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Research Article

Plasma Total Antioxidant Activity and Endothelium Dysfunction in Hypertensive Smokers

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Abstract: Although role of oxidative stress in the etiopathogenesis of hypertension (HT) development has been well documented, there is no far conclusive evidence regarding alteration in plasma total antioxidant activity (TAA) and nitric oxide (NO) levels in hypertensive smokers. Aims & objective of the study was to ascertain the plasma TAA, NO, uric acid and erythrocyte malondialdehyde (MDA) in normotensive and hypertensive smokers, and to determine their cumulative effect in disease pathology. Plasma TAA, NO, plasma uric acid and erythrocyte MDA levels were estimated in 120 subjects (30-55 years), categorized into three groups (40 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 and NO levels were significantly low ($p<0.05$, $p< 0.001$) in Group I and II, as compared to healthy controls where as plasma uric acid levels were increased insignificantly ($p<0.1$) in Group I and significantly ($p<0.05$) only in Group II. On the other hand, erythrocyte MDA levels were significantly high in both the study groups with respect to controls. Alteration in plasma TAA and uric acid along with endothelial dysfunction due to culprit effect of oxidative stress (via MDA production) may be responsible for development of HT in smokers. Therefore, change in dietary pattern and community based preventive approach should be encouraged in order to overcome the burden of hypertension as well as harmful effects of smoking.
Keywords: Nitric oxide, Lipid peroxidation, Total antioxidant activity, oxidative stress.

INTRODUCTION

Smoking is a curse to human beings, characterized by various sorts of health related complications including hypertension [1]. Hypertension (HT) is a major cause of cardiovascular disease and an important contributor to morbidity and mortality in general population [2]. Despite various mechanisms, oxidative stress caused by increased production of reactive oxygen species (ROS) such as superoxide anion ($O_2^{\cdot-}$) and its metabolites or by reduced bioavailability of antioxidant defenses in smokers, forecasting a grim scenario for the evolving HT and CVD complications.

ROS attack on polyunsaturated fatty acids (PUFA) in the membrane lipids and brings about lipid peroxidation in smokers, which is characterized by production of variety of end products including reactive aldehydes such as, malondialdehyde, (MDA). 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 [3].

In addition, Nitric oxide (NO), a versatile molecule, is produced in the body by the isoenzyme nitric oxide synthase (NOS) using L-arginine, as a substrate. NO plays versatile roles in both intracellular and extracellular signaling mechanisms and maintains homeostasis of the cell. NO takes part in blood pressure control and regulates vascular tone [4]. Alteration in the levels of NO, a marker of endothelial dysfunction, exerts culprit effect in inducing hypertension and other patho-physiological complications in smokers.

ROS are well controlled by antioxidant defense system of the body. Total antioxidant activity (TAA) including co-operative action of widely recognized non-enzymatic antioxidants may have a

significant role in the regulation of physiochemical alterations during smoking and, received much attention in hypertensive smokers [5, 6]. Amongst various non-enzymic antioxidants, uric acid is an effective antioxidant in plasma as it scavenges superoxide radical, protects erythrocyte against peroxidative damage and free radical attack [7, 8]. However, emerging concepts reveal its relation with circulating inflammatory markers, vascular injury and endothelium dysfunction, and attract the researchers to clarify its role in smokers [9]. In this context, the objectives of present study was to ascertain the levels of plasma TAA and uric acid along with the markers of endothelium dysfunction (NO) and lipid peroxidation in normotensive and hypertensive smokers and to determine their cumulative effect in the etiopathogenesis of HT in smokers.

MATERIAL AND METHODS

In the present study, 120 subjects belonged to age group 30-55 years were included of which 40 subjects were healthy normotensive non-smokers (served as controls), 40 subjects were normotensive smokers (smoking 10-15 cigarette per day for about five years i.e. Group I) and 40 subjects were Hypertensive smokers (Group II) having characteristic high blood pressure (>120/80 mmHg) and smoking habit (10-15 cigarette/day).

Fasting blood samples were collected in plain vial and 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 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 thio barbituric acid reactive substances (TBARS). Antioxidants from the added plasma cause the suppression of TBARS production. The reaction was measured spectrophotometrically at 532 nm [10].

The measurement of plasma NO is difficult because this radical is poorly soluble in water and has a short half-life in tissue (10-60 s), but its half-life may be as long as 4 minutes in the presence of oxygen. For these reasons, the end products of the phenomenon, nitrate and nitrite, are preferentially used in clinical biochemistry. Plasma total nitrate and nitrite levels were measured with the use of Griess reagent as described earlier [11].

