



SERUM PARAOXONASE ACTIVITY AND OXIDATIVE STRESS IN METABOLIC SYNDROME.

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ABSTRACT

Metabolic syndrome is a group of risk factors that increase the risk of developing heart disease, stroke and Type II Diabetes. It's also known as Insulin Resistance Syndrome. MES is known to be associated with Oxidative stress. The enzyme paraoxonase being antiatherogenic and sensitive to oxidative stress was intriguing in MES. This study was conducted with objectives to determine paraoxonase activity in MES and to correlate paraoxonase activity to oxidative stress in MES. In this study there were a total of 100 male subjects which included 50 healthy male as control and 50 male with MES without complications. Serum paraoxonase (PON) is an enzyme that helps protect the body from oxidative damage, and may help prevent cardiovascular disease. PON hydrolyzes lipid peroxidase and prevent phospholipids from building up in oxidized low-density lipoprotein (LDL). It also hydrolyzes homocysteine thiolactone, which protects against N-homocysteinylated. PON is associated with atherosclerosis and coronary artery disease, endocrine disorders and liver disease. Serum malondialdehyde (MDA) is a biomarker of oxidative stress. High levels of MDA are associated with various diseases. Our study revealed that serum paraoxonase activity was significantly reduced in MES when compared to controls. Correlation between serum paraoxonase and malondialdehyde with routine biochemistry parameters in both MES 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 MES where oxidative stress is implicated.

KEYWORDS : Serum paraoxonase, Oxidative stress and MES.

INTRODUCTION

Paraoxonase1 (PON) is an enzyme that has antioxidant potential, which confers a protective effect against the atherosclerotic process. However, studies associating genetics dietary patterns and PON1 activity in individuals with cardiovascular disease (CVD) are scarce.

The paraoxonase (PON) are a group of enzymes, co coded by genes on chromosome 7. PON1 is the most studied member of this family can hydrolyze organophosphate compounds and has antioxidant potential. PON1 is synthesized by the liver and released into the bloodstream associated with high density lipoprotein (HDL) molecules. PON1 has a protective effect against the atherosclerotic processes; it inhibits oxidation of low density lipoprotein (LDL). In this context more than 160 single nucleotide polymorphism (SNPs) in the PON1 gene are known. The aim of this study was to determine serum PON aryl esterase (ARE) activities in metabolic syndrome (MES).

Metabolic syndrome (MES) a collection of cardiovascular risk factors including central obesity, hypertension, hyperglycemia, glucose intolerance and dyslipidemia associated with an increased risk of cardiovascular disease and diabetes. Human serum paraoxonase 1 (PON) is ~43-45kDa glycoprotein synthesized with high density lipoprotein (HDL) and protects low density lipoprotein (LDL) from oxidation by the hydrolysis of biologically active lipoperoxides. The activity of this enzyme is measured using paraoxon or is estimated from the activity of arylesterase (ARE) using phenyl acetate. It has been reported that ARE activity is not affected by the polymorphisms of PON1. PON1 is recognized as an antioxidant enzyme because it hydrolyzes lipid peroxide in oxidized lipoprotein. To the best of our knowledge, information regarding PON and ARE activities in MES is limited.

Serum paraoxonase (PON) is a calcium dependent esterase that is known to catalyze hydrolysis of organophosphates and is widely distributed among tissues including liver, kidney, and intestine. PON which is exclusively bound to high density lipoprotein (HDL), is recognized as an antioxidant enzyme since it hydrolyzes, lipid peroxides^[4]. It was reported that HDL associated PON inhibited not only low density lipoprotein oxidation modes (transition metal ions free radicals). In this study, we aimed to elevate PON1 activities and malondialdehyde (MDA) levels, one of the end products of lipid peroxidation induced by ROS in patients with BD in the active stage of the disease.

