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CIGARETTE SMOKING AND THE OXIDATIVE STRESS INDUCED BIOCHEMICAL CHANGES

***D. S. Jaya**

Department of Environmental Sciences, University of Kerala, Kariavattom Campus, P.O. Thiruvananthapuram. PIN-695581. Kerala, India

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****Corresponding author: D. S. Jaya***

ABSTRACT

An investigation was carried out to assess the health status of cigarette smokers, oxidative stress induced changes and prevalence of smoking among smokers of different age groups in Thiruvananthapuram city, situated in the southern part of Kerala, India. The 52 male volunteers, aged between 20 and 70 years who are residents in Ulloor ward of Thiruvananthapuram Corporation in Kerala state were selected for the study. Among these 52 subjects, 36 were current smokers and 16 were non-smokers. The body weight, height and duplicate blood pressure measurements at rest were taken. Fasting blood samples were collected to determine the changes in serum biochemical parameters like total cholesterol, malondialdehyde, sodium, potassium and selected heavy metals in smokers and non-smokers. Analysis of the results show that among the total participants, 69 % were smokers and of these smokers, 41.7 % were in the age group 36- 50 years. The body weight of the smokers was found to be less than that of the nonsmokers in the same age group. The BMI of 72.2% smokers were found to be less than that of the non-smokers. 77.8 % of the smokers in the study population were hypertensive and the blood pressure increases with age. Serum total cholesterol levels in smokers were found to be higher than that of non-smokers and this excess cholesterol may get deposited in the blood vessels and causes atherosclerosis. The smokers with BMI less than 25 recorded highest cholesterol levels. Serum sodium and potassium levels in smokers were higher than that of non-smokers. The increase in serum sodium content in smokers was highly significant compared to non-smokers. Concentrations of serum malondialdehyde, the lipid peroxidation product and the heavy metals like Cu and Cd were also found to be significantly high in smokers. The studyproves that smoking enhances free radical production which results in the formation of lipid peroxidation products and degradation of macromolecules, which may lead to cell damage or death. Results of this study also indicate that there is a positive correlation between mean cadmium content and blood pressure of smokers.

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INTRODUCTION

Oxidative stress is caused by an imbalance between production of reactive oxygen and a biological system's ability to readily detoxify the reactive intermediates or easily repair the resulting damage. All forms of life maintain a reducing environment within their cells. This reducing environment is preserved by enzymes that maintain the reduced state through a constant input of metabolic energy. Disturbances in this normal redox state can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell, including proteins, lipids and DNA (Khanna *et al*., 2009). Cigarette smoke contains over 7,000 chemicals, including 43 known cancer-causing (carcinogenic) compounds and 400 other toxins. Of the more than 7,000 chemicals in tobacco smoke, at least 250 are known to be harmful, including hydrogen cyanide, carbon monoxide and ammonia (Bauer, 1968). Smoking cigarettes produces a rapid distribution of nicotine throughout the body, and reaching the brain within seconds of inhalation. Others include benzene, radon, carbon monoxide, heavy metals, pesticides, hydrogen cyanide, ammonia etc. The carbon monoxide produced is quickly picked by the oxygen carrying hemoglobin of the blood and reduces its oxygen carrying ability. The human body is constantly under attack of free radicals. Once formed, free radicals attack cell structures within the body. The environmental stress factors like smoking accelerate the production of free radicals. In fact, each puff on a cigarette generates millions of free radicals. Studies by Tsuchiya *etal*. (1993) demonstrated that cigarette smoke contains superoxide and a large number of other oxygen reactive species (ROS). The major objective of the present study was to find out the biochemical changes and heavy metal content in the serum of cigarette smokers.

MATERIALS AND METHODS

Study Protocol: The study included 52 male volunteers who are residents of Thiruvananthapuram city, Kerala state, South India. A medical camp was conducted in Ulloor residential area near Medical College, Thiruvananthapuram. The entire adult population, aged between 20 and 70 yrs were invited to take part in the camp for this study. Participation was on a totally voluntary basis. Physical examination of the participants was done with the help of a Medical Partitioner. All the volunteers are abstained from taking drugs and alcohol twenty-four hours before the blood sample collection. They were requested to eat a light meal on the night before blood collection, and to consume no food in the morning of the blood collection day.

