Original Article www. pimr.org.in

Biomarkers of Oxidative stress in essential hypertension

Navid N Shah[1], Srinivas Ch[2]

[1]Associate Professor, [2]Post Graduate Student, Department of Physiology, Prathima Institute of Medical Sciences, Nagunur, Karimnagar District, Telangana-505417.

Address for correspondence: Dr Navid N Shah, Associate Professor, Department of Physiology, Prathima Institute of Medical Sciences, Nagunur, Karimnagar District, Telangana-505417.

Email: drnavid23@gmail.com

ABSTRACT

Introduction

There is a increasing amount of verification supporting the view that oxidative stress is involved and plays a important role in the pathophysiology of primary hypertension.

Objective:

This research examines the association of blood pressure with blood oxidative stress-related parameters in normotensive and hypertensive subjects.

Materials & Methods:

A cross-sectional design was applied to 32 hypertensive patients and 33 healthy normotensive subjects. All subjects were men between the ages of 35 and 60 years. Cases of dyslipidemia, diabetes mellitus, obesity, smoking and those taking medication were excluded from the study. In erythrocyte lipid peroxidation (malondialdehyde) and reduced/ oxidized glutathione ratio (GSH/GSSG) were determined. Parameters measured in the plasma of test subjects were plasma antioxidant status, plasma vitamin C, vitamin E, lipid peroxidation (8-isoprostane), blood pressure modulators renin, aldosterone, endothelin-1, and homocysteine.

Results: Daytime systolic and diastolic blood pressures of hypertensives were negatively correlated with plasma antioxidant capacity (r=–0.54, p=0.001 and r=–0.60, p<0.001), plasma vitamin C levels (r=–0.47, p=0.006 and r=–0.43, p=0.01), erythrocyte activity of antioxidant enzymes, and erythrocyte GSH/GSSG ratio, with hypertensives showing higher levels of oxidative stress.

Conclusion: Blood pressures showed a positive correlation with both plasma and urine 8-isoprostane. These results show a strong association between blood pressure and some oxidative stress-related parameters and propose a probable role of oxidative stress in the pathophysiology of essential hypertension.

Keywords: Antioxidants, essential hypertension, 8-isoprostane, oxidative stress, vitamin C

INTRODUCTION

Recently, many evidence has involved oxidative stress in the mechanism of hypertension. Vascular oxidative stress could be involved in the pathogenesis of hypertension[1,2]. Imbalance between the generation of reactive oxygen species (ROS) and the antioxidant defense results in Oxidative stress[3,4]. The initially formed ROS is superoxide anion radical, produced from NADPH oxidase (NOX), an enzyme subjected to regulation by hormones.

Also, mechanical stimuli known to occur in blood pressure elevation further contribute to increased ROS production. Furthermore, increased intracellular calcium concentration may result from ROS induced vasoconstriction, hence increasing the development of hypertension[5]. Oxidative stress probably causes endothelial dysfunction, however it is unknown whether this abnormality is a primary event or a consequence of increased blood pressure[6]. It is well-known that superoxide rapidly inactivates endothelium-derived nitric oxide (NO), the most significant endogenous vasodilator, thus promoting vasoconstriction[7,8].

Moreover, exogenous administration of antioxidants improve the vascular function and reduce the blood pressure in animal models[9,10] and in human hypertension[11,12]. The available data are not decisive and the correlation between blood pressure and oxidative stress in humans remains to be revealed. The purpose of the present study was to investigate the association of blood pressure with oxidative stress in volunteer male normotensive and essential hypertensives.

METHODS AND MATERIALS

Study Design

The study protocol was approved by the Ethics Committee of the Prathima Institute of Medical Sciences. A cross-sectional design was conducted to 32 hypertensive patients and 33 healthy normotensive subjects. All participants signed a written consent form and no complications were encountered during the study.

