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COMPARATIVE STUDY OF OXIDATIVE STRESS IN CIGARETTE AND BIDI SMOKERS

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ABSTRACT

The aim of the study was to determine the extent of oxidative stress and the antioxidant status in smokers as compared to non-smokers and to know which type of smoking (cigarette or bidi) is more injurious to health. For this purpose 90 male subjects (age group 25-40 years) were studied of which 30 were non-smokers, 30 cigarette smokers, and 30 bidi smokers. Oxidative stress was assessed by estimating malondialdehyde (MDA), a lipid peroxidation product in the form of thiobarbituric acid reactive substances. Antioxidants in the form of superoxide dismutase (SOD), glutathione, vitamin C and E were measured in all the groups. On comparative evaluation, plasma SOD, glutathione, vitamin E and vitamin C levels were significantly decreased (p < 0.001) whereas plasma MDA levels were significantly increased (p < 0.001) in smokers as compared to non-smokers which showed that smokers are exposed to greater risk of oxidative stress. Further it was concluded that bidi smoking produces more oxidative stress as compared to cigarette smoking.

Key Words: Smokers, Cigarette, Bidi, Oxidative Stress, Lipid Peroxidation, Antioxidants

INTRODUCTION

Cigarette smoking is probably the most addictive and dependence producing form of object-specific, self-administered gratification known to man. According to present estimates, tobacco is responsible for causing more than 5 million deaths every year (World Health Organization, 2008). About 19% of tobacco consumption in India is in the form of cigarettes, while 53% is smoked as bidis (Gupta and Asma, 2008). Both smokeless tobacco users and smokers face a higher risk of dying from cardiovascular disease than nonusers. Regular smoking doubles the risk of stroke in men (Asplund *et al.*, 2003). Not only the smoking individual, but surrounding individuals can be harmed by tobacco smoke (Sikorska *et al.*, 2012). It has been estimated that 10^{16} radicals are present in one puff of cigarette smoke (Church and Pryor, 1985). Free radicals can oxidize lipid, protein and carbohydrate molecules, damaging cell membranes and DNA, thereby altering cellular structure and function. Cell membranes are rich sources of polyunsaturated fatty acids (PUFAs), which are readily attacked by oxidizing radicals causing lipid peroxidation (Svingen *et al.*, 1979).

Since some free radical production in human cells is inevitable and because they can be very damaging, defences against the deleterious actions of free radicals have evolved. These are known as antioxidant defences (Halliwell, 2012). Superoxide dismutase (SOD) catalyses the conversion of two superoxide molecules to form hydrogen peroxide and oxygen. Glutathione peroxidase is the enzyme whose role is to safely decompose peroxides. As an electron donor, vitamin C is a potent water-soluble antioxidant in humans. Vitamin E is a fat-soluble antioxidant that stops the production of reactive oxygen species formed when fat undergoes oxidation.

With the increasing acceptance of free radicals as commonplace and important biochemical intermediates, they have been implicated in a very large number of human diseases including ischaemic heart disease, cancer, diabetes mellitus, cataract, respiratory diseases and ageing (Gomes *et al.*, 2002). Therefore, the present study was undertaken to assess the levels of lipid peroxidation and antioxidants (superoxide dismutase, glutathione, vitamin C and vitamin E) in smokers and non-smokers. At present very little data is available regarding comparison of oxidative stress caused by cigarette and bidi smoking, so our study also evaluated the relative oxidative stress in cigarette smokers as compared to bidi smokers.

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MATERIALS AND METHODS

The present study was carried out in the Department of Physiology, Adesh Institute of Medical Sciences and Research, Bathinda with approval of ethical committee. A total of 90 male subjects in the age group of 25-40 years were selected of which 30 were non-smokers (Group I), 30 cigarette smokers (Group II) and 30 bidi smokers (Group III). The smokers who were smoking 10 or more cigarettes/bidis for more than 5 years were included in the study. The selected subjects, smokers as well as controls, were not on any supplementation such as vitamins or minerals, which may affect oxidative stress. Further, persons suffering from any disease causing oxidative stress like diabetes mellitus, rheumatoid arthritis, malignancy, tuberculosis, hypertension, any metabolic disorder or chronic disease were excluded from the study. Most of the subjects were from Security Department, labourers and Class IV employees of our Institute. A detailed history including the history of smoking was taken and general physical and systemic examination was done.