MATERIAL AND METHODS

The study was carried out on 50 healthy normotensive controls and 50 MES subjects in North Bengal Medical College & Hospital. The diagnosis of hypertension was established in accordance with the recommendation of world health organizations, and international society of hypertension. The inclusion criteria included male subjects in the age in the age group of 40 to 60 years. Exclusion criteria included any underlying medical disorder such as cardiovascular disease, renal disease, diabetes mellitus, smoking and alcohol abuse determine by comprehensive physical examination and routine laboratory investigation. Informed consent was taken from both cases and control. Subject demographics medical history in details and physical examination data were obtained from both cases and controls.

Malonadehyde (MDA) a reactive aldehyde s a product of lipid peroxidation. MDA in serum react with thiobarbituric acid (TBA) to form pink colored complex of TBA-MDA adduct. It was then centrifuged at 3000 rpm for 10 mints. The upper clear supernatant fluid was transferred to a cuvette and the absorbance was measured at 530nm 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 1.0mmol/ L of phenyl acetate and 0.9mmol/L of calcium chloride in 20mmol/L, this HCL at PH 8 at 25 degree Celsius. Non enzymatic hydrolysis. To this 5 micro liter of serum was added and mixed. Absorbance was noted phenol determined the arylesterase activity which was measured spectrophotometrically at 270nm. Serum glucose was estimated by standard methods. Chi- square test and fisher exact test were used to find the statistical significance of proportion between case and control. Student t test (independent two tailed) was used to find the significance of anthropometry, hemodynamics and laboratory investigation between cases and control. The effect sizes due to Cohen were computed to find the effect of hypertension on laboratory parameters over the control group.

Criteria For Diagnosis Of The Metabolic Syndrome According To The National Cholesterol Education Program (NCEP) ATP III

Table-1

CRITERION NCEP ATP III	NCEP ATP III
Waist (cm) :-	
Male	102

Female	88
HDL (mg/dl):-	
Male	40
Female	50
Triglyceride (mg/dl)	150
Fasting glucose (mg/dl)	100
Blood pressure (mmHg)	130/85
THREE OF THE FIVE REQUIRED	

Table-2 Laboratory Investigation

Parameters	Controls (n=50)	Cases (n=50)	P Value	Effect size
SBP (mm/hg)	120.01±8.55	143.64±12.30	0.000**	
DBP(mg/dl)	74.68±5.76	95.10±5.84	0.000**	
FBG(MG/DL)	85.41±12.08	112.50±56.44	<0.001	0.050*
Triglyceride (mg/dl)	172.15±39.35	213.80±47.80	< 0.001	
Total Cholesterol (mg/dl)	171.52±39.15	171.41±38.30	< 0.0001	
HDL (mg/dl)	44.84±6.19	41.08±7.88	< 0.0001	
LDL (mg/dl)	101.71±33.48	126.9±44.19	< 0.0001	
Paraoxonase (nmol/min/ml)	204.16±39.18	170.54±52.91	0.020*	0.6
Malondialdehgde (nmol/ml)	2.81±2.09	4.04±3.08	0.109	0.41

DISCUSSION

Neutrophils, lymphocytes and macrophages release cytokines, growth factors and ROS upon activation. An imbalance between the pro-oxidant and anti-oxidant system may result in formation of excessive ROS; these ROS molecules are highly reactive and capable of attacking almost every cell. This mechanism has a crucial and probably a causative role in the pathogenesis of various acute and chronic diseases including inflammation, rheumatoid arthritis and atherosclerosis. There are several reports suggesting that increased ROS production in patients with BD causes cell and tissue injury. ROS can attack double bonds in poly-unsaturated fatty acids, and thus induce lipid peroxidation, which in turn results in further oxidative damage.^[6]

ROS mediated oxidation of cell membrane lipids leads to the formation of lipid peroxidation products such as MDA^[7]. Increased plasma MDA levels in patients with BD have been shown in previous studies^[8]. We obtained similar result in the present study when compared with healthy controls. We found a statistically significant increase in serum MDA levels ($p < 0.001$), which is the evidence of increased lipid peroxidation during the active stage of the disease.

