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Oxidative imbalance in smokers with and without hypertension

Meera K.S.

Department of Biochemistry, M S Ramaiah Medical College, Bangalore - 560054, Karnataka, India.

Abstract

Cigarette smoking is a leading risk factor for coronary and vascular disease. Smokers are exposed to increased load of reactive oxidants which can promote peroxidation of lipids and lipoprotein resulting in increased arterial pressure. Therefore we intended to determine the ROS mediated endothelial dysfunction by assessing the extent of lipid peroxidation and to study the possible role of erythrocyte catalase activity and serum total bilirubin in smokers with and without hypertension. The study group included essential hypertensive smokers (n=22) and nonsmokers (n=22) and normotensive smokers (n=22) as cases and nonsmokers (n=22) as controls. Fasting blood sample were collected from both cases and controls. Erythrocyte catalase activity, serum total bilirubin, serum malondialdehyde (MDA) level were measured. ANOVA and Pearson's correlation were used for statistical analysis. The present study showed significantly decreased erythrocyte catalase activity and serum total bilirubin was on the higher side of the physiological range. There was a significant rise in MDA levels in smokers with and without hypertension as compared to controls. The present study showed progressive increase in oxidative stress in smokers and hypertensives.

Key Words: Smokers, Catalase, MDA, Total bilirubin.

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Introduction

Cigarette smoking is an established leading risk factor for coronary and vascular disease. Smoking adversely affects the prognosis in patients with previous myocardial infarction or angina pectoris by enhancing the effects of hypertension, hypercholesterolemia and metabolic perturbation of insulin resistance. The acute effects of smoking include transient increase in heart rate, blood pressure, decrease in serum high density lipoprotein level, impaired glucose tolerance and altered insulin sensitivity. [1]

Cigarette smoke is a complex mixture of toxic agents and included among these are free radicals, redox cycling agents, cytotoxic aldehydes and other carcinogens like polycyclic aromatic hydrocarbons, benzpyrenes and nitrosamines. Each puff of cigarette smoke contains more than 10^{14} low molecular weight free radicals which can directly or indirectly initiate and propagate lipid peroxidation [2]. Cigarette smoking and hypertension increase the predisposition for the development of atherosclerosis and its clinical complications. A dysfunctional endothelium is due to reduced nitric oxide [NO] availability and increased production of reactive oxygen species (ROS) like superoxide ion (O2−) and hydrogen peroxide (H_2O_2).They are considered an early indicator of atherothrombotic damage and of cardiovascular events [3]. Increased load of reactive oxidants promotes peroxidation of lipids and lipoproteins. Several defense mechanism exists which can reduce the damages brought about by the ROS. These defense mechanisms are crucial to reduce the detrimental effect of ROS and preserving the cellular function at the optimum. ROS are unstable and have very short life span, therefore by products of lipid peroxidation or depletion of endogenous antioxidants have been used as a marker of free radical generation.

Serum malondialdehyde (MDA) a three carbon compound reflects both autoxidation and oxygen mediated peroxidation of poly unsaturated fatty acids in particular. It reflects the oxidative status of the biological system. MDA causes damage to low density lipoproteins (LDL) which in turn can be taken up by macrophages via scavenger receptors and forms foam cells. Due to increased production of ROS and increased oxidative stress, lipid peroxidation products are found to be elevated in smokers [4]. Catalase (E.C.1.11.1.6) is a major antioxidant defense component directly catalyzing the decomposition of H_2O_2 toH_20 and sharing the function with glutathione peroxidase. Increased erythrocyte catalase activity is found in smokers and hypertensives. Bilirubin, the downstream product of heme degradation has a very effective antioxi-

dant and anti inflammatory properties. The antioxidant properties of bilirubin are responsible for reduced risk for cardiovascular disease in individuals with slightly increased serum bilirubin [5].

Therefore we intended to determine the ROS mediated endothelial dysfunction by assessing the extent of lipid peroxidation and study the possible role of erythrocyte catalase activity and serum total bilirubin in smokers with and without hypertension.