Erythrocyte malondialdehyde (MDA) levels were measured as thiobarbituric acid reactive substances, after preparation of hemolysate (12). The heat induced reaction of MDA with thio barbituric acid (TBA) in the acid solution formed a trimethine coloured substance, which was measured spectrophotometrically at 532 nm.

Plasma uric acid levels were estimated by Caraway's method in which uric acid reacted with phosphotungstic acid in alkaline medium forming a blue color complex which was measured at 700 nm [13].

Statistical analysis

The data collected from study group subjects were entered separately in Microsoft Excel sheet of windows 2010 and values were expressed as Mean ± SD. The significance of mean difference between study group subjects was compared by using Student's t test and distribution of probability (P).

RESULTS

In the present study, the mean blood pressure and anthropometric indices of the study group subjects are depicted in Table 1. The observation made reveal significant changes in the levels of plasma TAA, uric acid, NO and erythrocyte malondialdehyde (Table 2) in Group I and Group II subjects with respect to control group. Plasma total antioxidant activity and NO levels were found to be significantly low ($p<0.05$ & $p<0.001$) in both the study groups 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 respectively. However, plasma uric acid levels were increased insignificantly in Group I ($p<0.1$) and significantly in Group II ($p<0.05$) subjects as compared to healthy controls.

Table 1: Demographic profile of control group, Group I and Group II subjects (Mean±SD)

Sl. No.	Particulars	Control group (n=40)	Group I (n=40)	Group II (n=40)
1)	Age (years)	42.6 ± **7**.0	43.5 ± **9**.0	43.0 ± 8.0
2)	Height (meter)	1.56 ± 0.05	1.59 ± 0.07	1.59 ± 0.06
3)	Weight (Kg)	56.5 ± 2.6	58.0 ± 4.2	60.5± 3.0
4)	B.M.I. (Kg/m^2)	23.6 ± 1.8	23.5 ± 2.0	24.2 ± 1.9
5)	Systolic blood pressure (mmHg)	106 ± 4.2	110 ± 6.0	130.5 ± 8.4
6)	Diastolic blood pressure (mmHg)	78.0± 2.5	80.5± 3.0	90.0± 4.0

Table 2: Plasma Total antioxidant activity, Uric acid, NO and erythrocyte Malondialdehyde levels in Control group, Group I and Group II subjects.(Mean ± SD)

Sl. No.	Particulars	Control group (n=40)	Group I (n=40)	Group II (n=40)
1)	TAA level (m mol/L)	1.18 ± 0.15	0.82 ± 0.10**	0.70 ± 0.08***
2)	Uric acid (mg%)	4.5 ± 0.62	5.20 ± 0.95*	5.70 ± 1.05**
3)	NO (µ mol/L)	8.02 ± 1.56	6.40 ± 1.34**	5.15 ± 1.20***
4)	Malondialdehyde (µmol MDA/ml)	2.82 ± 0.25	3.46 ± 0.28**	3.87 ± 0.34***

Where, * p<0.1 : Non-significant; ** p<0.05 : Significant; *** p<0.001 : Highly significant

DISCUSSION

Association of oxidative stress with the etiopathogensis of cardiovascular complications, musculoskeletal diseases and various age related complications are well documented [14-16]. In particular, assessment of risk of hypertension in smokers has renewed the interest of researchers due to common etiopathological events caused by free radicals. In smokers, the most prominent cytotoxic effects related to HT development include damage to cell membrane via lipid peroxidation, electrolyte imbalalnce and endothelial dysfunction [3, 17].

In this context, erythrocyte malondialdehyde levels, the most abundant reactive aldehyde derived from lipid peroxidation, were significantly high in both normotensive and hypertensive smokers in association with significantly altered levels of plasma NO in study group subjects (Table 2) which authenticate the contention that development of HT in smokers is closely associated with oxidative stress characterized by enhanced lipid peroxidation and endothelial dysfunction These findings were in agreement with that of previous studies in smokers, hypertensives and other CVD complications which authenticated the fact that oxidation of LDL inhibits endothelial production of Nitric oxide, well known vasodilator and inhibitor of platelet aggregation; and, thus, exerts the culprit effect of smoking mediated lipid peroxidation in inducing vascular disorders [17-19].

In addition to enhanced MDA production, development of HT due to endothelial dysfunction may be closely associated with alteration in antioxidant defense system as observed in marked depletion of plasma TAA in normotensive and hypertensive smokers. Consisting findings have been documented in a recent study on Indian older population as well as in smokers belonged to Nigeria, who were susceptible to develop HT complications [5, 20]. According to them, reduction in TAA indicates the disturbance in the antioxidant defense system of the body, which could be due to decrease in individual antioxidants including uric acid, as observed in present study. In addition, depletion of plasma TAA in smokers leads to develop various sorts of complications related to cardiovascular health due augmented oxidative stress.

