



Study of Oxidative Stress Parameters in Hypertension and their Correlation with Blood Pressure

Shashank Deoghare*, Kirtida Tandel, Ashishkumar Zala, Prakash Malam, Abhinav Sharma, Paresh Solanki

Department of Pharmacology, Government Medical College, Surat, Gujarat, India.

*Corresponding author's E-mail: shashank.deo12@gmail.com

Accepted on: 24-12-2013; Finalized on: 28-02-2014.

ABSTRACT

Increasing evidence has underlined the importance of oxidative stress in the Pathophysiology of hypertension and its contribution to the associated complications. However the correlation between oxidative stress parameters and blood pressure levels is not clearly understood. Thus present study was done to evaluate the oxidative stress parameters in patients with hypertension and to check the correlation, if any, between these parameters and systolic and diastolic blood pressure. Plasma malondialdehyde, superoxide dismutase and catalase levels were measured in sixty hypertensive and forty normotensive subjects. Pearson's correlation analysis was performed to study the correlation between these parameters and systolic and diastolic blood pressure. Enhanced oxidative stress was observed in hypertensive subjects as denoted by raised plasma malondialdehyde levels and reduced superoxide dismutase and catalase levels. Plasma malondialdehyde correlated positively whereas superoxide dismutase and catalase showed negative correlation with systolic and diastolic blood pressure in hypertensive subjects. No significant correlation was observed between these parameters and blood pressure levels in normotensive controls.

Keywords: Oxidative stress, Hypertension, Correlation, Malondialdehyde, Catalase, Superoxide dismutase.

INTRODUCTION

Hypertension has emerged as a major public health problem in most parts of the world including India over the last few decades. It is the most common cardiovascular disorder that contributes to significant morbidity and mortality.¹ Pathophysiology of hypertension is complex involving interaction between multiple factors. Oxidative stress has emerged as one of the significant factor that contributes to various complications of hypertension.²

Oxidative stress is defined as a state of imbalance between oxidants and antioxidants in favour of the oxidants, potentially leading to cellular damage.³ Increased oxidative stress has been demonstrated in various animal models of hypertension as well as in human hypertension.⁴ It is suggested that activation of specific signaling pathways in hypertension leads to overproduction of reactive oxygen species and free radicals including superoxide ions, hydroxyl radicals and hydrogen peroxide. Moreover the enzymatic and non-enzymatic antioxidant defenses of the body are also exhausted. The resultant oxidative stress consequently leads to vascular injury, atherosclerosis, renal dysfunction, and hypertensive end-organ damage.⁵

Consequently, a few definitive markers of oxidative status have gained importance. These biomarkers include Malondialdehyde (MDA) which is a secondary product of lipid peroxidation and antioxidant enzymes like superoxide dismutase (SOD) which catalyzes the dismutation of superoxide into oxygen and hydrogen peroxide, and catalase which further detoxifies hydrogen peroxide.⁶

Increased plasma level of malondialdehyde and decreased activity of antioxidant enzymes like catalase and superoxide dismutase in human hypertension has been demonstrated previously.^{7,8} Nevertheless, the available data is not conclusive and the association between human blood pressure and oxidative stress remains to be elucidated. So the present study was undertaken to assess malondialdehyde, catalase and SOD activities in randomly selected normotensive and essential hypertensive subjects and to study the relationship, if any, between arterial blood pressure levels and these biomarkers of oxidative stress.

MATERIALS AND METHODS

Cross sectional design was used for this study. The study protocol was approved by Institutional Ethics Committee.

Subjects

The study included sixty newly diagnosed hypertensive patients never treated previously for hypertension. All patients were between the ages of 45 to 65 years. Hypertension was defined according to the criteria of the VII Joint National Committee (SBP >140 mmHg and DBP > 90 mmHg). Forty normotensive healthy volunteers served as controls. Blood pressure was measured using a mercury sphygmomanometer according to the recommendations of the British Hypertension Society. After explaining the study details, informed consent was obtained from all the participants. Patients with cerebrovascular or coronary artery disease, congestive heart failure, diabetes, renal or liver disease and any active viral or bacterial infection were excluded from the study. Smokers, alcoholics and subjects taking any



medication known to affect oxidative stress were also excluded.

