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Original Research Article

Circulating Lipid Peroxide and Antioxidant Status in Cigarette Smokers: An Oxidative Damage Phenomena

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ABSTRACT

Background: Oxidative stress plays an important role in the pathogenesis of some diseases such as lung cancer, chronic obstructive pulmonary disease, and atherosclerosis. Smoking may enhance oxidative stress not only through the production of reactive oxygen radicals in smoke but also through weakening of the antioxidant defense systems.
Aim: The present study was conducted to determine the effect of cigarette smoking on changes in lipid peroxidation and antioxidant status in cigarette smokers.
Materials & Methods: The study population consisted of 150 male subjects divided into two groups; 88 smokers and age- and sex-matched non-smokers 62 subjects were selected. Biochemical parameters such as lipid peroxidation thiobarbituric acid reactive substances malondialdehyde (MDA) and antioxidants (superoxide dismutase (SOD),catalase (CAT), reduced glutathione (GSH), vitamin A, vitamin C and vitamin E were measured by colorimetric methods.
Results: Enhanced lipid peroxidation with concomitant depletion of antioxidants was observed smokers as compared to non-smokers. In addition, the activities of enzymatic and non-enzymatic antioxidants were more significantly altered in smokers than nonsmokers.
Conclusion: These findings may suggest that, smokers have evidence of oxidative stress and an impaired oxidant defense system. Alterations observed in smokers that increased oxidative stress can represent a risk factor for the development of chronic disease in earlier future.

Key words: Smokers, Lipid peroxidation, Antioxidants

INTRODUCTION

Cigarette smoking, here after referred to as 'smoking', is the largest single risk factor for premature death in developed countries. It is the avoidable cause of death in our society and most important health issue of our time. [(1)] Hence prevention and quitting smoking are major public health goals. The World Health Organization predicts that tobacco deaths in India may exceed 1.5 million annually by 2020. In recent years, large household surveys have shown that in middle age, more than one third of men and a few percent of women smoke tobacco and that there are about 120 million smokers in India. [(2,3)] Free radical-

induced oxidative damage has been suggested to play a major role in the pathogenesis of numerous smoking-related disorders. [4,5] It has been argued that, the increased production of reactive oxygen species associated with smoking may exceed the capacity of oxidant defense system, resulting in oxidative damage. [6]

Tobacco smoke is a rich source of oxidants. The main addictive component of cigarette smoke is nicotine, which was first prescribed as medical drug to treat rodent ulcer and constipation. But now it is common knowledge that, nicotine does harm to our body. Smoking is an easy way to administer multiple doses of psychoactive drug nicotine. [6,7] Two major phases were identified in cigarette smoke: a tar phase and a gas phase; both phases are rich in oxygen-centered, carbon-centered and nitrogen-centered free radicals as well as non-radical oxidants. From the analysis of each phase, it was estimated that a single cigarette puff contains approximately, 1014 free radicals in the tar phase, and 1015 radicals in the gas phase. These include various compounds, which are capable of causing an increase in the generation of various reactive oxygen species (ROS) like superoxide (O_2^-) hydrogen peroxide (H_2O_2), hydroxyl (OH.) and peroxyl (ROO.) radicals. These reactive oxygen species in turn are capable of initiating and promoting oxidative damage in the form of lipid peroxidation. [8, 9] The present study was therefore undertaken to assess the extent of lipid peroxidation and the status of antioxidants in cigarette smokers & non smokers.

MATERIALS AND METHODS

Study population

The present study was carried out in the department of Biochemistry, Mahatma Gandhi Institute of Medical Sciences (MGIMS) and Kasturba Hospital, Sewagram. The study population consisted of 150 male (age-matched) subjects divided into two groups viz. 88 smokers and age-sex matched 62 non-smokers (healthy volunteers), On the basis of smoking habits we further categorized smokers (n=88) into moderate(15 to 20cigarrate/day, n=40) & heavy smokers (>20to39cigarrate/day, n=48).

Demographic and smoking habits information

Each subject was interviewed and asked to provide demographic and smoking habit information. The demographics included age. Smoking habits included smoking period (years) and number of cigarettes smoked. A written informed consent was taken from the subjects. The protocol of this study was approved by the Institutional Human Ethics Committee.

Collection and hemolysate preparation

Blood samples were collected by venous puncture in plain bulb & heparinized tubes. Serum was separated and used to estimate SOD & GSH. The plasma was separated by centrifugation at 1000 g for 15 minutes. It was used to estimate MDA, vitamin c, beta carotene & vit E. After centrifugation, the buffy coat was removed and the packed cells were washed three times with physiological saline. A known volume of the erythrocytes was lysed with hypotonic phosphate buffer (pH 7.4). The hemolysate was separated by centrifugation at 2500× g for 15 min at 2°C for catalase estimation.

ESTIMATIONS

Estimation of Malondialdehyde

MDA was assessed in plasma by modified TCA - TBA method of Stater T. F. *et* al. [10] The assay utilizes TBA and is based on the acid catalysed decomposition of lipid hydroperoxides to malondialdehyde (MDA) was measured at 530 nm.

