

STUDY ON THE LEVELS OF OXIDANT AND ANTI-OXIDANT ENZYMES IN HYPERTENSIVE PATIENTS

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ABSTRACT

Background: Hypertension is a hallmark risk factor for coronary heart disease, cerebrovascular stroke, congestive heart failure, cardiac arrhythmia, cardiomyopathy and abnormal renal function. Free radicals are involved in the pathogenesis of hypertension by altering endothelial function via oxidative stress.

Aims & Objective: To assess the level of antioxidant enzymes superoxide dismutase (SOD), Catalase, Glutathione peroxidase (GPX) and lipid peroxidation product malondialdehyde (MDA) in hypertensive patients.

Materials and Methods: 100 hypertensive patients and 25 healthy controls were included. Serum levels of enzymes and MDA were estimated by spectrophotometric methods.

Results: The level of antioxidant enzymes were significantly lower and MDA was significantly higher in hypertensive patients as compared to controls ($p < 0.05$). For SOD, significant difference could not be obtained between controls and stage I hypertensives with drugs ($p > 0.05$). We also compared the enzymes and MDA level within the hypertensive groups. Significant results were obtained ($p < 0.05$, 0.01). On comparison between stage I and stage II hypertensive without and with drugs, we found that hypertensive patients on drug have significantly higher level of antioxidant enzymes and lower level of MDA than those without anti-hypertensive drugs ($p < 0.01$).

Conclusion: Our study shows that hypertension is associated with oxidative stress. Adequate control of blood pressure and antihypertensive therapy decrease the oxidative stress, improves antioxidant status and endothelial function.

Key Words: Oxidative Stress; Malondialdehyde (MDA); Glutathione Peroxidase (GPX); Superoxide Dismutase (SOD); Catalase

Introduction

Cardiovascular disease caused 2.3 million deaths in India in 1996 and this is predicted to double by the year 2020.^[1] Hypertension is one of the major causes of morbidity and mortality in human population. It is a hallmark risk factor for coronary heart disease, cerebrovascular stroke, congestive cardiac failure, cardiac arrhythmia, cardiomyopathy and abnormal renal function.^[2] Hypertension is responsible for 57% of all stroke death and 24% of all coronary artery disease in India.^[1]

The term hypertension denotes a systolic blood pressure (SBP) of >140 mmHg, diastolic blood pressure of >90 mmHg or a patient taking antihypertensive medication. In most cases hypertension is idiopathic and is referred to as essential or primary hypertension. The pathogenesis of essential hypertension may include a number of factors such as heredity, increased fluid volume, renal sodium transport deficiency, increased sympathetic activity and increased vascular tone, involvement of renin aldosterone system, chronic stress, diminished activity of vasopressor hormones (PGs, ANF) etc. In addition, factors such as obesity, physical

inactivity, occupation, smoking, alcoholism etc. may also contribute.^[3]

Essential hypertension is associated with endothelial cell dysfunction, which is defined as the imbalance between the production and bioavailability of endothelium derived relaxing factor or nitric oxide (NO) and endothelium derived contractile factors (EDCFs) associated with oxidative stress (OS).^[2,4]

Oxidative stress is a condition in which cellular antioxidant defence are inadequate to completely inactivate ROS (Reactive Oxygen Species) and reactive nitrogen species generated because of their excessive production, loss of antioxidant defence or both.^[5] The important ROS detectable within the vasculature includes superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical ($\cdot OH$) and reactive nitrogen species peroxynitrite ($ONOO^-$).^[6]

ROS can be produced by the action of NADPH oxidase, uncoupled nitric oxide synthase (NOS), mitochondrial electron transport, xanthine oxidase, cyclooxygenase, lipoxygenase, heme oxygenase and cytochrome P450 monooxygenase. Of these, NADPH oxidase, xanthine

oxidase, uncoupled NOS and mitochondrial electron transport are the major producers of ROS in the vascular wall.

