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Study of Oxidant (MDA) and Antioxidants (SOD & Vitamin E) in Hypertensive Patients and Normotensive Individuals

Shashi Prabha Singh[1*], Manish Kumar Verma[1], Pratibha Tripathi[1], Durgesh Singh[2]

[1]Department of Biochemistry, Integral Institute of Medical Sciences & Research Lucknow, India

[2]Department of Anatomy, Integral Institute of Medical Sciences & Research Lucknow, India

ABSTRACT- This study was undertaken to evaluate the serum levels of Oxidant (MDA) & antioxidant (SOD & Vitamin E) and compare oxidative stress (MDA) level among normotensive and hypertensive subjects. Oxidative stress has been related to mechanisms of EH (essential hypertension). A total number of 70 subjects were taken, including both sex (Men and Women) between the ages of 35-70 years taken in this study. Exclusion criteria were chronic diseases, alcohol consumer, obesity, smoking/tobacco consumer and current use of any medication. Antioxidant enzyme activity and lipid peroxidation (malondialdehyde) were determined in serum. In 70 subjects out of 35 were found as a controls normotensive individuals and the cases 35 hypertensive patients. Serum MDA levels were highly significantly elevated in hypertensive patients in compared to normotensive individuals (4.39±0.98 µmol/l vs 1.51±0.70µmol/l and $p < 0.0001$). SOD acts as an antioxidant was highly significantly decrease in hypertensive patients in compared to normotensive individuals (0.44±0.06U/mg protein/min vs 0.96±0.04 U/mg protein/min and $p <0.0001$). Vitamin E, which acts as a biomarker of hypertensive was significantly higher decrease in hypertensive in compared to normotensive individuals (0.69±0.08 vs 1.06±0.25 and $p < 0.001$). These findings demonstrate the strong association of SOD and Vitamin E level decrease in hypertensive patients and by MDA level increase in hypertensive patients. Oxidative stress in hypertensive patients, increasing over time may play a role in the improvement of atherosclerosis and cardiovascular disease, should be considered in further research.

Key-words: Hypertensive, MDA, Normotensive individuals, SOD, Vitamin E

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INTRODUCTION

Hypertension (HT) is a major health problem worldwide. Individuals with hypertension are at an increased risk for stroke, heart disease, and kidney failure. Although the etiology of essential hypertension has a genetic component, lifestyle factors such as diet play an important role. The excess of sugar and salt or deficiencies of antioxidant vitamins in diet play a vital role in the etiology of hypertension.

Corresponding Address

*Dr. Shashi Prabha Singh
Department of Biochemistry
Integral Institute of Medical Sciences & Research Lucknow, India
E-mail: dr.spsingh084@gmail.com

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Cardiovascular disease counted 2.3 million deaths in India in the year 1990; this is predicted to double by the year 2020. Hypertension is directly responsible for 57% of all stroke deaths and 24% of all coronary heart disease in India. [1]

Studies demonstrate that hypertension may develop as a result of increased reactive oxygen species [2, 3] and that a variety of antioxidant therapies ameliorate hypertension. Hypertensive effects of oxidative stress are mostly due to endothelial dysfunction resulting from disturbances of vasodilator systems, particularly the degradation of nitric oxide (NO) by oxygen-free radicals. [4,5]

Reduced antioxidant capacity also promotes cellular oxidative stress and is implicated in cardiovascular and renal oxidative damage in hypertension. Superoxide dismutase (SOD) activities are reduced in hypertensive patients. An antioxidant SOD

catalyses the conversion of superoxide radicals to H_2O_2 and O_2 protecting the cells against potential toxicity of reactive oxygen. H_2O_2 is further detoxified by catalase.[6] Hypertension, on the other hand, may lead to tissue damage through lipid per oxidation and other oxidative mechanisms. *In vivo* oxidation of low-density lipoproteins by oxygen-free radicals may increase hypertension-related atherogenesis, and antioxidants may be beneficial in this regard. Studies concerning associations between serum levels of antioxidants and hypertension have been inconsistent. [7] As recommended by the American Institute of Nutrition, the daily amount of vitamin E in humans is 30 IU/d or 0.43 IU/kg per day. Current clinical criteria for defining hypertension generally are based on the average of two or more seated blood pressure readings during each of two or more outpatient visits.[8] Based on the seventh report of the Joint National Committee on prevention, detection, evaluation and treatment of high blood pressure (JNC VII report).

