

Paraoxonase Activity in Prehypertension and its Relation to Oxidative Stress

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ABSTRACT

Hypertension (HTN) is a major cause of stroke, ventricular hypertrophy, congestive heart failure, atherosclerosis, end stage renal disease and peripheral vascular disease. Since, Prehypertension is one step towards hypertension, the same are the consequences. Serum paraoxonase (PON 1) is an HDL bound enzyme exhibiting antiatherogenic properties. PON 1 activity is inversely related to the risk of developing atherosclerosis. Free O₂ radicals react with membrane lipids to form lipid hydroperoxides, a destructive process known as lipid peroxidation. Lipid hydroperoxides decompose to form a variety of products including Malondialdehyde (MDA), which is used as an indicator of oxidative damage of cells and tissues. Endogenous antioxidant enzymes such as superoxide dismutase (SOD) counteract the oxidative damage preventing Oxidative stress. Recently hypertension has been considered a state of oxidative stress that can contribute to the development of atherosclerosis and other hypertension induced organ damage. In this study, serum paraoxonase activity and oxidative stress were monitored in 100 patients with Prehypertension and 100 sex matched healthy controls. Prehypertensives are again classified into obese and smokers. The activity of PON was significantly ($p < 0.001$) low as well as SOD was also significantly ($p < 0.001$) low in both obese and smokers with Prehypertension where as MDA level was significantly ($p < 0.001$) increased in both obese and smokers with Prehypertension as compared to controls. Our study showed the increased oxidative stress and decreased paraoxonase antioxidant activity in Prehypertensives when compared to controls. Thus, the estimation of Paraoxonase activity and Oxidative stress will prove to be an emerging marker in prognosis of Prehypertension.

Key words: Paraoxonase, Superoxide dismutase, Malondialdehyde.

INTRODUCTION

Serum Paraoxonase (PON 1) E.C.3.1.8.1. is an arylesterase synthesized in the liver and is an HDL associated enzyme which is responsible for the antioxidant properties of the HDL^{1,2}. This enzyme plays an important role in preventing low density lipoprotein (LDL) oxidation, it is considered to protect against the development of atheromatous plaques³. The serum HDL concentration is inversely correlated with atherosclerosis risk⁴. Many studies showed an association between activity of serum paraoxonase and atherosclerosis. Hypertension is one of the major cause of atherosclerosis. Since, Prehypertension which is one step towards hypertension, So

diagnosis of Prehypertension at early stage is of more significance. PON 1 activity is inversely related to the risk of developing an atherosclerotic lesion, which contains Cholesterol-loaded macrophage from cells. Increased vascular oxidative stress could be involved in the pathogenesis of hypertension^{5,6}, a major risk factor for cardiovascular disease mortality. Oxidative stress occurs when there is an imbalance between the generation of reactive oxygen species (ROS) and the antioxidant defense systems so that the latter become overwhelmed^{7,8}. In hypertension, ROS may increase due to a diminution of the activity of antioxidant enzymes⁹. The importance of ROS in vascular function and the development of hypertension have been recently

reviewed^{10, 11}. It is known that superoxide rapidly inactivates endothelium-derived nitric oxide (NO), the most important endogenous vasodilator, thereby promoting vasoconstriction^{12,13}. Thus oxidative stress may account for endothelial dysfunction, but it is unknown whether this abnormality is a primary event or a consequence of increased blood pressure¹⁴. Endogenous antioxidant enzymes such as superoxide dismutase (SOD) counteract the oxidative damage to prevent oxidative stress. Malondialdehyde (MDA) is a breakdown product of peroxidation of long chain fatty acids which accumulate when lipid peroxidation increases¹⁵. The present study was undertaken to investigate any relationship which may be hypothesized between PON 1 and oxidative stress markers (SOD and MDA) in prehypertensives.

MATERIALS AND METHODS

The study consisted of 100 patients having systolic blood pressure between 120-139 mmHg and diastolic blood pressure between 80-89 mmHg (prehypertensives), who attended the OPD of the Medicine department of Hamidia Hospital associated with GMC Bhopal. Patients are further sub grouped as smokers and non smokers according smoking habit, obese and non obese whose body mass index $>30 \text{ kg/m}^2$. Patients with blood pressure $\geq 140 \text{ mmHg}$ and with secondary hypertension, past history of stroke, coronary artery disease (CAD), myocardial infarction, peripheral vascular disease, diabetes mellitus and taking antioxidant vitamin supplements were excluded. The control group consisted of 100 healthy adults, who had been matched for age, sex, BMI, socio-economic status having blood pressure $\leq 120 \text{ mmHg}$ were selected for study during routine health check-up program, Hamidia Hospital. Arterial blood pressure (BP) was measured with a mercury sphygmomanometer. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were averaged by using three readings measured at 5 minute intervals. Patients who had mean systolic/diastolic blood pressures within the range of 120-139 mmHg/80-89 mmHg and they had never been told that they have high BP levels were considered as pre-hypertensive. Fasting blood samples were collected from both control and patients for a series of laboratory investigation using standard protocols and after taking consent from patients. The study was

approved by the Institutional Ethical Committee, for Biomedical research.