Reduced antioxidant capacity also promotes cellular oxidative stress and is implicated in cardiovascular and renal oxidative damage in hypertension. Superoxide dismutase (SOD), catalase and glutathione peroxidase (GPX) activities are reduced in hypertensive patients.^[7-9] As antioxidants SOD catalyses the conversion of superoxide radicals to H₂O₂ and O₂ protecting the cells against potential toxicity of reactive oxygen. H₂O₂ is further detoxified by catalase.^[10]

ROS generated due to endothelium dysfunction can bring about peroxidation of PUFAs, a constituent of cellular membrane leading to production of toxic and reactive aldehyde metabolite such as MDA. MDA is a marker of peroxidant status of biological system and causes damage to LDL. The altered LDL taken up by macrophages via scavenger receptors forms foam cells, thereby predisposing to atherogenesis.^[5] Thus the aim of our study was to determine the ROS induced endothelial dysfunction in the hypertensive patients by assessing the levels of lipid peroxidation product (MDA) and antioxidant enzymes (SOD, catalase, GPX).

Materials and Methods

The study included 100 hypertensive patients and 25 healthy controls. The patients included in the study, were selected from OPD, emergency ward & from indoor patients, admitted in ward of Department of Medicine, Gold Field Institute of Medical Sciences & Research, Chhainsa, Faridabad, Haryana. The individuals were categorized into 5 different groups on the basis of blood pressure (BP) (Table – 1).

Table-1: Groups of patient based on blood pressure

Groups	Systolic BP (mmHg)	Diastolic BP (mmHg)
Normotensive (25)	120	80
Pre-hypertensive(20)	120-139	80-89
Stage I hypertension (40)	140-159	90-99
Stage II hypertension (40)	≥160	≥100

Stage I and stage II hypertensive groups were further subdivided into those taking antihypertensive drug and those who are not taking the antihypertensive drugs. Blood pressure was measured by sphygmomanometer. Individuals with the history of smoking, diabetes mellitus, renal failure, chronic liver disease and other chronic diseases were excluded. Serum levels of antioxidant enzymes like SOD^[11], catalase^[12], GPX^[13],

and level of lipid peroxidation product (MDA)^[14], were estimated by spectrophotometric method.

Statistical Analysis

It was done by SPSS 16. Comparison of mean values of enzymes and MDA between control and hypertensive groups was done by student's t test. p value of <0.05 was considered statistically significant.

Results

Statistically significant decrease in the levels of antioxidant enzymes was observed in hypertensive groups as compared to controls (p<0.001) except for SOD in case of stage I hypertension with drug (p>0.05). Similarly MDA level was significantly increased in all the hypertensive groups (p<0.001) (table 2). The mean values of antioxidant enzymes and MDA were significantly different in both stage I and Stage II hypertensive patients taking drugs as compared to those without drugs (p<0.01). However it was not significant for SOD in case of stage I hypertensive groups (p>0.05) (table 4).

Discussion

Oxygen is important to sustain life. It is non-reactive in ground state. Cells use oxygen to generate energy but during the process free radicals are generated ^[15] which are highly unstable and reactive. The tissues are protected against these oxidants by enzymatic antioxidant (SOD, Catalase, GPx) and non-enzymatic anti oxidants (vit C, A etc).^[16]

In the present study, levels of SOD, catalase and GPx were significantly decreased in all hypertensive groups except for SOD in case of stage I hypertensive groups (p>0.05). Our finding was similar to that of KS Meera et al.^[17] Moreover, MDA levels in this study were increased significantly as compared to normal controls (P<0.05) and this was in accordance with that of Nwanjo HO et al.^[18]

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.^[19]

Martinez et al have reported that MDA can exacerbate

Table 2: Comparison of antioxidant enzymes and MDA between control and hypertensive groups (mean \pm SD)