Subjects and Methodology: The bio-data and information on personal habits of 52 male volunteers (smokers and non-smokers) was obtained from the self-prepared questionnaire. Among the 52 volunteers included in this study, thirty-two volunteers are habitual cigarette smokers and the remaining were healthy control subjects who were non-smokers. Anthropometric measurements like body weight and body height of the volunteers were also noted. Body weight in kilogram was taken using a portable foot weighing balance (Libra Scales, manufactured by Edryl, India). Body height in metres was measured using a non- elastic cloth tape. The body mass index (BMI) or Quetelet index was calculated dividing the body weight in kg by square of body height in metre (WHO, 1995). The systolic and diastolic blood pressure at rest was measured using a Mercury Sphygmomanometer (Elko, New Delhi). All chemicals and biochemicals used for the experiments were of analytical grade and of high degree of purity. Blood sample (10 ml) was collected from each participant by venipuncture of the antecubital vein with the help of a Medical Laboratory Technician. 1 ml blood was added to a tube with anticoagulant for hemoglobin estimation. The remaining blood was immediately collected in a plain tube, allowed to clot for 15 minutes and serum was separated.

The serum samples were used for the estimation of different biochemical parameters. Serum malondialdehyde (MDA) was measured spectrophotometrically by the thiobarbituric acid assay (Yagi, 1992). Serum total cholesterol was determined spectrophotometrically by Ferric chloride method (Chawala, 2003) and serum total protein by the Biuret method using clinical kits purchased from Nice Chemicals Pvt. Ltd., Kochi. The sodium and potassium concentrations in the serum was determined (Hawk, 1954) by using the flame photometric method (Systronics, Mediflame 127). The heavy metal, copper in the serum samples was determined by atomic absorption spectrophotometry (Alan and GowenLock, 1996) using an Atomic Absorption Spectrophotometer (GBC, model 1190). The cadmium content in the serum samples was determined (Ostapczuk, 1992) by Aniodic Stripping Voltammetry (Metrohm 757 VA Computrace). All tables and figures are composed by the author.

Statistical Analysis: The data were subjected to different statistical analyses with the help of a personal computer using Statistical Package for Social Sciences (SPSS 10) software.

Table 1. Anthropometric measurements

Age Group		Body Height (m)		Body Weight (Kg)		Body Mass Index (BMI)	
		Smokers	Nonsmokers	Smokers	Nonsmokers	Smokers	Nonsmokers
≤ 35 yrs	Mean	1.675	1.730	62.077	73.571	22.010	24.510
	± SEM	0.022	0.034	3.386	3.316	0.954	0.500
36 - 50 yrs	Mean	1.676	1.700	67.867	78.750	24.183	27.347
	± SEM	0.015	0.035	2.853	4.270	0.988	1.800
≥ 51 yrs	Mean	1.595	1.668	63.500	61.200	25.034	21.957
	± SEM	0.016	0.011	2.771	4.705	1.258	1.543

