

## Study of Oxidative Stress in Essential Hypertension

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### ABSTRACT

Essential hypertension is associated with an elevation of reactive oxygen species (ROS) which reacts with membrane lipids to form lipid hydroperoxides that decomposes to form Malondialdehyde (MDA) an indicator of oxidative stress. Endogenous antioxidant enzyme, Superoxide dismutase (SOD) counteracts oxidative stress. This study aims at understanding the role of oxidative stress in essential hypertension. The study comprised of 50 confirmed cases of hypertension and 50 age and sex matched controls. Inclusion criteria includes patients with blood pressure  $\leq$  140/90 mm of Hg, while patients with secondary hypertension, stroke, CAD, MI and diabetes mellitus are excluded. Serum SOD estimated spectrophotometrically by Mishra H.P. & Fridovich I, 1972 method and Plasma MDA by colorimetric method of Satoh K. *et al.* SOD activity was statistically significantly ( $p < 0.0001$ ) decreased while MDA level was statistically significantly ( $p < 0.0001$ ) increased in hypertensives compared to controls. Patients suffering from hypertension have increased ROS activity which oxidizes nitric oxide (NO) and affect vascular tone. Lassegue *et al.* (2004) also found convincing evidence that ROS is an intrinsic part of pathology of hypertension. If oxidative stress is indeed a cause or consequence of hypertension, then reduction in oxidative damage may result in a reduction in blood pressure. Antioxidants like Vit.A, Vit.C & Vit E which are present in vegetables, citrus fruits & oils respectively are able to trap ROS and thus may be capable of reducing oxidative damage and possibly blood pressure. Estimation of oxidative stress markers (SOD & MDA) is simple and inexpensive; it can be used to predict the development of atherosclerotic disease like coronary artery disease, cerebrovascular disease and renal complications associated with essential hypertension. Oxidative stress markers estimation may also be helpful in assessing the usefulness of antihypertensive drugs in prevention of associated complications.

**Key words:** Superoxide dismutase, Malondialdehyde, Reactive Oxygen Species.

### INTRODUCTION

Essential hypertension is one of the most prevalent diseases of developed Western societies and is an unequivocal risk factor for cardiovascular morbidity and mortality. The criteria for hypertension were a blood pressure measurement of systolic blood pressure  $\geq$  140 mm of Hg or Diastolic blood pressure  $\geq$  90 mm of Hg<sup>1</sup>. Recently hypertension has been shown to be associated with oxidative stress, which is involved in enhanced vascular growth, vascular inflammation and impaired endothelium<sup>2</sup>. The mechanisms producing

the oxidative stress status and its contribution to the deregulation of the factors and or mechanisms controlling normal vascular tone, and the implications in hypertension –induced target organ damage by oxidative stress –derived products remain to be known. This study aims at understanding the role of oxidative stress in essential hypertension.

### MATERIAL AND METHODS

The present study entitled “Study of Oxidative stress in Essential Hypertension” has

been done in the Department of Medical Biochemistry, Gandhi Medical College, Bhopal (M.P.) in association with department of Medicine, Hamidia Hospital, Bhopal (M.P.). The study includes 50 hypertensive patients and 50 healthy sex matched controls. 5 ml Fasting Blood sample was collected, 2ml was collected in EDTA vial and the rest 3 ml was collected in plain vial. The blood samples were centrifuged at 3000 RPM for 10 min. After which the serum was separated for the estimation of enzyme Superoxide dismutase (SOD). Plasma was separated for the estimation of Malondialdehyde (MDA).

#### Measurement of blood pressure.

Two readings of BP were measured on the right arm, five minutes apart with a mercury sphygmomanometer (cuff size 12.5 X 40 cm) with auscultator method of BP measurement. BP readings were confirmed in the contra lateral arm at the same time. The SBP and DBP were read to the nearest 2mm Hg. First and fifth phases of Korotkoff's sounds were taken as criteria for SBP and DBP respectively. The average of the two consecutive readings was recorded.

#### Estimation of SOD

SOD was estimated by spectrophotometric method of Mishra H.P. and Fridovich I, 1972<sup>3</sup>. In this method, the assay mixture consists of 0.5 ml sodium carbonate buffer (pH 10.2), 0.5 ml EDTA, 0.5 ml D.W., 0.5 ml adrenaline bi tartarate are added to 0.5 ml serum. After mixing the contents absorbance is

read after every 30 seconds till 5 min at 480 nm (greenish blue filter) by using spectrophotometer. Mean absorbance was calculated. The values are expressed in terms of SOD units/mg.protein/ml.

