DOI: 10.7860/JCDR/2013/5829.3091

Original Article

Physiology Section

The Role of the Endogenous Antioxidant Enzymes and Malondialdehyde in Essential Hypertension

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ABSTRACT

Context: Oxidative Stress is caused by an imbalance between the production of reactive oxygen species and a biological system's ability to readily detoxify the reactive intermediates.

Aims: 1. To compare the levels of Malondialdehyde (MDA), in hypertensive and normotensive subjects.
2. To compare the levels of the antioxidant enzymes, namely, Catalase, Glutathione peroxidase (GPX) and Superoxide Dismutase (SOD) in hypertensive and normotensive subjects.
3. To determine the correlation between the MDA levels and the mean arterial pressure (MAP) among hypertensive subjects.
4. To determine the correlation between the antioxidant enzyme levels and MAP among the hypertensive subjects and to evaluate the effect of 6 months of antihypertensive therapy with a tight blood pressure control on the MDA levels.

Materials and Methods: In this cross sectional study, 25 normotensive and 40 hypertensive subjects were recruited. The hypertensive subjects were further subdivided into three subgroups: Prehypertensives, Stage I hypertensives and Stage II hypertensives. All the subjects underwent a blood pressure measurement and the markers of oxidative stress in their sera were estimated. The subjects of Stage I hypertension and Stage II hypertension were given antihypertensive treatment for 6 months and their blood pressures were tightly regulated and brought to the normotensive state. After 6 months, the estimations of the markers of oxidative stress were done again.

Results: The MDA levels were significantly increased in the stage I and stage II hypertension groups as compared to those of the control group ($p<0.05$). The antioxidant enzymes (SOD, Catalase and GPX) were significantly decreased ($p<0.05$) in the prehypertension and in the stage I and stage II hypertension groups as compared to those in the control group. There was a significant increase in the levels of the antioxidant enzymes after 6 months of a tight regulation and bringing of the blood pressure to the normotensive state by giving antihypertensive therapy.

Conclusion: On comparison of the present study with other studies in which the use of antioxidants were found to be ineffective in the blood pressure reduction, it can be concluded that oxidative stress is an effect rather than a cause of essential hypertension.

Key Words: Essential hypertension, Oxidative Stress, Catalase, Glutathione peroxidase, Superoxide Dismutase Antihypertensive therapy

INTRODUCTION

Reactive oxygen species (ROS) are highly reactive intermediates of the oxygen metabolism, which are constantly being generated and destroyed, both by the environment and the endogenous system. When there is an imbalance between the generation of ROS and the antioxidant defense system so that the latter becomes overwhelmed, oxidative stress occurs [1]. Traditionally, macrophages had been assumed to be the source of the most ROS in the vessel wall, However, it has become clear that virtually all the cells in the vessel wall (endothelial cells and smooth muscle and adventitial cells) produce ROS in varying amounts and in response to diverse stimuli, which can act in an autocrine or paracrine fashion to modulate the cellular function [2]. The ROS play a physiological role in the vessel wall and they participate as second messengers in the endothelium-dependent function in the smooth muscle and endothelial cell growth and survival and in the remodeling of the vessel wall. Each of these responses, when they are uncontrolled, contribute to vascular disorders and they play an important role in the development of cardiovascular disorders [3].

Animal studies have supported the hypothesis that an increased blood pressure is associated with increased oxidative stress [4]. However, the findings in humans are inconsistent. Also, it is unknown whether this abnormality is a primary event or a consequence of the increased blood pressure. An attempt which was made to counteract the hypertensive effect of ROS has led to the use of an exogenous administration of antioxidants, in an attempt to improve the vascular function and to reduce the blood pressure in animal models and in human subjects. However, the recent data are inconsistent and not conclusive and so the relationship between blood pressure and oxidative stress in humans remains to be elucidated.

Our study was conducted with the following aims:

1. To compare the levels of Malondialdehyde (MDA), which is a marker of the lipid peroxidation, in hypertensive and normotensive subjects.
2. To compare the levels of the antioxidant enzymes, namely, Catalase, Glutathione peroxidase (GPX) and Superoxide Dismutase (SOD) in hypertensive and normotensive subjects.
3. To determine the correlation between the MDA levels and the mean arterial pressure (MAP) among hypertensive subjects.

