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Research Article

Assessment of Plasma Total Antioxidant Activity in Hypertensive Smokers

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Abstract: Oxidative stress has been found to be involved in the development of endothelial dysfunction leading to hypertension. In addition, smoking has been found to be associated with increased production of free radicals & endothelial dysfunction. The objectives of present study were to ascertain the plasma TAA and erythrocyte malondialdehyde (MDA) levels in normotensive and hypertensive smokers, and to determine their cumulative effect in the development of HT in smokers. In the present study, aforesaid parameters were estimated in 90 subjects (30-60 years), categorized into three groups (30 subjects in each group) depending upon their smoking habit and blood pressure i.e. Healthy non smokers (Control group), normotensive smokers (Group I) and Hypertensive smokers (Group II); and compared it statistically by using student's t- test. Plasma TAA levels were significantly low ($p<0.05$, $p< 0.001$) in Group I and II, as compared to healthy controls where as erythrocyte MDA levels were significantly high in both the study groups ($P<0.05$ & $P<0.001$) with respect to controls. Our findings indicate that alteration in plasma total antioxidant status along with oxidative stress (via MDA production) may be responsible for biomolecular deterioration and disturbance in homeostatic control leading to the etiopathogenesis of HT in smokers. Therefore, preventive approach against smoking along with incorporation of antioxidant rich diet could be effective in reducing the incidence of hypertension amongst smokers.
Keywords: Free radical, Hypertensive smokers, Lipid peroxidation, Total antioxidant activity.

INTRODUCTION

It is well accepted that smoking is an emerging and independent risk factor for the development of HT. However the underlying mechanism is not clear. Smoking has been associated with dyslipidemia, endothelial dysfunction and increased leukocyte activation [1,2]. Moreover, increased production of free radicals in smokers either in combination with these events or alone may lead to the development of secondary complication such as HT and CVD [3,4].

Association of oxidative stress with the etiopathogensis of cardiovascular complications, musculoskeletal diseases and various age related complications are well documented [5,6]. Oxidative stress ensues when large amount of reactive oxygen species are produced in the cells during smoking that can evade or overwhelm the antioxidant protective mechanism of cells and tissues, and produce major interrelated impaired cell metabolism including DNA strand breakage, rises in intracellular free Ca^{2+}, damage to membrane ion transporters and other specific proteins leading to cell death [7]. Prime target to free radicals attack are the polyunsaturated fatty acids in the membrane lipids, causing lipid peroxidation, have been found to be a major event in the production of vascular disorders and other cardiovascular complications. Malondialdehyde is the most abundant among the reactive aldehydes derived from lipid peroxidation. It has been suggested that these aldehydes released from cell membrane and increase the risk of HT not only by disturbing endothelial cells of the blood vessels but also by inducing oxidative modification to the cell and in LDL [8].

These free radicals are efficiently removed by antioxidant defense system, which includes antioxidant enzymes and antioxidants. Total antioxidant activity (TAA) is a complex trait reflecting homeostasis of redox metabolism, affected by the relative contribution of each antioxidant and the stress of oxidative free radicals [9]. TAA may have a significant role in the physiochemical alterations in smokers and received much attention in preventing smoking associated complications such as HT. Interestingly, there is no far conclusive evidence on alteration in plasma total

antioxidant status in relation with lipid peroxidation in normotensive and hypertensive smokers. Therefore, the overall objectives of present study were to ascertain the plasma levels of TAA and erythrocyte MDA levels in normotensive and hypertensive smokers and to determine their cumulative effect in the etiopathogenesis of HT in smokers.

MATERIAL AND METHODS

The present study was male oriented due to low incidence of female smoking in local population and hence the female subjects have been excluded from the study. In the present study, 90 subjects belonged to age group 30-60 years were included of which 30 subjects were healthy normotensive non-smokers (served as controls), 30 subjects were normotensive smokers (smoking 10-15 cigarette per day for about five years i.e. Group I) and 30 subjects were Hypertensive smokers (Group II) having characteristic high blood pressure (>120/80 mmHg) and smoking habit (10-15 cigarette/day).

All subjects were included after taking their informed consent and approval of protocol by the ethics committee of the college. Fasting blood samples were collected in EDTA vial from anticubital veins avoiding venostasis from each subject after collecting the information of age, sex, height, weight, blood pressure and confirmation of smoking habit. Height and weight were measured with subject barefoot and light dressed. The body mass index (B.M.I.) was calculated as B.M.I. = *weight (Kg) / Height (metre^2)*. Obese (B.M.I > 25), alcoholics and subjects taking antioxidants or lipid lowering drugs were excluded from the study. Samples were processed immediately for plasma and serum separation. Plasma total antioxidant activity was estimated spectrophotometricaly by the method involving reaction of standardized solution of iron EDTA complex with hydrogen peroxide, i.e. Fenton type reaction, leading to the formation of hydroxyl radicals. This reactive oxygen species degrades benzoate, resulting in the release of TBARS. Antioxidants from the added plasma cause the suppression of production of TBARS. The reaction was measured spectrophotometrically at 532 nm [10].

