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ASSOCIATION OF OXIDATIVE STRESS WITH ESSENTIAL HYPERTENSION IN ASIAN INDIANS

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ABSTRACT

In India, a large group of people with hypertension age between 40-60 years. We aimed at assessing the relationship between essential hypertension and oxidative stress in this age group. The plasma levels of Malondialdehyde, Catalase and erythrocyte Superoxide dismutase were determined in 210 patients with essential hypertension and 149 healthy control subjects, using standard procedures. Levels of malondialdehyde and oxidant/antioxidant ratio was significantly higher in patients compared to controls. The same increased with age (>50/<50 years) in patients, but not in controls. The increase in oxidative stress might be contributed by decline in the levels of Superoxide dismutase and Catalase activity in patients. This study, first of its kind, from India suggested that higher level of oxidative stress is prevalent in patients with essential hypertension even at age group below 60. The study also suggests that people who do not develop hypertension upto 60 years of age have been safe because they constitutively possessed more effective mechanisms to keep the levels of oxidative stress at a lower level with increasing age. Thus oxidative stress is a key risk factor for hypertension irrespective of age.

Key words: Antioxidant status, Catalase, Essential hypertension, Malondialdehyde, Oxidative stress, Reactive oxygen species, Superoxide dismutase.

INTRODUCTION

Hypertension is a major risk factor for coronary artery disease. Complex gene-environment interactions appear to underlie the pathophysiology of hypertension. In India, epidemiological studies show a steadily increasing trend in the prevalence of Coronary artery diseases (CAD) and hypertension over the last 40 years, more in urban than in the rural areas [1]. Over 90% of the patients with hypertension suffer from essential hypertension [2]. Etiology of the disease remains far from clear. Romero *et al*.,, [3] has hypothesized that high blood pressure is a pathological state associated with oxidative stress. Oxidative stress is caused due to imbalance in the generation of the reactive oxygen species (ROS) or deficiency in ROS scavenging mechanisms [4]. However, it has never been clearly established whether the enhanced oxidative stress observed in those conditions is primary or secondary to the pathological process. ROS includes oxygen free radicals, nitrogen free radicals, lipid peroxides, and non-radicals like hydrogen peroxide and hypochlorous acid [5]. Several factors inherent to hypertensive status, such as enhanced activity of angiotensin II, hyperinsulinemia, and increased NADPH oxidase activity have been implicated in inducing altered oxidative state in hypertension [6]. Mechanisms by which ROS may effect hypertension is being increasingly understood. Oxidative stress has been seen to promote the entry of Ca^{2+} into vascular myocytes, stimulating neointimal hyperplasia and vasoconstriction [7]. An age-based analysis of hypertension prevalence in Americans documented that over 60% of the non-Hispanic and Hispanic patients with hypertension

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belonged to the age group of 60 years and above [8]. Another survey from India revealed that 60-70% of the patients of hypertension were aging >60 years [9]. Oxidative stress increases with age and is associated with age related disorders including hypertension [10]. Disturbance of free radical homeostasis is a major reason for increased oxidative stress. Unsaturated fatty acids are early targets of free radical action. Free radicals attack the double bond to produce malondialdehyde (MDA), which propagates oxidative chain reactions. Amongst different antioxidant mechanisms, superoxide dismutase (SOD) and Catalase play important role in diminishing the levels of free radicals [7,9,11]. Though the prevalence of hypertension is highest in people aging above 60, a large number of patients lie in the age group 40-60years [1,9]. Level of oxidative stress and its relationship with essential hypertension in this age group, however, was less investigated. Such an investigation is important to assess whether imbalance of free radical homeostasis is a key factor contributing to hypertension in a large section of patients suffering from this disease. With no data available from India, in this investigation, we compared the levels of MDA, SOD and Catalase activity, and their correlations in healthy individuals and patients with essential hypertension to assess the association of oxidative stress with the disease in the individuals, age group 40-60 years.

METHODOLOGY

210 patients with essential hypertension were recruited from the Department of Cardiology, AIIMS, New Delhi, INDIA. Inclusion criteria of hypertensive patients were (i) Age 40-60 years (ii) B.P. > 140 mm Hg Systolic and or > 90 mm Hg diastolic, (iii) visiting the hospital for the first time with no previous treatment. 149 control subjects were of similar age and sex of the patients. Selection criteria of controls were – (i) age 40-60 years (ii) B.P. < 140/90 mm Hg. Blood Pressures were measured using Mercury sphygmomanometer and JNC 7 criteria were followed for the diagnosis of essential hypertension. Approval of ethical committee of All India Institute of Medical Sciences, New Delhi was obtained, and its guidelines were followed.

