

Original Article:**Antioxidant status in patients with metabolic syndrome as measured by ferric reducing ability of plasma (FRAP) assay**

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ABSTRACT

Background: Oxidative stress is involved in the pathophysiology of diabetes and cardiovascular complications of metabolic syndrome. Endothelial dysfunction which is the key feature of metabolic syndrome and its vascular complication is intimately linked to insulin resistance. This relationship is partly due to oxidative stress.

Methods: Twenty five patients with metabolic syndrome (mean age 47.3 ± 2.6 years, 13 males) diagnosed on the basis of National Cholesterol Education Programme/Adult Treatment Panel (NCEP/ATPIII) criteria along with 25 age and gender matched healthy controls (mean age 42.1 ± 1.8 years, 11 males) were studied. Malondialdehyde (MDA), as an index of changes in lipid peroxidation was estimated as thiobarbituric acid reactive substances (TBARS) along with plasma total antioxidant capacity as ferric reducing ability of plasma (FRAP).

Results: A significant increase ($p=0.001$) in MDA levels in the study group was observed when compared to the control group, where as FRAP levels were decreased in the study group compared to the control group ($p=0.001$). Among the components of metabolic syndrome hyperglycaemia, hypertriglyceridaemia, hypertension and waist circumference positively correlated with MDA levels whereas hyperglycaemia, hypertriglyceridaemia and waist circumference correlated negatively with FRAP.

Conclusions: The findings of the present study suggest the presence of oxidative stress in patients with metabolic syndrome which further increases the cardiovascular risk in these patients. Antioxidant therapy monitored with a simple assay like FRAP would definitely add to the existing measures like reducing abdominal obesity in preventing the cardiovascular sequelae and hence CVD risk in these patients.

Keywords: Oxidative stress, Malondialdehyde, Ferric reducing ability of plasma, Metabolic syndrome

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INTRODUCTION

Metabolic syndrome constitutes a cluster of various disorders, such as, hypertension, obesity, dyslipidaemia and hyperglycaemia.^{1,2} It is considered as a coronary artery disease (CAD) equivalent in National Cholesterol Education Programme/Adult Treatment Panel (NCEP/ATPIII) guidelines³ as individuals with metabolic syndrome have an almost 20% chance of developing CAD in 10 years. Various components of metabolic syndrome like obesity, hypertension, hyperglycaemia and dyslipidaemia have been found to be associated with insulin resistance.⁴

Oxidative stress results due to disturbed equilibrium between prooxidants and antioxidants and plays a role in pathophysiology of diabetes mellitus and cardiovascular disease (CVD).⁴ Some factors of metabolic syndrome, such as hyperglycaemia and increased inflammation may lead to increased production of reactive oxygen species (ROS). Obesity is closely associated with metabolic syndrome including hyperglycaemia, dyslipidaemia and hypertension.⁵

Obese patients with metabolic syndrome have been found to have decreased concentrations of antioxidants especially, vitamins E, C and carotenoids, compared with controls.⁶ Simi-

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larly, Indian patients with metabolic syndrome have been shown to have decreased concentrations of antioxidant vitamins.⁷ Also, recently it has been found that there exists a racial difference in the levels of oxidative stress in patients with metabolic syndrome.⁸ However, there are very few publications⁹⁻¹¹ which have studied the total antioxidant capacity as an index of antioxidant defense in patients with metabolic syndrome with conflicting results. The present study was thus taken up to evaluate the oxidative stress including total antioxidant status in patients with metabolic syndrome.

MATERIAL AND METHODS

In the present study 25 patients (13 males) attending the outpatient service of the Department of Endocrinology, Sri Venkateswara Institute of Medical Sciences (SVIMS), Tirupati, diagnosed to have metabolic syndrome as per NCEP-ATP III guidelines³ and 25 age- and gender matched healthy controls were included. Patients on lipid lowering drugs, vitamin supplements, hormone replacement therapy and those with a history of smoking, alcoholism, infections, abnormal renal function, and malignancy were excluded from the study. Written informed consent was obtained from all patients participating in the study. The study was cleared by the Institutional Ethical Committee.