Estimation of Catalase

Catalase was estimated in erythrocytes is based on the fact that

dichromate in acetic acid reduced to chromic acetate when heated in presence of H2O2 with formation of perchromic acid as an unstable intermediate. [11] The chromic acetate is measured colorimetrically at 570nm.

Estimation of Superoxide dismutase

SOD was assayed in serum utilizes the principle of inhibition of auto-oxidation of pyrogallol by SOD enzyme using the method of Marklund S, Marklund G. [12]

Estimation of Reduced Glutathione

GSH was assayed in serum by the method of Beutler. [13] GR catalyses the reduction of oxidized glutathione with simultaneous oxidation of NADPH.

Estimation of plasma ascorbic acid

Vitamin C was assayed by colorimetric method as described by Aye Kyaw. [14]

Estimation of beta carotene

Plasma proteins were precipitate with ethanol and beta carotene from plasma was extracted into light petroleum. The intensity of yellow color due to carotene was estimated by Bradley &Hornbeck method. [15]

Estimation of alpha tocopherol

This in based on Emmerie Engel procedure [16] in which tocopherol is oxidized to tocopheryl quinine by ferric chloride & resultant ferrous ions is complexed with 2,2' dipyridyl to produced a red colored compound.

RESULTS

The mean age, body weight, height and Body Mass Index of the subjects were statistically similar between smokers and non-smokers.

The level of plasma MDA and enzymatic and non- enzymatic antioxidant in smokers compared with non-smokers was depicted in Table I. The extent of lipid peroxidation (MDA) in a smoker was significantly increased compared to non-smoking subjects ($p<0.01$). A significant decrease in the activities of SOD, CAT was seen in smokers compared with nonsmoking subjects. However significant decrease in activities of SOD and Catalase of the smoking subjects suggesting pro-oxidant role of oxidative stress, which was confirmed by the decreased activities of all the enzymes in the smokers when compared to the non-smoking group. On the other hand, the level of non-enzymatic antioxidants b-carotene, vitamin C, vitamin E and GSH significantly decreased in smokers compared with non-smokers ($p<0.01$). The level of oxidant and antioxidants in moderate & heavy smokers was depicted in Table II. The MDA level in heavy smokers were found to be significantly high compared to moderate smokers ($p<0.01$). However, enzymatic as well as non enzymatic antioxidant levels in moderate smokers were found to be low compared to moderate smokers. However in all antioxidants beta carotene, catalase , SOD& Vitamin E showed significant positive correlation in heavy smokers than moderate smokers ($p< 0.01,0.05$), while GSH & Vitamin C did not show significant correlation in heavy and moderate smokers.

Table 1: Circulatory lipid peroxide and antioxidant status in non-smokers and smokers subjects

Parameter	Non-Smokers	Smokers
MDA (nmole/ml)	2.00±0.76	3.04±0.70***
SOD (Unit/ml)	3.40±0.85	2.19±0.74***
CAT (µ/ mg/protein)	69.44±8.90	63.72±9.39***
GSH (g/Hb)	8.82±1.29	8.29±1.47**
B carotene (mg/dl)	68.01±9.15	53.40±10.72***
Vitamin C (mg/dl)	0.89±0.12	0.71±0.11**
Vitamin E (mg/dl)	0.83±0.09	0.77±0.11**

Values are given as mean ± SD.
***Significant (P value <0.001)
** Significant (P value <0.01)
MDA- Malondialdehyde , SOD- Superoxide dismutase, CAT- Catalase, GSH-Glutathione reductase

Table 2: Circulatory lipid peroxide and antioxidant status in types of smokers

Parameter	Smokers	
	Moderate	Heavy
MDA (nmole/ml)	2.85±0.71	3.21±0.65**
SOD (Unit2 mg/Hb)	2.41±0.82	2.01±0.62**
CAT (Unitb mg/Hb)	66.84±10.26	61.11±7.79**
GSH (Unitc mg/Hb)	8.21±1.38	8.36±1.45 (NS)
B carotene (mg/dl)	56.69±11.86	50.66±8.89**
Vitamin C (mg/dl)	0.73±0.11	0.69±0.10 (NS)
Vitamin E (mg/dl)	0.80±0.12	0.75±0.10*

Values are given as mean ± S.D
Smokers compared with non-smoking subjects
** Significant (P value <0.01)
* Significant (P value <0.05)
NS- Non significant
MDA- Malondialdehyde , SOD- Superoxide dismutase, CAT- Catalase, GSH-Glutathione reductase