Materials and Methods

The study population consisted of 88 males who were grouped as follows:
Group 1: 22 normotensive nonsmokers as controls
Group 2: 22 normotensive smokers
Group 3: 22 nonsmokers with Essential hypertension
Group 4: 22 smokers with Essential hypertension

These are the individuals who visited M S Ramaiah medical college teaching hospital, Bangalore. The study was approved by institutional Ethical board. The clinical history of the study population was taken including details of personal habits like smoking and alcohol intake. The number of cigarette smoked per day varied in smokers but in all cases were above 8 cigarettes per day and the reported length of smoking was greater than 12 months. Newly diagnosed essential hypertensive patients recruited for the study had diastolic pressure greater than 90 mmHg and/or systolic pressure greater than 140 mmHg. Secondary form of hypertension was excluded by routine diagnostic procedures. The study groups were not on any drug regimen like anti-hypertensives, lipid lowering drugs, antibiotics, NSAID group of drugs, multivitamins and antioxidant supplementation. Patients with diagnosed diabetes mellitus, cardio-vascular disease, impaired renal function, gastrointestinal, liver diseases and other chronic diseases were excluded from the study.

Blood samples were collected after overnight fasting in appropriate vacutainers. Hemoglobin was determined immediately after collecting whole blood sample by Drabkins method. The serum and the erythrocyte sediments were separated and various parameters were analysed. Erythrocyte catalase activity was assayed in hemolysate by the UV-method described by Aebi[6].The catalase activity was expressed as k(rate constant of first order reaction, absolute activity) and k/gmHb(specific activity).Serum MDA,TBA-reactive substance was estimated using 0.67% TBA and 40% TCA. The pink color adduct was measured spectrophotometrically at 530 nm. The MDA content was calculated using the molar extinction coefficient coefficient $1.56x10^5$[7]. Serum total bilirubin

Statistical Analysis
The results are expressed as Mean±S.D. ANOVA and Post hoc tukey test were used for statistical analysis. Pearson's correlation coefficient was calculated and for all determinants $p<0.05$ was considered significant. All statistical analysis was performed using SPSS 15.0 version software.

Results

The age distribution of the various subjects studied are shown in Table1,with normotensive nonsmokers (Group I), normotensive smokers(Group II), Essential hypertensive nonsmokers(Group III) and Essential hypertensive smokers(Group IV).There was not much difference in the mean age between the various study groups.

The mean systolic and diastolic blood pressures in normotensive smokers were higher than normotensive non smokers. Similarly, Essential hypertensive smokers had higher systolic and diastolic blood pressure than Essential hypertensive nonsmokers as shown in Table2. Erythrocyte catalase activity was significantly reduced and serum MDA level was significantly raised in smokers and non smokers with hypertension. Normotensive smokers had increased MDA levels and reduced catalase activity as compared to normotensive non smokers. As compared to group I total bilirubin gradually increased and hemoglobin gradually decreased in all other groups. (Table 2).Pair wise comparison shows a significant difference in diastolic pressure between Group I and Group II. However, there is a significant difference in both systolic and diastolic pressure between Group I and Group III, Group I and Group IV, Group II and Group III and between Group II and Group IV. There was significant reduction in erythrocyte catalase activity and significant increase in S.MDA and total bilirubin in hypertensive smokers as compared to normotensive smokers and non smokers. There was increase in S.MDA and total bilirubin in hypertensive smokers as compared to nonsmoker hypertensive. Hemoglobin was reduced in cases as compared to controls (Table 3).

Significant correlation was found between MDA and systolic blood pressure in hypertensive smokers($r=0.454$, $p<0.05$) (Fig 1).There was positive correlation between catalase activity and systolic blood pressure in hypertensive smokers ($r=0.474$, $p<0.05$)(Fig 2). Similarly there is inverse correlation between total bilirubin and diastolic blood pressure in normotensive smokers ($r=-0.473$, $p<0.05$) (Fig 3) and positive correlation between MDA and diastolic blood pressure($r=0.514.p<0.05$). There is negative correlation between serum MDA and erythrocyte

catalase activity in normotensive smokers (r= -0.543, p<0.05) (Fig 4).

