

## OXIDATIVE STRESS IN HYPERTENSION : ASSOCIATION WITH ANTIHYPERTENSIVE TREATMENT

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**Abstract :** There is growing evidence that oxidative stress contributes to the pathogenesis of hypertension. Our aim was to measure oxidative stress in hypertensive subjects, and assess the potential confounding influences of antihypertensive therapy. Serum malondialdehyde and antioxidant levels were estimated in patients at the time of presentation and also after a antihypertensive therapy for 3 months. During the period of study no antioxidant/s was given to the patients and control subjects. Mean blood pressure values were altered in the hypertensive patients following antihypertensive therapy from their respective values observed at the time of presentation. Serum malondialdehyde levels were significantly higher in the hypertensive patients in comparison to control cases. The antioxidant activity of enzymes super oxide dismutase, glutathione and non enzymatic antioxidant levels of vitamins E and C were significantly lower in patients compared to controls. After 3 months of antihypertensive treatment all the above parameters showed reversal in the respective levels of serum malondialdehyde and antioxidant activity. Antihypertensive medications lower the blood pressure and thereby results in reduced oxidative stress which indicates that oxidative stress is not the cause, but rather a consequence, of hypertension.

**Key words :** oxidative damage antihypertensive therapy hypertension

### INTRODUCTION

Hypertension is associated with increased vascular oxidative stress; however, there is still a debate whether oxidative stress is a cause or a result of hypertension. Animal studies have generally supported the hypothesis that, increased blood pressure is associated with increased oxidative stress;

however, human studies have been inconsistent (1). Oxidative stress promotes vascular smooth muscle cell proliferation and hypertrophy and collagen deposition, leading to thickening of the vascular media and narrowing of the vascular lumen (1). In addition, increased oxidative stress may damage the endothelium and impair the endothelium-dependent vascular relaxation

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and increases vascular contractile activity (1). All these effects on the vasculature may explain how increased oxidative stress can cause endothelial dysfunction. Treatment with antioxidant supplements have failed to show any consistent benefit (1). Our aim was to measure markers of oxidative stress in hypertensive patients, and to assess the influence of antihypertensive therapy.

#### MATERIAL AND METHODS

The present study includes 50 cases of hypertensive patients selected from the outpatient Department of Medicine, Sir Sunder Lal Hospital, Banaras Hindu University, and Varanasi according to the criteria of the VI Joint National Committee (2). Clinical assessment included medical history and physical examination. 20 healthy, normotensive individuals matched for age and sex with blood pressure  $\leq 140/90$  mm Hg were selected as a control group. Patients were on  $\beta$ -blockers and diuretics (antihypertensive medication) without any antioxidants for a period of three months. The antihypertensive therapy comprised of 50 mg atenolol combined with 12.5 mg hydrochloro thiazide. Both control and patients were counseled for adequate nutrition through a balanced and varied diet comprising of diet rich in high potassium 7 g/day, low in saturated fat, sugar and salt. Patients as well as controls were advised high potassium food such as fruits, vegetables, and low fat diary foods-low in saturated fat, total fat, and cholesterol with reduced salt and sodium in the diet. Informed consent of each participant was obtained for induction into purely scientific enquiry necessitating blood samples.

#### *Blood pressure measurement*

Blood pressure of the patients was measured with a mercury sphygmomanometer in the sitting position after 5 minutes of rest in a quiet environment following the recommendations of the British Hypertension Society (3). Mean of 3 readings of systolic blood pressure (SBP) and diastolic blood pressure (DBP) (Korotkoff phase I and phase V, respectively) were taken at 5-minutes interval.

#### *Collection of blood sample*

About 7 ml of fasting venous blood sample was collected from each patient as well as control subjects. Serum malondialdehyde level along with enzymatic (superoxide dismutase, glutathione peroxidase) and non-enzymatic (vitamin E and C) antioxidant levels were assayed from the serum of each sample.

#### *Oxidative stress, enzymatic antioxidants and vitamin assay*

Serum malondialdehyde levels in the patients and control were assayed by thiobarbituric acid technique of Philpot (4). Assay of super oxide dismutase (SOD) was based on the ability of the enzyme to inhibit the auto oxidation of pyrogallol (5). The enzyme inhibition caused by the serum was calculated and the enzyme activity was expressed in mg protein/ml of serum. Glutathione peroxidase (GSH) determination was performed using Ellman's reagent (6). The value of GSH was expressed in  $\mu$ M of DTNB conjugated/mg of protein. Assay of vitamin E was estimated using the method of Baker and Frank (7). Serum tocopherol

level was assayed by the technique of Denson and Bower (8). Student's t test was employed for the statistical analyses of data to compare each group. The data were presented as mean  $\pm$  SEM. Pearson's correlation coefficient was used to compare the correlations.