Patients

We selected untreated essential hypertensive outpatients(stage 1)[13]. All subjects were males between the ages of 35and 60 years. Hypertension was defined as mean

daytime blood pressure values =135 mmHg systolic or =85 mmHg diastolic, by ambulatory blood pressure monitoring [14]. Exclusion criteria were obesity, smoking, (body mass index [BMI] >30 kg/m2), diabetes, hypercholesterolemia, current use of any medication, including dietary supplements and chronic diseases and Normotensive volunteer subjects participated as controls. The potential participants were subjected to a selection protocol consisting of clinical history, physical examination and appropriate tests. Patients showing evidence of target-organ-damage were excluded from the study. Cardiovascular damage (myocardial hypertrophy and/ or valve dysfunction) was detected by echocardiographic examination[15]. In addition, plasma creatinine levels and urine analysis were used to detect renal damage.

Ambulatory Blood Pressure Monitoring

Blood pressures were determined through ambulatory monitoring on a regular workday (over a period of 24 h beginning at 9:30 AM) with an oscillometric monitor previously checked for accuracy against simultaneous measurements with a mercury sphygmomanometer. This device fulfils the validation criteria of the British Hypertension Society protocol[16] and satisfies the criteria of the Association for the Advancement of Medical Instrumentation (AAMI)for studies under ambulatory conditions[17]. The blood pressure analyzed was the mean daytime pressure value.

Assessment of Oxidative Stress–Related Parameters

Plastic tubes for 8-isoprostane samples were previously treated with butylated hydroxytoluene (final concentration1 mmol/L). Venous blood samples were obtained in ice-cold vacutainers. Erythrocyte lysates, plasma and urine samples were stored at -70°C.

Vitamin C was analyzed by a fluorometric method[18], and the inter Interassay and intra-assay coefficients of variation were 4.9% and 2.7%, respectively. Plasma antioxidant status was assessed by ferric reducing ability of plasma (FRAP), with a detection limit of 10 µmol/L[19]. The inter-assay and intra-assay coefficients of variation for FRAP were 3.0% and 1.0%, respectively. The plasma uric acid levels were also assayed. Lipid peroxidation was estimated through plasma and urine 8-isoprostane concentrations, a reliable biomarker of oxidative stress in vivo[20], using an ELISA kit. The inter-assay and intra-assay coefficients of variation were 9.5% and 10.7%, respectively. Renin and aldosterone were determined by radioimmunoassay, and homocysteine, folic acid and vitamin B12, endothelin-1 by ELISA. Vitamin E was analyzed by high performance liquid chromatography (HPLC)[21], and the inter assay and intra-assay coefficients of variation were 6.4% and 4.5%, respectively.

Assessment of Erythrocyte Antioxidant Status

Homogenized Lysates of erythrocytes were used for the fluorometric determination of reduced glutathione (GSH)[22]. The GSH/GSSG ratio was determined. In addition, superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) activities were also Determined [23].

Table 1 : Clinical characteristics of essential hypertensive patients and normotensive subjects

Characteristics	Hypertensive patients (n=32)	Normotensive subjects (n=33)	P value
Age (years)	47.5±7.3	46.3±9.2	0.56
Body mass Index (kg/m2)	27.4±3.4	26.7± 4.1	0.45
Serum glucose (mmol/L)	5.13±0.2	4.92 ± 0.006	<0.001
Creatinine (µmol/L)	83.3±2.4	80.5 ± 1.7	<0.001
Total cholesterol (mmol/L)	5.90±0.15	4.52±0.15	<0.001
HDLcholesterol (mmol/L)	1.23±0.05	1.30±0.05	<0.001
LDL cholesterol (mmol/L)	3.0±0.13	2.65±0.15	<0.001
Serum Triglycerides (mmol/L)	1.55±0.12	1.35±0.007	<0.001
Daytime SBP (mmHg)	139.6±4.3	121±5.7	<0.001
Daytime DBP (mmHg)	93.9±3.5	79.6±1.5	<0.001
Heart rate (beats/min)	75.6±5.3	73.8±4.5	0.07

Table2. Plasma Blood Pressure Modulator Levels in the Study Participants

Modulator	Hypertensive patients (n=32)	Normotensive subjects (n= 33)	P value
Renin activity (pmol/L/Hr)	20.79±2.62	25.09±390	<0.001
Aldosterone (nmol/L)	0.24±0.03	0.23±0.01	0.07
Endothelin- (pmol/L)	2.43±0.32	2.74±0.27	<0.001
Homocysteine (µmol/L)	9.90±0.41	8.83±0.29	<0.001
Folic acid (nmol/L)	46.2±1.25	43.46±1.46	<0.001
Vitamin B12 (pmol/L/)	232.6±7.83	2.28±9.5	0.03

