Scholars Journal of Applied Medical Sciences (SJAMS)
Abbreviated Key Title: Sch. J. App. Med. Sci.
ISSN 2320-6691 (Online)
ISSN 2347-954X (Print)

A Unit of Scholars Academic and Scientific Society, India
www.saspublishers.com

Biochemistry

Assessment of Plasma Paraoxonase Activity in Metabolic Syndrome Patients

Dr. Md Masood Ahmed Shareef[1], Dr. Nitin Faldessai[2*], Dr. Rahul Saxena[3], Dr. Ijen Bhattacharya[4]
[1]Assistant Professor, Department of Biochemistry Kamineni Institute of Medical Sciences, Narketpally, Nalgonda India
[2]Associate Professor, Department of Biochemistry, Kamineni Institute of Medical Sciences, Narketpally, Nalgonda India
[3]Assistant Professor, Department of Biochemistry, School of Allied Health Sciences Sharda University, Greater Noida, U.P., India
[4]Professor, Department of Biochemistry, Rama Medical College, Hospital & Research, Hapur, U.P., India

Original Research Article

***Corresponding author**
Dr. Nitin Faldessai

Article History
Received: 04.02.2018
Accepted: 15.02.2018
Published: 28.02.2018

DOI:
10.36347/sjams.2018.v06i02.028

Abstract: Metabolic syndrome (MS) is a major escalating public-health and clinical challenge worldwide. MS is characterized by various sorts of cardiometabolic risk factors. However, the role of oxidative stress in developing components of MS needs further investigations. Therefore, the objective of present study was to estimate the plasma paraoxonase (PON) activity in MS patients and to determine the relation of PON with dyslipidemia in MS subjects. 50 patients of either sex (30-50 years age group) suffering from MS as defined by the criteria of the Third Report of the National Cholesterol Education Program Adult Treatment Panel III and 50 normal healthy individuals served as control; were recruited. Plasma PON activity along with serum lipid profile was estimated by using standard methods and data from patients and controls were compared by using Student's t-test. In addition to dyslipidemia, significantly low ($p<0.001$) activity of plasma PON was observed in MS group subjects. PON activity was negatively correlated with the components of lipid profile except HDL-cholesterol. Therefore, depletion of PON activity plays a crucial role in the development of cardiovascular complications in MS patients. The present study also suggested that treatment of dylipidemia should be incorporated with regular monitoring of PON activity in order to predict and timely overcome with the burden of cardiac complication in MS patients.
Keywords: LDL cholesterol, body mass index, .oxidative stress, hypertension, cardiovascular disease.

INTRODUCTION

Metabolic syndrome is a major and escalating public-health and clinical challenge worldwide in the wake of urbanization, surplus energy intake, increasing obesity, and sedentary life habits.

MS confers a 2-fold the risk of developing cardio vascular disease and 5-fold increase in the risk of type 2 diabetes mellitus over the next 5 to 10 years[1].

It has been well documented that abnormal lipid profile or dyslipidemia in general population is an alarming condition of future health complications predominantly cardiovascular diseases (CVD) such as myocardial infarction, atherosclerosis etc [2,3]. Although precise etiology of this syndrome is poorly understood, the increased prevalence and manifestation of CVD in MS patients has renewed the interest of researchers to identify various other unidentified risk factors. Amusingly, the presence of oxidative stress in MS patients, as reported in previous studies further enhances the frequency to develop CVD [4].

Excessive production of reactive oxygen species (ROS) in combination with depleted antioxidant reserve leads to the development of oxidative stress. Oxidative stress is characterized by toxic effects of ROS which includes a cascade of deleterious events such as damage to endothelium, oxidation of LDL (lipid peroxidation), protein oxidation and DNA strand breakage. These events lead to uncontrolled metabolic profile and thereby development of MS components related complications [5,6]. In order to protect lipoproteins against oxidative modification, the role of paraoxonase (PON), a glycoprotein, synthesized mainly in the liver, as HDL-associated lipo-protective enzyme carried on apo A-I, is well documented. PON also hydrolyzes organophosphates like pesticides, neurotoxins, and arylesters [7]. Previous studies have shown that PON level alters in various MS related

complications such as cardiovascular diseases, hypertension and obesity [8-10].

