

Serum Paraoxonase activity and Oxidative Stress in Primary Hypertension

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Abstract

Hypertension is one of the most prevalent diseases of the world and is an unequivocal risk factor for cardiovascular morbidity and mortality. Hypertension is known to be associated with oxidative stress. The enzyme paraoxonase being antiatherogenic and sensitive to oxidative stress was intriguing in hypertensives. This study was conducted with objectives to determine paraoxonase activity in primary hypertension and to correlate paraoxonase activity to oxidative stress in primary hypertension. In this study there were a total of 60 male subjects which included 30 healthy males as controls and 30 male primary hypertensives without complications of hypertension or any other underlying medical disorder as cases. Our study revealed that serum paraoxonase activity was significantly reduced in hypertensives when compared to controls. Correlation between serum paraoxonase and malondialdehyde with routine biochemistry parameters in both hypertensives and controls was not found to be significant. This study indicated that assessment of serum paraoxonase activity can serve as a predictive factor for atherosclerotic complications in primary hypertensives where oxidative stress is implicated.

Keywords: Serum Paraoxonase, Oxidative Stress, Hypertension.

1. Introduction

Hypertension is one of the most prevalent diseases of developed Western societies and rapidly increasing in developing world. It is a major risk factor for cardiovascular morbidity and mortality.

National health and nutrition examination surveys (NHANES) in United States have reported a hypertension prevalence rate of 20% of entire US population in 1991. The prevalence rate varied from 4% in age group 18-24 years to 60% in age group of 65-74 years. In India several regional small surveys with varying protocols have reported a prevalence which varies widely from 3.80 to 15.63% in men to 2 to 15.38% in women in urban areas and from 1.57 to 6.93% in men and 2.38 to 8.80% in women in rural areas. These surveys were based on casual reading taken only on one occasion and used criteria of $\geq 140/90$ mm of Hg as hypertension. In a survey in Mumbai, the overall prevalence based on average of three readings was 26.9%. The criteria

for hypertension were blood pressure measurements of SBP ≥ 140 mm of Hg or DBP ≥ 90 mm of Hg.[1] The underlying pathophysiological abnormalities that lead to the development of the complications due to elevated arterial pressure remain elusive. Hypertension has been shown to be associated with oxidative stress, which is involved in enhanced vascular growth, vascular inflammation and impaired endothelium.[2] However, the role of oxidative stress in the pathogenicity of hypertension and its complications is still not well understood. The mechanisms contributing to the dysregulation of normal vascular tone and the implications in hypertension-induced target organ damage by oxidative stress-derived products remains to be understood.[3] The enzyme paraoxonase being antiatherogenic and sensitive to oxidative stress was intriguing.[4]

This study was aimed at determining the paraoxonase activity and to understand the role of oxidative stress in hypertension and its relation to serum paraoxonase activity in hypertensives. Correlation of paraoxonase activity to oxidative stress in hypertensives was studied.

2. Material and methods

The study was carried out on 30 healthy normotensive controls and 30 hypertensive patients. The diagnosis of hypertension was established in accordance with the recommendations of world health organizations, and international society of hypertension.[5] The inclusion criteria included male subjects in the age group of 40 to 60 years. Exclusion criteria included any underlying medical disorders such as cardiovascular disease, renal disease, diabetes mellitus, smoking, and alcohol abuse determined by a comprehensive physical examination and routine laboratory investigations. Any treatment medication was noted. Informed consent was taken from both cases and controls. Subject demographics, medical history in detail and physical examination data were obtained from both cases and controls. 5ml of random venous sample was collected from the subjects. The samples were immediately centrifuged. Serum was separated and stored at 4°C until analysis. A random urine sample was collected and stored at 4°C until analysis. The blood samples were analyzed for plasma malondialdehyde, serum paraoxonase activity, serum glucose, serum urea, and serum creatinine and serum uric acid. Urine samples were analyzed for glucose and protein. All the estimations were done within eight hours of collection of specimen and separation of serum.

Malonaldehyde (MDA), a reactive aldehyde is a product of lipid peroxidation. MDA in serum reacts with thiobarbituric acid (TBA) to form pink colored complex of TBA-MDA adduct.[6] It was then centrifuged at 3000 rpm for 10 minutes. The upper clear supernatant fluid was transferred to a cuvette and the absorbance was measured at 530 nm with a spectrophotometer. For estimation of serum paraoxonase arylesterase activity, serum was incubated with buffered P-nitro phenyl acetate as a substrate. The assay mixture contained 1.0 mmol/L of phenyl acetate and 0.9 mmol/L of calcium chloride in 20 mmol/L Tris HCl at pH 8 at 25degree Celsius. Non enzymatic hydrolysis of phenyl acetate was subtracted from total rate of hydrolysis. To this 5 micro liter of serum was added and mixed. Absorbance was noted immediately using UV spectrophotometer at 270nm at 0, 30 and 60 seconds. The rate of formation of P-nitro phenol determined the arylesterase activity which was measured spectrophotometrically at 270 nm. Serum glucose, urea, uric acid and creatinine were estimated by standard methods. Urinary protein was estimated by turbidimetric

method, urine glucose was measured by reagent-strip method.

