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STUDY OF LIPID PEROXIDATION PRODUCT, SULPHYDRYL PROTEINS (-SH) AND ANTIOXIDANT STATUS IN SMOKERS

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ABSTRACT

Lipid peroxide plays an important role in smokers. Increased epithelial permeability produced by cigarette smoke is likely to be mediated through depletion of sulphydryl proteins (-SH) and antioxidant capacity. The oxidant burden in the lungs is enhanced in smokers by the release of ROS from macrophages and neutrophils. Oxidants present in cigarette smoke can stimulate alveolar macrophages to produce ROS some of which attack neutrophils and other inflammatory cells into lungs. 60 smokers with smoking history 20 pkts /year were included in the study.100 healthy non-smokers' were served as controls. Their base line clinical examination, malondialdehyde (MDA), sulphydryl proteins (-SH), superoxide dismutase (SOD) and total antioxidant capacity (TAC) were measured. The mean malondialdehyde levels in the patients at base line were high (P< 0.001) than Controls. The sulphydryl proteins, superoxide dismutase and total antioxidant capacity were low (P<0.001) in the smokers compared to controls. We found decreased levels of sulphydryl proteins and increased levels of lipid peroxide (MDA) in smokers. Thus the present study confirmed the existence of oxidative stress and alteration in enzymatic and nonenzymatic antioxidant (-SH Proteins) status.

Keywords: Sulphydryl proteins, Malondialdehyde, Antioxidant status, Superoxide Dismutase, Smokers', Reactive Oxygen Species (ROS).

INTRODUCTION

The lungs are a pair of sponge like organs in the chest which are primarily responsible for the exchange of oxygen and carbon dioxide between the air we breathe and the blood. [(1).] It is a unique organ in terms of its direct exposure to high levels of oxygen and reactive compounds [(2)] Inhaled ozone induces toxic processes that impair lung function. The oxidant burden in the lungs is enhanced in smokers' by the release of ROS from macrophages and neutophiles. Oxidants present in cigarette smoke can stimulate alveolar macrophages to produce reactive oxygen species (ROS). Some are weakly attack neutophiles and other inflammatory cells into the lungs [(3)] A number of abnormalities and measurement of biomarkers have suggested that increased oxidative stress is occurring and is detrimental in cigarette smokers'. The most convincing way to determine the involvement of oxidative stress in smokers' is to directly measure oxygen radicals. However direct measurement is difficult. The alternative has been to measure damage by oxygen radicals upon various lung biomolecules usually lipids [(4)]. Lipid peroxidation can be defined as a chain of reaction of oxidative deterioration of polyunsaturated fatty acids, initiated by free radicals[(5)]. Exposure to cigarette smoke has been

shown to increase the oxidative metabolism of macrophages and appear to be more prevalent in he lungs of smokers' and are responsible for the increased O_2^- production by smokers' macrophages. Sulphydryl proteins (-SH) and redox states of intracellular and extracellular compartments is critical in the determination of protein structure, regulation of enzyme activity and control of transcription factor activity and binding of-SH / disulfide redox buffer as metal chelators, as radical quencher and as specific reductants of individual protein disulfate's bonds. Thus the increased level of –SH proteins have been associated with increased tolerance to oxidant stresses in all these systems. Sulphydryl proteins (-SH) groups are essential in the protection against the deleterious effect of reactive species [(6)] Cigarette smoking increases oxidation of plasma proteins. Exposure to gas phase cigarette smoke caused increased, lipidperoxidation. It is likely that α - ß unsaturated aldehyde, which are abundantly present in cigarette smoke, may react with sulphydryl (-SH) groups leading to the formation of a protein bound aldehyde functional group [(7).]

To minimize oxidant damage to biologic molecules the human lung is endowed with an integrated antioxidant system of enzymatic and expendable soluble non enzymatic antioxidants. This system includes several antioxidant defense mechanisms that detoxify reactive products and convert them to products that are quenched by other antioxidants. If the oxidant burden is sufficiently great, the reactive species may overwhelm or inactive the antioxidant system. The resulting excess oxygen species can damage major cellular components, including membrane lipids, proteins and DNA etc. The pathophysiological consequences of this injury are inflammation and widespread tissue damage. Thus overall alters the antioxidant status [(8).]

AIMS AND OBJECTIVES

1-The present work was planned to study -SH proteins as well as oxidant/antioxidant balance in smokers.

Following parameters were studied-

1. To explore the existence of possible peroxidative damage in smokers by estimating the level of serum malondialdehyde as an index of lipid peroxide.
2. To evaluate alteration in enzymatic antioxidant such as erythocytic superoxide dismutase (SOD).
3. To study possible alteration in antioxidant status in smokers by estimating concentration of non enzymatic antioxidant sulphydryl proteins.
4. To make global assessment of antioxidant defense by measuring total antioxidant capacity in smoker'.