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Estimation of Catalase, MDA and SOD activity

Plasma Catalase activity [12], MDA, [13] of the study subjects was determined by standard methods and the concentration of SOD in erythrocytes was measured by kit (R&D Systems, Abington, UK).

Oxidant /antioxidant ratio

Ratios of the mean values for MDA/SOD and MDA/Catalase were determined.

Statistical analysis

The levels of parameters in the study groups are presented as mean ± S.D. using student's t-test, a value of $p<0.05$ being considered significant. The spearman's rank tests were used to analyze any correlation. Logistic regression analysis regressed for age and sex was performed at 95% confidence interval to determine the antioxidant status. All computations were carried out with STATA program, version 8.0.

RESULTS

Table 1 represents the baseline characteristics of the study groups. Average age of the controls (N=149) and patients (N=210) was comparable. Study subjects were further divided in two categories, age ≤50 and age >50. The number of males was higher than females in both the groups. The individuals who smoked heavily were categorized under smokers. Number of smokers was significantly higher in patients as compared to controls.

Table 2 displays the plasma MDA, Catalase and erythrocyte SOD levels in the patients and controls. Levels of MDA were significantly higher (+40%) in patients as compared to controls. The mean value for SOD activity was lower by 60%, in patients as compared to controls. The mean value for Catalase activity was lower by 41%, in patients as compared to controls. Further analysis was done on the age related variation in the levels of MDA, SOD and Catalase in patients and controls.

The ratio of MDA to SOD activity was higher in controls of age group ≤50 years as compared to those in the age group >50 years. The ratio of MDA to SOD activity was 2.7 times higher in patients of age category ≤50 years as compared to controls.

The ratio of MDA to Catalase activity is lower in controls at the age group >50 years as compared to age group ≤50 years. The MDA / Catalase activity ratio was 1.5 times higher in patients representing age group >50 years in comparison to those aging ≤50 years. This ratio was 3.8 times higher in patients representing the age group >50 years as compared to controls.

Table 3 represents the correlation studies among different parameters (plasma Catalase activity and MDA, erythrocyte SOD activity) using Spearman's rank correlations. Highly significant inverse correlations were found between levels of MDA and NO in both patients (N=210) and controls (N=149).

Table 4 represents logistic regression analysis of various parameters in the study subjects. Multivariate logistic regression analysis (regressed for age, sex and smoking status) reveals that MDA was at 1.3 times higher odds (OR at 95% C.I) to develop essential hypertension.

Table 1.Baseline characteristics of the study subjects

Parameters	Patients [210] Mean/ no., (%)	Controls,[149] Mean/ no., (%)	P value
Age, years	48.2+-4.6	48.9+/-4.9	0.11
≤50	123, (58.6)	86, (57.7)	0.87
>50	87, (41.4)	63, (42.3)	
Gender,			
Male	155, (73.8)	118, (79.2)	0.24
Female	55, (26.2)	31, (20.8)	
Blood pressure,			
Systolic, mm Hg	147.9±14.7	120±3.3	<0.001**
Diastolic, mm Hg	96±8.5	80.6±2.6	<0.001**
Smokers	67, (41.9)$^{\uparrow\uparrow}$	36, (24.2)$^{\uparrow}$	<0.001**

Patients groups were compared with controls with t-test of significance
or by chi-square test. Data are available only in $^{\uparrow}$137 and$^{\uparrow\uparrow}$176 samples.

Table 2. Age related variation in the levels of MDA, Catalase and SOD in the study subjects

Parameters	Patients		Controls		
	N	Mean ±SD	N	Mean ±SD	p-value
MDA (µM)					
Total	210	5.9±3.5	14	4.2±3.4	<0.001
Age , years ≤ 50	123	5.4±3.7	9	4.8±3.8	0.062
>50	87	6.5±3.0	86	3.4±2.5	<0.001**
p-value		0.034*	63	0.012*	
SOD (units/mg Hb)					
Total		2.1±1.0	14	5.3±2.6	
Age, years≤50	184	2.2±1.0	9	5.3±3.4	<0.001**
>50 years	115	1.9±1.0	86	5.4±2.0	<0.001**
p-value	69	0.2	63	0.9	<0.001**
Catalase (KU/L)			14		
Total	210	75.3±63.8	9	128.4±83.7	<0.001**
Age, years ≤ 50	123	82.6±64.6	86	125.3±70.5	<0.001**
>50	87	65±59	63	132.7±99	<0.001**
p-value		0.046*		0.59	

oxidant-antioxidant Ratio+				
	MDA/SOD		MDA/Catalase	
	Controls	Patients	Controls	Patients
Total	0.79	2.80	0.033	0.078
Age, years	0.9	2.45	0.038	0.065
≤ 50	0.63	3.42	0.026	0.1
>50				