Chi-Square test and Fisher Exact test were used to find the statistical significance of proportion between cases and control. Student t test (independent two tailed) was used to find the significance of anthropometry, haemodynamics and laboratory investigations between Cases and Control. The Effect Sizes due to Cohen was computed to find the effect of hypertension on laboratory parameters over the control group.

3. Results

The study subjects that were hypertensives and controls were matched for age and sex. The blood pressure values of systolic, diastolic and the mean for the subjects are presented in Table-1. The systolic blood pressure in the hypertensives (142.33±11.35) were higher than the normotensive controls (122.00±8.77). The diastolic blood pressure was also significantly elevated in the hypertensives (94.2±6.11) than the normotensive controls (77.67±5.94).

Table 1: Haemodynamics

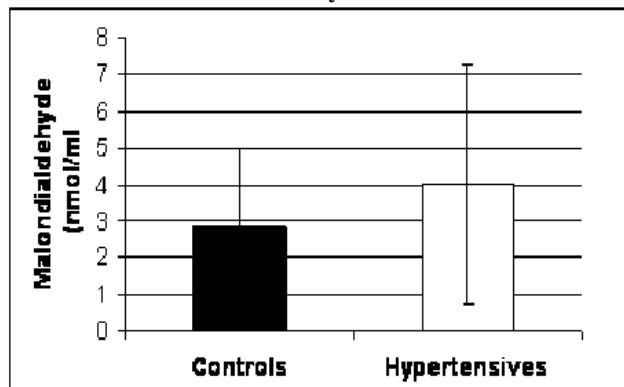
Haemodynamics (Mean ± SD)	Controls (n=30)	Hypertensives (n=30)	P value
SBP	122.00±8.77	142.33±11.35	0.000**
DBP	77.67±5.94	94.20±6.11	0.000**

Table 2: Laboratory Investigations

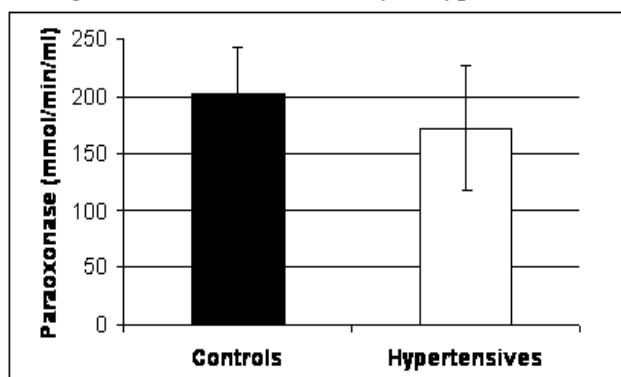
Lab Investigations (Mean ± SD)	Controls (n=30)	Hypertensives (n=30)	P value	Effect Size
Paraoxonase (nmol/min/ml)	201.84±41.13	171.84±54.91	0.020*	0.61
Malondialdehyde (nmol/ml)	2.83±2.17	4.01±3.25	0.109	0.44
Plasma Glucose (mg/dl)	85.23±17.83	95.97±23.30	0.050*	0.51
Plasma Urea (mg/dl)	27.00±6.01	23.27±6.80	0.028*	0.57
Serum Creatinine (mg/dl)	1.11±0.17	1.10±0.21	0.946	0.05
Uric acid (mg/dl)	6.05±1.04	6.12±1.13	0.813	0.06
Urine Protein (mg/dl)	7.83±17.52	32.17±48.13	0.000**	0.76

Serum glucose, serum urea and serum creatinine: The values of serum glucose, serum urea, serum creatinine, and their mean of the study subjects are represented by Table-2. All the values were within the normal reference range and no significant difference was found. The individual and mean Urine protein levels in hypertensives (32.17±48.13 mg/dl) showed significant elevation compared to the control group (7.83±17.52 mg/dl).