An excess of lipid peroxidation products is present and relevant in the pathogenesis of human hypertension had not received definitive support^[9]. In the present study lipid peroxidation was not significantly increased in hypertension when compared with normotensive controls but statistical analysis showed moderated size in Kohen. Higher level of malondialdehyde (MDA) and lipid peroxide and decreased antioxidant activity have been measured in hypertensive subject compared to normotensive controls^[10]. Previous study have reported increase plasma H₂O₂ in hypertensive compared to normotensive and also reduced antioxidant factors like super oxide dismutase (SOD) and glutathione peroxide (GPx)^[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 result of the present study is in the agreement with these studies. Treatment with ACE inhibitor, Ang-11 receptor blocker or calcium antagonist has been shown to reduced nonspecific markers of oxidative stress and also increase endogenous antioxidant of SOD, GSSH, GPx^[13].

The role of enzyme paraoxonase in the reserve cholesterol transport is well known. It is also found that paraoxonase act as an antioxidant by hydrolyzing phospholipid hydro peroxides and cholesterol ester hydro peroxides and reduces lipid hydro peroxides to respective hydroxide and also degrades H₂O₂^[14].

In the present study there was a significant difference in the paraoxonase activity between hypertensive and normotensive controls ($p = 0.023$). Also correlation studies between MDA and paraoxonase showed a significant negative correlation in both hypertensive and controls. But the correlation was more significant in controls than

hypertensive; this may be explained by the possible effects of antihypertensive drugs on the serum level of malondialdehyde and paraoxonase activity. There are very few studies on paraoxonase activity in hypertension recent study have been show decrease in paraoxonase activity and increased malondialdehyde levels in sustained hypertension^[15].

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 the 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 atherosclerosis disease in the hypertensive is yet to be well established. The possible beneficial effect of the certain group of antioxidant supplements on the paraoxonase activity needs to be investigated.

We found decreased serum PONI activity in MES patients in comparison with healthy controls during the activation stage of the disease, no other study have been elicited in the literature dealing with serum PONI activity in MES. The mechanism of the observed decreased in serum PON activity in our MES patients is unclear. Serum PONI activity is generally considered to vary in response to the consumption of PONI for the prevention of oxidation. The decrease in serum PON activity in our MES patients might have resulted from the increased inactivation of PONI according to increased generation of ROS in MES^[16].

In our study the MES patients consisted of those who had the complete type of the disease with all the major criteria determining PON levels in a large series of MES patients also with incomplete type of disease at various stages could help reveal the underlying pathogenesis. Further studies may also reveal whether this enzyme could be used in the differential diagnosis of the suspected cases, or used as a specific marker of the approaching activation of the disease.

In conclusion, endothelial damage and increased PMN leukocyte activity in the active stage of the disease in MES may result in a pro-oxidation environment which in turn. Result in a decreased antioxidant PON activity and increased lipid peroxidation as evidenced by increased MDA levels.

CONCLUSION

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 CVD. Estimation of serum paraoxonase activity is simple and inexpensive. Reduced serum paraoxonase activity can be used as a predictive factor for the development of atherosclerosis disease like coronary artery disease and 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 longer scale to know the exact cause of full in the paraoxonase activity in case of the hypertensive subjects compared to the normotensive controls as seen in the present study. It is well known that in general population. MES is associated with increased cardiovascular morbidity and mortality and high prevalence of type 2 diabetes mellitus. Human PONA a HDL associated enzyme is capable of preventing LDL oxidation. According to this and previous results, reduced PON activities in MES might be an independent risk factor for cardiovascular disease in these patients. However, more studies in diverse populations are needed for clear confirmation.

Declaration Of Interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Disclosure

No potential conflict of interest relevant to this article was reported.

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