4. To determine the correlation between the antioxidant enzyme levels and MAP among the hypertensive subjects and to evaluate the effect of 6 months of the antihypertensive therapy with a tight blood pressure control on the MDA levels.

MATERIAL AND METHODS

This study was conducted at a tertiary care teaching hospital. The period of the study was from July 2011 to June 2012. 40 hypertensive patients (including prehypertension and stage I and stage II hypertension, according to JNC 7) who were diagnosed in the medicine outpatients department were included in this study. Only those patients who were not on any antihypertensive treatment previously were included. Twenty five healthy normotensive subjects served as the control group. All the participants signed written consents before being included in the study; the study protocol was approved by the ethics committee of the institution. The exclusion criteria were secondary causes of the hypertension, smoking, diabetes, asthma, COPD, malignancies, chronic diseases and the current use of any medications, which included dietary supplements. The potential participants were subjected to the taking of the clinical history, a physical examination and appropriate investigations. Their blood pressures were recorded by an auscultatory method by using a Sphygmomanometer on the left arms, with the patients in the sitting position at room temperature. On the basis of the blood pressure levels, the following groups were formed according to the JNC-VII classification.

Group	Systoloc Blood Pressure (Mm Hg)	Diastolic Blood Pressure
Normotensive (Control)	<120	<80
Prehypertension (Case)	120-139	80-89
Stage I Hypertension (Case)	140-159	90-99
Stage Ii Hypertension (Case)	≥160	≥100

Under aseptic conditions and with the prior consents of the subjects, 5ml of blood samples was drawn from the peripheral veins and they were centrifuged at 3000rpm for fifteen minutes. The sera were subjected to estimations of Malondialdehyde (MDA), which is a marker of lipid peroxidation and the antioxidant enzymes, namely, Catalase, Glutathione peroxidase (GPX) and Superoxide Dismutase (SOD) by a prior adopted method [5-8]. All the subjects in stage I hypertension and stage II hypertension were given antihypertensive treatment in the form of various combinations of β-blockers, ACE inhibitors and calcium channel blockers and their blood pressures were tightly regulated for 6 months and brought to the normotensive state. After 6 months, their venous blood was again sampled and the sera were subjected to the estimations of MDA and the antioxidant enzymes.

The protein contents of the sera were estimated by the method which was put forth by Lowry [9]. The antioxidant enzymes were expressed with respect to the serum protein levels.

STATISTICAL ANALYSIS

The statistical analysis was done by using the Statistical Package for Social Sciences (SPSS 17). The results were expressed as Mean±Standard Deviation (SD). The differences between the groups were analyzed by using the Student's "t"-test and one way ANOVA with the post hoc Tukey test and Pearson's correlation was applied to determine the relationships between the variables. The statistical significance was defined at a p value of <0.05.

RESULT

The clinical characteristics and the serum levels of the markers of oxidative stress in the control and the case groups have been shown in [Table/Fig-1] and [Table/Fig-2]. The antioxidant enzymes (SOD, Catalase and GPX) were significantly decreased (p<0.05) in prehypertension and stage I and stage II hypertension as compared to those in the control group. The MDA level was significantly increased in the stage I and stage II hypertension groups as compared to that in the control group (p<0.05) but there was no significant difference in the MDA levels between the prehypertension and the control groups (p>0.05) [Table/Fig-2], thus indicating an increase in the oxidative stress levels in the hypertensive subjects as compared to those in the normotensive individuals.

	Control (N=25)	Hypertensive (N=40)
Age (Years)	33.36 ± 9.74	48.47 ± 5.38*
Height (Cm)	161.72 ± 6.23	163.45 ± 6.20
Weight (Kg)	61.04 ± 9.28	65.52 ± 7.14
BMI (Kg/M²)	23.25 ± 2.83	24.45 ± 1.82*
Systolic BP (mmHg)	114.32 ± 2.68	150 ± 15.33*
Diastolic BP (mmHg)	75.04 ± 2.16	96 ± 12.04*
Map (mmHg)	88 .08 ± 2.27	114.30 ± 13*

[Table/Fig-1]: Age, anthropometric and basal cardiovascular profile of different study group subjects
Data presented are mean±SD. Analysis of data was done by unpaired t- test. The * depicts p <0.05.