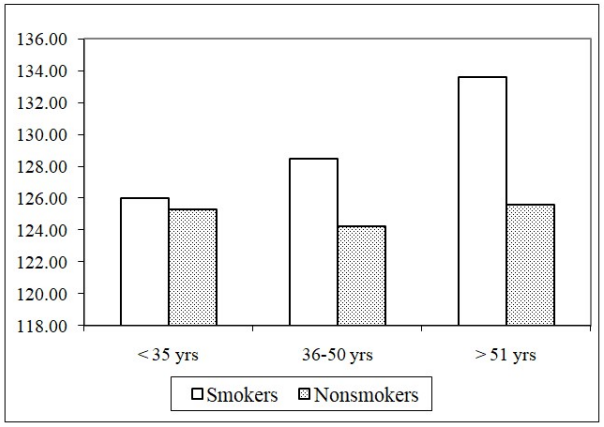

Figure 1. Systolic Blood Pressure (mm Hg) of smokers and non-smokers

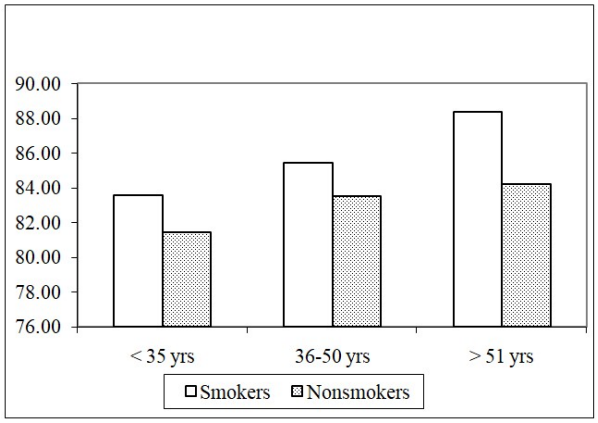

Figure 2. Diastolic Blood Pressure (mm Hg) of smokers and non-smokers

Data was compiled and grouped into smokers and non-smokers. Data were normally expressed as mean ± SEM. The difference between the means for the groups, smokers and non-smokers was assessed using Student's 't' test. Chi-square analysis with Odd's ratio were also computed for smoking and non-smoking groups regarding different parameters (Snedecor & Cochran, 1967).

RESULTS

Analysis of the data showed that of the 52 male volunteers attended the study, 36 were current smokers (those smoking at least one cigarette a day for 5 years or greater) (72.2%) and 16 were non-smokers. The age groups of smokers and non-smokers in the study population and their anthropometric measurements (mean body weight, body height and BMI) were given in Table-1. Among the current smokers, 11 were heavy smokers smoking 20 or more than 20 number of cigarettes, and 25 were light smokers taking 1 or more cigarettes daily. The study population was divided into three age groups: 20 to 35 years, 36 to 50 years and above 50 years. Body weight of smokers in the age group 20-35 yrs and 36-50 yrswere less than that of the non-smokers in the same age group. It was also found that the prevalence of smoking was high (41.7 %) in the age group 35 to 50 years. The BMI of smokers in the age group 35 to 50 years were also less than that of non-smokers in the same age group as given in Table-1.