#### Estimation of MDA

MDA was estimated by colorimetric method of Satoh K. *et al.*. In this method, 2.5 ml of 20% trichloroacetic acid and 1.0 ml of 0.67% TBA are added to 0.5 ml of serum then the mixture is heated in boiling water bath for 30 min. The resulting chromogen is extracted with 4.0 ml of n-butyl alcohol and the absorbance of organic phase is determined at the wavelength of 530nm. The determined values expressed in terms of malondialdehyde (mmol/L) used as a reference method-1,1,3,3-tetraethoxypropane.

#### Statistical analysis

Statistical Analysis was carried out by using student's unpaired 't' test. The  $p < 0.0001$  was considered significant.

#### Observations

Table no.1 indicates demographic characteristics of control group and hypertensives which includes age, sex and duration of hypertension

Table no.2 indicates anthropometry parameters of control group and hypertensives which includes weight, height, BMI (body mass index) and Waist-hip ratio.

**Table 1. Demographic Characteristics**

| Demographic Characteristics | Controls   | Hypertensives | P value |
|-----------------------------|------------|---------------|---------|
| Age in years (Mean±SD)      | 48.67±7.19 | 50.70±4.87    | 0.20    |
| Sex (M/F)                   | 27/23      | 29/21         | -       |
| Duration of Hypertension    | -          | 3.83±4.30     | -       |

**Table 2. Anthropometry Parameters**

| Anthropometry (Mean± SD) | Controls (n=50) | Hypertensives (n=50) | P value |
|--------------------------|-----------------|----------------------|---------|
| Weight (kg)              | 67.57±7.39      | 72.17±6.36           | 0.012*  |
| Height (cm)              | 166.70±6.23     | 164.87±5.16          | 0.220*  |
| BMI (kg/m <sup>2</sup> ) | 24.20±1.47      | 26.49±1.82           | 0.000** |
| Waist –Hip ratio         | 0.91±0.02       | 0.93±0.03            | 0.016*  |

Table no.3 indicates Haemodynamics of control group and hypertensives which includes SBP (systolic blood pressure) and DBP (diastolic blood pressure)

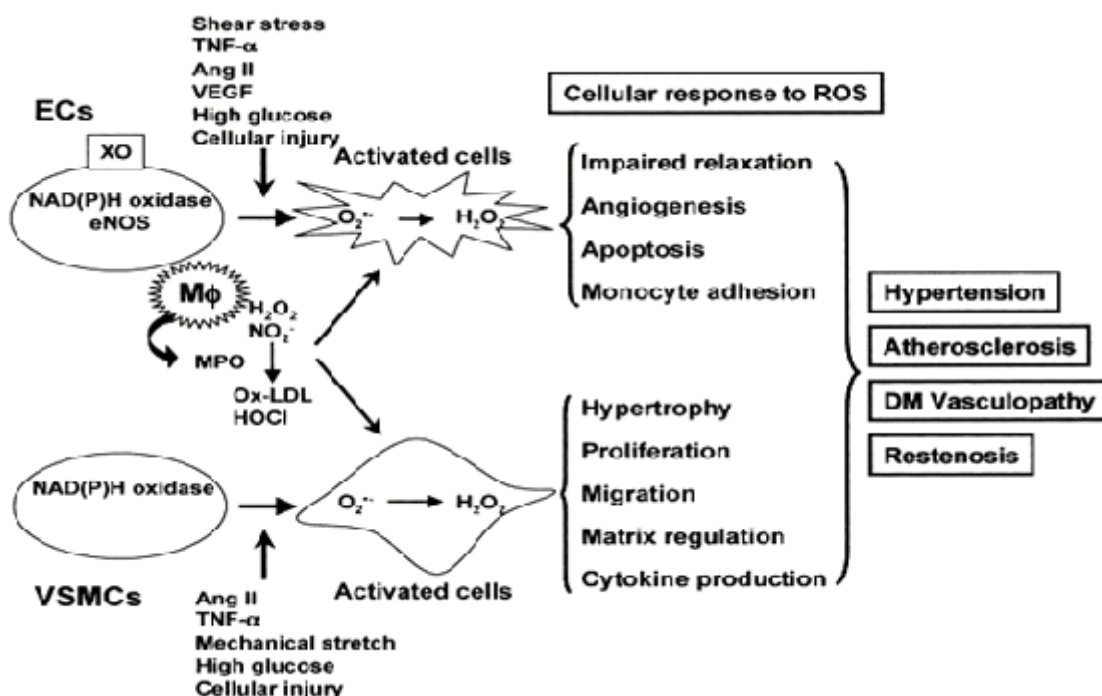
Table no.4 indicates comparison of SOD and MDA between hypertensives and controls.