Parameters	Control	Pre-hypertension	Stage I HT with drug	Stage I HT without drug	Stage II HT with drug	Stage II HT without drug
SOD	9.27 \pm 0.29	9.09 \pm 0.1**	8.51 \pm 1.99	8.84 \pm 0.11**	8.83 \pm 1.1**	8.64 \pm 0.08**
Catalase	9.07 \pm 0.07	8.99 \pm 0.06**	8.9 \pm 0.09**	8.74 \pm 0.15**	8.65 \pm 0.08**	8.49 \pm 0.03**
GPX	57.09 \pm 0.33	56.03 \pm 0.3**	54.19 \pm 0.39**	53.4 \pm 1.13**	51.1 \pm 0.78**	49.36 \pm 0.48**
MDA	1.24 \pm 0.03	1.26 \pm 0.02**	1.28 \pm 0.02**	1.33 \pm 0.07**	1.38 \pm 0.07**	1.5 \pm 0.04**

* p < 0.05: statistically significant; ** p < 0.01: highly significant

Table-3: Comparison of antioxidant enzymes and MDA between the hypertensive groups

Parameters	Hypertensive Groups	Pre-hypertension	Stage I HT with drug	Stage I HT without drug	Stage II HT with drug	Stage II HT without drug
SOD	Pre-hypertension	-	>0.05	<0.001	<0.01	<0.01
	Stage I hypertension with drug	>0.05	-	>0.05	>0.05	>0.05
	Stage I hypertension without drug	<0.01	>0.05	-	>0.05	<0.01
	Stage II hypertension with drug	<0.01	>0.05	>0.05	-	<0.01
	Stage II hypertension without drug	<0.01	>0.05	<0.01	<0.01	-
Catalase	Prehypertension	-	<0.05	<0.01	<0.01	<0.01
	Stage I hypertension with drug	<0.05	-	<0.01	<0.01	<0.01
	Stage I hypertension without drug	<0.01	<0.01	-	<0.05	<0.01
	Stage II hypertension with drug	<0.01	<0.01	<0.05	-	<0.01
	Stage II hypertension without drug	<0.01	<0.01	<0.01	<0.01	-
GPX	Prehypertension	-	<0.01	<0.01	<0.01	<0.01
	Stage I hypertension with drug	<0.01	-	<0.05	<0.01	<0.01
	Stage I hypertension without drug	<0.01	<0.05	-	<0.01	<0.01
	Stage II hypertension with drug	<0.01	<0.01	<0.01	-	<0.01
	Stage II hypertension without drug	<0.01	<0.01	<0.01	<0.01	-
MDA	Prehypertension	-	<0.05	<0.05	<0.01	<0.01
	Stage I hypertension with drug	<0.05	-	<0.05	<0.01	<0.01
	Stage I hypertension without drug	<0.05	<0.05	-	<0.05	<0.01
	Stage II hypertension with drug	<0.01	<0.01	<0.05	-	<0.01
	Stage II hypertension without drug	<0.01	<0.01	<0.01	<0.01	-

* p < 0.05: statistically significant; ** p < 0.01: highly significant

Table-4: Comparison of antioxidant enzymes and MDA between the stage I and Stage II hypertensive groups with and without drugs (mean \pm SD)

Parameters	Stage I hypertension			Stage II hypertension		
	Without drug	With drug	p value	Without drug	With drug	p value
SOD	8.84 \pm 0.11	8.51 \pm 1.99	>0.05	8.64 \pm 0.08	8.83 \pm 1.1	<0.01
Catalase	8.74 \pm 0.15	8.9 \pm 0.09	<0.01	8.49 \pm 0.03	8.65 \pm 0.08	<0.01
GPX	53.4 \pm 1.13	54.19 \pm 0.39	<0.01	49.36 \pm 0.48	51.1 \pm 0.78	<0.01
MDA	1.33 \pm 0.07	1.28 \pm 0.02	<0.01	1.5 \pm 0.04	1.38 \pm 0.07	<0.01

* p < 0.05: statistically significant; ** p < 0.01: highly significant

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 H₂O₂ concentration inactivates SOD leading to further rise in MDA levels.^[20]