5 ml of blood sample was drawn from antecubital vein and collected in a heparinized tube and centrifuged. Plasma was separated and was used for assessment of various parameters. Malondialdehyde (MDA) was used as an indicator of lipid peroxidation and was estimated in terms of thiobarbituric acid reactive species (TBARS) by the method of Satoh (Satoh, 1978). SOD activity was assayed based on the method of Marklund and Marklund (Marklund and Marklund, 1974) as modified by Nandi and Chatterjee (Nandi and Chatterjee, 1988). Glutathione levels were determined by the method of Butler *et al.*, 1963). Concentration of vitamin C was estimated in plasma according to the method of Natelson (Natelson, 1971) and concentration of vitamin E was estimated by method of Baker and Frank (Baker and Frank, 1968).

Statistical analysis was carried out by Student's paired't'-test. The data were expressed as Mean \pm SD and the p value < 0.05 was taken as significant.

RESULTS

Table1 shows the demographic profile of subjects under study. There was no significant variation in age or height of controls and smokers though a small decrease in weight was observed in smokers as compared to controls.

Parameters	Group I (Controls) Mean ± SD	Group II (Cigarette smokers) Mean ± SD	Group III (Bidi smokers) Mean ± SD
Subjects (n)	30	30	30
Age (years)	34.34 ± 4.18	33.70 ± 5.15	34.56 ± 4.55
Weight (Kg)	62.97 ± 2.76	57.32 ± 2.35	52.07 ± 2.12
Body Height	165.49 ± 5.87	164.66 ± 6.03	161.88 ± 7.95
No. of cigarettes/bidis smoked	Nil	15.94 ± 3.82	17.35 ± 2.44
per day			
Duration of smoking (years)	Nil	9.12 ± 1.10	8.38 ± 1.94

Table 1: Demographic and clinical data of control and smokers

Table 2: Plasma MDA, SOD, Glutathione, Vitamin-C and Vitamin-E in Study Groups

	Group I	Group II	Group III
Parameters	(Controls ; n=30)	(Cigarette smokers; n=30)	(Bidi smokers; n=30)
	Mean ± SD	Mean ± SD	Mean ± SD
MDA (nmol/ml)	1.61 ± 0.66	3.10 ± 0.41 **	5.13 ± 0.91** ++
SOD (U/ml)	4.47 ± 0.41	$3.06 \pm 0.29 **$	$2.21 \pm 0.33^{**}$
Glutathione (mg/dl)	52.63 ± 2.95	$34.22 \pm 2.98 **$	$22.43 \pm 4.69^{**}$ ++
Vitamin-C (mg/dl)	1.45 ± 0.19	$0.81 \pm 0.20 **$	$0.68 \pm 0.17^{st st}$
Vitamin-E (mg/dl)	1.33 ± 0.29	$1.12 \pm 0.19^{*}$	0.71 ± 0.13 ** ⁺⁺

Statistical comparison was done between: Group I and Group II; Group I and Group III; Group II and Group III.

* p < 0.01, ** p < 0.001 when compared with Group I;

 $p^+ p < 0.01$, $p^{++} p < 0.001$ when compared with Group II

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The amount and duration of smoking were more or less same between cigarette and bidi smokers. Table 2 shows the mean \pm SD of the various parameters studied in smokers and controls. There was a statistically significant increase in plasma MDA levels in smokers as compared to controls. The activities of antioxidant enzymes SOD, Glutathione, Vitamin C and Vitamin E were significantly decreased in group II and III as compared to group I. On comparing Group II with Group III, it was observed that MDA levels were significantly increased while levels of antioxidants were significantly reduced in bidi smokers (Group III) as compared to cigarette smokers (Group II).

