

## Study of interaction of cigarette smoke with thiol (-SH) group of sulfhydryl proteins in smokers

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### Abstract

The cigarette smoke contains more than 4000 chemical including 43 cancer causing carcinogens and more than 400 toxins these includes nicotine, tar, carbon monoxide, ammonia, hydrogen cyanide, arsenic, nitric oxide and many others. Cigarette smoke oxidants cause oxidative damage and increases the protein oxidation by rapid proteolytic degradation of the oxidized proteins, as there is loss of thiol groups. The present study was planned to study interaction of thiol (-SH) of sulfhydryl proteins as well as lipid peroxidation by studying MDA, total antioxidant capacity and level of vitamin C. 30 smokers of smoking history 10 to 20 cigarettes per day were included in the study whereas 40 healthy non-smokers who never had smoked were served as healthy controls. The base line clinical investigations malondialdehyde (MDA), Sulfhydryl proteins (-SH), total antioxidant capacity (TAC) & vitamin C were measured. The results indicate that the total antioxidant capacity (TAC), vitamin C and sulfhydryl proteins (-SH) were low ( $p < 0.001$ ) in smokers as compared to healthy controls. We found increased malondialdehyde (MDA) product of lipid peroxidation high ( $p < 0.001$ ) in smokers. Thus the present study confirmed the interaction of cigarette smoke with thiol (-SH) proteins.

**Keywords:** Thiol sulfhydryl proteins, Malondialdehyde (MDA), Total antioxidant capacity, Vitamin C and cigarette smoke (CS).

### Introduction

Cigarette smoking is one of the very important cause of early and preventable death and a significant public health concern throughout the globe.<sup>1</sup> Cigarette smoke is highly complex aerosol. Composed of thousands of chemicals in the tar and gas phase. When we smoke cigarettes, many chemicals enter in our body through our lungs. Burning tobacco produces more than 4,000 chemicals. Nicotine (NIK-uh-TEEN), carbon monoxide (muh-NOK-side), nitrosodimethylamine, N-Nitrosopyrrolidone, acetone, acrolein, vinyl chloride and tars are some of toxic compounds.<sup>2,3</sup>

Smoking has very serious effect on human health which include chronic obstructive pulmonary disease, atherosclerosis and even cancer. These changes take place slowly and it lasts long, often for the rest of the life. These are chronic changes. Emphysema is one of the good example of a chronic changes in lungs.<sup>4-6</sup> Complex inflammatory processes and changes in the immune system are crucial in the pathogenesis of smoking related disorders like chronic obstructive lung disease (COPD), lung cancer, and atherosclerosis.<sup>4</sup>

Since CS contains a wide variety of chemically reactive species that lead to the modification of macromolecules and due to increased oxidative burden depletion in vitamin C and total antioxidant capacity is demonstrated.

These results indicate that acute CS inhalation increases the oxidant burden on the lungs causing a transient depletion of GSH leading to thiol depletion in a variety of pools. Concurrently, the lungs may possess regulatory mechanism (s) which respond immediately by the uptake of CySH equivalents present in plasma disulfides which are kept in reduced state by protein disulfide isomerase.<sup>7</sup>

Worldwide the cigarette smoking is of prime concern. In India also we are facing cigarette smoking habitat problem a national issue specially in youngsters and middle aged group people, which is quite preventable. The very purpose of our study was to assess the role of antioxidant barrier of human body against tobacco smoke oxidative stress. We assessed different antioxidant indexes in plasma which include MDA, Thiol protein, TAC, and vitamin C and compared in both study group and healthy controls.

### Aims and Objectives

The present work was planned to study interaction of cigarette smoke with thiol proteins.

Following parameters were studied-

1. To know the presences of possible damage by cigarette smoke by analysing level of serum malondialdehyde as an index of lipid peroxidation.
2. To evaluate alteration due to cigarette smoke by estimating concentration of non-enzymatic antioxidants sulfhydryl proteins.
3. To make global assessment of antioxidant defence by measuring total antioxidant status.
4. To study effect of cigarette smoke on another non enzymatic antioxidant vitamin C.

### Materials and Methods

The present study was conducted in the Department of Biochemistry, Dr. Vithalrao Vikhe Patil foundation's Medical College and Hospital Ahmednagar. The patients selected for the present study were attending indoor/outdoor patient department from Dr. Vithalrao Vikhe Patil Memorial Hospital, Ahmednagar.

Exclusion criteria: Smokers with Hypertension, Malignancy, Cardiac failure, recent surgery, severe endocrine, hepatic, renal diseases or lung disorders were excluded.

Inclusion criteria: All smokers were active smokers without any disease. 30 smokers with smoking history of more than 10-20 cigarettes per day in the age group of 20-60 years were included. As well as 40 healthy controls were also included in the study. Informed consent was obtained from each participant in the present study.