CONCLUSION

Considering the above culprit events and documented evidences of previous studies, the present study authenticates the fact that smoking, an important modifiable risk factor, is injurious to health due to its intimate relation with future HT development via. oxidative stress mediated endothelial dysfunction and biomolecular deterioration as well. Therefore, cigarette cessation may prove to be an effective approach in HT prevention. In addition, antioxidant rich diet should be supplemented with regular check up of blood pressure in order to sustain the smoking mediated HT development.

REFERENCES

1. Mazzone A, Cusa C, Mazzucchelli I, Vezzoli M, Ottini E, Ghio S *et al*.; Cigarette smoking and hypertension influence nitric oxide release and plasma levels of adhesion molecules. Clin Chem Lab Med., 2001; 39(9): 822-826.
2. Bhattacharya I, Saxena R, Saxena R, Lal AM; Alteration in plasma tocopherol levels in the patient with different stages of Essential Hypertension. Sch J App Med Sci., 2014; 2(2D): 812-815.
3. Morrow JD, Frei B, Longmire AW, Gaziano JM; Increase in circulating products of lipid peroxidation in smokers. Smoking as a cause of oxidative damage. N Eng J Med., 1995; 332: 1198-1203.
4. Suneja S, Saxena R, Saxena R, Sharma D, Lal AM; Association between serum paraoxonase and plasma nitric oxide in pre-eclampsia. Int J Med., 2014; 1(1): 19-23.
5. Onyesom I, Osioma E, Ighodayenowho OK; Serum total antioxidant capacity of some nigerian cigarette smokers. JPBMS, 2012; 18 (14):1-2.
6. Saxena R, Jaiswal G; Total antioxidant activity and non-enzymic antioxidants levels in patients with different stages of Essential Hypertension. Saudi Germ Hosp Med J., 2009; 3(1): 2-10.
7. Bhattacharya I, Saxena R, Saxena R, Lal AM; Vitamin E supplementation and the markers of oxidative stress in Indian Acute Myocardial Infarction patients. Asian J Medical Sciences, 2014; 5(2): 46-53.
8. Ames BN, Cathcort R, Schwiers E, Hochstein P; Uric acid provides an antioxidant defense in

humans against oxidant and radical caused aging and cancer. Proc Natl Acad Sci U.S.A., 1981; 78(11): 6858 – 6862.
9. Ward JH; Uric acid as an independent risk factor in the treatment of Hypertension. Lancet, 1998; 352: 670-671.
10. Koracevic D, Doracevic G, Djordjevic A, Cosic V; Method for measurement of antioxidant activity in human fluids. J Clin Pathol., 2001; 54: 356 - 361.
11. Sinnhuber RO, Yu TC, Yu TC; Characterization of the red pigment formed in the thiobarbituric acid determination of oxidative rancidity. Food Res., 1958; 23: 626-630.
12. Moshage H, Kok B, Huizenga JR, Jansen PL; Nitrite and nitrate determinations in plasma: a critical evaluation. Clin Chem., 1995; 41: 892-896.
13. Caraway WT; Standard methods of Clinical Chemistry. Academic press, New York, 1963; 4: 239-240.
14. Sen CK; Oxygen Toxicity and antioxidants: state of the art. Ind J Physiol Pharmacol., 1995; 39(3): 177-196.
15. Saxena R, Bhattacharya I, Saxena R; Susceptibility of Knee Osteoarthritic patients to develop Cardiovascular disease. Asian J Medical Sciences, 2013; 4(3): 62-68.
16. Bhattacharya I, Saxena R, Gupta V; Efficacy of vitamin E in knee osteoarthritis management of North Indian Geriatric population. Therap Adv Musculo Dis., 2012; 4(1):11-19.
17. Gupta V, Saxena R, Bhattacharya I, Thakur RK, Mishra M; Lipid peroxidation mediated electrolyte imbalance and altered antioxidant status in Hypertensive smokers. Saudi Germ Hosp Med J., 2011; 5(1): 7-17.
18. Yanbaeva DG, Dentener MA, Creutzberg EC, Wesseling G, Wouter EF; Systemic effect of smoking. Chest, 2007; 131:1557-1566.
19. Rahkola-Soisalo P , Mikkola TS, Vuorento S, Ylikorkala O, Väisänen-Tommiska M; Smoking is accompanied by a suppressed cervical nitric oxide release in women with high-risk human papillomavirus infection. Acta Obstet Gynecol Scand., 2013; 92(6): 711-715.
20. Saxena R, Mehrotra V; Prediction of hypertension and cardiovascular disease risk in North Indian geriatric population: a conundrum of senescence. Int J Community Med Public Health, 2014; 1(1): 18-23.