DISCUSSION

Tobacco smoke contains >4000 compounds, most of which are well-known sources of free radicals (Church and Pryor, 1985). In our study we observed that cigarette smoking was not associated with a reduction in height. The slightly lower body weights of the smokers were probably secondary to a lower caloric intake in this group than in the non-smoking group. The levels of MDA (which is a product of lipid peroxidation) were significantly increased in smokers as compared to controls. Results of our study are akin to other authors who found increased lipid peroxidation in smokers (Kashinakunti *et al.*, 2011). The free radicals produced by cigarette smoke attack polyunsaturated fatty acids (PUFAs) in the cell membranes which can result in loss of membrane integrity (Esterbauer *et al.*, 1991). The oxidative destruction of PUFAs, known as lipid peroxidation, is particularly damaging because it proceeds as a self-perpetuating chain-reaction (Svingen *et al.*, 1979). The possible endothelial cell injury would lead to a host of changes in the endothelial lining culminating in formation of lesions of atherosclerosis (Santanam *et al.*, 1997). Atherosclerosis would lead to coronary artery and other occlusive peripheral arterial diseases in smokers.

The present study shows that activity of SOD and glutathione were significantly decreased in cigarette and bidi smokers than in controls. Similar reports of decreased SOD and glutathione activities have been reported in smokers by other authors (Yildiz *et al.*, 2002 and Hemalatha *et al.*, 2006). The antioxidant enzyme superoxide dismutase (SOD) serves as primary line of defence in destroying free radicals. SOD reduces the radical superoxide (O_2^-) to form hydrogen peroxide (H_2O_2) and oxygen (O_2). Glutathione being an important cellular reductant helps in repair of oxidized DNA and destruction of oxidized lipids. Oxidized glutathione is converted to reduced glutathione with the help of enzyme glutathione reductase which requires NADPH for its activity. The levels of NADPH may be depleted in smokers because of increased production of lipid peroxides which can be a reason for decreased levels of reduced glutathione (Sarkar, 1995).

We observed a significant decrease in the levels of plasma vitamin C and E in smokers as compared to controls. Other eminent authors also reported a significantly decreased activity Vitamin C and E in smokers (Kashinakunti *et al.*, 2011 and Song *et al.*, 2009). The decrease in the levels of these vitamins in smokers might be due to their increased consumption to counteract the increased oxidative stress and to inhibit membrane lipid peroxidation. It has been found that smoking directly lowers plasma vitamin C concentrations by mechanisms that do not depend on dietary vitamin C intake, such as impaired vitamin C absorption or decreased turnover (Chow *et al.*, 1986). As a water-soluble antioxidant, vitamin C destroys aqueous peroxyl radicals before these destructive substances have a chance to damage the lipids. Vitamin E is the most important lipid-soluble antioxidant and it protects cell membranes from oxidation by reacting with lipid radicals produced in the lipid peroxidation chain reaction (Traber and Atkinson, 2007).

Among smokers groups we observed that bidi smoking produces more oxidative stress than cigarette smoking and that antioxidant defences in the plasma of bidi smokers are more compromised than in cigarette smokers. Because of low combustibility of the tendu leaf wrapper, bidi smokers inhale more often and more deeply, breathing in greater quantities of tar and other toxins than cigarette smokers. The semiquinone components of tar reduce dioxygen forming superoxide radicals and hydrogen peroxide (Borish *et al.*, 1985). The levels of steam-volatile phenol, hydrogen cyanide and benzopyrene in bidis are higher than in cigarettes (Pakhale *et al.*, 1990). This can be responsible for increased production of reactive oxygen species in bidi smokers as compared to cigarette smokers. Bidi smoke raises the risk of oral cancer, cancer of the lung, stomach and esophagus, heart disease, chronic lung disease, asthma and tuberculosis (Rahman and Fukui, 2000).

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Conclusion

It is evident from our study that there is enhanced oxidative stress and decreased antioxidant defences in smokers as compared to non-smokers which can play an important role in the pathogenesis of the diseases most frequently associated with cigarette smoking. Therefore discontinuation of smoking may minimize the risk of smoke related diseases. Also general awareness needs to be created that bidi smoking is more harmful and is not a safer alternative of cigarette smoking.

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