Erythrocyte malondialdehyde (MDA) levels were measured as thiobarbituric acid reactive substances, after preparation of hemolysate. In this method, the heat induced reaction of malondialdehyde (MDA) with thio barbituric acid (TBA) in the acid solution forms a trimethine colored substance, which was measured spectrophotometrically at 532 nm [11]. The data from both the study group subjects and controls were expressed as Mean ± SD and compared by using Student's t-test and distribution of probability (P).

RESULT

In the present study, the mean blood pressure and anthropometric indices of the study group subjects are depicted in Table 1.0. The observation made reveal significant changes in the levels of plasma TAA and erythrocyte malondialdehyde (Table 2.0) in Group I and Group II subjects with respect to control group. Plasma total antioxidant activity was found to be significantly low ($p<0.05$ & $p<0.001$) in both the study groups i.e. 26.7 % and 37.06 % low as compared to controls. On the other hand, erythrocyte MDA levels were increased significantly ($p<0.05$ & $p<0.001$) in both the study groups i.e. 31.5 % high in Group I and 38.9 % high in Group II respectively.

Table-1.0: Anthropometry, duration of smoking and blood pressure of control group, Group I and Group II subjects (Mean±SD).

S.No.	Particulars	Control group (n=30)	Group I (n=30)	Group II (n=30)
1)	Age (years)	38.5 ± 6.0	40.0 ± 7.0	42.8 ± 7.0
2)	Height (meter)	1.58 ± 0.05	1.57 ± 0.07	1.60 ± 0.06
3)	Weight (Kg)	56.0 ± 3.4	58.2 ± 4.0	62.8 ± 3.6
4)	B.M.I. (Kg/m^2)	22.40 ±1.6	23.50 ± 2.0	24.4 ±1.5
5)	Systolic blood pressure (mmHg)	104 ± 4.8	112 ± 6.8	132.2 ± 10.4
6)	Diastolic blood pressure (mmHg)	76.0± 2.0	80.2± 3.6	94.0± 6.0

Table-2.0: Plasma Total antioxidant activity (TAA) and erythrocyte Malondialdehyde (MDA) levels in Control group, Group I and Group II subjects. (Mean ± SD)

S.No	Particulars	Control group (n=30)	Group I (n=30)	Group II (n=30)
1)	TAA level (m mol/L)	1.16±0.18	0.85±0.11**	0.73±0.09***
2)	Malondialdehyde (µmol MDA/ml)	1.49±0.12	1.96±0.15**	2.07±0.13***

where, ** P<0.05 : Significant; *** P<0.001 : Highly significant

DISCUSSION

Free radicals that are produced in increased amount in smokers have been implicated in the pathogenesis of many disease process such as HT, CVD and cancer etc. [4,7]. In smokers, excessive production of superoxide radical is occur via activation of NADPH oxidase activity in neutrophils that leads not only the depletion of antioxidant enzymes as reported in previous studies [1,12] but also amplify further deterioration by producing H_2O_2, highly reactive hydroxyl radical, peroxynitrite anion and hypochlorus acid (HOCl). The mechanisms whereby these free radicals may exert cytotoxic effect related to HT development in smokers include damage to cell membrane via lipid peroxidation, endothelial dysfunction and electrolyte imbalance.

Lipid peroxidaion is a deleterious process leading to structural modification of complex lipid protein assemblies associated with cellular malfunction[6]. Prithviraj & Mishra reported that oxidation of LDL inhibits endothelial production of Nitric oxide and prostacyclin, well known vasodilators and inhibitors of platelet aggregation; which reflects the role of lipid peroxidation in vascular disorder leading to hypertension [13]. In the present study, malondialdehyde levels, the most abundant reactive aldehyde derived from lipid peroxidation, were also found to be significantly high in both normotensive and Hypertensive smokers ($P<0.05$, $P<0.001$; Table 2.0) which authenticate the contention that development of HT in smokers is closely associated with lipid peroxidation mediated destruction in cell membranes, ion transporters, subcellular organelles and biomolecules.

Reduction in total antioxidant activity (TAA) indicates the disturbance in the antioxidant defense system of the body, which could be due to decrease in individual antioxidants [9]. In the present study, plasma TAA levels decrease continuously with subsequent increase in blood pressure ($P<0.05$, $P<0.001$) along with erythrocyte MDA levels, in both normotensive smokers and hypertensive smokers which clarify the contributory effect of reduced antioxidant status due to augmented oxidative stress.. Similarly, marked reduction in TAA in smokers of Nepal population and in hypertensive smokers are well documented [14, 15].

CONCLUSION

Our study has suggested that reduction in plasma total antioxidant activity due to their free radical scavenging action; and increased production of MDA may not only be an excellent marker of oxidative stress in smokers but also important for early diagnosis of HT and therapeutic interventions in smokers. Moreover, these events may contribute the development of HT and its related complications in smokers by inducing biomolecular deterioration. Thus, cigarette cessation may prove to be an effective approach in HT prevention and consumption of diet rich in antioxidants should be increased with increase in blood pressure. However, there is a need to carry out these studies in a larger sample size.

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