Table 1. *Age distribution of subjects studied*

Age in years	Group I		Group II		Group III		Group IV	
	No	%	No	%	No	%	No	%
21-30	0	0.0	2	9.1	0	0.0	0	0.0
31-40	11	50.0	10	54.5	1	4.5	3	13.6
41-50	7	31.8	8	36.4	12	54.5	9	40.9
51-60	4	18.2	2	9.1	8	36.4	8	36.4
61-65	0	0.0	0	0.0	1	4.5	2	9.1
Total	22	100.0	22	100.0	22	100.0	22	100.0
Mean ± SD	42.55±7.68		40.05±7.47		48.68±6.94		50.36±7.89	

Table 2. *Mean and SD of SBP, DBP , Catalase , MDA , T.Bilirubin and Hb*

Variables	Group I	Group II	Group III	Group IV	Significance
SBP (mm Hg)	122.18±6.47	126.45±5.38	153.14±8.91	157.82±13.32	F=88.694; P<0.001**
DBP (mm Hg)	77.18±6.64	82.09±3.68	93.82±3.59	96.45±6.29	F=68.153; P<0.001**
Catalase (k/gm Hb)	128.67±21.56	119.99±20.5	87.03±21.3	81.12±19.25	F=28.753; P<0.001**
MDA (nmoles/dl)	92.23±20.80	106.56±25.23	238.47±41.56	268.4±45.58	F=146.117; P<0.001**
T.Bilirubin (µmoles/dl)	8.84±1.92	9.14±1.69	10.33±1.8	11.76±2.09	F=10.971; P<0.001**
Hb (gm %)	11.85±1.2	11.22±1.18	10.81±1.34	10.54±1.43	F=4.310; P<0.001**

Table 3*: Pairwise comparison of SBP , DBP , Catalase, MDA , T.Bilirubin and Hb between groups*

Variables	I-II	I-III	I-IV	II-III	II-IV	III-IV
SBP (mm Hg)	0.404	<0.001**	<0.001**	<0.001**	<0.001**	0.322
DBP (mm Hg)	0.014*	<0.001**	<0.001**	<0.001**	<0.001**	0.348
Catalase (k/gmHb)	0.507	<0.001**	<0.001**	<0.001**	<0.001**	0.779
MDA (nmoles/dl)	0.527	<0.001**	<0.001**	<0.001**	<0.001**	0.028*
T.Bilirubin (µmoles/dl)	0.952	0.050*	<0.001**	0.163	<0.001**	0.063+
Hb (gm%)	0.365	0.043*	0.006**	0.720	0.310	0.901