The increase in MDA levels further inactivates the antioxidant enzymes (SOD, catalase and GPx) in untreated hypertension.^[21] Simic et al 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.^[20]

Reduced enzyme activity increases the production of hydroxyl radical which may contribute to the oxidative

stress thereby favoring lipid peroxidation. The superoxide ions can also react with NO to form peroxynitrite which in turn can induce lipid peroxidation and S-nitrosylation of thiol groups in proteins. NAD(P)H oxidase can be activated by ROS like H₂O₂ thereby enhancing the production of superoxide ions and contributing to vascular injury.^[22]

Armas Padilla et al reported an increase in lipid peroxidation in essential hypertension associated with increase superoxide mediated inactivation of nitric oxide.^[23] Nitric oxide is crucial for maintaining normal vascular function and inactivation of NO by excessive production of ROS has been implicated in several cardiovascular diseases.

ROS can act on angiotensin converting enzyme to increase its catalytic activity resulting in increase in

Angiotensin II production which, in turn, is a potent vasoconstrictor and is implicated in the pathogenesis of hypertension. Angiotensin II is a major endogenous inducer of NAD(P)H oxidase, increasing NAD(P)H concentration which subsequently increases vascular intimal ROS production with resultant increase in oxidative stress. Angiotensin II generates quinones which react with thiol group of GSH, thereby, decreasing its protective function and further increasing lipid peroxidation.^[24]

We also compared the antioxidant enzymes and MDA levels within the hypertensive groups. We found that for catalase, GPx and MDA, the levels were significantly different among the hypertensive groups, whereas, for SOD, significant difference could be obtained between prehypertensives and other groups except for stage I hypertension with drug.

When we compared SOD among Stage I hypertension with other groups, significant difference could not be obtained in any case. For stage I hypertension without drug and stage II hypertension with drug, significant results were obtained only in cases of pre-hypertensives and stage II hypertension without drug. In case of stage II hypertension without drug, significant difference was present for pre-hypertensive, stage I hypertensive without drug and stage II hypertensive with drug.

In our study, we also compared the level of enzymes and MDA among stage I and stage II hypertensives without drug with those taking antihypertensive drugs. We found that the levels of SOD, catalase and Gpx increased and MDA decreased significantly in those patients taking antihypertensive drugs. In case of stage I hypertensives, SOD levels were not significant between those taking drug and those not taking drugs.

In hypertension, vascular alteration in small and large arteries develops and contributes to the progression and its complication. Prevention of vascular alteration and improvement of function therefore could favourably affect the outcome of hypertension.^[25]

Antihypertensive drugs exert these effects via antioxidant, anti-inflammatory, anti-atherosclerotic or anti-fibrinolytic actions, thereby, improving endothelial function, reversing vascular remodelling and reducing cardiovascular complications.^[26] Antioxidant supplementation may be useful to improve the endothelial dysfunction. However, there are many

studies which have failed to show the reduction of blood pressure with antioxidant supplementation.

Kim et al observed no change in blood pressure after vitamin C supplementation.^[27] Palumbo et al^[28] also could not show the reduction in blood pressure after vitamin E supplementation whereas according to KS Meera et al, vitamin C has shown to improve endothelium dependent vasodilatation in hypertensives and increase in nitric oxide bioavailability.

Vitamin C influences blood pressure probably by its free radical scavenging property and preventing prostacyclin synthase inhibition. Lowering of blood pressure is associated with reduced oxidative stress. So, aggressive control of blood pressure can be helpful in reducing long term harmful effect of hypertension by reduction of free radical generation.^[17]

Conclusion

There is a marked increase in ROS production, decrease in antioxidant level and increase in lipid peroxidation in hypertension. ROS play a physiological role in vessel wall and participate as second messenger in endothelium dependent function of smooth muscle, endothelial cell growth, survival and remodelling of vessel wall.

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. Further the potential value of antioxidant supplement to reduce blood pressure reduction in oxidative stress is limited. More extensive study is required to check the association between hypertension and oxidative stress.

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