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ORGINAL ARTICLE

Evaluation of Oxidative Stress Presented in Patients with Diabetes Mellitus and Metabolic Syndrome

[1]B.Sai Ravi Kiran, [2]T.Mohana Lakshmi, [3]SLV. Sankeerthi.Ch, [4]G.Surya Prakash, [5]V.Seshadri Reddy, [6]S.Arul Murugan, [7]E.Prabhakar Reddy*

ABSTRACT

Diabetes Mellitus (DM) is a syndrome characterized by chronic hyperglycemia and the most common complications such as atherosclerosis, nerve damage, renal failure, male impotence and visual disturbance.

Metabolic syndrome(MetS) represents a cluster of several risk factors that includes dyslipidemia, obesity, increased blood pressure and hyperglycemia, proinflammatory state, prothrombiotic state.

Our aim is to evaluate oxidative stress due to reactive oxygen species(ROS) and assess the antioxidant status by measuring MDA (Malondialdehyde) and FRAP(Ferric reducing ability of plasma) in patients with DM and MetS. The study included 50 diabetic patients, 50 MetS patients and 50 Controls. MDA and FRAP were estimated by UV spectrophotometer. 5 ml of venous blood samples were collected from patients and controls. All the samples were collected overnight fasting of 12 hrs. Collected samples centrifuged under 2000 rpm for 20 min and after centrifugation of samples used for the determination of MDA, FRAP, Lipid profile, Bilirubin and Uric acid. Increased MDA levels were found in DM comparing with MetS and controls. Our data are in agreement with numerous reports of an increase in plasma peroxidation products in DM ($p < 0.01$). Excessive lipid peroxidation in plasma could be due to uncontrolled production of ROS, and we found depletion of total antioxidant capacity estimated in diabetic patients comparing with Mets and Controls. FRAP is significantly decreased and acts as a good marker in DM when compared with Mets and controls its favors the OS. Blood glucose, Uric acid, Bilirubin significantly increased like MDA. We found significant evaluation of FRAP shows with clinical characteristics of diabetes ($p < 0.001$), Mets patients($p < 0.001$).

KEY WORDS : Metabolic syndrome, FRAP, MDA, Oxidative Stress

Introduction

Diabetes mellitus is a group of metabolic disorder which includes hyperglycemia due to defects in insulin secretion, insulin action (or) both. Chronic hyperglycemia of diabetics is associated with long term damage, dysfunction, retinopathy, nephropathy and neuropathy. Majority of diabetic cases caterozied into type I and type II. Type I (insulin dependent) Type II (insulin independent) because of autosomal immune destruction of β cells of pancreas with consequent insulin deficiency.90–95% diabetes causes accounts for type II. Most of the type II diabetics patients are obese and obesity itself causes some degree of insulin resistance[1]. If diabetes is not diagnosed early and managed properly, patients are at enhanced risk of microvascular and macrovascular complications.

[1,3]Tutor of Biochemistry, [2]Assistant Professor of Microbiology, Sri Lakhmi Narayana Institute of Medical Sciences, Puducherry.
[4]Professor of Biochemistry, Anna Medical College, Mauritius,
[5]Assistant Professor, BPS GOVT. Medical College, Sonepat, Harayana,
[6]Assistant Professor of General Medicine,
[7]Associate Professor and Central Lab Head, Department of Biochemitry, Sri Lakhmi Narayana Institute of Medical Sciences, Puducherry.

***Corresponding Author**
Dr. E. Prabhakar Reddy,
Associate Professor and Central Labouratory Head
Sri Lakhmi Narayana Institute of Medical Sciences,
Osudu, Puducherry-605502, India.
Email-id: brvavikiran86@gmail.com
Telephone number: +917708873424

Metabolic syndrome (MetS) first described by Reaven in 1988.It represents a cluster of several risk factors that includes dyslipidimia, obesity, increased blood pressure and hyperglycemia [2-3]. MetS is a collection of cardiometabolic risk factors that includes obesity, hypertension, insulin resistance, Proinflammatory state, Prothrombiotic state. Obesity responsible for rising prevalence of metabolic syndrome[4]. Obesity is the central and casual components of MetS especially abdominal obesity, physical inactivity and atherogenic diet.

MetS is highly significant for type 2 DM and CVD.75-80% of adults diabetic patients death caused by CVD [5]. MetS also includes elevated level of fasting TG and decreased(HDL-C) cholesterol concentration, high level of VLDL concentration, abnormal postprandial lipemia, apolipo protein B and HbA1C . MetS have a five –fold higher risk of type 2 DM and a two to three –fold higher risk factor of atherosclerotic CVD[2-3].

The etiology of metabolic syndrome patients with CVD involves coronary atherosclerotic diseases, artery hypertension, left ventricular hypertrophy, diastolic dysfunction, endothelial dysfunction, coronary macro-vascular disease and autoimmune dysfunction diseases occurred[6]. MetS individuals seemingly are susceptible to other conditions notably polycystic ovary syndrome, fatty liver, cholesterol gallstones, asthma, sleep disturbance and some forms of cancer[7].