Numbers are P values obtained Post-hoc Tukey test

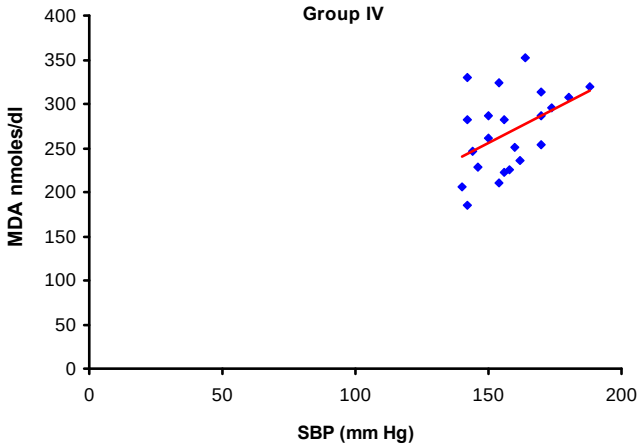

***Figure 1.**: Correlation between systolic blood pressure and S. MDA in Group IV*

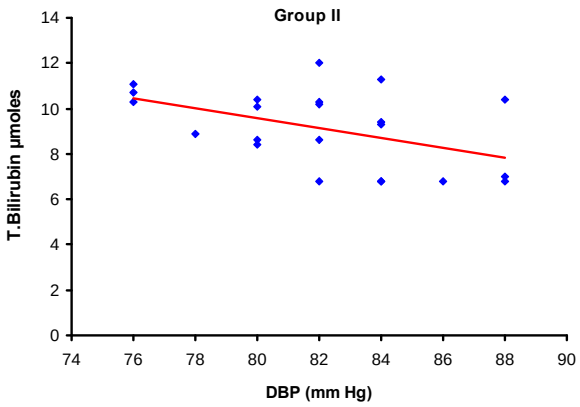

***Figure 3.** Correlation between diastolic blood pressure and S. Total bilirubin in Group II.*

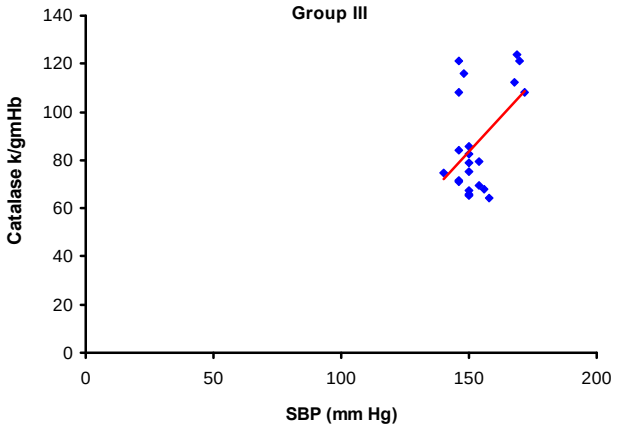

***Figure 2.** Correlation between systolic blood pressure and erythrocyte catalase activity in Group III.*

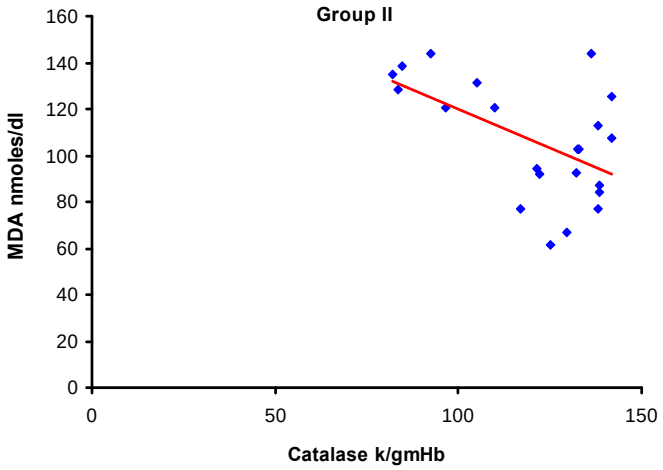

***Figure. 4.** Correlation between erythrocyte catalase activity and S. MDA in Group II*

Discussion

Cigarette smoking is associated with increased production of ROS which in turn can initiate lipid peroxidation and proceed as self perpetuating chain reactions. An increase in ROS generation especially reduces the bioavailability of NO by inactivating it and consequently increasing the vascular tone and blood pressure [8]. The other mechanism by which smoking can contribute to the elevation in arterial pressure includes α 1-adrenoreceptor mediated vasoconstricition, vasopressin release and direct toxic effect on endothelial cells by reducing prostacyclin production and increasing leucocytes adhesion to the endothelial cells ; which can predispose to the development of hypertension over a period of time [9,10].

Erythrocyte catalase activity is significantly reduced in both hypertensives and smokers. But the reduction is more marked in hypertensive smokers (Table 2). An increase in ROS generation especially O2− by endothelial and vascular smooth muscle cells results in oxidant damage of the tissues. Erythrocytes in blood act as a sink for H_2O_2 and O2− generated in tissue. CAT also protects erythrocytes against H_2O_2 which is generated by the dismutation of O2− and by auto oxidation of hemoglobin. CAT has higher Km for H_2O_2 and becomes more important at higher concentration of H_2O_2 than glutathione peroxidase during increased oxidative stress [11].The possible mechanism for decrease in CAT activity may be due to inhibition of the enzyme by O2− by generating ferroxy catalase, which does not decompose H_2O_2 rapidly thereby resulting in further damage to cells. The resulting increase in H_2O_2 concentration can inactivate superoxide dismutase leading to higher O2−.levels. The increase of O2− increases arterial pressure by inactivating NO and producing peroxy nitrite, a stronger and relatively long lived oxidant which is cytotoxic and can initiate lipid peroxidation without the requirement of transition metals[8][12]. The reduced capacity of CAT and superoxide dismutase to neutralize ROS results in increased generation of hy-

droxyl radical, which initiates the peroxidation of polyunsaturated fatty acids. Cigarette smoke contains peroxyl radical and acetaldehyde. Increased concentration of peroxyl radical induces lipid peroxidation and acetaldehyde is found to deplete the cells of reduced glutathione making the cells more vulnerable to peroxidative damage [13].