Estimation of Serum Paraoxonase activity

Paraoxonase was estimated spectrophotometrically by the method, described elsewhere with modification¹⁶. Briefly, the assay mixture consists of 500 μl of 2.2 mmol/l paraoxon substrate in 0.1 mmol/l Tris HCL buffer (pH 8.0) containing 2mmol/l CaCl₂, and 50 μl of fresh serum. After mixing the contents, kinetic measurements were taken immediately at every minute for 5 minutes, at 405 nm at 25°C. First absorbance reading is taken as 0 min reading and subsequent absorbance readings were taken as one minute to four minute readings. Corrected absorbance reading were obtained by subtracting 1 minute reading with 0 minute reading, likewise the latter minute reading was subtracted with previous minute reading. The mean absorbance was calculated. Mean absorbance was used to determine PON1 activity, and standard graph plotted using 1 mM P-nitrophenol. PON 1 activity expressed in international units (IU). One IU was defined as 1 μmol of p-nitro phenol formed/min/L at 25 °C.

Estimation of SOD

SOD was estimated by spectrophotometric method of Mishra H.P. and Fridovich I, 1972[17]. In this method, the assay mixture consists of 0.5 ml sodium carbonate buffer (pH 10.2), 0.5 ml EDTA, 0.5 ml D.W., 0.5 ml of adrenaline bitartrate are added to 0.5 ml serum. After mixing the contents absorbance is read after every 30 seconds till 0.5 minute at 480 nm (greenish blue filter) by using spectrophotometer. Mean absorbance was calculated. The values are expressed in terms of superoxide dismutase units / mg.protein/ml.

Estimation of MDA

MDA was estimated by colorimetric method of Satoh K .etal. In this method, 2.5 ml of 20 % trichloroacetic acid and 1.0 ml of 0.67 % TBA are added to 0.5 ml of serum then the mixture is heated in boiling water bath for 30 min. The resulting chromogen is extracted with 4.0 ml of n-butyl alcohol and the absorbance of organic phase is determined at the wavelength of 530 nm. The determined values expressed in terms of malondialdehyde

(nmole/ml) used as a reference standard – 1,1,3,3-tetraethoxypropane¹⁸.

Limitation of Study

Test samples were collected from patients who attended the OPD of Medicine, and diagnosed as prehypertensives. This study was subjected to 100 prehypertensives cases within 30-60 yrs of age. The laboratory of Biochemistry department is well equipped with semiautoanalyzer, colorimeter and spectrophotometer. All investigation methods used in this study are standardized in our laboratory.

Statistical analysis

All the data were analyzed by using the SPSS version 10.0.

Table 1: Classification of Hypertension

	Systolic (mmHg)	Diastolic (mmHg)
Normal	<120	<180
Prehypertension	120-139	80-89
Stage 1	140-149	90-99
Stage 2	≥160	≥100

Table 3 shows the demographic profile of Prehypertensive patients and controls.

Among 100 Prehypertensive patients, 70 were males and 30 were females. Among 100 controls 55 were males and 45 were females. There are 25 Prehypertensive patients with obesity and

Values presented are Mean±SD (standard deviation). To test the significance between the study groups were analyzed by a student's t-test. The p value ($p < 0.05$) was considered significant.

RESULTS

Table 1 shows the classification of hypertension, which is classified into Prehypertension and Hypertension.

According to the JNC VII hypertension is classified as Prehypertension, stage 1 and stage 2 as per the above table.

Table 2 shows the distribution of Risk factors in 100 Prehypertensive patients in the study.

Table 2: Risk factor distribution of the Prehypertensive patients in this study

S.No	Risk factor	N=100	%
1.	Parental occurrence	15	15%
2.	Smoking	35	35%
3.	Obesity	45	45%
4.	Alcohol consumption	25	25%

25 Prehypertensive patients were smokers. BMI was significantly high in Prehypertensive obese ($p < 0.001$) compared with Prehypertensive non obese and also significantly high in Prehypertensive smokers ($p < 0.001$) compared with Prehypertensive non smokers. SBP was significantly high in Prehypertensive obese ($p < 0.001$) compared with