	Control (n=25)	Pre HTN (n=15)	Stage I HTN (n=13)	Stage II HTN (n=12)	P value
SOD (U/mg of serum protein)	9.37±0.29	9.19±0.10**	8.80± 0.11**##	8.65± 0.04**##ff	P<0.05
CATALASE (U/mg of serum protein)	9.07±0.07	8.98±0.70**	8.66± 0.20**##	8.48± 0.02**##ff	P<0.05
GPX (NADPH/ min/mg of serum protein)	57.09±0.33	55.94±0.30**	53.02± 0.49**##	49.33± 0.36**##ff	P<0.05
MDA (nmol/ml)	1.24±0.03	1.26±0.01*	1.31± 0.08**##	1.49± 0.05**##ff	P<0.05

[Table/Fig-2]: Comparison of Marker of Oxidative Stress Between Control and Subgroup Hypertensive Subjects
Data presented are mean±SD. Analysis of data was done by one-way ANOVA and post-hoc by Tuky test. The * depicts comparison with control 1 and the # depicts comparison with pre htn , and the ƒ depicts comparison with stage I htn. ** P < 0.05; ## P < 0.05; ff P < 0.05.

As per [Table/Fig-3], among the hypertensive group, there was a negative correlation between the antioxidant enzymes and the mean arterial pressure (MAP). The MDA levels showed a positive correlation with the MAP. This showed that there was a positive correlation between oxidative stress and the levels of MAP.

As per [Table/Fig-4], there was a significant increase in the antioxidant enzyme levels after 6 months of a tight regulation and bringing of the blood pressure to the normotensive state by an antihypertensive therapy in the stage I and stage II hypertension groups as compared to their levels before the treatment (p<0.05).

The MDA levels showed a significant decrease after 6 months of the antihypertensive therapy in the subjects of stage II hypertension as compared to their levels before the treatment ($p<0.05$). However, there was no significant difference in the MDA levels before and after the treatment in the subjects of stage I hypertension ($p>0.05$).This suggested that a tight regulation of the blood pressure among the hypertensive subjects had led to a decrease in the oxidative stress among the hypertensive subjects.

Enzyme	R-Value
SOD	-0.773**
CATALASE	-0.766**
GPX	-0.936**
MDA	+0.849**

[Table/Fig-3]: Pearson's Correlation Between Mean arterial pressure and Markers of Oxidative Stress
** p< 0.05

Enzyme	Stage I HTN Before Treatment	Stage I HTN After Treatment	Stage II HTN Before Treatment	Stage II HTN After Treatment
SOD (U/mg of serum protein)	8.80 ± 0.11	9.05±0.12**	8.65±0.04	8.78±0.02##
CATALASE (U/mg of serum protein)	8.66 ± 0.20	8.90±0.92**	8.48±0.02	8.59±0.05##
GPX (NADPH/min/mg of serum protein)	53.02 ± 0.49	54.22±0.39**	49.33±0.36	50.91±0.65##
MDA (nmol/ml)	1.31 ± 0.08	1.27±0.01*	1.49±0.05	1.41±0.07##

[Table/Fig-4]: Comparison of Oxidative Stress markers before and after the treatment within Subgroup Hypertensive Subjects
Data presented are mean±SD. Analysis of data was done by paired t-test .
The * depicts comparison with stage I HTN before treatment and the # depicts comparison with stage II HTN before the treatment. ** $p < 0.05$; ## $p < 0.05$.

DISCUSSION

Free radicals have diverse roles in the vascular redox systems in the patients of hypertension, thus suggesting that the complexity of the redox signaling in the distinct spatial spectrums should be considered for a better understanding of the hypertension. Our study demonstrated a decrease in the endogenous antioxidant enzyme levels in the hypertensive cases, when they were compared with those of the normotensive control group. There was an increase in the MDA levels, which is marker of lipid peroxidation, in the hypertensive group in comparison to those in the control group. There was a negative correlation between the MAP and the endogenous antioxidant enzyme levels, whereas MDA showed a positive correlation with the MAP within the hypertensive subjects. Thus, it showed an impairment of the antioxidant defense systems in essential hypertension and there was also a positive correlation between oxidative stress and the blood pressure.

