ORIGINAL CONTRIBUTIONS

ALTERED OXIDANT-ANTIOXIDANT STATUS IN NON-OBESE MEN WITH MODERATE ESSENTIAL HYPERTENSION

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ABSTRACT

BACKGROUND: Although a wide number of experimental evidences are available regarding oxidant-antioxidant disturbance in hypertension, clinical data supporting it is lacking in men in early stages of hypertension. AIMS: The objective of the study was to evaluate oxidative status and antioxidant activities in males with stage I essential hypertension. MATERIALS AND METHODS: Thirty hypertensives and 21 normotensives were included in the study. Protein carbonyl, reduced glutathione, glutathione peroxidase, catalase and fasting glucose were assessed in both the groups. STATISTICAL ANALYSIS: Results were analyzed by student's 't' test and linear regression analysis test. RESULTS: Plasma protein carbonyl and glutathione peroxidase were significantly increased, and catalase and GSH were significantly reduced in the hypertensive group compared to normotensive subjects. There was a significant negative correlation between glutathione peroxidase and catalase in the test group. CONCLUSIONS: The data from the present study indicates an alteration in oxidant-antioxidant status in non-obese men in early stages of essential hypertension.

Key words: Antioxidants, essential hypertension, oxidative stress

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The prevalence of hypertension has been ! increasing in developing countries; and ! community surveys have documented that it ! is more prevalent among the Indians between ! the third and sixth decades of their life.[1] ! Hypertension is a major modifiable risk factor ! for cardiovascular disease, which accounts ! for 57 and 24% of all deaths due to stroke and ! coronary heart disease respectively.[2] !

Oxidative stress, which results from either ! overproduction of free radicals or depletion of ! antioxidant reserve, has been implicated in ! the development of cardiovascular disorders ! including hypertension.[3] Previous !studies ! have assessed oxidative stress byproducts like ! protein carbonyls and antioxidant activities in ! elderly hypertensive subjects and indicated an ! altered oxidant-antioxidant balance in them.[4] ! This hypothesis has been supported by several ! experimental studies, which documented an ! increased oxidative stress in animal models ! - like renovascular hypertension and obesity-! related hypertension[5,6] - and its subsequent ! reduction on treatment with antioxidants.[7]!

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It has been reported that at young age the prevalence of hypertension is higher among males compared to females.[1] This can be attributed to the fact that females are more protected from oxidative stress through estrogen.[8] Since the reports related to oxidant-antioxidant disturbance in men with moderate (stage I) essential hypertension are lacking, the present study was undertaken to evaluate oxidative stress parameters such as protein carbonyls and antioxidant enzymes in relatively young male essential hypertensive subjects.

MATERIALS AND METHODS

The current study was conducted in the Department of Biochemistry and the Department of Physiology, JIPMER, Pondicherry. Our subjects included the nonteaching staff belonging to our institute and outpatients who visited our laboratory for blood pressure check-up. Blood pressure was measured using a mercury sphygmomanometer (Diamond, India) with the patients in the sitting position after 5 min of rest in a quiet environment - according to the recommendations of the British Hypertension Society. They were classified as normotensive and hypertensives as per the recommendation of the Joint National Committee (JNC) 7 report.[9] Newly diagnosed hypertensive subjects were defined as subjects who were diagnosed with a sustained elevation of blood pressure during the course of the study and were neither taking any medications nor were under any form of lifestyle modification. If the systolic and diastolic blood pressure were in different categories, the higher of the two was used in the classification.

Thirty newly diagnosed stage I hypertensive [systolic blood pressure (SBP mm Hg) = 140-159 or diastolic blood pressure (DBP mm Hg) = 90-99] and 21 normotensive (SBP <120 mmHg and DBP <80 mm Hg) men in the age group of 25-55 years were enrolled in the study. Subjects with history of diabetes, renal disease, endocrine dysfunction, coronary heart disease, after infections, smokers and alcoholic and those who were on any kind of medication were excluded from the study. A written informed consent was obtained from all the subjects. The study was approved by the Human Research and Ethics Committee of our institute.

Five milliliters of fasting blood was collected from all the subjects in Ethylene diamine tetraacetate bottles. Whole blood was used to analyze reduced glutathione (GSH) and hemoglobin. Plasma was collected by centrifuging rest of the sample at 5,000 rotation per minute (rpm) for 5 min at 4°C and was used for the estimation of protein carbonyl, lipid profile parameters and glucose. Erythrocytes were washed with 0.9% saline and lysed with cold distilled water. Antioxidant enzymes were estimated using the lysate.