In all subjects, anthropometric measurements, including height, weight and waist circumference measurements; systolic and diastolic blood pressure were recorded. After overnight fasting, peripheral venous blood sample was collected into a plain (5mL) and fluoride (2 mL) vials from the study subjects. The samples were then centrifuged at 2000 rpm for 20 minutes. The separated serum(plain vial) and plasma(flouride vial) were stored at -80°C until further analysis. Plasma glucose (oxidase peroxidase method, Coral diagnostics, Goa, India), cholesterol (oxidase peroxidase method, Futura systems, Rome, Italy), triglycerides

(glycerol phosphate oxidase/peroxidase method, Span diagnostics, Surat, India) and high density lipoprotein (HDL)-cholesterol (immunoinhibition method, Beckman U.S.A) were estimated on fully automated analyzer(synchron CX9 from Beckman USA). Malondialdehyde (MDA) was analyzed as thiobarbituric acid reactive substances (TBARS).¹² Total antioxidant capacity was determined by ferric reducing ability of plasma (FRAP) method in which a colourless ferric tripyridyltriazine complex at low pH is reduced to a blue ferrous complex by the antioxidants in the plasma. The FRAP values are obtained by comparing the absorbance change at 593 nm in test reaction mixtures with those containing ferrous ions in known standards.¹³

Statistical analysis

All values were expressed as mean \pm standard error of mean (SEM). Independent samples 't' test was used to test the significance of difference in means between study group and controls. A p-value less than 0.05 was considered as statistically significant. Correlation of oxidative stress parameters with components of metabolic syndrome was assessed by Spearman's rank correlation analysis. Statistical analysis were done by using Microsoft Excel (Microsoft Corporation; Redmond) and SPSS for windows version 11.5 (SPSS, Inc., Chicago).

RESULTS

The mean \pm standard error of mean (SEM) age of patients with metabolic syndrome (study group) was 47.3 ± 2.6 years; there were 13 males. The mean \pm SEM of the control group was 42.1 ± 1.8 years; there were 11 males. Table 1 shows comparison of the components of metabolic syndrome in the study and control group. The control group was essentially free of the components of metabolic syndrome. As shown in the Table 2 the mean MDA levels were significantly higher in the study group compared with the control group ($p=0.001$).

Also, the mean FRAP levels were significantly lower in the study group compared with the control group ($p=0.001$). On correlating oxidative stress parameters with components of metabolic syndrome, MDA levels showed a positive correlation with fasting blood glucose (FBG), systolic and diastolic blood pressures, serum triglyceride (TG) levels and waist circumference (WC), while FRAP levels showed a significant negative correlation with FBG, TG and WC (Table 3). The serum uric acid levels were comparable among patients with metabolic syndrome and control subjects (4.3 ± 0.9 vs 3.8 ± 0.8 mg/dL, $p=0.100$).

DISCUSSION

Metabolic syndrome, first described by Reaven⁴ in 1988 is characterized by a constellation of cardiovascular risk factors, including atherogenic dyslipidaemia, abnormal glucose tolerance, hypertension and visceral obesity, which are intimately associated with insulin resistance and hyperinsulinaemia. Oxidative stress is involved in the pathophysiology of diabetes and cardiovascular complications of metabolic syndrome. Four of the five criteria of metabolic syndrome defined in NCEP-ATPIII, namely, hypertriglyceridaemia, hypertension, hyperglycaemia, and abdominal obesity are independently characterized by elevated systemic oxidative stress.¹⁴

In the present study, MDA levels were found to be significantly higher in the study group when compared to the control group ($p=0.001$). This is in agreement with previous reports of increased MDA levels in patients with metabolic syndrome compared to controls.^{9,15-17} We observed a statistically significant lower FRAP levels in the study group when compared to controls ($p=0.001$). FRAP is a measure of the antioxidant power, based on the reduction of ferrous ions by the effect of the reducing power of plasma constituents, contributed by low molecular weight antioxidants of a hydrophilic and hydrophobic character especially vitamins