Table 3: Demographic profile of Prehypertensive patients and controls

Variable	Control (n=100)	Prehypertensive Cases (n=100)			
		Obese (n=25)	Non obese (n=25)	Smokers (n=25)	Non smokers (n=25)
Age (years)	50.80±14.84	49.63±16.15	48.62±15.14	51.08±13.62	50.14±14.32
Sex (M/F)	55/45	12/13	10/15	11/14	9/15
BMI (Kg/m ²)	22.54±1.99	25.24±2.73*	23.24±2.70	25.5±1.50*	22.72±1.65
SBP(mmHg)	120.38±8.69	129.5±9.5*	128±8	130.4±9.0*	127±8
DBP(mmHg)	75.56±5.48	84.5±4.5*	83.5±3.5	85.5±5*	82.5±4

(*p-value <0.001, **p-value <0.01, ***p-value <0.05)

Prehypertensive non obese and also significantly high in Prehypertensive smokers ($p < 0.001$) compared with Prehypertensive non smokers. DBP was significantly high in Prehypertensive obese ($p < 0.001$) compared with Prehypertensive non obese and also significantly high in Prehypertensive smokers ($p < 0.001$) compared with Prehypertensive non smokers.

Table 4 shows the serum paraoxonase activity, SOD and MDA level in Prehypertensive patients (obese and smokers) compared with controls. Serum paraoxonase activity was significantly ($p < 0.001$) more decreased in Prehypertensive obese and also in Prehypertensive smokers, SOD level was highly significantly ($p < 0.001$) more decreased in Prehypertensive obese and also in Prehypertensive

Table 4: Biochemical profile of Prehypertensive patients and controls

Variable	Control (n=100)	Prehypertensive Cases (n=100)			
		Obese (n=25)	Non obese (n=25)	Smokers (n=25)	Non smokers (n=25)
Paraoxonase (IU)	330.83±65.59	270.70±65.45*	281.30±64.58*	265.80±64.55*	280.41±55.5**
SOD units/mg. pro/ml.	9.37±0.29	9.09±0.20*	9.29±0.10	8.91±0.32*	9.20±0.13**
MDA (nmole/ml)	1.84±0.64	2.38±0.79*	1.89±0.60	2.73±0.81*	1.88±0.9

(*p-value <0.001, **p-value <0.01, ***p-value <0.05)

smokers, MDA level was highly significantly ($p < 0.001$) more increased in Prehypertensive obese and also in Prehypertensive smokers as compared with control group.

DISCUSSION

Prehypertension is not a disease, but a phase which progressively leads to Hypertension. Hypertension is a leading risk factor that predisposes to increased cardiovascular morbidity and mortality, and is additionally an important risk factor for development of chronic renal disease in the presence of obesity. Smoking is very common cause of hypertension. The positive associations between cigarette smoking and diseases such as lung cancer and coronary heart disease have been observed in population based study of 451 Australian women investigated between 1982 and 1984 (Andre et al 1988) Thus, in the present study we have examined the effects of smoking on pre hypertension. We have found its significant role in Prehypertension. Obesity is closely associated with metabolic syndrome including hyperglycemia,

Dyslipidemia and hypertension¹⁹. The study done By Kearney *et al.*, (2005) also showed significant positive correlation between Oxidative stress and BMI²⁰.

The root cause of hypertension is mainly atherosclerosis. Atherosclerosis is a process for which there is substantial evidence of a role of oxidative stress. Increased production of free radicals and the declining activity of the antioxidant defense system are two possible factors which may lead to increased oxidative stress. Antioxidant enzymes are the major defense system of cells in normal aerobic reactions. In present study, the lower enzymatic activity of CuZn-SOD in pre-hypertensive compared to normotensive was found. The study done by I Rahman and W Mac NEE (1996), P. Padmawati *et al.*, (2009)^{21,22} also showed significant correlation of smoking with SOD.

There was an increase in the MDA levels, which is marker of lipid peroxidation, in the Prehypertensive group in comparison to those in the control group. 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^{22,23}.

In the present study, Serum PON 1 activity was significantly decreased in Prehypertensive patients. This might play a central role in atherosclerotic process, because HDL-PON activity modulates the susceptibility of HDL to atherogenic modifications such as glycation and homocysteinylation^{24,25}. PON 1 associated with HDL-C in plasma is thought to protect LDL-C oxidation²⁶. Purnima Dey Sarkar *et al.* (2006) also showed a significant decrease in PON1 activity in patients with premature coronary artery disease²⁷.

CONCLUSION

Our study shows that Prehypertension is associated with decreased Paraoxonase activity with increased Oxidative Stress. The fall in PON 1 and SOD levels are observed in smokers as well as obese prehypertensives, where as MDA levels are found to be increased in both subgroups. The present study confirms that there is elevated oxidative stress and reduced antioxidant capacity of PON1 in Prehypertensive patients compared to controls emphasizing the importance of these parameters in assessing these markers for early diagnosis and therapeutic interventions. We can check damage caused by free radicals and other oxidants by giving antioxidants in the diet to the patient and prevent progress of disease by giving proper education to patient about healthy lifestyle and also advising them to practice yoga, aerobics, walk, etc.

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