Table-1: Blood Pressure is classified into the following stages:

Category	Systolic blood Pressure (SBP) mm of Hg	Diastolic blood pressure (SBP) mm of Hg
Normal	<120	<80
Prehypertension	120-139	80-89
Hypertension, stage I	140-159	90-99
Hypertension, stage II	≥ 160	≥100

Production of reactive oxygen species is a particularly destructive aspect of oxidative stress. Such species include free radicals and peroxides. Some of the less reactive of these species (such as superoxide) can be converted by oxido-reduction reactions with transition metals or other redox cycling compounds (including quinones) into more aggressive radical species that can cause extensive cellular damage.[9]

METHODOLOGY

The criteria used for selection of both hypertensive and normotensive controls were performed by well-established diagnostic criteria as recommended by 7th Joint National Committee. The present study was conducted on cases of 35 hypertensive patients and was control 35 normotensive. The study was approved by the Institute Ethics Committee, Integral Institute of Medical Sciences & Research Lucknow, India and informed consent was obtained from all the cases and control subjects. A venous blood sample was collected by using disposable syringes. A volume of 3ml of blood is collected by venipuncture under aseptetic conditions in a sterile clot activator/ plain vial from selected subjects by the investigator. Blood in the tube was allowed to clot at room temperature for 10-15 minutes and then centrifuged at (4000 rpm) for approx 2-3 minutes. After centrifugation, supernatant (serum) was collected and split into 3 micro tubes for the study of MDA, SOD and vitamin E. The serum samples were used for the analysis of various parameters:

Estimation of Malondialdehyde by Satoh K. (1978) method [10]

The TCA –TBA-HCl solution was freshly prepared by mixing equal volume of 15% TCA, 0.375% TBA and 0.25N HCl. 0.8 ml of serum +1.2 of TCA-TBA-HCl reagent. Mixed immediately + kept in a boiling water bath for 10 minutes. Cooled +2ml of 1N NaOH (freshly prepared) to eliminate centrifugation. O.D at 535nm against blank, which contained normal saline in place of serum The MDA concentration was calculated according to the following formula: $\mathbf{MDA\ (\mu mol/l) = OD_{532} \times 1.75/0.15}$

Estimation of Superoxide dismutase (SOD) using Nitroblue tetrazolium (NBT) method [11]

The assay mixture contained 1.2ml of sodium pyrophosphate buffer, 0.1ml of PMS, 0.3ml of NBT, 0.2ml of the enzyme preparation and water in a total volume of 2.8ml. The reaction will be initiated by the addition of 0.2ml of NADH. The mixture will be incubated at 30°C for 90 seconds and arrested by the addition of 1.0ml of glacial acetic acid. The reaction mixture will be then shaken with 4.0ml of n-butanol, allowed to stand for 10 minutes and centrifuged. The intensity of the chromogen in the butanol layer was measured at 560nm in a spectrophotometer. One unit of enzyme activity is defined as the amount of enzyme that gave 50% inhibition of NBT reduction in one minute.

One unit of SOD activity is the amount of the enzyme

that inhibits the rate of auto oxidation of NBT by 50% and was expressed as Units /mg protein/min. The enzyme unit can be calculated by using the following equation :

Rate (R) = (final OD - initial OD) / 3 min,

% of inhibition = {(blank OD - R) / blank OD} x 100

Enzyme unit (U) = (% of inhibition / 50) x common dilution factor

[50% inhibition = 1 U]

Specific activity = (U / mg) protein

Estimation of Vit E by Emmoria engel reaction method [12]

1 ml of plasma is thoroughly mixed with 1 ml of redistilled 95% ethanol in a 15 ml centrifuge tube (stoppered). 3 ml of petroleum ether is added to the tube and shaken vigorously for 3 minutes and stoppered. 2 ml of clear supernatant in a clean dry cuvette is taken and the O.D is measured at 450 nm for carotenes. The petroleum ether is evaporated at low temperature (50^0 C) and low pressure. The residue is redissolved in 1 ml of chloroform. 1 ml of 95% ethanol is added, followed by 1 ml of α,α- dipyridyl followed by 0.1 ml of 0.1% $FeCl_3$ exactly after 15 minutes. O.D is read at 520 nm and the concentration is calculated using the formula.

$$\frac{\text{OD at 520 nm} - (\text{OD at 450 nm} \times 0.29) \times \text{amount of standard}}{\text{OD of standard at 520 nm}} \times \frac{100}{\text{volume of test}}$$

Statistical analysis

The results are presented in mean ±SD and percentage. The unpaired t-test was used to compare the study parameters between cases and controls. The pearson correlation coefficient was calculated among the study parameters. P-value <0.05 was considered significant.