Smoking increases endothelial angiotensin II production. Angiotensin II activates NADH/NADPH oxidase and protein kinase C activity in vascular cells thereby increasing O2− production and decreasing NO availability which also may attribute to endothelial dysfunction[8][14]. MDA, a marker of oxidative stress due to increased peroxidation of lipids is significantly increased in both smokers and hypertensives in the present study. However, it is increased three-fold in smokers with hypertensives as compared to normotensives. The increased production of MDA may be due to increased formation of reactive oxidants by smoking. Peroxidation of lipids brings about changes in the molecular structure of the lipids and these changes becomes more marked when the damaged lipids are the constituents of the biological membrane disrupting the cohesive lipid layer arrangement and structural organization. The lipid peroxides in general, enhances prostaglandin synthesis which is another source of free radical and associated decrease in NO production are well known risk factor for atherosclerotic complication [15]. Increased serum MDA in hypertensives suggests an association between increased oxidative stress. The slight increase of MDA level in normotensive smokers as compared to non-smokers indicates the non specific nature of MDA as a marker of any disease.

The significant positive correlation between both diastolic and systolic blood pressure with MDA in hypertensive smokers suggests the role of lipid peroxidation in causing endothelial dysfunction and increasing arterial pressure. The inverse correlation between MDA and erythrocyte catalase activity can be explained by the fact that as catalase activity is reduced, lipid peroxidation is favored and consequently serum MDA level raises. The increased MDA level further inactivates antioxidant enzymes like catalase and superoxide dismutase under oxidative stress.

Bilirubin by virtue of its radical scavenging property reduces the risk of cardiovascular disease. Its antioxidant activity and cardio protective potential are attributable to both unconjugated and conjugated bilirubin[16]. Bilirubin acts as co-antioxidant with α-tocopherol and inhibits oxidation of LDL. Smoking reduces the antioxidant potential of bilirubin by oxidant damage and hence erasing some of the beneficial effect of bilirubin [17]. However, in the present study high bilirubin levels (in the upper limit of the reference range) as found in smokers and hypertensives can be reasoned out as due to increased induction of heme oxygenase (HO-1). HO-1 can be induced by heme cytokines, oxidative stress and others [16,18]. Heme primes the endothelial for oxidant damage by the release of catalytically active iron into the aqueous environment of the tissue. Iron, a transition metal can generate O2− and other ROS. Increased expression of HO-1 in endothelial and smooth muscle cells generate bilirubin which renders protection against oxidants. The induction of HO-1 is even more beneficial when catalase and superoxide dismutase activity is compromised or glutathione levels are reduced. The increased level of bilirubin within physiological limits can reduce arterial pressure by scavenging O2− in the vasculature, inhibiting NADPH oxidase and protein kinase C activity [19]. The other possible mechanism by which HO-1 induction can reduce arterial pressure is by decreasing vasculature resistance by the HO-driven carbon monoxide. Ju chin et.al has reported a negative correlation between bilirubin and the incidence of hypertension [19]. In the present study a negative correlation was found between bilirubin and diastolic pressure in smokers. In the other groups significant correlation was not found between bilirubin and arterial pressure which may be reasoned out, as some of the other antioxidants may have been used up which may have sparing action on lipid soluble antioxidants.

Hemoglobin is found to be reduced in both smokers and hypertensives as compared to normotensives (Table2).This can be explained as either due to poor diet or smoking or both inspite of high bilirubin level, the antioxidant role of bilirubin in the present study appears to be ineffective as shown by elevated serum MDA level. The elevation of MDA levels may be due to increased ROS in smokers and hypertensives. The decrease in erythrocyte catalase activity leads to ineffective breakdown of H_2O_2 which can further increase lipid peroxidation and impair endothelial dysfunction thereby initiating the process of atherogenesis.

In conclusion, the present study indicates marked increase in ROS production as reflected by elevated MDA levels with concomitant decrease in erythrocyte catalase activity in smokers and hypertensives. Increase in total bilirubin level within physiological limits is not able provide defense to cellular damage by ROS in smokers and hypertensives. Smoking further compounds the endothelial dysfunction and oxidative stress associated with hypertension. However, additional studies using larger sample size with the inclusion of various other antioxidants which contribute to the radical trapping antioxidant parameter (TRAP) and other clinically relevant endothelium dysfunction markers are needed to substantiate these studies.

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