Table 3. Plasma, Erythrocyte and Urine Oxidative Stress–Related Parameters of the Study Participants

Parameter p value	Hypertensive patients (n=32)	Normotensive subjects (n= 33)	P value
Plasma FRAP (µmol/L)	305.4±9.5	436.2±14.7	<0.001
Vitamin C (µmol/L)	34.6±2.0	41.68±2.0	<0.001
Uric acid (µmol/L)	306.9±12.0	295.4±10.2	<0.001
Vitamin E (µmol/L)	15.2±1.1	15.4±1.1	0.46
8-Isoprostane (pmol/L)	114.32±3.5	76.6±3.5	<0.001

Parameter p value	Hypertensive patients (n=32)	Normotensive subjects (n= 33)	P value
Erythrocytes Catalase (k/g Hb)	215.2±2.2	272.15±2.4	<0.001
Superoxide dismutase (U/g Hb)	1,185±6	1,362±7	<0.001
Glutathione peroxidase (U/g Hb)	5.29±0.03	6.29±0.03	<0.001
GSH/GSSG ratio	5.4±0.5	7.5±0.6	<0.001
Malondialdehyde (nmol/g Hb	365.29±2.5	310.5±2.9	<0.001
Urine 8-Isoprostane (nmol/µmol creatinine)	179.4±10.8	125.6±8.9	<0.001

Statistical Analysis

The essential sample size was considered using blood pressure as the primary endpoint to detect a difference of about 20% between the blood pressure levels and oxidative stress–related parameters of control and study at a power of 80% and a p value of 0.05. The source of variation was assessed by unpaired Student's t-test for normally distributed parameters. The association of variables was studied by Pearson correlation test. Descriptive statistics of variables used the mean and standard error of mean.

RESULTS

Clinical Characteristics

Table 1 shows the clinical individuality of the 65 study participants. All parameters were in the normal ranges and showed no considerable differences between the two groups except for the considerably higher daytime SBP and DBP in the hypertensive group ($p<0.001$).

Blood Pressure Modulators and Oxidative Stress–Related Parameters

Aldosterone, plasma renin, endothelin-1 and homocysteine levels (Table 2) were not considerably different between hypertensive and control subjects. But, the blood antioxidant activity as assessed by erythrocyte plasma FRAP, GSH/GSSG ratio and the vitamin C levels were 30% ,28% and 17% lower in hypertensives than normotensives, respectively (Table 3). Also, the erythrocyte activities of SOD CAT, and GSH-Px of hypertensives were 13%, 21%, and 16% lower than the respective normotensive values. In addition, the lipid peroxidation of hypertensives, as assessed by erythrocyte malondialdehyde (MDA) concentrations plasma and urine 8-isoprostane, and were 15% 33%, and 30% higher respectively, compared to normotensives.

In hypertensives SOD activity was negatively correlated with only SBP, whereas in normotensives neither relation was significant. In normotensives only the correlation between daytime SBP and CAT activity was statistically significant. Whereas the relation between daytime SBP and DBP were both negatively correlated with the activity of CAT in hypertensives.

Negative correlations between daytime SBP or DBP and the plasma FRAP for both normotensives and hypertensives. In normotensives only the correlation between plasma vitamin C and SBP was significant whereas in hypertensives, plasma vitamin C levels were negatively correlated with SBP and DBP. The erythrocyte GSH/GSSG ratio was not significantly related to either SBP or DBP in normotensives. But, the erythrocyte GSH/GSSG ratio was negatively correlated with SBP and DBP in hypertensives. Particularly, there was a strong positive correlation between plasma and urine 8-isoprostane concentrations and both SBP and DBP in both groups.

DISCUSSION

The present results demonstrate a strong association between blood pressure and some oxidative stress–related parameters. The increased oxidative stress levels that were observed in essential hypertensive patients are consistent with the findings of several previous studies[24-26]. In some studies, however, no significant increase in plasma 8-isoprostane levels was observed [27-28] as these studies were performed either in early stages of disease or with patients receiving statin medication

As per the previous data essential hypertensive subjects showed an impairment of the antioxidant defense system as estimated by a diminution of plasma and erythrocyte antioxidant status[29-31].