Whole blood glutathione (GSH) was estimated using Ellman's reagent by Beutle's method.[10] Hemoglobin levels were estimated spectrophotometrically at 546 nm by using Drabkin's reagent (E. Merck, Mumbai, India). Erythrocyte glutathione peroxidase and catalase were estimated by methods of Wendel *et al.* and Aebi *et al.* respectively.[11,12] Protein carbonyl was estimated by Dinitrophenylhydrazine method.[13] Fasting glucose, total cholesterol and triglycerides were estimated by enzymatic methods and HDL cholesterol was estimated by the 'phosphotungstate magnesium acetate'

method using reagent kits (Agappe's Diagnostics, India) adapted to 550 express plus random access autoanalyzer (West pole, Canada). LDL cholesterol was calculated by Friedwald's formula. Fasting insulin was estimated by radioimmunoassay (RIAK-1 kit, Board of Radiation and Isotope Technology, Mumbai) using gamma counter (Wallac, Germany).

Statistical analysis

The results were expressed as mean (S.D.) and analyzed by using student's '*t*' test. Linear regression analysis was used to assess the association between oxidants and antioxidants. A *P* value of less then 0.05 was considered significant.

RESULTS

Table 1 shows mean and standard deviation of age, BMI, protein carbonyl, antioxidants, fasting insulin and lipid profile parameters in hypertensive subjects and controls. Protein carbonyl and glutathione peroxidase were significantly increased, and catalase and GSH were significantly decreased in hypertension. Since diabetes and obesity are commonly associated with hypertension, we estimated BMI and fasting plasma glucose in both the groups and found that there was no significant difference in these parameters between the two groups. These findings suggest a state of oxidative stress in nondiabetic and non-obese hypertensive subjects. Also in the hypertension group, fasting insulin, total cholesterol, triglycerides and LDL cholesterol were significantly increased, and HDL cholesterol was significantly decreased in comparison with control.

Figure 1 shows linear regression analysis of catalase and glutathione peroxidase, which was found to be significant (β = -0.444, R^2 = 0.197, *P* = 0.014). These results show an altered antioxidant status in early stages of hypertension.

Table 1: Mean and standard deviation of age, blood pressure, fasting glucose, BMI, fasting insulin, lipid profile, protein carbonyl and antioxidants in hypertensive subjects and controls

Parameters	*Control (n =21)*	*Hypertension (n = 30)*	*P value*	*95% CI lower*	*95% CI upper*
Systolic blood pressure (mm Hg)	114 ± 4	141 ± 10	<0.001	- 31.34	- 22.05
Diastolic blood pressure (mm Hg)	72 ± 4	92 ± 4	<0.001	- 22.14	- 17.50
Age (yrs)	36 ± 11	42 ± 12	0.100	- 11.76	1.07
BMI (kg/m²)	23.57 ± 3.21	24.86 ± 2.24	0.097	- 2.82	0.24
Waist/Hip ratio	0.91 ± 0.06	0.94 ± 0.04	0.076	- 0.05	- 0.003
Plasma Glucose (mg/dl)	85.66 ± 18.85	93.00 ± 13.58	0.112	- 16.45	1.78
GSH (mg/gHb)	3.68 ± 1.50	2.76 ± 1.24	0.021	0.14	1.69
Glutathione -Peroxidase (u/gHb)	74.13 ± 44.02	105.62 ± 49.46	0.023	- 58.54	- 4.43
Catalase (k/ml)	27.92 ± 14.08	19.81 ± 9.7	0.019	1.41	14.81
Protein carbonyl (nmol/mg protein)	1.85 ± 0.68	2.36 ± 0.73	0.017	- 0.91	- 0.09
Total protein (g/dl)	5.2 ± 0.84	4.59 ± 1.80	0.133	- 0.20	1.49
Fasting Insulin (µu/ml)	21.79 ± 10.48	42.07 ± 28.74	0.003	- 33.48	- 7.06
Total cholesterol (mg/dl)	170.80 ± 27.18	190.20 ± 36.20	0.043	- 38.15	- 0.62
Triglycerides (mg/dl)	106.85 ± 54.80	142.30 ± 60.84	0.038	- 68.86	- 2.02
HDL- cholesterol (mg/dl)	50.38 ± 11.44	40.06 ± 12.41	0.004	3.43	17.19
LDL- cholesterol (mg/dl)	99.57 ± 29.38	122.36 ± 33.65	0.016	- 41.08	- 4.50
VLDL- cholesterol (mg/dl)	20.85 ± 10.41	28.43 ± 12.10	0.024	- 14.11	- 1.03