Abdominal obesity is strongly associated with the MetS. It present clinically as increased waist circumference (Men->40 inch-women>35inch) Obesity strongly associated with increased blood pressure in insulin–resistant persons. Increased CRP level, clinically observed in proinflammatory persons with MetS. Obesity is one of the causes for elevation of CRP levels due to excess adipose tissues release, inflammatory cytokines that may elicit higher CRP levels. Increased Plasma plasminogen Activator Inhibitor (PAI) and fibrinogen associated with the metabolic syndrome.

Metabolic syndrome is a powerful determinant of diabetics and CVD. There are few prospective data, however on the extent to which this syndrome (or) its consistutents components predicts incidence of type-2 diabetics.

Metabolic syndrome is often characterized by oxidative stress, a condition in which an imbalance results between the production and inactivation of reactive oxygen species. Oxidative stress defined as increased formation (or) insufficient removal of highly reactive molecules that is reactive oxygen species/ reactive nitrogen species and decreased antioxidant defenses (disturbed balance between prooxidants and antioxidants)[7]. Increased systemic oxidative stress characterized by low level of serum Vit C, α- tocopherol and reduced superoxide dismutase activity in the diabetic condition [8-10]. Oxidative stress impairs glucose uptake in muscle, fat and decreased insulin secretion from pancreatic β cells and atheroscterosis by directly affecting vascular wall cells. OS stress involved in the pathophysiology of hypertension, DM & Cardiovascular diseases of Mets.

Imbalanced production of adipocytokines [TNFα, teptin, adiponectin, resistin, Plasmogen Activator Inhibitor PAI-1] is the pathogenesis of obesity-associated metabolic syndrome[11]. Malondialdehyde is produced by lipid per oxidation is the best marker for free radical tissues damage and oxidative stress and it increased in metabolic syndrome.

Dietary antioxidant could have favorable effects on prevention of oxidative stress. Specific antioxidants are VitE and Vit C. Its consists of antioxidant property but did not have

significant effects on MetS[12]. Antioxidant enzymes including malondialdehyde Superoxide, Catalase, Glutathioneperoxide observed modified levels in metabolic syndrome[13].

Specific micronutrients have anti-inflammatory and antioxidant capacity by reducing oxidative stress and also detected many chronic diseases including type 2DM, CVD, Rhematological condition and carcinogenic[14-17].

There are very few studies which have studied the total antioxidant capacity as an index of antioxidant defense in DM patients with Mets incompatible results. Uricacid and bilirubin act as a non- enzymatic antioxidant biomarkers[18-19] as they prevent free radical reactions. Provides the primary extracellular defense against the oxidative stress sequestering the transition metal ions by chelating plasma [20]. The present study focused on oxidative stress along with total antioxidant status DM patients with Metabolic syndrome.

Objective of the Study

Our aim is to evaluate oxidative stress due to reactive oxygen species(ROS) and assess the antioxidant status by measuring MDA (Malondialdehyde) and FRAP(Ferric reducing ability of plasma) in patients with DM and MetS.

Materials and Methods

The study was conducted at SLIMS, Puducherry. The study included 50 diabetic patients, 50 MetS patients and 50 Controls. MDA and FRAP were estimated by UV spectrophotometer. 5 ml of venous blood samples were collected from patients and controls. All the samples, and this separated seum were collected overnight fasting of 12 hrs. Collected samples centrifuged under 2000 rpm for 20 min and after centrifugation of samples used for the determination of MDA, FRAP, Lipid profile, Bilirubin and Uric acid.

Patients on Insulin, Smokers, Alcholics, Tobacco chewers were excluded from the study.

Fasting blood glucose, Fasting lipid profile (Total cholesterol, TG, HDL), Uric acid and Total bilirubin were done by using enzymatic kits on siemens™ autoanalzyer. LDL- C values done by using the friedwald formula.

Malonaldehyde(MDA) is estimated as thiobarbaturic acid reactive substances (TBARS).

The total antioxidant capacity of the were estimated by using FRAP(Ferric reducing ability of plasma) assay. The FRAP assay as global marker of the antioxidants as reductants in a redox-linked colorimetric method. In this assay, at low pH, a ferric-tripyridyltriazine complex is reduced to the ferrous form, which is blue and monitored by measuring the change in absorption at 593 nm. The change in absorbance is directly proporational to the reducing power of the electron donating antioxidants present in plasma. The absorbance change is translated into a FRAP value by relating the change of absorbance at 593 nm of test sample to that of a standard of known FRAP value[21].

Results

Serum cholesterol, LDL-C,TG levels were significantly increased in diabetic patients when compared with Mets and controls, and HDL-C level was significantly decreased.

Increased MDA levels were found in DM comparing with MetS and controls. Our data are in agreement with numerous reports of an increase in plasma peroxidation products in DM ($p < 0.01$)(Table.No.1) Excessive lipid peroxidation in plasma could be due to uncontrolled production of ROS, and we found depletion of total antioxidant capacity

estimated in diabetic patients comparing with Mets and Controls. FRAP is significantly decreased and acts as a good marker in DM when compared with Mets and controls. We found significant evaluation of FRAP shows with clinical characteristics of diabetes (p <0.001), Mets patients(p <0.001). Blood glucose, Uric acid, Bilirubin significantly increased like MDA(Table No.1).