$^{+}$Ratio of mean values; *Significant; ** Highly significant;

Table 3. Correlations among different parameters in the study subjects

Parameters	Patients			Controls		
	Total	≤50 years	>50years	Total	≤ 50 years	>50 years
MDA and NO	-0.227**	-0.27**	-0.25*	-0.211**	-0.2**	-0.19*
MDA and Catalase	-0.27**	-0.37**	-0.03	-0.32**	-0.35**	-0.29*
MDA and SOD	-0.33**	-0.47**	-0.08	-0.23**	-0.17	-0.26*
SOD and Catalase	0.2**	0.21*	0.13	0.14	0.16	0.14

correlation at the significance of *0.05 level and ** 0.01 level.

Table 4. **Regression analysis of the parameters in the study subjects**

Parameters	Odds ratio (95% CI)	p-value
Multiple logistic regression analysis[a]**:**		
MDA	1.33[1.11-1.60]	<0.01**
SOD activity	0.069[0.03-0.16]	<0.001**
Catalase activity	0.97[0.96-0.98]	< 0.001**

[a]after adjusting for age and sex; **highly significant

DISCUSSION

Association of low nitric oxide bioavailability, enhanced activity of the renin–angiotensin–aldosterone system, enhanced levels and endothelin receptor expression, and salt sensitivity have been reported with the essential hypertension [14-15]. Prevalence and association of any of these factors with phenotypic expression of essential hypertension may be different in residents of different geographical, sociocultural background and genetic makeup. Animal studies had suggested association of oxidative stress with endothelial dysfunction and essential hypertension [16]. 60- 70% of the patients suffer from hypertension age >60 years, majority of the remaining patients age between 40-60years [1, 9]. This age group, however did not receive much attention in elucidation of the etiopathogenesis of the disease. In a previous study, we found lower than normal levels of plasma nitric oxide in a sample of northern Indian patients age 40-60 years suffering from essential hypertension [17]. Increased oxidative stress could be a reason for this decline in the levels of NO [18]. In this case-control study, we, therefore, assessed the state of oxidative stress in the same milieu of patients and controls with further expansion of the sample size. We found a highly significant increase in the levels of plasma MDA in patients as compared to controls. This suggested higher levels of free radicals in patients. Earlier Kashyap *et al*., [9] and Russo *et al*., [19] reported higher than normal levels of MDA in patients with essential hypertension and suggested enhanced oxidative stress in the patients. Kumar *et al*., [20] suggested that superoxide anion may affect vascular resistance and can cause vasoconstriction and increase in peripheral resistance. Reports also suggest that Angiotensin II mediated hypertension, to a great extent is mediated by increase in free radicals [18]. During the development of hypertension, free radicals are generated by endogenous sources, notably NADPH Oxidase enzymes and uncoupled nitric oxide synthase [6]. Nitric oxide a potent vasodialator and regulator of vascular tone, scavenges oxygen radicals [21]. In a previous study, we found a significant decline in the levels of NO as compared to controls [16] and suggested higher levels of NO as protective. In this study we did not find any significant difference in the levels of NO in different age groups both in patients and in controls (data not shown).We found a significant negative correlation of NO levels with the levels of MDA. This suggested that increased oxidative stress may partially account for the decline in the levels of NO [18] in the patients with essential hypertension. The cause–effect relationship, however, is not yet clear. Levels of NO in patients and controls were comparable with our previous report [17], hence data not shown. SOD has got predominant role in maintaining free radical homeostasis [22]. We found a significant decline in SOD activity in patients as compared to the controls. This decrease in SOD activity may either be due to increased oxygen-derived free radicals on SOD, as it is known that lower O^{2-} concentration induce the SOD activity while higher oxidative stress and O^{2-} concentrations inhibit the same [20] or due to decrease in the production of superoxide dismutase as had been documented by two previous investigators in separate studies [9,20]. We found a strong negative correlation between the levels of MDA and SOD activity. It has earlier been reported that in-vitro generation of increased superoxide flux results in Catalase inactivation [23-24]. Levels of plasma Catalase activity were also significantly lower in our patients than the controls. Catalase activity correlated very significantly with the levels of MDA. Although the decline in both SOD and Catalase were highly significant in patients as compared to controls, decline in SOD was more marked than Catalase. At present, we do not know whether lower than normal levels of Catalase in patients represents an inherent deficiency of this enzyme or an effect of higher levels of reactive oxygen species. It has earlier been reported that in-vitro generation of increased superoxide flux results in Catalase inactivation [25-26]. It is further stressed that each of the above contributors is influenced by more than one factor.