95% CI = 95% confidence interval of difference

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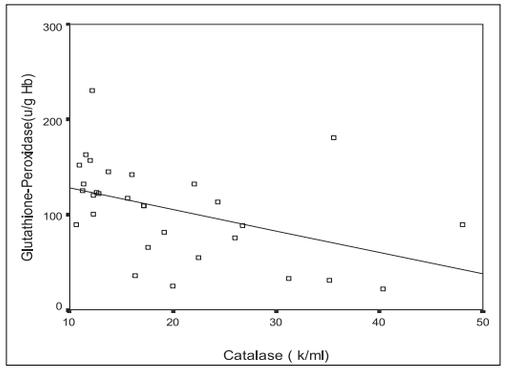

Figure 1: Linear regression analysis of catalase and glutathione peroxidase among hypertensive subjects (β = -0.444, R^2 = 0.197, P= 0.014)

DISCUSSION

Oxidative stress has been implicated in the pathogenesis of various cardiovascular disorders including hypertension. Oxidative stress stimulates vascular smooth muscle proliferation and reduces nitric oxide bioavailability, causing endothelial dysfunction, which plays a crucial role in the pathogenesis of hypertension by reducing endothelium-dependent vasodilatation.[14,15]

In contrast with the huge number of experimental studies, clinical studies supporting the involvement of oxidative stress in the pathogenesis of essential hypertension are lacking. In the present study, oxidative stress markers such as protein carbonyls were significantly increased in hypertensive cases compared to controls. This finding was supported by previous studies which reported increased protein carbonyls in different stages of hypertension.[16] Increase in plasma protein carbonyl in this study indicates that 'free radical'-mediated oxidative damage of proteins occurs at an early stage of hypertension and could increase significantly in later stages.

Antioxidant defense mechanisms are altered in response to generation of free radicals. Several investigators have reported contradictory findings regarding antioxidant status in essential hypertension.[4,16] In our study, we found a significant increase in erythrocyte glutathione peroxidase (GPX) levels and reduction in catalase and GSH levels. Also, we found considerable negative correlation between catalase and glutathione peroxidase. The primary catalytic cellular defense that protects cells and tissues against lipid peroxidation is the glutathione peroxidase enzyme.[17] It has been observed that glutathione peroxidase can be rapidly induced in some conditions when cells or organisms are exposed to oxidative stress.[18] The increased glutathione peroxidase activity in red blood cells of the test subjects may be interpreted as a compensatory mechanism due to the increased oxidative stress.

The levels of glutathione peroxidase may be related to the stages of hypertension. There are no reports which point out whether oxidative stress sets in first or hypertension. The decrease in catalase activity in our study may be attributed to its inactivation as a result of continuous exposure to hydroperoxides and hydrogen peroxide. This decrease can also be due to a down-regulation of its expression. The depletion of glutathione and the accumulation of free radicals could induce the enhanced expression of glutathione peroxidase, as observed in the present study. This decrease in catalase and an increase in glutathione peroxidase explain the negative correlation found between them. Because it has been shown that glutathione peroxidase is more potent on a molar basis

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than catalase and other antioxidant enzymes to protect cells from oxidative stress,[19] it can be hypothesized that body tends to combat stress by overexpressing glutathione peroxidase gene as the first line of defense in essential hypertension. As the severity of hypertension advances into stage II and III, even the defenses of glutathione peroxidase may deteriorate because of the increased production of free radicals. This may be the reason for the decreased levels of glutathione peroxidase observed by Kedziora *et al.*[4]

CONCLUSION

Our results point towards an imbalance in the oxidant / antioxidant ratio in hypertensive patients. Although more than one factor is implicated in the development of hypertension, the hypothetical role of oxidative stress *per se* in the development of hypertension cannot be ruled out. Future epidemiological, polymorphic studies to identify candidate antioxidant genes that are altered in essential hypertension are warranted. This will increase our understanding of the genetic modulation of antioxidant enzymes in these subjects, which will be useful for the development of molecular interventions in them. Further studies are also required to define whether dietary or supplemental antioxidants ameliorate these processes.

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