Additionally, the negative correlation between SBP and DBP and the erythrocyte GSH/ GSSG ratio suggest the importance of the blood antioxidant status in blood pressure modulation. Previous studies found a negative correlation of catalase activity with both daytime SBP and DBP in essential hypertensives, [24] in accord with our results.

Normotensives did not prove any relationship between blood pressure and the activity of most of the erythrocyte antioxidant enzymes whereas hypertensives did deserves special investigation. It is well known that exposure to ROS increases the expression of antioxidant enzymes [32, 33]. The blood pressure and the erythrocyte GSH/GSSG ratio in hypertensives were negatively correlated, this can indicate that GSH oxidation by increased ROS is not followed by a compensatory response of glutathione metabolism–related enzymes. However, this correlation was not significant in normotensives probably because of lack of ROS increase that may be responsible to change the erythrocyte GSH/GSSG ratio. Primary decrease in the antioxidant defense system activity or an elevation of ROS concentration leads to oxidative stress. This disorder leads to oxidative damage to the antioxidant enzymes, thus causing oxidative stress in hypertensives, and not in normotensives. Elevations of blood pressure can also increase of ROS, thus enhancing the mechanism of ROS-mediated hypertension through a multifaceted dependency.

On the other hand, the elevated plasma 8-isoprostane levels in hypertensives has been proposed to be a contributory factor in the increase of vascular peripheral resistance[34], though it needs confirmation. The strong positive association of both SBP and DBP, with the plasma and urine 8-isoprostane concentration indicate that lipid peroxidation should be considered as a risk factor for blood pressure elevation. The increase in 8-isoprostane may be from a primary metabolic derangement or from the elevation of blood pressure itself. In addition, decreased plasma FRAP levels in hypertensives and their strong negative correlation with SBP and DBP levels in both groups suggests a role of the plasma antioxidant status in the modulation of blood pressure. The result in hypertensives are with low plasma levels of vitamin C, a contributor to FRAP. The relationship of vitamin C and blood pressure is in accord with previous studies that were performed on an epidemiologic scale[35, 36].

Even though our data were obtained from a lesser sample, our access allowed us to study the role of oxidative stress in the modulation of blood pressure. It is notable that the effects of antioxidants and vitamin C in human hypertension remain questionable[37,38]. Also, it has also been recorded that the antioxidant adrenomedullin may show antihypertensive effects derived from its antioxidant properties [39]. The suggestion that oxidative stress share the etiology of essential hypertension is further supported by our present finding that the plasma levels of the various modulators of blood pressure were not considerably different between normotensives and hypertensives.

It was noteworthy that 8-isoprostane and FRAP had the highest correlations with blood pressure among the oxidative stress–related parameters considered. Additionally, the administration of candesartan and valsartan has been proved to cause a decrease in oxidative stress in essential hypertensives [2,40]. Antioxidant vitamins have been shown to exert antihypertensive effects in hypertensive rats, even if the extensibility of these results to human beings remain controversial [41], and hopes the conclusion of large scale clinical trials that are currently in progress. The use of only male subjects restricts the value of the results of the present study. It is recognized that in women endogenous and exogenous estrogens and progestogens, could be subjected to time-dependent variations, be able to modulate renal sodium metabolism as well as the activity of the RAAS [42].Yet, future studies could be done to analyze these issues.

CONCLUSION

Finally, these data provide evidence of blood pressure modulation by measurable oxidative stress–related parameters and give unique description of a functional dependence between these parameters. Hence, oxidative stress may be considered as an adjuvant therapeutic approach for the therapy of essential hypertension.

