD the endogenous antioxidant viz. reduced glutathione (GSH) estimated from whole blood was comparable to that of control group, whereas in patients with emphysema, bronchiectasis and bronchial asthma, GSH concentration was increased to that of control group. The activity of enzyme superoxide dismutase (SOD) was significantly decreased in all study groups. Pulmonary function tests were found to have no correlation with MDA and antioxidants

KEY WORDS

Vitamin antioxidants, Endogenous antioxidants, Respiratory disorders, MDA

INTRODUCTION

Recent research suggests that oxygen and its related species (oxidants) may contribute to the pathogenesis of a number of important lung diseases. The lung exists in a high-oxygen environment and, together with its large surface area and blood supply, is susceptible to injury mediated by these oxidants. Oxidants or free radicals are atoms or molecules capable of independent existence that contain one or more unpaired electrons, making these species highly reactive (1). Oxidant-mediated injury or oxidant stress may occur through the reactions of these radicals and also those of nonradicals. Exogenous sources of oxidants include cigarette smoke, toxic gases, and ozone, while the main source of endogenous oxidants is inflammatory cells (1,2). These agents may cause direct tissue oxidation, release of endogenous oxidants, and inactivation of antioxidant defense mechanisms, which interact with other protective agents, such as antiprotease.

The potential of oxidants to damage pulmonary tissue is dependent on the local antioxidant defense mechanisms. Antioxidants are physiologic substances that are derived from

both endogenous and exogenous sources and that act against oxidant stress. Antioxidants may delay or prevent direct oxidation of oxidizable substrates or scavenge oxidant free radicals and thus neutralize the physiologic oxidant burden created by both exogenous and endogenous free radicals (1). Various cells of the respiratory tract, including macrophages and epithelial cells, produce antioxidant enzymes such as superoxide dismutase, and catalase (3). The dominant antioxidant in the epithelial lining fluid of the lung is glutathione (3). (Poly) aromatic substances; which are obtained mainly from the diet, include vitamins and their precursors such as vitamin A (beta carotene), vitamin E (α -tocopherol), and vitamin C (ascorbic acid). It is believed that in order to maintain normal lung function, there must be a balance between toxic oxidants and protective antioxidants. Further research suggests that oxidant stress may contribute to the pathogenesis of many different lung disorders, including asthma, COPD, cystic fibrosis (CF), pulmonary fibrosis, and pneumoconiosis, lung cancer, and the acute respiratory distress syndrome (ARDS) (4).

The aim of the present study was, to study the plasma oxidant and antioxidant status in patients with COPD, emphysema, bronchiectasis and bronchial asthma and values were compared with normal healthy controls..

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MATERIALS AND METHODS

Hundred and elevens patients included for the study were

from respiratory OPD of Lokmanya Tilak Municipal Medical College and General Hospital, Sion, Mumbai. These patients were sub grouped depending upon the diagnosis as : 24 COPD (Age range:35 to 65 years), 20 Emphysema (Age range:35 to 72 years), 32 Bronchiectasis (Age range:20 to 65 years) and 35 Bronchial asthma (Age range:18 to 55 years). The control group included 50 (Age range:22 to 55 years) healthy, non-smokers without any history of lung disease and had normal pulmonary function tests.

Fasting venous blood samples were collected for the study of various parameters and taken in EDTA vial, in plain vial (without anticoagulant) and in heparinized vials. Samples were used for the estimations of plasma vitamin C, Plasma vitamin E, whole blood reduced glutathione, red cell SOD activity and Serum MDA.

Plasma vitamin C was estimated by 2,6 dichlorophenolindophenol titration method (5). 2,6-Dichlorophenolindophenol is red in acid solution and on titration with a solution of ascorbic acid is reduced to the colourless leucobase, the ascorbic acid being oxidized to dehydroascorbic acid.

Plasma vitamin E was estimated by using the Emmeric-Engel reaction (6). This method is based on the reduction by tocopherols of ferric to ferrous ions, which then form a red complex with α,α' -dipyridyl. Tocopherols and carotenes are then extracted into xylene and the extinction read to 460nm to measure the carotenes. A correction is made for these after adding ferric chloride and reading at 520nm.