Moreover, the study pertains to assessment of PON activity and lipid profile in MS patients along with characteristic factors such as increased blood pressure and body weight is not available to the best of our knowledge. Therefore, the aim of present study was to evaluate the plasma paraoxonase (PON) activity and lipid profile along with hypertension in MS patients and to determine the association of PON activity with dyslipidemia in order to predict the risk of CVD in MS patients.

MATERIALS AND METHODS

In the present study, 100 subjects belonged to age group 30 -50 years, were included. These subjects were categorized as Group I (Control group) comprised of 50 normal healthy individuals and Group II (Patient group) comprised of 50 individuals who were age and sex matched patients suffering from metabolic syndrome. A general information or pre-experimental questionnaire regarding demographic information, family history and limited physical examination including blood pressure measurement was completed from all the subjects after taking their informed consent and approval of protocol by ethics committee of college.

INCLUSION CRITERIA

A total number of 50 MS patients fulfilling the National Cholesterol Education Program Adult Treatment Panel III criteria (NCEP- ATP III) and above 30 years of age were included [11]. A general information or pre-experimental questionnaire regarding demographic information, family history and limited physical examination was completed from all the subjects. In all subjects, anthropometric measurements, including height, weight and waist circumference measurements; systolic and diastolic blood pressure were recorded. Height and weight were measured with subject barefoot and light dressed. The body mass index (B.M.I.) were calculated as B.M.I. = weight (Kg) / Height (metre^2).

Exclusion criteria

Patients, aged above 50 and below 30 years, under vitamin supplements, hormone replacement therapy and those with a history of infections, abnormal renal function and malignancy were excluded from the study.

Fasting blood samples (4 ml) of both the study group subjects were collected in plain vial (2 ml for serum separation) and EDTA vial (2 ml for plasma separation). Plasma paraoxonase activity was estimated by Gan *et al*. method using p-nitrophenyl acetate (5.5 mM/L) as a substrate [12]. The increase in absorbance of p-nitrophenol formed at 412 nm was measured spectrophtometrically. The activity of PON was measured in Tris buffer (20 mM/L; pH 8.0) containing 1mM $CaCl_2$. The generated product p-nitrophenol was calculated by using molar extinction coefficient of 17000 per mole/cm at pH 8.0 and results were expressed as Units/ml.Serum lipid profile contents (total Cholesterol, Triglycerides& HDL cholesterol) were analysed enzymatically. Serum LDL-cholesterol and VLDL-cholesterol levels were calculated by Friedwald's formula[13].

LDL-C = TC - [(TG/5)+HDL-C]
VLDL cholesterol = Total chol. – (HDL + LDL)

STATISTICAL ANALYSIS

The data collected from study group subjects were entered separately in Microsoft Excel sheet of windows 2007 and values were expressed as Mean ± SD. The significance of mean difference between study group subjects was compared by using Student's t test. P value <0.05 and <0.001 were considered as significant and highly significant respectively. In addition, correlation analysis between aforesaid parameters was performed by using Pearson correlation test.

RESULTS

In the present study, age, anthropometry and clinical profile of the control group subjects and MS patients are depicted in Table 1. MS is more prevalent in middle age group subjects. To avoid the potential confounding factor i.e sex difference (males sex are more prone to CVD risk), subjects of both the sex in 1:1 ratio were taken in account. Systolic blood pressure ($p< 0.05$; 11.36% high), diastolic blood pressure ($p< 0.05$, 12.5% high), BMI ($p<0.001$, 18.58% high) and waist circumference ($p<0.05$, 17.99% high) were significantly high in MS patient than healthy controls (Table 1).