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

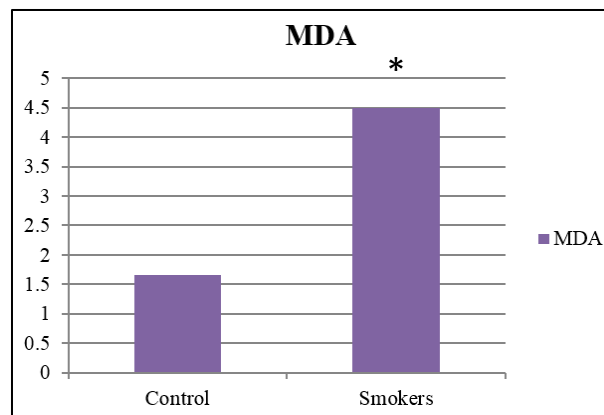
10 ml blood was collected from each subject. 5ml was collected in EDTA bulb and 5 ml was collected in plain bulb. Plasma and serum were collected from respective bulbs by centrifugation at 3000 rpm for 10 minutes at room temperature. All samples were analyzed on the same day of collection.

Serum MDA levels were measured reacting with thiobarbituric acid at high temperature to form pink coloured complex which was measured at 530nm.<sup>8</sup> Plasma thiol protein were measured at 412 nm using A.F.S.A. method.<sup>9</sup> Total antioxidant capacity was measured at 593 nm by using FRAP analysis.<sup>10</sup> Serum vitamin C was measured at 500 nm by using Caraway's method.<sup>11</sup>

Statistical analysis was carried out using student unpaired 't' test. Probability values <0.05 was considered as significant. Also data were expressed in mean  $\pm$  SD form.

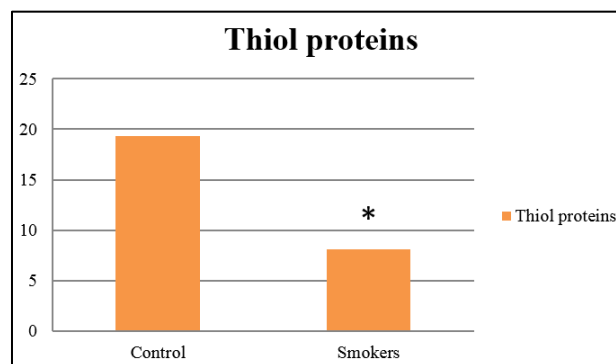
## Results

In this study, we studied different parameters to understand interaction of cigarette smoke as oxidative stress. As shown in table 1, the indicator of lipid peroxidation i.e. malondialdehyde (MDA) was significantly higher in smokers as compared to healthy controls (Fig. 1;  $P < 0.0001$ ). At the same time the major antioxidant weapon at the molecular level i.e. thiol-group-related antioxidant proteins (-SH) was also significantly decreased in smokers as compared to healthy controls (Fig. 2;  $P < 0.001$ ). The concentration of total vitamin C (ascorbic acid) in plasma was lower in smokers than in healthy controls (Fig. 3;  $P < 0.001$ ). We also studied total antioxidant capacity (TAC) and found that TAC was significantly decreased in smokers as compared to healthy controls (Fig. 4;  $P < 0.001$ ).



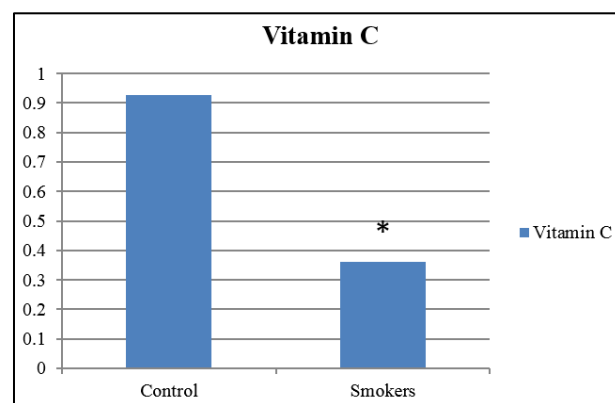
**Fig. 1: The level of malondialdehyde (MDA) ( $\mu\text{mol/L}$ ) in healthy controls and smokers**

\* $P < 0.001$  as compared to healthy controls.



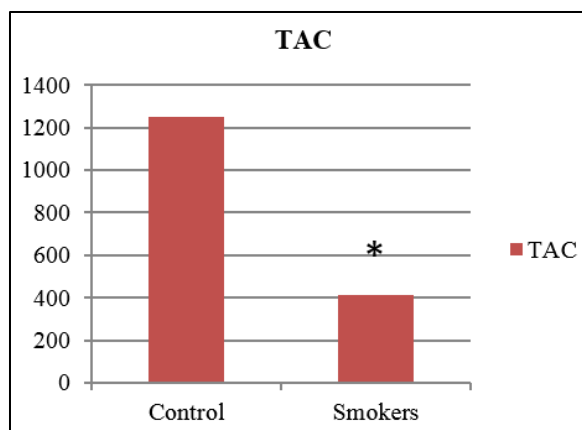
**Fig. 2: The level of serum thiol proteins ( $\mu\text{mol/L}$ ) in healthy controls and smokers**

\* $P < 0.001$  as compared to healthy controls.