The amount of GSH in the whole blood was estimated by the method of Beutler, Duron and Kelly (7). The method is based upon the development of a relatively stable yellow colour when 5,5'-dithiobis-(2-nitrobenzoic acid) is added to sulphhydryl compounds.

The assay of SOD was done according to the procedure of C.C.Winterbourn et al (8). This method is based on the method of Beauchamp and Fridovich and depends on the ability of the enzyme to inhibit the reduction of NBT by superoxide, which is generated by the reaction of photoreduced riboflavin and oxygen. Results are expressed as units of superoxide dismutase per gram of hemoglobin and 1 unit is defined for a particular system as that amount of enzyme causing half the maximum inhibition of NBT reduction.

Serum MDA was estimated in terms of thiobarbituric acid reactive species (TBARS) by the method of M.Sasikala et al

(9). The thiobarbituric acid reaction is used to measure serum MDA. In this test, the chromogen is formed by the reaction of one molecule of MDA with two molecules of TBA. The method involves heating the sample (serum) with trichloroacetic acid and thiobarbituric acid under acidic conditions. To increase sensitivity, the MDA-TBA adduct formed is extracted into an organic solvent (butanol) and reading the absorbance of the MDA-TBA adduct at 532 nm.

Pulmonary function tests were performed in all patients and in control subjects. Forced expiratory volume in one second (FEV_1) and forced vital capacity (FVC) were measured with Ocean Win Spiro Software. FEV_1 and FVC were expressed as a percentage of the predicted values for age, sex, and height.

Statistical analysis was carried out using unpaired 't' test. P value less than 0.05 ($P < 0.05$) was considered as significant.

RESULTS AND DISCUSSION

Results are summarised in Table 1. Oxidants-antioxidants balance is essential for the normal lung function. Both, an increased oxidant and/or decreased antioxidant may reverse the physiologic oxidant-antioxidant balance in favor of oxidants, leading to lung injury. A number of diseases involving the lung, such as COPD, emphysema, bronchiectasis and bronchial asthma have been associated with a disturbance of these balances (1). Vitamin E is a lipid soluble chain breaking antioxidant (10). It converts $O_2^{\cdot-}$, $^{\cdot}OH$ and lipid peroxy radicals to less reactive forms. While vitamin C is a water soluble free radical scavenger. Vitamin C can directly scavenge $O_2^{\cdot-}$ and $^{\cdot}OH$ with the formation of semidehydroascorbate-free radical that is subsequently reduced by GSH to generate dehydroascorbate and GS and it can also neutralize several oxidants present in the blood of smokers (11). In the present study, it was found that concentration of vitamin C significantly decrease in patients with COPD, emphysema and bronchiectasis, but was comparable in patients with Bronchial asthma to that of control group. Concentration of vitamin E was significantly decreased in all study groups as compared to that of control group. Similarly, Zhou et al. reported decreased plasma vitamin C and plasma vitamin E in smokers (17).

The reduced form of glutathione functions as an efficient intracellular and extracellular antioxidant by scavenging H_2O_2 (10). Cells are protected by extracellular GSH from oxidants produced and released by inflammatory cells, and by intracellular GSH from oxidants generated in normal biochemical processes, as well as from xenobiotics (1). In the present study, in patients with COPD, GSH concentration was

Table 1
Comparisons of Vitamin C, Vitamin E, GSH, SOD and MDA between study and control groups.