REFERENCES

1. Rodrigo R, Passalacqua W, Araya J, Orellana M, Rivera G. Implications of oxidative stress and homocysteine in the pathophysiology of essential hypertension. J Cardiovasc Pharmacol 2003; 42: 453–461.
2. Miyajima K, Minatoguchi S, Ito Y, et al. Reduction of QTc dispersion by the angiotensin II receptor blocker valsartan may be related to its anti-oxidative stress effect in patients with essential hypertension. Hypertens Res 2007; 30: 307–313.
3. Becker LB. New concepts in reactive oxygen species and cardiovascular reperfusion physiology. Cardiovasc Res 2004; 61: 461–470.
4. Juránek I, Bezek S. Controversy of free radical hypothesis: reactive oxygen species?cause or consequence of tissue injury? Gen Physiol Biophys 2005; 24: 263–278.
5. Paravicini TM, Touyz RM. Redox signalling in hypertension. Cardiovasc Res 2006;71:247–258. DOI: 10.1016/j.cardiores.2006.05.001.
6. John S, Schmieder RE. Potential mechanisms of impaired endothelial function in arterial hypertension and hypercholesterolemia. Curr Hypertens Rep 2003; 5: 199–207.
7. Dusting GJ, Akita K, Hickey H, Smith M, Gurevich V. Cyclosporin A and tacrolimus (FK506) suppress expression of inducible nitric oxide synthase in vitro by different mechanisms. Br J Pharmacol 1999; 128: 337–344.
8. Zicha J, Dobesova Z, Kunes J. Relative deficiency of nitric oxide-dependent vasodilation in salt-hypertensive Dahl rats: the possible role of superoxide anions. J Hypertens 2001; 19: 247–254.
9. Chen X, Touyz RM, Bae Park J, Schiffrin EL. Antioxidant effects of vitamins C and E are associated with altered activation of vascular NADPH oxidase and superoxide dismutase in stroke-prone SHR. Hypertension 2001; 38: 606–611.
10. Hoagland KM, Maier KG, Roman RJ. Contributions of 20-HETE to the antihypertensive effects of Tempol in Dahl salt-sensitive rats. Hypertension 2003; 41(3 Pt 2): 697–702.
11. Duffy SJ, Gokce N, Holbrook M, et al. Effect of ascorbic acid treatment on conduit vessel endothelial dysfunction in patients with hypertension. Am J Physiol Heart Circ Physiol 2001; 280: H528–H534.
12. Boshtam M, Rafiei M, Sadeghi K, Sarraf-Zadegan N. Vitamin E can reduce blood pressure in mild hypertensives. Int J Vitam Nutr Res 2002; 72: 309–314.

13. Chobanian AV, Bakris GL, Black HR, et al. Seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. Hypertension 2003; 289: 2560–2572.

14. Myers MG, Tobe SW, McKay DW, Bolli P. New algorithm for the diagnosis of hypertension. Am J Hypertens 2005; 18: 1369–1374.

15. ACC/AHA: Guidelines for the Clinical Application of Echocardiography. Circulation 1997; 95: 1686–1744.

16. O'Brien E, Mee F, Atkins N, O'Malley K. Accuracy of the Spacelabs 90207 determined by to the British Hypertension Society Protocol. J Hypertens 1991; 9: 573–574 (Short Report).

17. Groppelli A, Omboni S, Parati G, Mancia G. Evaluation of noninvasive blood pressure monitoring devices Spacelabs 90202 and 90207 versus resting and ambulatory 24-hour intra-arterial blood pressure. Hypertension 1992; 20: 227–232.

18. Chung WY, Chung JK, Szeto YT, Tomlinson B, Benzie IF. Plasma ascorbic acid: measurement, stability and clinical utility revisited. Clin Biochem 2001; 34: 623–627.

19. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem 1996; 239: 70–76.

20. Milne GL, Musiek ES, Morrow JD. F2-isoprostanes as markers of oxidative stress in vivo: an overview. Biomarkers 2005; 10 (Suppl 1): 10–23.

21. Sowell AL, Huff DL, Yeager PR, Caudill SP, Gunter EW. Retinol, alpha-tocopherol, lutein/zeaxanthin, betacrytoxanthin, lycopene, alpha-carotene, trans-beta-carotene, and four retinyl esters in serum determined simultaneously by reversed-phase HPLC with multiwavelength detection. Clin Chem 1994; 40: 411–416.

22. Hissin PJ, Hilf R. A fluorometric method for determination of oxidized and reduced glutathione in tissues. Anal Biochem 1976; 74: 214–226.

23. Videla LA, Rodrigo R, Orellana M, et al. Oxidative stress-related parameters in the liver of non-alcoholic fatty liver disease patients. J Clin Sci (Lond) 2004; 106: 261–268.