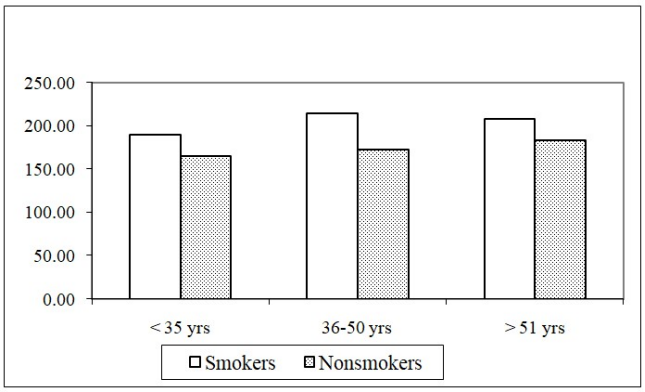

Figure 3. Serum Total Cholesterol content (mg%) in Smokers and Non-smokers

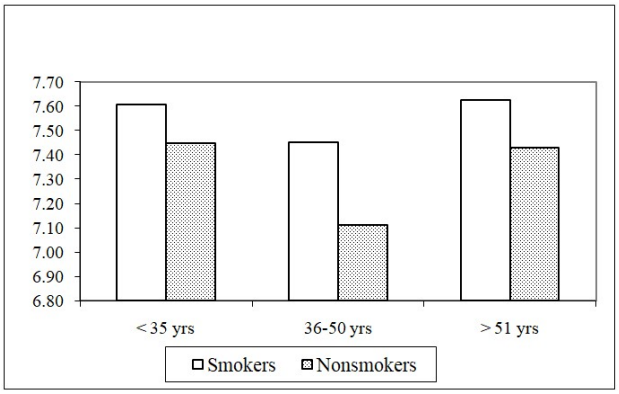

Figure 4. Serum Protein content (gm%) in Smokers and Non-smokers

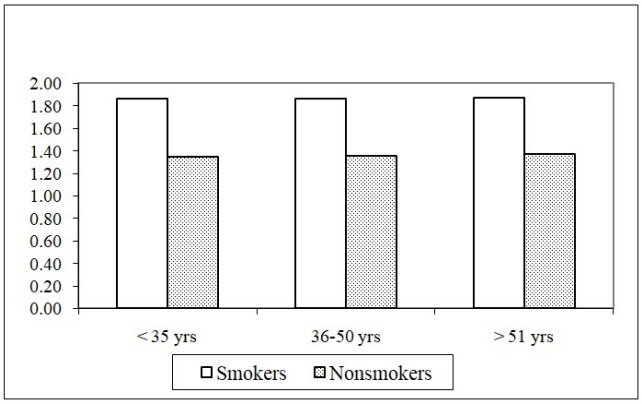

Figure 5. Serum Malondialdehyde content (nmol/L) in Smokers and Non-smokers

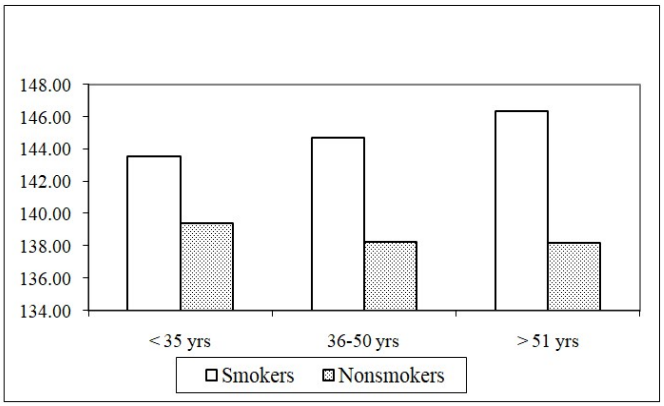

Figure 6. Serum Sodium content (mM/L) µin smokers and Non-smokers

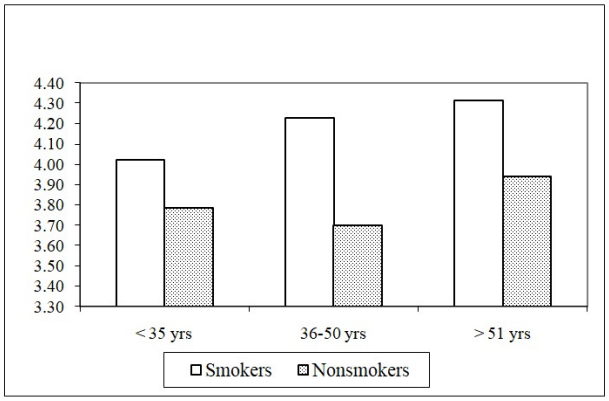

Figure 7. Serum Potassium content (mM/L) in Smokers and Non-smokers

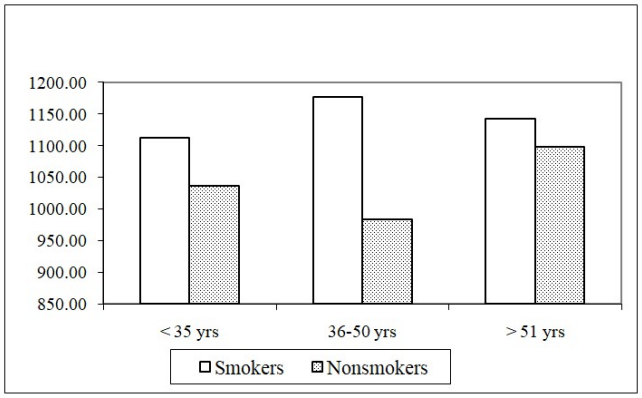

Figure 8. Serum Copper content (µg/L) in Smokers and Nonsmokers

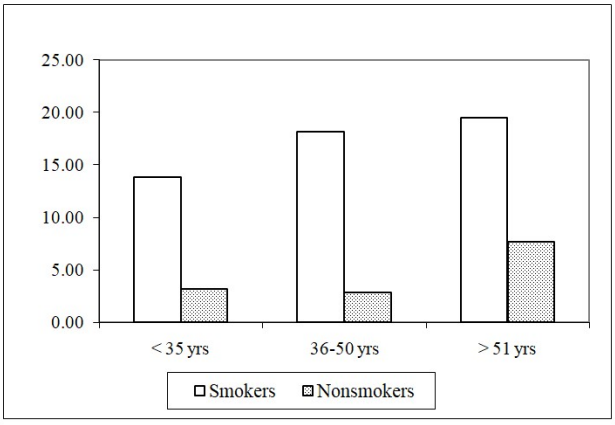

Figure 9. Serum Cadmium content (µg/L)) in Smokers and Non-smokers

Analysis of the data also showed that the number of participants with BMI < 25 was among the smokers compared to non-smokers.The changes in diastolic and systolic blood pressure in smokers and non-smokers in different age groups are given in Figure 1 and 2 respectively. Diastolic and systolic blood pressure of smokers was found to be more than that of non-smokers in all the age groups studied. The number of subjects with diastolic and systolic blood pressure higher than the normal B.P range was also found to be more in the case of smokers compared to non-smokers.The changes in serum cholesterol, protein and malondialdehyde was given in Figure 3, 4 and 5 respectively. Analysis of the results showed that the concentration of total serum cholesterol, protein and malondialdehyde were high in smokers compared to non-smokers in the same age groups. Statistical analysis showed that there was a significant increase ($p<0.01$) in serum cholesterol and MDA content in smokers compared to