MATERIALS AND METHODS

1. The level of serum total lipid peroxide in terms of Malondiadehyde (MDA) was determined by Kei Satoh method.[(9)]
2. Serum sulphydryl proteins (-SH) was determined by A.F.S.A.method[(10)].
3. RBC – Superoxide dismutase activity was estimated by Najwa Cortas and Nabil Wakid method [(11)]
4. Total antioxidant capacity in plasma (TAC) was assayed by FRAP analysis. [(12)]

STUDY PROCEDURE

The present study was conducted in the Department of Biochemistry Dr. Vikhe Patil Medical College and Hospital Ahmednagar. Smokers' with Hypertension, Malignancy, overt cardiac failure, recent surgery, severe endocrine hepatic or renal diseases and use of anticoagulant medicine and the lung disorders were excluded. All smokers' were active smokers' without any disease. Smokers' with smoking history of >20 pkts /year in the age group of 25-60 years of age were included in the study and 100 healthy controls were also included in the study . Informed consent was obtained from each participant in the study.

STUDY DESIGN

Distribution of these subjects was as follows.

Sr. No.	Group	Types	No. of Subjects
1	Controls	Healthy subjects	100
2	Smokers'	Smoking history > 20 pkts/years	60

The control subjects were completely healthy non smokers and showed no abnormality on clinical examinations and were completely symptom less. The study was cleared by institutional ethics committee.

10 ml blood was collected from each patient. 5ml of it was collected in EDTA bulb and 5ml was collected in plain bulb. Plasma and serum were separated from respective bulbs by centrifugation at 3000 rpm for 10 minutes at room temperature. All the samples were analyzed on the same day of collection.

Serum MDA levels were measured reacting than with thiobarbituric acid at high temperature to form pink colored complex which was measured at 530 mn[(9)]. Erythrocyte SOD activity was measured by Kajari Das method [(10)] which is based on the ability of SOD to inhibit nitrite formation. Plasma -SH protein were measured at 412 nm using A.F.S.A.method [(11)]. And total antioxidant capacity was measured at 593 nm by using FRAP analysis. [(12)]

The statistical analysis was performed by using student t test and P values < 0.001 were interpreted as statistically significant. The values were expressed as mean ± SD.

RESULTS

Table No. 1 Illustrate the levels of MDA, SOD,-SH, TAC in the healthy controls and smokers

Sr No.	Parameters	Healthy controls n=100	Smokers' n=60
1.	Sr.MDA (µmol/L)	1.66±0.28	4.5±2.76 *
2.	Sr. SOD (U/mg of Hb)	1.38±0.129	0.40±0.1 *
3.	Sr. –SH Proteins (µmol/L)	19.37±1.7	8.1±1.15 *
4.	Sr.TAC (umol /L)	1253.12±170.22	411.09±72*

n = number of cases

All values are expressed in mean ± SD

* = Significant when compared with control group

DISCUSSION

Table No. 1 display serum total lipid peroxide (MDA) levels in healthy controls and smokers'

Significantly higher levels of serum total lipid peroxide (MDA) ($P<0.001$) were observed in smokers' as compared to healthy controls .The elevated MDA levels observed in the present study suggest increased ROS production and thereby lipid peroxidation in smokers'.

Reactive oxygen species (ROS), such as the superoxide anion liberated by phagocytes recruited to sites of inflammation, are proposed to be a major cause of the cell and tissue damage, including apoptosis, associated with many chronic inflammatory diseases Lung cells, in particular alveolar epithelial type II cells, are susceptible to the injurious effects of oxidants. Lungs are continuously exposed to oxidants, either generated endogenously by metabolic reactions or exogenously, such as air pollutants or cigarette smoke.

Cigarette smoking a environmental hazard, also delivers oxidants and free radicals to the lungs. Cigarette smoke contains many oxidants and free radicals, both in the gas and the tar phase (13) and causes sequestration of neutrophils into the pulmonary microcirculation and accumulation of macrophages in respiratory bronchioles (14). Once recruited, these cells become activated and generate ROS in response to a sufficient level of stimulus (threshold concentration). The mechanism for this may involve neutrophil adhesion to endothelium and upregulation of CD18 integrins (15,16), which is known to upregulate the reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, hydrogen peroxide-generating system.

Activation of macrophages, neutrophils and eosinophils generates superoxide anion, which is rapidly converted to H2O2 by superoxide dismutase (SOD), and hydroxyl radicals, formed nonenzymatically in the presence of Fe2+ as a secondary reaction. In neutrophils, myeloperoxidase also catalyses the formation of the potent oxidant hypochlorous acid from H2O2 in the presence of chloride ions. ROS, which may also be released by lung epithelial cells, may also stimulate inflammatory cells directly, thereby amplifying lung Inflammatory and oxidant events. Changes induced by reactive oxygen species may result in inactivation of antiproteases, epithelial cell injury, apoptotic and necrotic cell death, mitochondrial dysfunction, disturbance of extracellular matrix repair and maintenance of airway inflammation [(17)].