Plasma PON activity and serum lipid profile data showed significant abnormalities in the patients group as represented in Table 2. Plasma PON activity was significantly low (21.25 %; $p<0.05$) in MS patients as compared to healthy controls as depicted in Graphs1. Serum total cholesterol, triglyceride, LDL and VLDL levels were found to be significantly high ($p<0.001$) in metabolic syndrome patients. However, HDL levels were found to be reduced significantly ($p<0.05$; 19.24% low) in the MS patients as compared to healthy controls.

In addition, we observed a significant correlation between plasma paraoxonase activity and lipid profile components, as shown in Table 2. PON activity was negatively correlated with total cholesterol, triglycerides, LDL and VLDL levels ($p < 0.001$); whereas PON activity was positively correlated with serum HDL levels in MS patients. These results clarify the role of oxidative stress in enhancing the CVD risk in MS patients most probably by its relation with abnormal lipid profile.

Table-1: Anthropometric and clinical profile of controls (Group I) and MS (Group II) subjects. (Mean ± SD)

S.No.	Particulars	Group I (N= 50)	Group II (N=50)
1	Age (years)	39.58 ± 7.2	40.5 ± 7.8
2	Systolic pressure (mmHg)	110 ± 8.0	122.5 ± 8.6**
3	Diastolic pressure (mmHg)	75.2 ± 4.8	84.6 ± 6.2**
4	Height (meter)	1.66 ± 0.7	1.64 ± 0.5
5	Weight (kg)	63.58 ± 5.4	71.42 ± 6.2
6	BMI	22.6 ± 2.1	26.80 ± 2.5***
7	Waist Circumference (cm)	80.24 ± 8.5	94.68 ± 9.0**
8	Hip (cm)	90.24 ± 7.4	105.20 ± 7.9
9	Waist-hip ratio	0.86 ± 0.06	0.92 ± 0.08

where,
* p < 0.1: Non-significant;
**p<0.05: Significant,
*** p < 0.001 : Highly Significant,
BMI= Body mass index

Table-2: Plasma paraoxonase activity and serum lipid profile of study group subjects. (Mean ± SD)

S.No.	Particulars	Group I N= 50	Group II N=50
1	Paraoxonase(IU/gm Hb)	222.50 ± 30.54	175.2 ± 25.40*
2	Total cholesterol(mg/dl)	154.8 ± 24.5	196.0 ± 30.2**
3	Triglycerides(mg/dl)	83.4 ± 15.8	145.2 ± 18.5***
4	LDL (mg/dl)	98.4 ± 14.2	124.2 ± 16.5**
5	HDL(mg/dl)	45.2 ± 5.1	36.5 ± 4.5**
6	VLDL (mg/dl)	19.2 ± 3.5	30.8 ± 4.1***

where,
* p < 0.1: Non-significant,
** p < 0.05: Significant,
*** p < 0.001: Highly significant

Table-3: Correlation coefficient (r) between Paraoxonase and lipid profile components in MS patients

Particulars	TC	TG	HDL	LDL	VLDL
PON	- 0.524**	-0.465 *	0.628**	-0.610**	-0.526**

Where,
* p < 0.05 : Significant; ** p < 0.001 : Highly significant
PON : Paraoxonase;
TC: Total Cholesterol;
TG: Triglycerides;
HDL: High Density Lipoprotein
LDL: Low Density Lipoprotein;
VLDL: Very Low Density Lipoprotein