24. Simic DV, Mimic-Oka J, Pljesa-Ercegovac M, et al. Byproducts of oxidative protein damage and antioxidant enzyme activities in plasma of patients with different degrees of essential hypertension. J Hum Hypertens 2006; 20: 149–155.

25. Laffer CL, Bolterman RJ, Romero JC, Elijovich F. Effect of salt on isoprostanes in salt-sensitive essential hypertension. Hypertension 2006; 47: 434–440.

26. Kedziora-Kornatowska K, Czuczejko J, Pawluk H, et al. The markers of oxidative stress and activity of the antioxidant system in the blood of elderly patients with essential arterial hypertension. Cell Mol Biol Lett 2004; 9: 635–641.

27. Cracowski JL, Baguet JP, Ormezzano O, et al. Lipid peroxidation is not increased in patients with untreated mild-tomoderate hypertension. Hypertension 2003; 41: 286–288.

28. Ward NC, Hodgson JM, Puddey IB, Mori TA, Beilin LJ, Croft KD. Oxidative stress in human hypertension: association with antihypertensive treatment, gender, nutrition, and lifestyle. Free Radic Biol Med 2004; 36: 226–232.

29. Moreno MU, Jose GS, Fortuno A, Beloqui O, Diez J, Zalba G. The C242T CYBA polymorphism of NADPH oxidase is associated with essential hypertension. J Hypertens 2006; 24: 1299–1306.

30. Kashyap MK, Yadav V, Sherawat BS, et al. Different antioxidants status, total antioxidant power and free radicals in essential hypertension. Mol Cell Biochem 2005; 277: 89–99.

31. Muda P, Kampus P, Zilmer M, et al. Effect of antihypertensive treatment with candesartan or amlodipine on glutathione and its redox status, homocysteine and vitamin concentrations in patients with essential hypertension. J Hypertens 2005; 23: 105–112.

32. Talalay P, Dinkova-Kostova AT, Holtzclaw WD. Importance of phase 2 gene regulation in protection against electrophile and reactive oxygen toxicity and carcinogenesis. Adv Enzyme Regul 2003; 43: 121–134

33. Bae I, Fan S, Meng Q, et al. BRCA1 induces antioxidant gene expression and resistance to oxidative stress. Cancer Res 2004; 64: 7893–7909.

34. Hozawa A, Ebihara S, Ohmori K, et al. Increased plasma 8- isoprostane levels in hypertensive subjects: the Tsurugaya project. Hypertens Res 2004; 27: 557–561.

35. Ness AR, Khaw KT, Bingham S, Day NE. Vitamin C status and blood pressure. J Hypertens 1996; 14: 503–508.

36. Sakai N, Yokoyama T, Date C, Yoshiike N, Matsumura Y. An inverse relationship between serum vitamin C and blood pressure in a Japanese community. J Nutr Sci Vitaminol (Tokyo) 1998; 44: 853–867.

37. Touyz RM, Schiffrin EL. Reactive oxygen species in vascular biology: implications in hypertension. Histochem Cell Biol 2004; 122: 339–352.

38. Kim MK, Sasaki S, Sasazuki S, Okubo S, Hayashi M, Tsugane S. Lack of long-term effect of vitamin C supplementation on blood pressure. Hypertension 2002; 40: 797–803.

39. Ishimitsu T, Ono H, Minami J, Matsuoka H. Pathophysiologic and therapeutic implications of adrenomedullin in cardiovascular disorders. Pharmacol Ther 2006; 111: 909–927.

40. Dohi Y, Ohashi M, Sugiyama M, Takase H, Sato K, Ueda R. Candesartan reduces oxidative stress and inflammation in patients with essential hypertension. Hypertens Res 2003; 26: 691–697.

41. Rodrigo R, Guichard C, Charles R. Clinical pharmacology and therapeutic use of antioxidant vitamins. Fundam Clin Pharmacol 2007; 21: 111–127.

42. ESHRE Capri Workshop Group: Hormones and cardiovascular health in women. Hum Reprod Update 2006; 12: 483–

How to cite this article : Shah Navid, Srinivas Ch. Biomarkers of Oxidative stress in essential hypertension. Perspectives in Medical Research 2018; 6(3):18-23.

Sources of Support: Nil,Conflict of interest:None declared