C and E, serum bilirubin and serum uric acid. Hence, FRAP can be said to provide more biologically and clinically relevant information on antioxidant capacity than that provided by individual antioxidant measurements. This describes the dynamic equilibrium between pro- and antioxidants in the plasma.¹⁸

Patients with metabolic syndrome have been shown to have decreased concentrations of antioxidant vitamins.^{6,7} This decrease has been shown to be more in obese patients compared to non-obese metabolic syndrome patients.^{7,15} In addition, high concentration of serum uric acid observed in patients with metabolic syndrome, can lead to pro-oxidant effects, causing a further decrease of the plasma antioxidant capacity.¹⁹ Serum bilirubin levels have been shown to be negatively associated with metabolic syndrome and abdominal obesity.^{20,21} Measurement of total antioxidant capacity as FRAP can reflect all these and hence is a better measure of antioxidant status than measurement of the individual antioxidants.

Similar findings of increase in oxidant parameters and decrease in antioxidants have been reported in previous studies.^{10,22,23} Findings from the Third National Health and Nutrition Examination Survey show a significant decrease in the serum levels of antioxidants in patients with metabolic syndrome.^{6,24} Ford et al⁶ hypothesized that increased use of antioxidants probably contributed to the reduced antioxidant concentrations among participants with metabolic syndrome. A likely mechanism for this could be due to the high levels of oxidative stress which deplete endogenous and exogenous pools of antioxidants. Contrary to this view,⁶ Marcus et al¹¹ found no alteration in the total antioxidant capacity was noted in obese Asian Indian metabolic syndrome patients compared to controls which was attributed to be due to a probable increase in serum uric acid levels, one of the components measured by the FRAP assay. We, however, did not find any

Table 1: Comparison of the components of metabolic syndrome in the study and control groups

Parameter	Study group	Control group	p-value
	n=25	n=25	
SBP (mm Hg)	135.6 ± 4.5	121.2 ± 2.0	0.007
DBP (mm Hg)	86.9 ± 1.9	76.0 ± 1.4	0.001
Waist circumference (cm)	111.7 ± 6.1	84.3 ± 1.3	0.001
FBG (mg/dL)	173.7 ± 17.2	95.4 ± 4.9	0.001
T CHOL (mg/dL)	196.0 ± 12.6	174.6 ± 6.9	0.146
HDL-C (mg/dL)	47.1 ± 1.2	51.6 ± 1.7	0.038
TG (mg/dL)	251.6 ± 27.8	146.4 ± 15.4	0.004

All data are expressed as mean ± standard error of mean

SBP=systolic blood pressure; DBP=diastolic blood pressure; FBG=fasting blood glucose; TCHOL= serum total cholesterol; HDL-C= serum HDL- cholesterol; TG=serum triglycerides

Table 2: Comparison of oxidative stress markers in study and control groups

Parameter	Study group	Control group	p-value
	n=25	n=25	
MDA (µmol/L)	3.44 ± 0.47	0.67 ± 0.062	0.001
FRAP (mmol/L)	0.34 ± 0.039	1.19 ± 0.064	0.001

All data are expressed as mean ± standard error of mean

MDA= malondialdehyde; FRAP=ferric reducing ability of plasma

Table 3: Correlation between markers of oxidative stress with components of metabolic syndrome

	MDA	p-value	FRAP	p-value
	Correlation coefficient (p)		Correlation coefficient (p)	
Systolic BP	0.549	0.022	0.452	0.059
Diastolic BP	0.516	0.034	0.444	0.065
WC	0.781	0.001	-0.787	0.001
FBG	0.741	0.001	-0.586	0.001
TG	0.328	0.030	-0.453	0.002

MDA= malondialdehyde; FRAP=ferric reducing ability of plasma; BP = blood pressure; WC=waist circumference
FBG=fasting blood glucose; TG=triglycerides

change in uric acid levels in our metabolic syndrome patients compared to controls ($p=0.100$). The FBG levels were found to positively correlate with MDA levels ($p=0.001$) and negatively with FRAP ($p=0.001$) (Table 3). Hyperglycaemia seen in these patients can cause increased production of ROS as a result of protein glycation and glucose auto-oxidation. Also, the dyslipidaemia of metabolic syndrome further contributes to this oxidative stress. Hypertriglyceridaemia seen in these patients acts as a source of free-fatty acids which is the substrate for ROS. Our findings of a significant positive correlation between TG and MDA ($p=0.030$) and a significant negative correlation between TG and FRAP ($p=0.002$) supports this view.