DISCUSSION

Cigarette smoking is a serious health problem and most important avoidable causes of death in world. The risk of disease increases with increasing intensity and duration of smoking. Smoking($p<0.01$) is associated with a variety of diseases. Cigarette smokes contain a range of xenobiotics, including oxidants, and oxygen free radicals that can increase lipid peroxidation. [17,18] Its toxicity may be further enhanced by the stimulation of reactive oxygen species (ROS) production by neutrophil. [19] Analysis of MDA in plasma is a widely used method for the evaluation of lipid peroxidation. The concentration of MDA in plasma was higher in smokers than in non-smoking control subjects. These results are consistent with previous studies [20], which reported that the oxidative stress biomarker (MAD) was significantly higher in smokers than in non-smokers. Nicotine, a major toxic component of cigarette smoke, is a well established procarcinogen. However, it has been reported that nicotine disrupts the mitochondrial respiratory chain leading to an increased generation of superoxide anion and hydrogen peroxide. This may lead to oxidative damaged macromolecules including lipid, DNA, RNA, antioxidant enzyme in subsequent cell through disruption of cellular functions and integrity. [5,21]

Antioxidants constitute the foremost defense system that limit the toxicity associated with free radicals. It is known that plasma antioxidant capacity decreases and oxidative/antioxidative balance shifts to the oxidative side in smokers. [22] SOD along with CAT and GPx, the preventive antioxidants, plays a very important role in protection against lipid peroxidation. In this study, SOD & CAT activities were significantly lower smokers than in nonsmokers. SOD is the first enzyme in antioxidant defense that scavenges superoxide radicals to form H2O2 and hence diminishes the toxic effects of the radical. Decreased activity of SOD has been reported in pathological conditions. The quinonesemiquinone radicals from the tar phase of cigarette smoke are capable of reducing molecular oxygen to superoxide radicals whose excessive generation inactivates this enzyme. Hence, a decrease in SOD activity upon smoke exposure could have resulted from its inactivation by tar phase oxidants. CAT is involved in the detoxification of high concentrations of H_2O_2. CAT has been suggested to play an important role in the protection of the erythrocyte against oxidative stress. [23] The presence and production of the free radicals from smoke lower this enzyme, leading to accumulation of H2O2 and lipid hydroperoxides further worsening the damage. Present study suggests the inability of host antioxidant defense to meet the oxidative stress following chronic exposure to cigarette smoke.

GSH; a widely distributed cellular reductant is a metabolic regulator and putative indicator of health. Blood glutathione levels are believed to be predicators of morbidity and mortality. GSH plays a key role in protecting cells against electrophiles and free radicals. GSH can act

directly as a free radical scavenger by neutralizing hydroxyl radicals, or indirectly by repairing initial damage to macromolecules inflicted by hydroxyl radicals. It is essential in the maintenance of protein and non-protein SH group in reduced form. Smoking induced depletion of GSH level has also been reported. This depletion was directly associated with elevation in lipid peroxidation which could be attributed to its protection against ROS generated by smoke, besides its consumption by the antioxidant enzymes GPx. Acetaldehyde, a major aldehyde from the smoke has been shown to deplete the cells of their GSH by conjugating with it, which further makes the cells more vulnerable to peroxidative damage. [24,25] The present study also revealed depletion in the levels of non-enzymatic antioxidants such as vitamin C, E and beta carotene in smokers as compared to nonsmokers. [26] In the present study, we found a significant inverse correlation between the concentration of serum MAD and β- carotene in the smokers and non-smokers. Thus may be more effective to scavenge free radical and prevent peroxidation in smokers. [27] Vitamin E, an important lipophilic antioxidant has an effective role in maintaining the cell structure against oxidative damage through blocking the chain reaction of free radicals. Vitamin E reacts with peroxyl radicals present in the smoke and terminates lipid peroxidation and vitamin A effectively quenches singlet oxygen. [28] Vitamin C is the first strong reductant in the aqueous phase that readily reacts with cigarette smoke oxidants and affords considerable protection to the cells. Studies involving different types of oxidative stress have shown that under all types of oxidative stress, ascorbic acid successfully prevents detectable oxidative damage and therefore it would be helpful in prevention of diseases in which oxidative stress plays a causative or exacerbation role. [29] Hence, the decrease in GSH levels could possibly be related to the inability of host tissue to synthesize GSH that is reflected from decrease in vitamin C, E and A. GSH and these vitamins are tightly linked to each other in a way that it helps to replenish vitamin C which in turn regenerates vitamin E and A. Beside that we have demonstrated the imbalance between oxidants and antioxidants is more pronounced in heavy smokers than moderate smokers, which provides the evidence of enhanced free radical mediated process corresponded with more advanced exposure.

CONCLUSION

However, smokers are constantly overexposed to free radicals through inhalation of long-lived carbon- and oxygen-centered radicals that subsequently deplete the plasma and tissue stores of these micronutrients. [26,30] The results of present study clearly show that cigarette smoking induces an oxidative stress in smoking by augmenting lipid peroxidation and diminishing both enzymatic and nonenzymatic antioxidant status. Further studies are required to define whether dietary or supplemental antioxidants ameliorate these processes. Meanwhile, major portions of public research resources directed at this problem should continue to be targeted toward interventions designed to reduce public consumption of tobacco products.

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