OXIDATIVE STRESS AND SERUM α_1 – ANTITRYPSIN IN SMOKERS

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ABSTRACT

The present study was undertaken to evaluate the levels of serum lipid peroxide, nitric oxide end products, erythrocytic superoxide dismutase activity and serum α_1 -antitrypsin in smokers. Total 90 active cigarette smokers were subdivided into Group I (subjects with smoking habit of less than 10 cigarettes per day) and Group II (with smoking habit of more than 10 cigarettes per day). In both groups lipid peroxide and nitric oxide end products were significantly increased with significantly decrease in erythrocytic superoxide dismutase activity and serum α_1 -antitrypsin as compared to controls. Our findings show enhanced oxidative stress and reduced α_1 -antitrypsin in cigarette smokers. Further increase in number of cigarettes per day exacerbates the oxidative stress with decrease in α_1 -antitrypsin.

KEY WORDS

Lipid peroxide, Nitric oxide, Superoxide dismutase, α_1 - antitrypsin, Smokers.

INTRODUCTION

Cigarette smoking is serious public health problem world wide with increased mortality and morbidity (1). Cigarette smoke contains a complex mixture of toxic free radicals in both tar and gas phase which include quinone, semiquinone, hydroquinone, alkyl, oxides of nitric oxide, peroxy radicals, superoxide anion ($O_2^{\cdot-}$), nicotine, carcinogens etc (2,3). The respiratory tract is an organ for molecular, cellular and tissue damage from inhaled radicals because of its massive surface area. Reactive oxygen species (ROS), pollutants, cigarette smoke and also products of inflammatory cells, polymorphonuclear leukocytes, alveolar macrophages, proteases etc may contribute lung damage (4).

Polyunsaturated fatty acids in cell membranes are major target for free radical attack resulting in lipid peroxidation (3). Free radicals produced in the respiratory chain are the main culprit of lipid peroxidation (5). Depletion of antioxidants reduces the

protection against cigarette smoke induced plasma membrane peroxidation (3). Oxidants can effectively inactivate molecule required to maintain integrity of pulmonary tissue eg. antiproteases or α_1 -antitrypsin (6).

To view the above concepts, the present study was designed to evaluate the role of oxidants, antioxidant and α_1 -antitrypsin in cigarette smokers in comparison with normal control subjects.

MATERIALS AND METHODS

The present study was carried out in the Department of Biochemistry, Dr. V.M. Govt. Medical College, Solapur with approval of ethical committee. Total 60 male volunteers with a history of active smoking were selected for present study. Active smokers were subdivided into Group I (subjects with smoking habit of less than 10 cigarettes per day) and Group II (subjects with smoking habit of more than 10 cigarettes per day) 30 subjects each. The selected subjects, smokers as well as controls, were not on any supplementation such as vitamins or minerals, which may affect oxidative stress. Further, they were thoroughly screened for clinical history of diabetes mellitus, cardiovascular disease, alcoholism, inflammatory disorders and such conditions which would alter the study parameters were strictly excluded. 5ml venous blood was drawn under aseptic condition, 3ml of which was collected in

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plain bulb to obtain serum and 2ml was collected in a heparin bulb. The RBCs were used for determination of erythrocytic superoxide dismutase (RBC-SOD) activity (7). Serum sample was used for estimation of total lipid peroxide as malondialdehyde (MDA) (8) and nitric oxide end products (NO $\cdot$) (9) and α_1 -antitrypsin by Kits from Bioscientifica S.A, Argentina. The data were analyzed by using student's 't' test.

RESULTS AND DISCUSSION

Table 1 shows significantly elevated levels of serum MDA in Group I and Group II as compared to control Group, where as serum MDA was further increased in Group II in comparison with Group I. This observation strongly support the concept that increased production of oxygen derived free radicals contributes to lipid peroxidation in chronic smokers (10,11). In smokers, lungs are persistently exposed to oxidants, which are inhaled into airspace or those released from inflammatory leukocytes (5). Oxygen free radicals exert cytotoxic effects by peroxidation of membrane phospholipids which alter membrane fluidity leading damage to lung interstitium (11). Further, in our study MDA levels show significant rise in Group II in comparison with Group I suggesting increase in number of cigarettes per day enhances lipid peroxidation.