Samples

After enrollment, 5 ml venous blood sample was collected in Na-EDTA (1 mg/ml) tubes from each subject and centrifuged immediately at 3000 rpm for 15 minutes. The separated plasma was used for estimation of MDA. Cells were washed with normal saline three times and RBCs were subjected to lysis by adding 3 ml ice cold distilled water. Hemolysate was then precipitated by adding 1 ml ethanol and 0.6 ml chloroform. The mixture was mixed thoroughly on vortex and centrifuged at 3000 rpm for 15 minutes. Supernatant was used for estimation of SOD and catalase activity.

Methods

Plasma MDA was estimated as per the method described by Ohkawa et al.⁹ 0.5 ml plasma, 0.5 ml of normal saline, 1 ml of 20% trichloroacetic acid (TCA) and 0.25 ml TBA reagent (200 mg of Thiobarbituric acid in 30 ml distilled water and 30 ml of acetic acid) were added in a test tube and kept in a boiling water bath at 95°C for one hour. The tube was centrifuged at 3000 rpm for 10 minutes and optical density of supernatant was measured in a spectrophotometer at 535 nm. MDA level was expressed in terms of nmol/ml of plasma.

SOD activity was measured by method of Marklund and Marklund¹⁰ with some modifications described by Nandi and Chatterjee.¹¹ Briefly, 50 µl hemolysate was added to 1ml of air equilibrated Tris-HCl buffer (pH 8.2) in a cuvette and allowed to incubate at room temperature. The reaction was started by adding 10 µL of freshly prepared 2.6 mM pyrogallol solution (252 mg pyrogallol and 10 µM HCl added to 100ml distilled H₂O). The rate of increase in the absorbance was recorded for a period of 2 minutes, from 1 minute 30 sec to 3 minute 30 sec at 420 nm. The 50% inhibition of auto-oxidation of pyrogallol was measured and activity of SOD was expressed as U/gHb.

Measurement of catalase was done by method described by Sinha.¹² In brief, 0.1 ml hemolysate was added to 1ml of phosphate buffer (pH 7.0) in a test tube. From 6% H₂O₂ solution, 0.4 ml (800 micromoles) was then added to it. The reaction was stopped exactly after one minute, by adding 2 ml potassium dichromate solution (5% potassium dichromate and glacial acetic acid in 1:3 proportions). Test tube was heated for 10 minutes in water bath and optical density was measured at 570 nm. Total number of moles of H₂O₂ consumed was determined and catalase activity was expressed in terms of µmol of H₂O₂ consumed/min/mg Hb.

Statistical analysis

Data were expressed as mean ± SD. Student t test was used to assess statistical differences between the groups. Pearson's correlation analysis was performed to study the correlation between parameters and systolic and diastolic

blood pressure. Differences were considered statistically significant at $p < 0.05$.

RESULTS

Sixty hypertensive and forty age matched normotensive controls were included in the study. Baseline characteristics of both groups are shown in table 1. In hypertensive group, SBP and DBP were significantly higher than the control group ($p < 0.001$) (Table 1).

Levels of oxidative stress biomarkers in both groups are shown in Table 2. Plasma MDA was increased significantly in hypertensive group compared to controls ($p < 0.01$). The activity of SOD was significantly lower in hypertensive group ($p < 0.01$). Similarly, activity of catalase was also significantly reduced in hypertensive group compared to the controls ($p < 0.01$). (Table 2)

Table 1: General characteristics of study population

Variable	Control (n=40)	Hypertension (n=60)
Age	57 ± 6	55 ± 7
Gender	Male	46
	Female	14
Systolic BP	118.7 ± 6.5	159.7 ± 10.8*
Diastolic BP	77.9 ± 5.3	102.2 ± 8.9*

Data shown are means ± SD, * $p < 0.01$ vs. control

Table 2: Oxidative stress parameters in normotensive and hypertensive subjects

	Control (n=40)	Hypertension (n=60)
Malondialdehyde (nmol/ml plasma)	4.19 ± 0.59	5.27 ± 1.27*
SOD (U/gHb)	1071 ± 128	892 ± 166*
Catalase (U/gHb)	5109 ± 418	4583 ± 638*

Data shown are means ± SD, * $p < 0.01$ vs. control

We further studied the relationship between these three parameters and systolic and diastolic blood pressure levels in both groups. Pearson correlation coefficients between the different parameters assessed and systolic and diastolic BP. Plasma MDA correlated positively with SBP and DBP in hypertensive subjects (fig. 1a). SOD and catalase showed negative correlation with SBP and DBP in hypertensive subjects (fig 1b and 1c). No correlation was observed between any of these parameters with SBP and DBP in controls (fig 1d, 1e and 1f).