### RESULTS

Fifty cases of hypertension in the age group of  $56 \pm 9$  years were compared with 20 healthy normotensive control individuals. Mean blood pressure of the patients in the studied group at the time of presentation varied from 150/96 to 200/120 mm Hg. Five patients (10%) had mild hypertension and were asymptomatic. 23 (46%) patients had moderate hypertension and 22 (44%) were having severe hypertension in the studied group. Headache was the most common symptom in the hypertensive patients, followed by restlessness, palpitation, dizziness, dyspnoea and easy fatigability. Dyslipidemia was present in 19 (38%) patients. Most of the patients had increased levels of LDL and cholesterol, and decreased levels of HDL.

Before the antihypertensive therapy serum malondialdehyde level was significantly raised in hypertensive patients in comparison to the control cases (Table I). Antioxidant enzyme activity of superoxide dismutase, glutathione peroxidase and non enzymatic antioxidant activity levels of vitamin E and vitamin C were significantly lower in patients (Table I). Antihypertensive medications for a period of three months to the hypertensive patients lowered their blood pressure along with the serum malondialdehyde levels. Also, there was a

TABLE I: Serum MDA level and antioxidant Enzyme activities in control and essential hypertension cases without and with antihypertensive treatment.

Variables	Control (n=20)	Hypertensive Patients (n=50)	
		Pre-treatment	Post-treatment (after 3 months)
MDA (m Mol/L)	0.21 $\pm$ 0.53	0.33 $\pm$ 0.07*	0.23 $\pm$ 0.06#
SOD (mg protein/ml of serum)	20.12 $\pm$ 3.65	6.93 $\pm$ 1.35*	10.66 $\pm$ 2.91#
GSH ( $\mu$ M of DTNB conjugated/ml of protein)	0.13 $\pm$ 0.03	0.05 $\pm$ 0.01*	0.09 $\pm$ 0.02#
Vitamin E (mg/L)	11.84 $\pm$ 1.21	9.58 $\pm$ 1.46*	11.02 $\pm$ 1.44#
Vitamin C (mg/dl)	2.12 $\pm$ 1.81	1.89 $\pm$ 0.23*	1.97 $\pm$ 0.24#

Pre-treatment vs. control \*P<0.01.  
Post-treatment vs. pre-treatment #P<0.01.

decline in the elevated values of superoxide dismutase, glutathione peroxidase, vitamin E and C activity.

### DISCUSSION

The present study demonstrates antihypertensive therapy causes decrease in the blood pressure and serum MDA levels of the hypertensive patients. Following antihypertensive medication there was reduction in the oxidative stress. Similar studies using nonspecific markers of oxidative damage have observed higher superoxide and hydrogen peroxide production in hypertensive subjects, which returned to levels observed for control subjects after blood pressure reduction (9). Russo et al. (10) showed that essential hypertension is

associated with greater than normal lipoperoxidation and an imbalance in antioxidant status, suggesting that oxidative stress is important in the pathogenesis of essential hypertension or in arterial damage related to essential hypertension. Reductions in superoxide dismutase and glutathione peroxidase activity have been observed in newly diagnosed untreated hypertensive subjects compared with control subjects, with superoxide dismutase activity being inversely correlated with blood pressure within the hypertensive group, but not control subjects (11). Higher production of hydrogen peroxide has also been observed in treated and untreated hypertensive subjects compared with normotensive subjects, with a significant correlation between hydrogen peroxide levels and systolic blood pressure (12).

There is growing evidence that increased oxidative stress and associated oxidative damage are mediators of vascular injury in cardiovascular pathologies, including hypertension, atherosclerosis, and ischemia-reperfusion (13). Increased production of superoxide anion and hydrogen peroxide has been demonstrated in experimental and human hypertension. This development has evoked considerable interest because of the possibilities that therapies targeted against reactive oxygen intermediates by decreasing generation of reactive oxygen species and/or by increasing availability of antioxidants, may be useful in minimizing vascular injury and hypertensive end organ damage (13). The endothelial dysfunction associated with hypertension and diabetes leads to an increase in free radical formation and enhances oxidation of low density lipoproteins

[LDL] (14,15). Conventional oxidizability assay is based on measurement of conversion of polyunsaturated fatty acid of LDL to hydroperoxides. Lipid hydroperoxides decompose to form a variety of products including malondialdehyde. Malondialdehyde is used as an indicator of oxidative damage of cells and tissues.

Unlike the findings in animal models, the association between oxidative stress and hypertension in humans is less consistent, and results vary depending on the marker of oxidative damage being investigated (16). Thus, the evidence to support using antioxidants as a blood pressure-lowering agent is limited. It is note worthy that antihypertensive drug therapy, in addition to the blood pressure-lowering properties, also has beneficial effects on both oxidative stress and endothelial function. Treatment with a  $\beta$ -blocker or angiotensin receptor blockers has been shown to reduce both blood pressure and markers of oxidative damage (17). Similarly, other studies have reported beneficial effects on blood pressure, oxidative stress, and endothelial function after treatment with ACE inhibitors (18) or calcium antagonists (19).

In conclusion oxidative stress is associated with hypertension; however, it is unclear whether reactive oxygen species initiate the development of hypertension, or if they are a consequence of the vascular damage observed in hypertension. The potential value of antioxidant supplements to reduce blood pressure via reductions in oxidative stress is limited. In some cases, their use may even be detrimental.

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