An increased vascular oxidative stress had been demonstrated in many forms of experimentally induced hypertension in animal models [10]. Studies have also shown that the activities of SOD, Catalase and GPX were significantly lower in the whole blood in hypertensive patients as compared to those in the normotensive subjects [11]. The decrease in the antioxidant enzymes could be due to their inactivations as the result of a continuous exposure to hydrogen peroxide, hydrogen peroxynitrite and other free radicals [12]. Studies have shown that this reduction could also be due to the down regulation of their gene expressions [13].

In accordance with the results of our study, Nwanjo et al., and Mahdi et al., also demonstrated an increase in the MDA levels in the essential hypertension cases [14, 15] Cracowski et al., found that in never-treated mild-to-moderate hypertension, the lipid peroxidation was not increased, thus suggesting that the ROS may not be critical in the early stages of human hypertension, but that they could be more important in severe hypertension, which was consistent with the findings of our study, as an increase in the MDA levels in the prehypertensive subjects was not significant as compared to those in the control group [16].

Our study also demonstrated that a tight regulation and bringing of the blood pressure to the normotensive state by antihypertensive drugs not only decreased the blood pressure but that they also decreased the MDA levels and increased the levels of the antioxidant enzymes like SOD, Catalase and GPX. Thus, there was a decrease in the oxidants and an increase in the antioxidants after the antihypertensive treatment, thereby leading to an overall improvement in the oxidative stress.

In hypertension, vascular alterations develop in the small and the large arteries in parallel and they interact, thus contributing to its progression. Corrections of the small artery and the large artery structures and functions could therefore favourably affect the outcome of hypertension [17]. The antihypertensive agents may exert these actions through their antioxidant, anti-inflammatory, anti-atherosclerotic and anti-fibrinolytic effects, thus improving the endothelial function, reversing the vascular remodeling, and reducing the cardiovascular complications [18]. These effects may improve the arterial function, reduce the peripheral vascular resistance and reverse the structural changes in the small and large arteries.

Galley et al., showed in a small group of normotensive and hypertensive subjects, that a short term oral high dose combination antioxidant therapy reduced the blood pressure, possibly via an increased availability of nitric oxide [19]. However, the evidence which supports the use of antioxidants as blood pressure-lowering agents is limited and the clinical studies are less convincing. One of the largest studies which were undertaken by the Heart Protection Collaborative Group saw no improvement in the blood pressure after a treatment with a Ascorbic acid, synthetic vitamin E and β-Carotene combination versus placebo after 5 years, in the subjects who were thought to be at a high risk of cardiovascular disease [20]. Kim et al., observed no reduction in the blood pressure after a vitamin C supplementation [21]. Similarly, Palumbo et al., failed to show the blood pressure reduction effect of a Vitamin E supplementation (300mg/day) at clinic and the 24 hour ambulatory blood pressure in 142 treated hypertensive patients [22].

Thus, the potential value of the antioxidant supplements for reducing the blood pressure via a reduction in the oxidative stress is limited. On the other hand, lowering the blood pressure is definitely associated with a reduction in the oxidative stress. Because many blood pressure-lowering agents reduce the oxidative stress,

it seems logical that lowering the blood pressure per se, rather than the agents which are used, reduce the oxidative stress. Therefore, it seems that oxidative stress is not the cause but the effect of hypertension. Thus, in conclusion, our study demonstrated that reduction of the blood pressure to the normotensive state in hypertensive subjects led to a definite reduction in the free radicals generation and that this could help in preventing the long term complications of the hypertension which was mediated by the free radicals. The major limitation of our study was the small sample size. Financial constraints were the main cause of choosing a small sample size. Multi centric studies with larger numbers of subjects are required to extrapolate these results to the general population.

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FINANCIAL OR OTHER COMPETING INTERESTS:

None.

Date of Submission: **Feb 08, 2013**
Date of Peer Review: **Mar 06, 2013**
Date of Acceptance: **Mar 26, 2013**
Date of Online Ahead of Print: **Apr 12, 2013**
Date of Publishing: **Jun 01, 2013**