DISCUSSION

The present study was done to evaluate the oxidative stress parameters in the patients with essential hypertension and to assess the relationship, if any, between these parameters and systemic blood pressure

levels. We observed that malondialdehyde levels were significantly raised in the patients of essential hypertension and there was concomitant reduction of antioxidant enzymes superoxide dismutase and catalase.

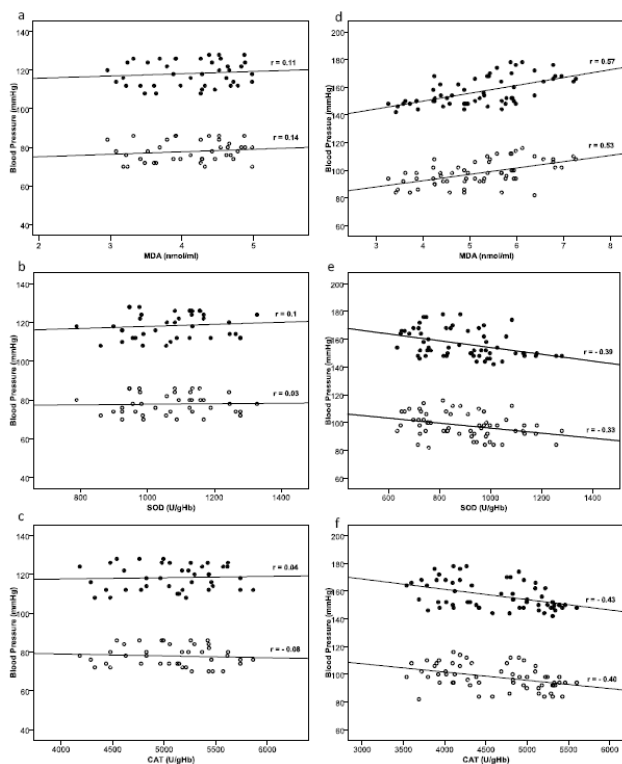


Figure 1: Pearson Correlation (r) between SBP or DBP and Malondialdehyde, Superoxide Dismutase and Catalase in normotensive (1a, 1b, 1c) and Hypertensive subjects (1d, 1e, 1f)

Increased production of free radicals together with increased lipid peroxidation and decreased antioxidant enzymes in hypertension was demonstrated in previous studies.^{13,14} It is suggested that in hypertension, activation of enzymes involved in free radical synthesis leads to overproduction of superoxide anion, hydrogen peroxide and other reactive oxygen species.¹⁵ Due to their highly reactive nature and nonspecific binding, ROS can attack almost all biomolecules including lipid membranes. MDA is a product of long chain fatty acid peroxidation that accumulates due to increased oxidative stress. MDA further accelerates peroxidation itself by synergizing with free radicals.¹⁶

Superoxide dismutase and catalase are among the primary antioxidant defense against oxidative stress. Augmented expression of these enzymes may be expected in face of increased production of reactive oxygen species in hypertension. However, the decreased level of antioxidant enzymes observed in the present study may be explained by the fact that excessive ROS inactivate these enzymes and also suppress the expression of genes encoding for these enzymes.¹⁴ The present study showing statistically significant increase in MDA and concomitant decrease in antioxidant enzyme levels in hypertensive patients confirms the results of previous studies. However whether oxidative stress is the

cause or the consequence of hypertension is not clear and it needs further evaluation.

We further examined the possible relationship between these parameters and systemic blood pressure levels. Positive correlation was observed between plasma malondialdehyde and systolic and diastolic blood pressure levels in hypertensive subjects. A negative correlation was observed between antioxidant enzyme levels and systolic and diastolic blood pressure in hypertensive subjects. These results suggest that the generation of free radicals increases proportionately with rise in blood pressure levels above normal. These findings point to the fact that severity of oxidative stress parallels the degree of rise in blood pressure in patients with hypertension.