**Fig. 3: The level of serum thiol Vitamin C (mg/dl) in healthy controls and smokers**

\* $P < 0.001$  as compared to healthy controls.



**Fig. 4: The level of Total Antioxidant capacity (TAC) (µmol/L) in healthy controls and smokers**

\*P<0.001 as compared to healthy controls

**Table 1: The levels of antioxidant parameters in both healthy controls and smokers**

S. No.	Parameters	Healthy control	Smokers
1.	Age (years)	39.12 ± 3.45	38.89 ± 3.95
2.	Serum lipid peroxide (MDA) (µmol/L)	1.66 ± 0.289	4.5 ± 2.76*
3.	Serum Thiol proteins (-SH) (µmol/L)	19.37 ± 1.7	8.1 ± 1.15*
4.	Serum Vitamin C (mg/dl)	0.927 ± 0.126	0.36 ± 0.07*
5.	Total antioxidant capacity (TAC) (µmol/L)	1253.12 ± 170.22	411.09 ± 72*

Data is expressed in mean ± SD format.

\*P< 0.001 compared to healthy controls.

## Discussion

The main affected system due to acute or chronic cigarette smoking (CS) is the respiratory system. Due to toxic chemicals in smoke it leads to metaplastic and dysplastic changes in respiratory epithelial cells. It is expressed by elevated expression of adhesion molecules. It also causes higher expression of many cytokines stimulating immune cells aggregation. In the respiratory tract macrophages are elevated. Apart from this the macrophages shows changes in surface markers with improper phagocytic and antigen presenting properties. It is been observed by various studies that chronic exposure to tobacco smoke leads to higher production of enzyme metalloproteinases (MMP) by macrophages and proteolytic enzymes by neutrophils. The obvious effect of the proteolytic enzymes is the increased destruction of alveolar cells and increased apoptosis of lung parenchyma results in aggregation of foreign material which in turn causes stimulation of immune system in lungs.<sup>12,13</sup>

The purpose of the present study was to understand the acute effects of cigarette smoke on utilization of external thiols by cigarette smoke. Cigarette smoke decreased GSH levels significantly (50%) without change in glutathione disulphide (GSSG). In lungs, protein thiol groups (protein-SH) decreased significantly without a significant change in protein-GSH mixed disulfides.<sup>12</sup>

Inhalation of Tobacco smoke Caused significant depletions in the free glutathione (GSH). These depletions were nonrecoverable even with dithiothreitol (DTT), which is used as protecting agent that prevents oxidation of thiol

groups. This suggest irreversible conjugation between GSH and CS-borne electrophiles. Corresponding lung cysteine (CySH) components were unaffected by CS inhalation.<sup>14</sup>

Cigarette smoking is the most common risk factor for chronic obstructive pulmonary disease, a disease that has been leading cause of death worldwide. Free radical generate the lipid peroxidation process in an organism. Malondialdehyde (MDA) is one of the final products of PUFA peroxidation in the cells. An increase in free radicals causes overproduction of MDA. Malondialdehyde level is commonly known as a marker of oxidative stress and the antioxidant status in cancerous patients.<sup>15,16</sup> In other study, it suggests an increased oxidative stress in both hypothyroid and subclinical states, which can be explained by both the insufficient increase in the antioxidant status and the altered lipid metabolism in these cases.<sup>17</sup>

Thiols are a class of organic compounds that contain a sulfhydryl group (-SH), known as thiol group. The thiol-group related antioxidants, including fGSH, tGSH, GSHPx and GST, constitute one of the major free radical scavenging systems involved in the clearing of the smoking mediated prooxidants.<sup>18</sup> Glutathione, a sulphide-containing tripeptide, is present in all cell types and can scavenge H<sub>2</sub>O<sub>2</sub>, OH<sup>°</sup>, O<sub>2</sub><sup>°</sup> and chlorinated oxidants. Due to its high content of oxidants, the cigarette smoke causes a prooxidant/antioxidant imbalance in the blood and tissues of smokers. Different investigators found variable results over antioxidant capacity in smokers. In one of the study<sup>19</sup> found that the erythrocytes from cigarette smokers contain more glutathione and protected endothelial cells against H<sub>2</sub>O<sub>2</sub> better than did erythrocytes from nonsmokers. In this study result suggest that tobacco smoking causes oxidation of

thiol protein (-SH) groups in plasma significantly as compared to healthy controls. Our study also reemphasized the previous findings that cigarette smoking lowers plasma vitamin C. Thus suggesting that smoking lowers blood vitamin C concentrations by impaired vitamin C absorption or decreased synthesis.<sup>20-22</sup>

### Conclusion

The present study confirmed cigarette smoke dependent interaction in the thiol moiety of protein by altering the values of thiol proteins, vitamin C, total antioxidant capacity and levels of toxic molecule of free radical related MDA. We found decreased level of non-enzymatic thiol proteins, vitamin C and overall total antioxidant capacity with increased in the free radical related toxicity of malondialdehyde (MDA) in the smokers.

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