Table 1: Depicts the Mean $\pm$ SD of serum lipid peroxide (MDA), nitric oxide (NO $\cdot$) end products, erythrocytic superoxide dismutase activity (RBC-SOD) and α_1 -antitrypsin in Smokers and Controls

Parameters	Controls	Study group	
		Group I (Smokers <10 cigarettes / day)	Group II (Smokers >10 cigarettes / day)
MDA nmol/ml	1.65 $\pm$ 0.65	2.64 $\pm$ 0.28*	4.47 $\pm$ 0.82**
NO $\cdot$ end product mmol/L	56.8 $\pm$ 8.0	81.2 $\pm$ 14*	93.3 $\pm$ 21**
RBC-SOD U/mg Hb	2.67 $\pm$ 0.37	2.03 $\pm$ 0.30*	1.67 $\pm$ 0.29**
α_1 - antitrypsin mg/dl	90 $\pm$ 37	81.7 $\pm$ 20*	71 $\pm$ 25**

*P < 0.001 as compared with controls;

**P < 0.001 as compared with Group I

Significant increase in levels of serum NO $\cdot$ end products were observed in both Groups I and Groups II, as compared to controls. Where as Group II showed significant increase in serum NO $\cdot$ end products in comparison with Group I. The possible reason behind this may be the smoke which itself is an ultimate source of oxides of NO $\cdot$, superoxide anion (O $_2^{\cdot-}$) peroxy radical, carbon centered radicals (2). NO $\cdot$ is unstable free radical with very short half life and is likely to react with O $_2^{\cdot-}$ to form peroxynitrite (ONOO $\cdot$) (12). Peroxynitrite formed in cigarette smoke fractions participates in neurotoxicity and

its action is mediated by lipid peroxidation (13). Furthermore, NO $\cdot$ may bind to and activate proinflammatory enzymes such as cyclo-oxygenase and certain metalloprotease, which in turn may contribute to tissue injury (14).

The present study shows that activity of SOD in erythrocytes was significantly decreased in Group I and Group II than that of controls. The significant fall in SOD activity was observed in Group II as compared with Group I. SOD catalyses dismutation of O $_2^{\cdot-}$ to hydrogen peroxide (H $_2$ O $_2$) and water. Alenjandro et al suggest that decrease in SOD may be due to a progressive enzyme inactivation by its product H $_2$ O $_2$ or due to an increase in the glycation of SOD (15). It was also reported that, in smokers, RBCs are more susceptible to H $_2$ O $_2$ induced peroxidation (16). Mammalian cells are protected against free radicals by enzymatic and non-enzymatic antioxidants. Antioxidants transform free radicals into less reactive free radical by limiting their toxic effects. The potential effects of oxidative damage on pulmonary tissues can be ameliorated by antioxidant defense mechanism (6).

A significant decline in serum α_1 -antitrypsin has been found in Group I and Group II as compared to control group, further significant decrease was observed in Group II as compared to Group I. Direct exposure of α_1 -antitrypsin to gas phase cause loss of elastase inhibitory capacity leading to formation of reactive free radicals from smoke that inactivate α_1 -antitrypsin by oxidizing methionine 358 terminal amino acid (17). Experimental study shows cigarette smoke inactivates α_1 -antitrypsin resulting in functional α_1 -antitrypsin deficiency (6,17,18). In smokers, neutrophils and macrophages accumulate in alveoli. Accumulated neutrophils are activated and release their granules rich in a variety of cellular proteases resulting in tissue damage. Importantly, both oxidants and proteases can damage lung tissues directly, by inactivating antioxidants and antiproteases (19).

It is evident from our study that, increase in number of cigarettes per day make smokers more susceptible to enhanced oxidative stress along with decline in α_1 -antitrypsin. Therefore, discontinuance of smoking by public awareness, supplementation of antioxidants as well as α_1 -antitrypsin may minimize the risk of smoke-related diseases. However, further studies are needed to better understand the role of oxidative damage and α_1 -antitrypsin in the etiology of smoking induced complications.

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