Accumulating evidence suggests a potential role for adipose tissue in the causation of oxidative stress in patients with metabolic syndrome. ROS production has been shown to be increased as a result of increased activity of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and decreased expression of antioxidant enzymes in response to increased fatty acids.²⁵ The oxidative stress in turn decreases antiinflammatory adiponectin²⁶ and increases proinflammatory adipocytokines leading to a systemic inflammatory state.²⁷ This is confirmed from the observation of an increased oxidative stress in obese individuals with metabolic syndrome compared with nonobese individuals.^{11,28} Findings of the present study support this observation as seen from the positive correlation between WC and MDA ($p=0.001$) and negative correlation between WC and FRAP ($p=0.001$) (Table 3).

Hypertension is a cause as well as effect of oxidative stress.²⁹ Hypertension results due to reduced bioavailability of nitric oxide converting nitric oxide to peroxynitrite. Also, endothelial nitric oxide synthase (eNOS) can undergo uncoupling in the presence of peroxynitrite which is diverted towards lipid peroxidation,³⁰

thus leading to the bidirectional effect. In the present study we found a positive correlation between systolic and diastolic blood pressure with MDA ($p=0.022$ and $p=0.034$ respectively; Table 3).

The findings of the present study show that the individual components of metabolic syndrome especially, hyperglycaemia, hypertriglyceridaemia, hypertension and obesity are related to oxidative stress. This is in agreement with previous studies.^{24,31-33} However, Abdilla et al³³ reported that the contribution of components of metabolic syndrome towards oxidative stress in metabolic syndrome is minimal and the oxidative stress observed is mainly due to hypertension. As the aim of the present study was to understand the utility of monitoring the antioxidant capacity, we did not estimate the interactions of the influencing components in producing oxidative stress.

Contribution of oxidative stress to CVD risk has been shown to have racial differences with African Americans having higher levels of oxidative stress than whites.⁸ Indian Atherosclerosis Research Society (IARS) have recently shown oxidized low density lipoprotein (LDL) to be a predictor for metabolic syndrome in Asian Indian population.³⁴ The findings of the present study of increased oxidative stress in the metabolic syndrome patients as indicated by, increased MDA levels and decreased total antioxidant status also shows that Indian patients with metabolic syndrome have increased oxidative stress. The oxidative stress contributes to the development and progression of atherosclerosis by causing oxidative modifications of the LDL particles. Hyperglycaemia, dyslipidaemia and obesity seem to contribute independently or in combination. Though racial differences exist in the degree of oxidative stress in patients with metabolic syndrome, a common finding however is the low level of antioxidant vitamins, especially vitamins A, E and C and an elevated uric acid.^{6,7,19,24}

Since these vitamins and uric acid constitute major components of the FRAP assay, this can be a simple measure to assess the antioxidant status in these patients and can be done in any clinical biochemistry laboratory. Antioxidant therapy at large have not been shown to have any beneficial effect on decreasing CVD risk in western studies,³⁵⁻³⁷ data with this respect are however lacking from India. Also, majority of the studies have not monitored the improvement in antioxidant status with antioxidant therapy.^{6,24} We believe antioxidant therapy along with monitoring of host antioxidant defense would definitely help in blocking the effects of oxidative stress, one of the crucial mechanism of action of major components of metabolic syndrome, ie., abdominal obesity, hypertension, insulin resistance and dyslipidaemia. We propose that antioxidant therapy monitored with the help of FRAP assay along with appropriate lifestyle modification to decrease abdominal obesity would definitely prevent the cardiovascular sequelae and hence CVD risk in these patients.

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