We found depletion of total antioxidant capacity estimated in diabetic patients comparing with Mets and Controls. FRAP is significantly decreased and acts as a good marker in DM when compared with Mets and controls its favors the OS. We found significant evaluation of FRAP shows with clinical characteristics of diabetes (p <0.001), Mets patients(p <0.001).

S.No.	Parameter(mg/dl)	DM(n=50) Mean ± SD	MetS(n=50) Mean ± SD	Controls	p Value
1	Cholesterol	263± 32.62	245± 30.51	188.5 ±27.3	p<0.001
2	HDL	36± 6.23	30± 5.52	50± 8.25	p<0.001
3	MDA	6.73± 0.87	5.73± 0.98	0.07± 0.01	p<0.001
4	FRAP	0.62± 0.08	0.42 ± 0.09	1.32± 0.14	p<0.001
5	UA	8.2 ± 0.06	6.5± 0.42	5.22± 0.23	p<0.001
6	Bilirubin	1.2 ± 0.07	1.0± 0.04	0.07 ± 0.02	p<0.001
7	FBS	168.55± 4.92	156.55± 4.82	106.12± 1.68	p<0.001

Table. No.1: The Mean ± SD values of Cholesterol, HDL, MDA, FRAP, UA, Bilirubin, FBS in DM, MetS patients and Controls

Discussion

Increased MDA levels were found in DM comparing with MetS and controls. Our data are in agreement with numerous reports of an increase in plasma peroxidation products in DM (p <0.01). MDA is the stable end product of lipid peroxidation, and it's produced during the decomposition of polyunsaturated fatty acids. Due to Hyperglycemia autooxidation of glucose, glycation of proteins and the activation of polyol metabolism occurs[22]. These changes increase the generation of reactive oxygen species and accelerates the oxidative modifications of lipids and proteins[23]. The oxidation of lipids plays important role in generation of the atherosclerotic lesions in mets patients (damaging of the arterial walls)[24].

FRAP is the global marker of the antioxidant power. FRAP conclude the total activity of antioxidant vitamins and enzymes due to difficulty in separate estimation of each antioxidant component of plasma and of the interactions that take place among different components. So FRAP assay used as single test to estimate total antioxidant capacity of blood. Decreased total antioxidant capacity in plasma because of reduced antioxidant deference and hyper glycemia in diabetes may increase ROS Production through changes in the redox potential of glutathione[25]. The strength of antioxidant system inhibit (or) trap the free radical's produced under normal (or) pathological condition's was evaluated by measuring the level of total antioxidant status. This status reflect the status of extra cellular antioxidants [26]. These antioxidants inhibit (or) delay the oxidative process.

Uric acid is the end product of purine metabolism serum uric acid is a diprotic acid produced by the enzyme xanthine oxidase from xanthine and hypoxanthine. Xanthine oxidase uses molecular oxygen as electron acceptor and generates superoxide anion and other reactive oxygen oxygen species(ROS) [27]. Uric acid is chain breaking antioxidant, which is of great importance in plasma. Increased serum uric acid results gout, lesch nyhan's syndrome, and uric acid stones hyper ureacemic also found to be associated with insulin resistance and components of the Mets[28-30].

FRAP assay estimates the antioxidant capacity, not just the antioxidant strength of a single component. In this study total antioxidant status were significantly higher in mets patients compared to healthy controls serum(or)plasma. TAC levels have not found to be consistently reflect increased OS[31].

However, the results are controversial. The FRAP test shown statistically significant ($p<0.001$),but it may not have any clinical importance. in conditions of hyperuricemia, estimation of total antioxidant capacity in MetS patients may be inaccurate because 60% of total antioxidant capacity is due to uric acid. [32]. In this study Mets patients had significantly higher uric acid levels than individuals in the control group. Although we expected TAC to decrease, we found higher TAC in Mets patients.the antioxidant property of uric acid is contributed to this finding However, further studies are required to confirm the mechanism's concluding this effect.

In olden days bilirubin was believed only waste product of Heme catabolic pathway, a potentially toxic compound and have various biological functions. Recent studies showed mildly increased serum bilirubin levels are strongly associated with low prevalence of oxidative stress –mediated diseases and it's shows antioxidant property [33]. Total bilirubin (TB) concentration was inversly related with coronary artery diseases, hyper tension and Mets[34]. Bilirubin shows negative reaction ship with Dm and Mets, abdominal obesity[33].

Conclusion

The monitoring of MDA, and total antioxidant status by FRAP, Uri cacid as clinical effective levels of drug treatments along with aimed to reduce cardiovascular risk in diabetic patients and MetS should be considered. Antioxidants are important for the prevention of MetS and its complications, so more research is needed to differentiate the effects of major serum antioxidant on MetS.

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