Table-2: Comparison of MDA level between cases and controls

Study variable	Controls (N=35)	Cases (N=35)	t- value	p- value
MDA (µmol/L)	1.51±0.70	4.39±0.98	14.1476	<0.0001

Table 1 shows the comparison of MDA level between cases and controls. MDA was significantly (p=0.0001) higher among cases (4.39±0.98) than controls (1.51±0.70).

Table 2: Comparison of SOD level between cases and controls

Study variable	Controls (N=35)	Cases (N=35)	t- value	p- value
SOD(U/mg protein/min)	0.96±0.04	0.44±0.06	42.6615	<0.0001

Table-2 shows the comparison of SOD level between cases and controls. SOD was significantly (p=0.0001) lower among cases (0.44±0.06) as compared with controls (0.96±0.04).

Table 3: Comparison of Vitamin E level between cases and controls

Study variable	Controls (N=35)	Cases (N=35)	t-value	p- value
Vitamin E (mg/dl)	1.06±0.25	0.69±0.08	8.3392	<0.0001

Table-3 shows the comparison of vitamin E level between cases and controls. The vitamin E level was significantly (p=0.0001) lower among cases (0.69±0.08) as compared with controls (1.06±0.25).

Table-4: Pearson correlation coefficient among the study parameters in cases

		MDA	SOD	Vitamin E
MDA	Pearson Correlation	1	.42*	-.039
	Sig. (2-tailed)		.010	.823
	N	35	35	35
SOD	Pearson Correlation	.428*	1	.004
	Sig. (2-tailed)	.010		.984
	N	35	35	35
Vitamin E	Pearson Correlation	-.039	.004	1
	Sig. (2-tailed)	.823	.984	
	N	35	35	35

*. Correlation is significant at the 0.05 level (2-tailed).

Only SOD and MDA was moderately correlated (r=0.42, p=0.01) in cases (Table 4 & Fig. 4).

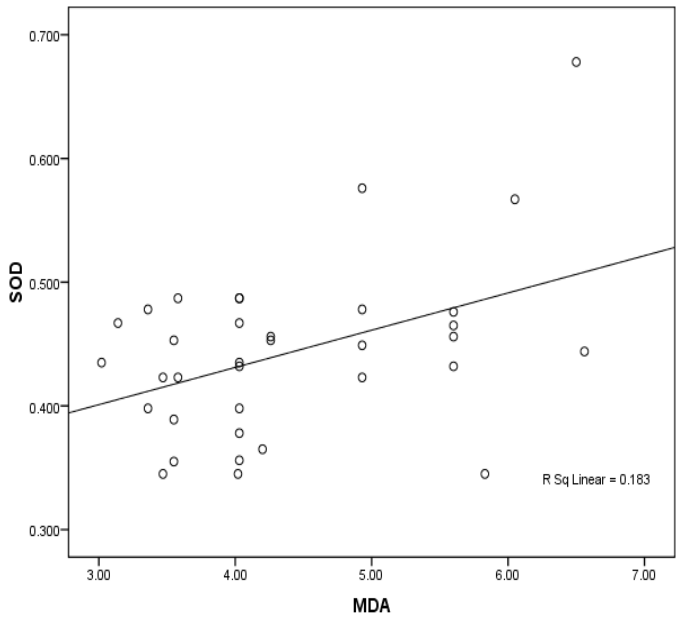

Fig. 4: Scatter diagram showing association between SOD and MDA in cases

Table 5: Pearson correlation coefficient among the study parameters in controls

		MDA	SOD	Vitamin E
MDA	Pearson Correlation	1	-.52**	.104
	Sig. (2-tailed)		.001	.565
	N	35	35	33
SOD	Pearson Correlation	-.52**	1	.124
	Sig. (2-tailed)	.001		.492
	N	35	35	33
Vitamin E	Pearson Correlation	.104	.124	1
	Sig. (2-tailed)	.565	.492	
	N	33	33	33

**. Correlation is significant at the 0.01 level (2-tailed).

Only SOD and MDA was as correlation (r=-0.52, p=0.001) in controls (Table 5 & Fig. 5).