non-smokers. In the case of serum protein content there was no significant difference between smokers and non-smokers. Comparison of the data of the smokers and non-smokers in the study population by Chi-square analysis showed that in the case of serum MDA, the odd's ratio is 2.095 and in the case of serum cholesterol, the odd's ratio is 8.889 which are highly significant.The serum sodium and potassium content in smokers and non-smokers are given in Figure 6 &7 respectively. The values of serum electrolytes Na^+ and K^+ was found to be high in smokers of all the age groups compared to that of non-smokers. Serum sodium concentration of 38.9 % smokers studied are greater than 145mM/L and the Odd's ratio is 5.639 which shows that the serum smokers Na content was significantly higher than that of non-smokers. There was no significant change in the serum potassium content of smokers and non-smokers. The changes in serum Cu and Cd were shown in Figure 8 and 9 respectively. The concentrations of copper and cadmium were found to be high in smokers of all age groups compared to that of the non-smokers in the same age groups. Analysis of the results show that there was significantly high cadmium content in smokers (Odd's ratio- 14.667) compared to that of non-smokers in the study population. Comparison of the mean values of copper and cadmium content in smokers and non-smokers by student's "t" test showed that the Cu and Cd content was significantly high ($p< 0.01$) in smokers.The relation of blood pressure and cadmium content in smokers was also determined using Chi-square Pearson correlation analysis. The results of this study show a significant positive correlation between cadmium content and blood pressure in smokers (Pearson Chi-Square value- 3.74).