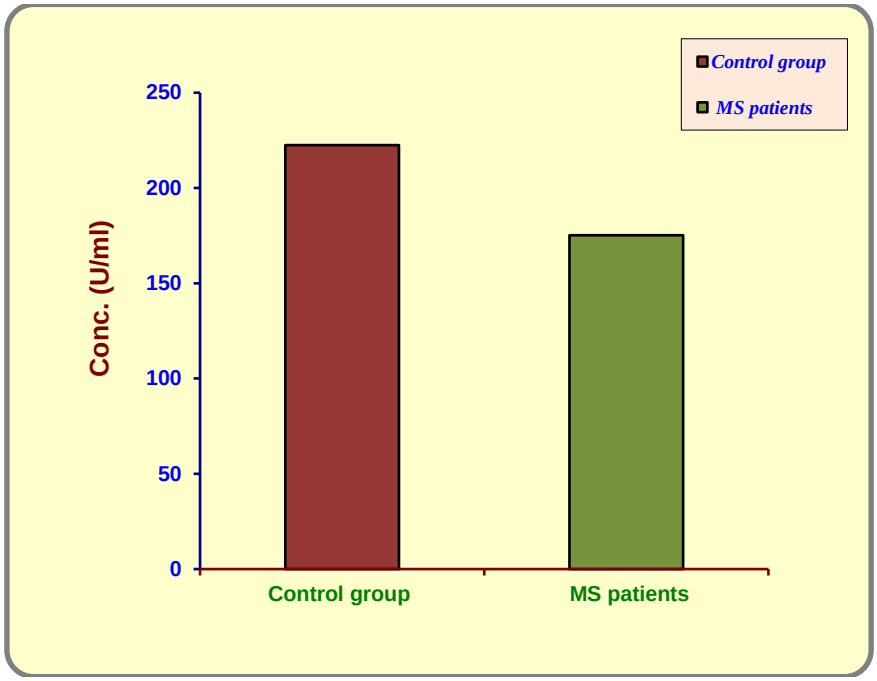

Fig-1: Plasma Paraoxonase activity in study group subjects

DISCUSSION

Dyslipidemia has been known to be associated with several clinical conditions such as cardiovascular disease, hypertension, diabetes and vascular diseases [3,14]. In this context, association of dyslipidemia with oxidative stress in MS patients has yet not be clarified and documented. It is speculated that occurrence of dyslipidemia in MS patients and its association with oxidative stress play a crucial role in solving the anonymity of future CVD incident in MS patients.

In particular, assessment of anti-atherogenic enzyme is another effective approach to predict CVD complications in metabolic syndrome patients. Recent studies on paraoxonase in MS related components such as hypertension, diabetes and in obese patients have received much attention [10,15]. PON enzyme found in association with HDL and contributing it to anti-atherogenic and antioxidant capability by regulating oxidation of LDL, by hydrolyzing specific oxidized phospholipids, cholesterol linoleate hydroperoxides, and by neutralizing hydrogen peroxide [7,9,16]. Alteration in the PON activity may have significant effect in inducing CVD complications in MS patients, possibly due to inability of enzyme to regulate the overproduction of reactive aldehydes. In the present study, plasma PON activity was found to be decreased significantly in MS patients which reflects toward the utilization of enzymes in reducing ROS mediated biomolecular deterioration as well as its inactivation due to interaction of oxidized lipids with the PON free sulphydryl group. Consistent findings have been reported by Kumar *et al.* in south asian elderly hypertensive patients and implicated the depletion of PON activity with CVD complications in future[17].

Similarly Senti *et al*. observed low PON activity in individuals with MS and its association with increased oxidative stress in MS patients. In addition, they also reported that the decrease in PON activity is associated with components of MS patients, as observed in our study, and thereby reinforcing the establishment of association of dyslipidemia with pro-oxidant condition mediated PON deficiency in MS patients [18]. Thus, the enhanced oxidative stress and its association with dyslipidemia in MS appear to be a major cause of cardiac complications.

CONCLUSION

It is obvious from the findings of present study that excessive production of free radicals followed by depletion of plasma PON activity along with abnormal lipid profile in MS patients plays a significant role in the development of CVD and its associated future complications. Therefore, management of normal lipid profile along with adoption of healthy life style and regular physical exercise should be recommended to MS patients. Furthermore, combined measurement of PON activity and lipid profile may be an efficient marker in early prediction of CVD incident in MS patients.