The correlation observed in this study assumes a great importance due to the fact that oxidative stress contributes to the complications of hypertension including endothelial dysfunction, atherosclerosis and end organ damage. Thus it seems logical that normalization of BP may considerably check oxidative stress and reduce the complications of hypertension. Some of the classic antihypertensive agents such as some β -adrenergic blockers, ACE inhibitors, AT1 receptor antagonists, and Ca^{2+} channel blockers reduce oxidative stress.¹⁷ In view of their ability to decrease the vascular oxidative stress in addition to their blood pressure lowering effect these agents may prove beneficial in long term treatment of hypertension.

CONCLUSION

Oxidative stress is increased in patients with hypertension as evidenced by the significant changes in the levels of oxidative stress parameters. Moreover, these parameters correlate with the systolic and diastolic blood pressure in hypertension. Thus attempts to lower blood pressure could prove beneficial in such patients by reducing the oxidative stress related long terms complications.

REFERENCES

- Gupta R, Trends in hypertension epidemiology in India, *J Hum Hypertens*, 18, 2004, 73–78.
- Oparil S, Zaman MA, Calhoun DA, Pathogenesis of hypertension, *Ann Intern Med*, 139, 2003, 761–776
- Seis H, Oxidative stress: introductory remarks. In: Seis H (ed), *Oxidative Stress*, Academic Press: London, 1985, pp 1–8.
- Touyz RM, Reactive Oxygen Species, Vascular Oxidative Stress, and Redox Signaling in Hypertension, What Is the Clinical Significance? *Hypertension*, 44, 2004, 248-252
- Paravicini TM, Touyz RM, NADPH oxidases, reactive oxygen species, and hypertension: clinical implications and therapeutic possibilities, *Diabetes Care*, 31(2), 2008, S170-S180.
- Griffiths NR, Moller L, Bartosz G, Bast A, Bertoni-Freddari C, Collins A, *Biomarkers*, *Mol Aspects Med*, 23, 2002, 101–208.

7. Armas-Padilla MC, Armas-Hernandez MJ, Sosa-Canache B, Cammarata R, Pacheco B, Guerrero J, Nitric oxide and malondialdehyde in human hypertension, *Am J Ther*, 14, 2007, 172–176.
8. Tandon R, Sinha MK, Garg H, Khanna R, Khanna HD, Oxidative stress in patients with essential hypertension, *Natl Med J India*, 18, 2005, 297–299.
9. Ohkawa H, Ohishi N, Yagi K, Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction, *Anal Biochem*, 95, 1979, 351-358.
10. Marklund S, Marklund G, Involvement of superoxide anion radical in the autoxidation of pyrogallol and a convenient assay of superoxide dismutase, *Eur J Biochem*, 47, 1974, 469-476.
11. Nandi A, Chatterjee IB, Assay of superoxide dismutase activity in animal tissue, *J Bio Sci*, 13, 1988, 305-315.
12. Sinha AK, Colorimetric assay of catalase, *Anal Biochem*, 47, 1972, 389-394.
13. Redon J, Oliva MR, Tormos C, Giner V, Chaves J, Iradi A, Sáez GT, Antioxidant activities and oxidative stress byproducts in human hypertension, *Hypertension*, 41, 2003, 1096-101.
14. Nwanjo HU, Oze G, Okafor MC, Nwasu D, Nwankpa P, Oxidative stress and non enzymic antioxidant status in hypertensive patients in Nigeria, *African Journal of Biotechnology*, 6, 2007, 1681-84.
15. Ceriello A, Possible role of oxidative stress in the pathogenesis of hypertension, *Diabetes Care*, 31, 2008, S181–S184.
16. Djordjevic VB, Pavlovic D, Pejovic M, Cvetkovic T, Lecic N, Deljanin-Ilic M, Changes of lipid peroxides and antioxidative factor levels in blood of patients treated with ACE inhibitors, *Clin Nephrol*, 47, 1997, 243-7.
17. Hamilton CA, Miller WH, Al-Benna S, Brosnan MJ, Drummond RD, McBride MW, Dominiczak AF, Strategies to reduce oxidative stress in cardiovascular disease, *Clin Sci (Lond)*, 106, 2004, 219-34.

Source of Support: Nil, **Conflict of Interest:** None.