DISCUSSION

In the present study, the body weight of the smokers in the age group 20-35 years and 36-50 years were found to be less than that of non-smokers or control subjects in the same age groups. Smokers on an average shows 9 kg less body mass than non-smokers in the same age group and this decrease in body weight was may be due to the decrease in appetite caused by nicotine in smokers. Other studies also showed an inverse relationship between smoking and bodyweight (Grunberg, 1990; French and Jeffery, 1995). The body mass indexof smokers were found to be less than that of non-smokers in the age groups 20-35 years and 36-50 years. BMI was used as a measure of body fatness. This study shows that 72.2% of the smokers are lean than the non-smokers in the same age group. The diastolic and systolic blood pressure was found to be high in smokers but not significant compared to that of the non-smokers in the same age groups. These results are consistent with the previous reports (Houterman,2003; Seltzer, (1974)on smoking subjects. Nicotine is known to contract the blood vessels and to release hormones that raise the blood pressure. Smoking and blood pressure in combination increases the risk of heart attack.The mean cholesterol content increases significantly in the serum of the smokers of all age groups compared to that of non-smokers. The carbon monoxide is a byproduct of tobacco smoke that has been found to boost cholesterol. Study by Houterman *et al*.(2003)also showed that cigarette smoking increases total cholesterol and lowers HDL- cholesterol levels. Smokers are more likely to develop a cerebral thrombosis (stroke) than smokers. In the present study, the serum protein concentration also increases in smokers of all age groups and the increase was significant in the smokers in the age group 36-50 years compared to the non-smokers in the same age group. This may be due to chronic infections occurring in smokers. Malondialdehyde is a lipid peroxidation product produced by the oxidative deterioration of polyunsaturated fatty acids in cell membranes by free radicals. Present study shows an increase in malondialdehyde content in the serum of smokers compared to non-smokers in the same age group and the increase in MDA content was significantly high ($p<0.01$). Cigarette smoke is one of the environmental factors that can stimulate free radical mediated oxidative processes in the body. The malondialdehyde measured is an indicator of whole body free radical activity. Studiesby Cheeseman & Slates (1993) also showed that high level of lipid peroxides are associated with diseases like cancer, heart disease, stroke, aging etc. Cigarette smokers have higher rates of *in vivo* and *in vitro* lipid peroxidation. These results support the hypothesis that the atherogenic effects of smoking are mediated in part by free radical damage to lipids (Miller *et al*., 1997).

In the present study the concentration of serum electrolytes, sodium and potassium show an increase in smokers compared to that in non-smokers, but the increase in serum potassium in smokers was not significant. There was a significant increase in serum sodium in smokers compared to the controls and this may result in hypernatraemia in smokers. It may be due to the lack of sufficient water intake owing to a lack of adequate thirst mechanism. The findings by Wannamethee *et al*. (1997) suggest that either raised potassium levels in association with smoking have an influence on the risk of death from non-cardiovascular disease, particularly lung cancer, or a raised serum potassium level is a marker for some other risk factor with associated smoking. Analysis of the heavy metal content in the serum of smokers in this study shows that there was an increase in the concentration of copper and cadmium in the smokers compared to non-smokers. The copper and Cd content was found to be highest in the smokers of age group 35-50 years compared to controls (non-smokers). This may be due to the higher number of heavy smokers i.e. taking 20 or more cigarettes daily, present in this age group. Similar studies (Pocock *et al*.,1998) also showed that 10% of the cadmium content of a cigarette is inhaled through smoking and on an average, smokers have 4-5 times higher blood cadmium concentrations than non-smokers. Ramakrishnan *et al*.(1995) found a significant accumulation of Cd in both the blood and lens of smokers. Therefore, heavy metals even in minute levels can cause health problems and it increases in smokers. Epidemiological evidence (Goyer, 1995) suggest that cadmium exposure causes pulmonary damage such as emphysema and lung cancer. Cadmium exposure enhances the production of oxygen reactive species which may result in increased lipid peroxidation (Zhang, 2019). In the present study also, smokers with high cadmium content showed significantly high malondialdehyde content, the main product of lipid peroxidation. The present study shows a significant positive correlation between cadmium content and blood pressure in smokers. Therefore, the cadmium causes hypertension in smokers. Studies by Perry (1972) noted that the possible role of Cd in hypertension should be considered in the context of environmental factors that modify the risk for development of hypertension.

CONCLUSION

The study showed increase in malondialdehyde content, in the serum of smokers and the cigarette smoke is one of the environmental factors that can stimulate free radical mediated oxidative processes in the body. The present study also showed a significantly high concentration of cadmium in smokers in all age groups. Cadmium exposure also enhances the production of oxygen reactive species which may result in increased lipid peroxidation. The present study also revealed that smoking causes hypertension. The study proves that smoking causes oxidative stress, harms nearly every organ of the body and deteriorates the overall health of human beings.

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