Study Groups		Control n=50	COPD n=24	Emphysema n=20	Bronchiectasis n=32	Bronchial Asthma n=35
Plasma Vitamin C mg/dl	Range	0.70-1.23	0.64-1.0	0.73-1.14	0.44-1.33	0.76-1.45
	Mean	1.00	0.85	0.87	0.83	0.94
	±SD	0.146	0.126	0.102	0.17	0.175
	p Value		p<0.001	p<0.001	p<0.001	NS
Plasma Vitamin E mg/dl	Range	0.75-1.9	0.4-1.75	0.6-1.5	0.53-1.75	0.5-1.7
	Mean	1.29	0.98	1.034	0.99	1.05
	±SD	0.308	0.346	0.237	0.315	0.337
	p Value		p<0.001	p<0.001	p<0.001	p<0.01
Whole blood Reduced glutathione mg/dl	Range	21.7-45.2	13.6-39.2	20.2-45.9	27.0-50.8	20.4-48.9
	Mean	30.6	30.2	33.6	35.7	33.4
	±SD	5.75	5.39	6.92	6.08	7.17
	p Value		NS	NS	p<0.001	NS
Superoxide dismutase (SOD) units	Range	1316-3125	1316-2632	962-2941	685-2778	980-2941
	Mean	2053.6	1775.1	1491.4	1585.4	1692.8
	±SD	517.12	355.84	459.05	530.86	600.64
	p Value		p<0.01	p<0.001	p<0.001	p<0.01
Serum Malondialdehyde (MDA) µmol/L	Range	0.16-0.64	0.37-1.11	0.31-1.04	0.35-0.96	0.40-1.14
	Mean	0.451	0.658	0.634	0.617	0.596
	±SD	0.111	0.234	0.238	0.190	0.197
	p Value		p<0.001	p<0.01	p<0.001	p<0.001

Values expressed as mean ± SD; NS= Not significant

comparable to that of control group, whereas in patients with emphysema, bronchiectasis and bronchial asthma, GSH concentration was increased than that of control group.

Toth and colleagues (1986) stated that among the red cell antioxidants involved in detoxification of hydrogen peroxide (H_2O_2), catalase and glutathione play prominent role. Greater amounts of these two substances were found in erythrocytes of smokers than in those of non-smokers, presumably because the erythrocytes of smokers are constantly exposed to oxidative stress and undergo induction of protein synthesis. These antioxidant-enhanced erythrocytes protect endothelial cells in culture from H_2O_2 more effectively than do those from SOD is thought to play a role in protecting living cells against toxic oxygen derivatives. The enzyme catalyzes the dismutation of two superoxide radicals ($O_2^{\cdot-}$) into O_2 and H_2O_2 (14). Toxic oxygen free radicals have been implicated as important pathological mediators in many clinical disorders (15). Enhancing the intracellular content of antioxidant enzymes (superoxide dismutase, glutathione peroxidase, and catalase) can provide means of limiting biological damage caused by oxygen free radicals. The oxygen free radicals and

changes of antioxidant enzymes are thought to play a role in pathogenesis of COPD (15). In the present study, in patients with COPD, emphysema, bronchiectasis and bronchial asthma, activity of enzyme SOD was significantly decreased than that of control group. Similarly, Zhao et al. reported decrease SOD in the blood of smokers. This indicates increase of oxidation and decrease of antioxidation (20). Tekin et al. (18) and Filip et al. (19) also reported decrease SOD activity in blood of asthma patients.

MDA is a representative of thiobarbituric acid reacting substances (TBARS). The amount of MDA is a measure of lipid peroxidation and its measure provides an estimate of free radical activity (16). In the present study MDA was found to be having statistically significantly higher values in all the study groups as compared to control group. Similarly, Nadeem et al. reported increased lipid peroxidation products in patients with bronchial asthma (21). Lee also reported increased serum and red blood cell thiobarbituric acid reactants in COPD patients (15).

In the present study we estimated pulmonary function

parameters of patients and control group. Correlation results showed that significant correlation ($r = 0.55$, $P < 0.05$) between oxidant, antioxidants concentration and pulmonary function tests was found only in some study groups. Altuntas et al. investigated the oxidant and antioxidant imbalance in smokers and in the pathogenesis of COPD. They also tried to find the relationship of imbalance with lung function tests. No significant correlation were seen between FEV_1 (predicted %), FEV_1/FVC (predicted %) and erythrocyte catalase, GSH and plasma MDA in all the three groups (22). Thus our study confirmed, the presence of an oxidant-antioxidant imbalance in patients with COPD, emphysema, bronchiectasis and bronchial asthma, supporting the concept of systemic oxidative stress in these conditions.

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