**Table 3. Haemodynamics**

| Haemodynamics(Mean ± SD) | Controls(n=50) | Hypertensives(n=50) | P value |
|--------------------------|----------------|---------------------|---------|
| SBP(mmHg)                | 122.00±8.77    | 142.33±11.35        | 0.000** |
| DBP(mmHg)                | 77.67±5.94     | 94.20±6.11          | 0.000** |

**Table 4. Values In Hypertensives Compared With Control Group**

| S.no | Parameter              | Cases Mean±SD | Control Mean±SD | T-value | P-value | Significance |
|------|------------------------|---------------|-----------------|---------|---------|--------------|
| 1.   | SOD (enz.u./mg pro/ml) | 6.96±2.34     | 12.8±1.70       | 14.22   | <.0001  | S            |
| 2.   | MDA (mmol/L)           | 4.01±3.25     | 2.83±0.17       | 15.47   | <.0001  | S            |



On extracellular stimuli enzymatically generated ROS activate resident vascular cells ,leading to altered cellular function .These changes in phenotype contribute to initiation and progression of cardiovascular diseases .EC's indicates endothelial cells :VSMC's -vascular smooth cells ,M-macrophages ,XO –Xanthine oxidase ,e NOS-endothelial nitric oxide synthase, MPO –myeloperoxidase, Ox LDL –oxidized low density lipoprotein, TNF- tumour necrosis factor ,Ang II –angiotensin II,VEGF-vascular endothelial growth factor ,DM- diabetes mellitus

**Fig. 1. Modulation of cellular function by ROS in cardiovascular diseases[5]:**

## RESULTS AND DISCUSSION

Hypertension is a complex multifactorial disease. Hypertension is characterized by an increase in systolic and/or diastolic blood pressure (SBP and DBP) than the upper limit of the optimal level as per the JNC (Joint National Committee) VII guidelines. In the present study, both the SBP and DBP of all the cases were significantly higher than that of controls. The findings of the present study demonstrated a strong association between blood pressure and oxidative stress parameters. The increased oxidative stress parameter levels observed in the hypertensive cases of our study is consistent with the findings of several previous studies.

SOD level in hypertensive cases had a mean value of  $6.96 \pm 2.34$  u/mg protein /ml. The difference was found to be statistically highly significant ( $p < 0.0001$ ) when compared to controls. It signifies that an imbalance in antioxidant status suggesting oxidative stress is important in the pathogenesis of essential hypertension Aquil Ahmed *et al.* (2013) reported it to be  $8.6 \pm 0.04$  u/mg protein /ml.

MDA level in hypertensive cases had a mean value of  $4.01 \pm 3.25$  mmol/L. The difference was statistically highly significant ( $p < 0.0001$ ) when compared to controls. It shows that essential hypertension is associated with greater

lipoperoxidation than normal Sadanand G (2013) reported it to be  $4.81 \pm 3.29$  mmol/L.

## CONCLUSION

The hypertensive subjects and control subjects were matched for age and sex. The hypertensive subjects were found to have significantly higher body mass index (BMI) and waist to hip ratio (WHR). The results of the study were independent of these factors.

The superoxide dismutase was significantly lower in the hypertensives compared to normotensive controls.

The mean serum malondialdehyde levels were higher in hypertensives compared to normotensive controls. There was a significant negative correlation between superoxide dismutase and serum malondialdehyde levels in both controls and hypertensives.

Estimation of oxidative stress markers (SOD & MDA) is simple and inexpensive; it can be used to predict the development of atherosclerotic disease like coronary artery disease, cerebrovascular disease and renal complications associated with essential hypertension.

Oxidative stress markers estimation may also be helpful in assessing the usefulness of antihypertensive drugs in prevention of associated complications.

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