REFERENCES

1. Shrestha S, Saxena R, Srivastava S, Thakur RK. Evaluation of cardio metabolic profile, endothelial dysfunction and oxidative stress in Metabolic Syndrome: A comparative perspective. Medical Science. 2016; 4(3): 334-340.
2. Miller M. Dyslipidemia and cardiovascular risk: the importance of early prevention. QJM: An International Journal of Medicine. 2009 Jun 4;102(9):657-67.

3. Shanmugasundaram M, Rough SJ, Alpert JS. Dyslipidemia in the elderly: should it be treated?. Clinical cardiology. 2010 Jan 1;33(1):4-9.
4. Kumar VS, Bhattacharya I, Praveena V, Saxena R. Study on dyslipidemia and oxidative stress in Elderly- A clinical approach to predict cardiovascular disease risk. SJAMS. 2017; 5(4): 1504-1508.
5. Goyal R, Nandkeoliar MK, Saxena V, Payal P, Saxena R. Assessment of Glutathione peroxidase and its correlation with the components of metabolic syndrome: A biochemical approach. SJAMS. 2017; 5(5A): 1770-1774.
6. Roberts CK, Sindhu KK. Oxidative stress and metabolic syndrome. Life Sci. 2009;84 (21-22):705-12.
7. Marchegiani F, Marra M, Olivieri F, Cardelli M, James RW, Boemi M, Franceschi C. Paraoxonase 1: Genetics and activities during aging. Rejuvenation Research 2008; 11:113-127.
8. Das D, Saxena R, Bhattacharya I. Alteration in plasma paraoxonase levels and its relation with Coronary Artery Disease. Sch J App Med Sci 2014; 2(5C): 1682-1687.
9. Suneja S, Saxena R, Saxena R, Sharma D and Lal AM. Association between serum paraoxonase and plasma nitric oxide in pre-eclampsia. Int J Adv Med. 2014; 1(1):19-23.
10. Kota SK, Meher LK, Kota SK, Jammula S, Krishna SVS, Modi KD. Implications of serum paraoxonase activity in obesity, diabetes mellitus, and dyslipidemia. Indian J Endocrinol Metab. 2013; 17(3): 402–412.
11. Grundy SM, Cleeman JI, Merz CN, Brewer HB, Clark LT, Hunninghake DB, Pasternak RC, Smith SC, Stone NJ. Implications of recent clinical trials for the national cholesterol education program adult treatment panel III guidelines. Circulation. 2004 Jul 13;110(2):227-39.
12. Gan KN, Smolen A, Eckerson HW, Bert NLD. Purification of human serum paraoxonase/arylesterase. Drug Metabol Disp 1991, 19: 100-106.
13. Friedewald WT, Levy RI, Friedrickson DS. Estimation of the concentration of LDL – cholesterol in plasma, without use of the preparative ultracentrifugation. Clin. Chem. 1972; 18 : 499 – 502.
14. Ferrieres J, Amber V, Crisan O, Chazelle F, Junger C, Wood D. Total lipid management and cardiovascular disease in the dyslipidemia international study. Cardiology. 2013;125(3):154–63.
15. Cheraghi M, Shahsavari G, Maleki A, Ahmadvand H. Paraoxonase 1 Activity, Lipid Profile, and Atherogenic Indexes Status in Coronary Heart Disease. Reports of biochemistry & molecular biology. 2017 Oct;6(1):1.
16. Saxena R, Mehrotra V. Prediction of hypertension and cardiovascular disease risk in North Indian geriatric population: a conundrum of senescence. Int J Comm Med Public Health. 2014; 1(1): 18-23.
17. Kumar A. Correlation of serum paraoxonase activities in known cases of 130 elderly hypertensive South Asian aged 56-64 years - a hospital based study. Asian Pac J Trop Dis 2014; 4(1): S330-S335.
18. Sentí M, Tomás M, Fitó M, Weinbrenner T, Covas MI, Sala J, Masiá R, Marrugat J. Antioxidant paraoxonase 1 activity in the metabolic syndrome. The Journal of Clinical Endocrinology & Metabolism. 2003 Nov 1;88(11):5422-6.