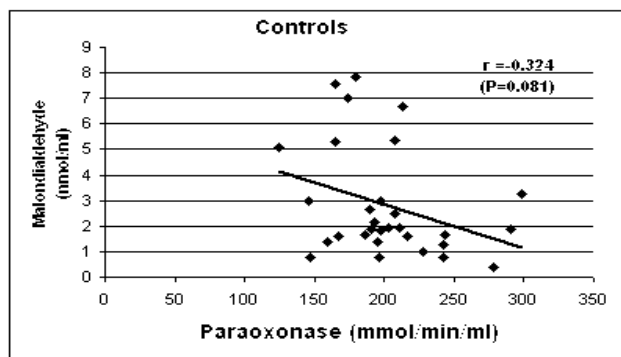
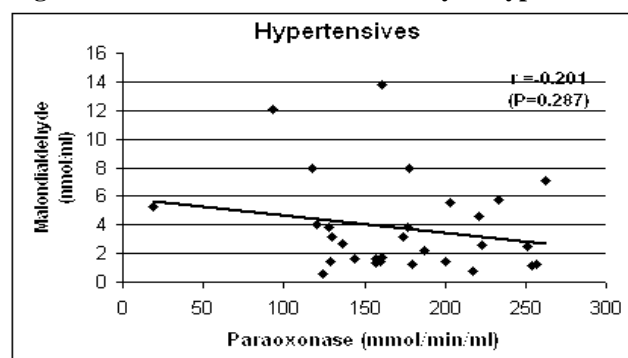
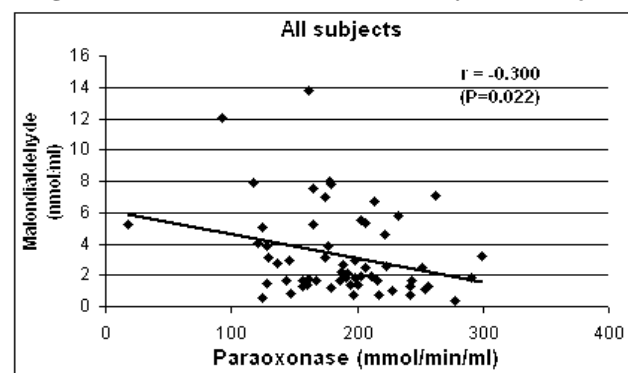
Plasma malonaldehyde levels were moderately increased in hypertensive cases, the mean levels being 4.01±3.25 nmol/ml. The mean and individual plasma malonaldehyde levels are depicted in figure 1.

Figure 1: The mean and individual plasma malondialdehyde levels

Paraoxonase activity in hypertensives was characteristically decreased in hypertensives as shown in table-2 and figures 2a and 2b. The mean serum paraoxonase activity was 201.84 ± 41.13 mmol/min/ml in controls and 171.84 ± 54.91 mmol/min/ml in hypertensive patients. The mean decrease in paraoxonase activity in hypertension was found to be statistically significant ($P=0.020$).

Figure 2: Paraoxonase activity in hypertensives

Correlation study of serum MDA and serum Paraoxonase activity showed mild to moderate negative correlation between the two in controls ($r = -0.324$) and a mild correlation in hypertensives ($r = -0.201$) as shown in Table 2 and Fig 3a, 3b, and 3c.

Figure 3a: Serum Paraoxonase activity in control**Figure 3b: Serum Paraoxonase activity in hypertensive****Figure 3c: Serum Paraoxonase activity in all subject**

4. Discussion

Oxidative stress in hypertension: Enhanced oxidative stress has been found to be associated with hypertension; studies have shown that there is an elevation of reactive oxygen species (ROS) and also impaired endogenous antioxidant system. However the view that an excess of lipid peroxidation products is present and relevant in the pathogenesis of human hypertension had not received definitive support.[7] In the present study lipid peroxidation was not significantly increased in hypertensives when compared with normotensive controls, but statistical analysis shows moderate effect size of Kohen. Higher levels of malondialdehyde (MDA) and lipid peroxides and decreased antioxidant activity have been measured in hypertensive subjects compared to normotensive controls.[8] Previous studies have reported increased plasma H_2O_2 in hypertensives compared to normotensives[9] and also reduced antioxidant factors like super oxide dismutase (SOD) and glutathione peroxidase (GPx).[10] In contrast to these, recent studies in untreated mild to moderate hypertensive subjects by Crakowski et al, it was found that there was no difference in urinary isoprostanes (a lipid peroxidation product) excretion compared to normotensive control subjects.[11] Another recent study on treated hypertensive subjects there was no difference in the excretion of urinary and plasma concentration F2 isoprostanes compared to the

normotensive controls.[12] The results of the present study is in agreement with these studies. Also the effect of drugs administered for the treatment of hypertension affecting oxidative stress. Treatment with ACE inhibitor, Ang-II receptor blocker or calcium antagonist has been shown to reduce nonspecific markers of oxidative stress and also increase endogenous antioxidant activity of SOD, GSSH, GPx.[13]