Thus, smokers' are clearly subjected to oxidative stress as indicated by increased lipid peroxidation.

To prevent free radical formation or limit their damaging effects, cells have developed a comprehensive array of defenses i.e. antioxidants. Table No. 1 show superoxide dismutase activity in healthy controls and smokers' significantly diminished SOD activity ($P<0.001$) was observed in smokers'.

In smokers' the excessive production of superoxide anions, by polymorphonuclear leukocytes, macrophages and monocytes disturb the oxidant and antioxidant balance. To scavenge these superoxide anions more amount of SOD gets utilized.

Cu-Zn-SOD has two identical subunits of about 32 kDa, each containing a active site constituted by a copper and a zinc atom bridged by a common ligand histidine [(18).]

It has been reported that Cu-Zn SOD is irreversibly inactivated by its product H_2O_2, and it is well known that mature erythrocytes are unable to synthesize de novo SOD (or other proteins). Under normal circumstances a minor loss of SOD due to oxidative inactivation may not be of physiological significance. During exposure to oxidative stress, however, substantial SOD inactivation may occur, thus seriously compromising the antioxidant defenses of the RBC [(19)].

SOD inactivation by H_2O_2 has been shown to involve oxidative modification of a histidine residue which is important in the binding of the copper moiety to the active site of the enzyme. H_2O_2-dependent structural changes in the active site region of SOD lead to a conformational change which is susceptible to proteolysis. Such modified SOD undergoes

further reactions to form protein fragments, which are rapidly degraded by intracellular peptidase. The low SOD activity could contribute to a failure in plasma antioxidant defense.

Table No.1depicts the levels -SH proteins in healthy controls and smokers' significantly lower levels of -SH proteins ($P<0.001$) were observed in smokers' as compared to healthy controls.

Protein sulphydryl groups contribute physiologically to overall redox balance and modulate oxidative stress by generating reversible semi-oxidized species (mixed disulphides with non-protein low molecular weight thiols). Protein sulphydryl groups are also responsible for antioxidant action in plasma because they can react with several oxidant radicals [(20).]

In smokers', oxidative stress oxidize thiols of various proteins and glutathione. Two series of oxidized species arise depending on the starting target, oxidation / nitration leads to the formation of protein thiolates ($cys–S^-$), can be readily oxidized to a sulphenic acid (cys-SOH), which is a relatively reactive form that can quickly form a disulphide with a nearby -SH. Strong oxidants will oxidize either $cys–S^-$ or cys–SH to sulphinic ($cys–SO_2H$) and /or sulphonic ($cyss–SO_3$ H) acid derivatives. This difference in the generation of a particular cysteine -SH species provides a basis for distinguishing redox signaling from oxidative stress. While oxidative stress generally involves nonspecific oxidation of those cysteines which are located in an environment promoting dissociation of -SH. The higher oxidation states in the form of sulphinic and sulphonic derivatives have essentially been considered as irreversible modification under biologically relevant condition and associated with oxidative injury thus lowering the -SH levels in smokers'[(21).]

It has been hypothesized that cigarette smokers were found to have 20 % more oxidized proteins in their plasma compared with non smokers'. Therefore -SH proteins measurement may be used as a prognostic marker test for cigarette smoke dependent lung diseases.

Total antioxidant capacity and smokers- The total antioxidative potential of the plasma reflects the ability of an individual to resist the oxidative stress. Ferric reducing ability of plasma (FRAP) evaluates plasma total antioxidant capacity due to known and unknown antioxidants in the plasma [(22)].

Table No. 1 describe total antioxidant capacity in healthy controls and smokers. As compared to healthy controls total antioxidant capacity of smokers was significantly decreased ($p<0.001$).

In smokers' free radical activity is increased which is associated with enzymatic and nonenzymatic antioxidant depletion. Although we measured the individual antioxidant concentration directly, the combined effect due to co-operativity between the antioxidants for example vitamin E, superoxide dismutase and any compensation mechanism due to relative deficiency in any one antioxidant can be better examined by measurement of the total antioxidant capacity.

Significant reduction in total ferric reducing ability of plasma may be due to increased free radical activity either because of inflammation or complications that results in imbalance between antioxidant capacity and proxidant affecting lung function. Extensively amplified oxidant burden and declined individual antioxidant levels might be responsible for the observed significant fall in total antioxidant capacity of smokers'. An inequity between oxidative stress and antioxidative capacity has been proposed to play an important role in smokers'.

CONCLUSION

Our study confirmed the existence of oxidative stress and alteration in –SH proteins studied as a non enzymatic antioxidant and total antioxidant capacity. We found decreased levels of enzyme antioxidants with increased levels of lipid peroxide in smokers'.

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