Since the levels of oxidant/antioxidant are intimately related and oxidative stress is defined as an imbalance in the homeostasis between the two [4], we assessed the level of oxidative stress in our subjects, also, as MDA/SOD and MDA/Catalase ratios. Ratios were calculated from the mean values of these parameters. The above ratios were found highly elevated in patients as compared to controls.

According to Harman's theory, oxidative stress is intensified with the process of aging, and in elderly, this is accompanied by a more common occurrence of essential hypertension [10]. It was interesting to note that levels of MDA were lower in controls of normal healthy individuals aging higher than 50 as compared to those aging ≤50 years. The lower levels of MDA might have conferred protection against hypertension to these individuals even at growing age (>50) which otherwise is associated with higher

prevalence of hypertension. Kashyap *et al*., [9] reported age to be positively correlated with Catalase in normotensives. According to some authors, the activity of antioxidative enzymes in erythrocytes negatively correlates with age in the case of SOD and positively in the case of Catalase [9,11]**.** We, did not find any significant difference in the Catalase and SOD activity in controls at >50 and ≤50 age groups.

Patients at the age group >50 had higher levels of MDA along with lower levels of Catalase compared to individuals with age category ≤ 50 years. The levels of SOD were comparable between the two groups of patients. Decreased SOD and Catalase activity and increased MDA level in the blood of arterial hypertensive patients were observed by Redon *et al*., [27]. Similar report was produced by Kornatowska *et al*., [11]. MDA/SOD ratio was 5.4 times higher in patients age group >50 years and 2.7 times higher in patients, age ≤50 years as compared to their respective controls. MDA/Catalase ratio was 3.8 times higher in patients, age >50 and 1.7 times higher in patients, age ≤50 years, as compared to their respective controls. Thus, the oxidative stress in terms of MDA/SOD ratio and MDA/ Catalase ratio was about 2 times higher in the higher age group (>50 years age) as compared to the lower age group (≤50years) patients.

Kornatowska *et al*., [11] studied the markers of oxidative stress and activity of the antioxidant system in age groups 39±7, 73±8 and 82±8.3 years. Their examination showed that hypertension in the elderly is associated with greater than normal levels of lipid peroxidation and imbalance in antioxidant status (MDA, SOD, Catalase, Glutathione Peroxidase and Glutathione S-transferase). However, only controls represented lower (39±7) age group in that study.

Logistic regression analysis (at 95% C.I.) of our data showed significant association of MDA levels with the disease (O.R. 1.33), SOD (O.R. 0.069) and Catalase (O.R. 0.97) were protective. Despite such associations, we did not find any correlation of MDA, SOD, Catalase with SBP and DBP. Redon *et al*., [27] reported an increase in oxidative stress and a reduction in the activity of antioxidant mechanisms in hypertensive subjects which appeared to be independent of the blood pressure levels. At present, we are not able to explain this phenomena, we can only suggest that since phenotypic expression of blood pressure is influenced by many different factors, a direct correlation of the blood pressure with some of these factors may get masked. Smoking is reported to influence the levels of oxidative stress [28] we did not find any difference in the levels of MDA, SOD and Catalase in smokers in comparison with non-smokers, both in controls and patients. Smoking, however, emerged as an independent risk factor for essential hypertension {O.R. at 95% C.I. 2.26 [1.38-3.69]}. We did not find any significant difference in the levels of different parameters between males and females (data not shown).

Kashyap *et al*., [9] found a negative correlation between some antioxidants like glutathione-S-transferase, glutathione peroxidase (GPx), ascorbic and non-protein thiols with systolic blood pressure, detailed patients profile, however had not been reported.

To the best of our knowledge, ours is the first case-control study to elucidate and demonstrate the prevalence and association of higher oxidative stress (> than normal levels of MDA, < than normal levels of NO, SOD, Catalase) in Asian Indian patients aging, 40-60 years. The oxidative stress, though prevailed in all the patients, increased with the age (≤50/>50), but only in the patients, not in controls. This suggested that people who do not develop hypertension even at the age or 60, have more efficient mechanism to keep the oxidative stress at a lower level with progressing age from 40-60 years. The study also emphasizes that oxidative stress is a major risk factor for hypertension irrespective of age. We however, did not study the people who age below 40. Study also suggests oxidant antioxidant ratio as an appropriate parameter to evaluate the oxidative stress and its association with essential hypertension in the Asian Indians. The above information may suggest a need to monitor the levels of oxidative stress in people at the younger age for timely prevention and management of hypertension.

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