Uric acid and hypertension: In the present study serum Uric acid levels were similar in both normotensive controls and hypertensives. No statistical significance was found between values of controls and hypertensives. Previous studies have shown that hypertension is associated with hyperuricemia. The primary mechanism for increased serum uric acid in hypertension is reduced renal excretion due to reduced renal blood flow and decreased glomerular filtration rate due to renal vasoconstriction.[14] One of the studies indicate uric acid is also shown to stimulate renal afferent arteriopathy and tubulo-interstitial disease leading to hypertension.[15] In previous studies on treated hypertensives, it was found that drugs like ACE inhibitors and Ang II receptor blockers reduced serum uric acid levels by decreasing urate absorption in proximal tubules. Other groups of drugs like β -blockers and calcium channel antagonists improved renal circulation and reduced serum uric acid.[16] The results of the present study are in agreement with these studies. The similar levels of uric acid in controls and hypertensives can be explained by the administration of antihypertensive therapy with ACE inhibitors, Ang II receptor blockers, calcium channel blockers, β adrenergic antagonists.

Serum paraoxonase activity in hypertension: The role of enzyme paraoxonase in the reverse cholesterol transport is well known. It is also found that paraoxonase acts as an antioxidant by hydrolyzing phospholipid hydro peroxides and cholesterol ester hydro peroxides and reduces lipid hydro peroxides to respective hydroxides and also degrades H_2O_2 . [17] In the present study there was a significant difference in the paraoxonase activity between hypertensives and normotensive controls ($P= 0.023$). Also correlation studies between malondialdehyde and paraoxonase showed a significant negative correlation in both hypertensives and controls. But the correlation was more significant in controls than hypertensives; this may be explained by the possible effect of antihypertensive drugs on the serum levels of malondialdehyde and paraoxonase activity. There are very few studies on paraoxonase activity in hypertension recent studies have shown decrease in paraoxonase activity and increased malondialdehyde levels in sustained hypertension.[18]

Urinary protein excretion and hypertension: In a healthy person the urinary protein excretion is less than

30mg per day. The major bulk of this protein is constituted by Tamm-Horsefall protein. It is normally produced by the renal tubules and shed into the urine. In hypertensive patients, arteriosclerotic lesions of afferent and efferent arterioles and the glomerular tufts are most common renal vascular lesions. Proteinuria and microscopic hematuria occur because of these glomerular lesions. The earliest evidence of nephropathy is appearance of microalbuminuria ($>30\text{mg}/24\text{hrs}$). In the present study the mean protein excretion was higher in hypertensives ($32.17 \pm 48.13 \text{ mg/dl}$) compared to the control subjects ($7.83 \pm 17.52 \text{ mg/dl}$). There was no significant correlation between serum malondialdehyde levels and urine protein excretion and also serum paraoxonase activity and urine protein excretion. Urine protein excretion seems to be independent of paraoxonase activity and malondialdehyde levels.

The present study has shown that there is a reduced level of serum paraoxonase activity, but the limiting factors in the study were that the study group was small and had both treated and untreated cases. Large scale study is needed to establish a relation between the paraoxonase activity by taking in to consideration the treatment aspect and the type of therapeutic agent administered. The significance of the paraoxonase activity in the development of atherosclerotic disease in the hypertensives is yet to be well established. The possible beneficial effect of the certain groups of antioxidant supplements on the paraoxonase activity needs to be investigated.

5. Conclusion

In this study the small group of hypertensive subjects and control subjects were matched for age and sex. The paraoxonase activity was significantly lower in the hypertensive subjects compared to the normotensive controls. The mean serum malondialdehyde levels were higher in hypertensives compared to normotensive controls. There was a significant negative correlation between paraoxonase activity and serum malondialdehyde levels in both controls and hypertensives. Estimation of serum paraoxonase activity is simple and inexpensive. Reduced serum paraoxonase activity can be used as a predictive factor for the development of atherosclerotic disease like coronary artery disease, cerebrovascular disease and renal complications associated with hypertension. Serum paraoxonase activity estimation may also be helpful in assessing the usefulness of antihypertensive drugs in prevention of associated complications. There is scope for further studies on larger scale to know the exact cause of fall in the paraoxonase activity in case of the hypertensive subjects compared to the normotensive controls as seen in the present study.

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