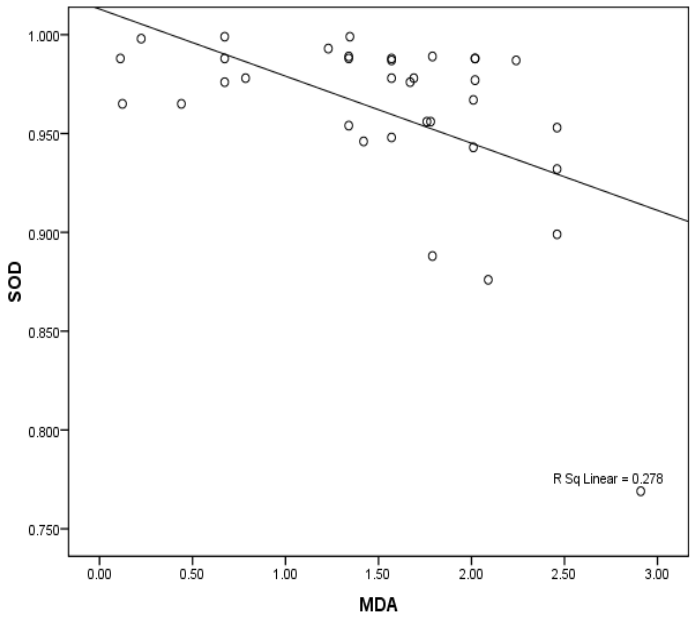

Fig.5: Scatter diagram showing association between SOD and MDA in controls

DISCUSSION

In the present study, MDA was significantly ($p<0.0001$) higher among cases (4.39±0.98) than controls (1.51±0.70) and this was in accordance with that of [13] Similar finding was also reported by [14] in which MDA was significantly higher in hypertensive patients as compared to controls ($p<0.05$). Essential hypertension is associated with increased production of ROS predisposing to increase in lipid peroxidation, which is a marker for cellular damage. An imbalance in the challenge posed by the increased production of free radical mainly superoxide ions or decreased production of nitric oxide may facilitate the development of functional arterial spasm. [15] MDA can exacerbate the actions of superoxide ions by impairing endothelium dependent relaxation and propagation of lipid peroxidation by chain reaction in membranes. Superoxide ions can inactivate calatalse enzyme, resulting in decreased dismutation of hydrogen peroxide, and consequently, increase in H_2O_2 concentration inactivates SOD leading to further rise in MDA levels. [16] The increase in MDA levels further inactivates the antioxidant enzymes (SOD) in untreated hypertension [16, 17] reported the increased damage of various proteins in essential hypertension. The consequences of such oxidative protein damage in hypertension may also be one of the causes for reduced enzymatic activity. SOD was significantly ($p<0.0001$) lower among cases (0.44±0.06) as

compared with controls (0.96±0.04). This finding was similar to [18, 19]. Proteins are another potential target of ROS, whose structure and function can be affected by modification. There are many side chain targets for protein oxidation including cysteine, methionine, and tyrosine. Carbonyls are the oxidation product of proteins and are reported as the potent biomarker of oxidative stress. [20] In the present study, Vitamin E level was significantly ($p<0.0001$) lower among cases (0.69±0.08) as compared with controls (1.06±0.25). This is in agreement with the study by [21] Vitamin E protect against damage to endothelial function from elevated blood sugar, particularly in subjects with hypertension. The endothelium is the lining of blood vessels, which plays an important role in blood vessel barrier function, inflammation, blood clotting, and vascular tone and blood pressure. The Centers for Disease Control and Prevention (CDC) report that approximately 67 million American adults, about one in three, have high blood pressure. [22] One of the limitations of this study was smaller sample size, the studied with larger sample size is being recommended for better interpretation of the results.

CONCLUSIONS

This study has shown that the levels of oxidant namely MDA is significantly increased and the levels of enzymatic (SOD) and non-enzymatic (Vit E) antioxidants are also markedly decreased in hypertensive patient. The increased concentration of oxidant MDA and decreased concentration of antioxidants SOD and Vitamin E, supports the hypothesis that lipid peroxidation is an important causative factor in the pathogenesis of hypertension. Finally, the alteration in the function of endothelium along with antioxidant/pro-oxidant imbalance in hypertension can lead to detrimental consequences and long term adverse effects like atherosclerosis and cardiovascular disease. More extensive study is required to check the association between hypertension and oxidative stress.

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