Research Article

Total Antioxidant Status and Oxidative Stress in Diabetes Mellitus and Metabolic Syndrome

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ABSTRACT

Total antioxidant capacity assays have been designed to determine overall antioxidant power of samples contributed by antioxidant and their interactions. Diabetes mellitus is a group of metabolic disorder which includes hyperglycemia due to defects in insulin secretion, insulin action and both. Metabolic syndrome is characterized by insulin resistance and the presence of risk factors for cardiovascular diseases and Diabetes mellitus. Oxidative stress impairs glucose uptake in muscle, fat and decreased insulin secretion from pancreatic beta cells and atherosclerosis by directly affecting vascular wall cells. Oxidative stress involved in the pathophysiology of diabetes mellitus and cardiovascular diseases of metabolic syndrome. We assessed MDA, FRAP, Lipids in 200 patients of diabetes mellitus, 200 patients of metabolic syndrome and 200 healthy controls. Increased total antioxidant status may not always represent ones healthier condition and condition with a low oxidative stress, suggesting that total antioxidant status may be used as sole indicator of oxidative stress.

Keywords: Total antioxidant status (TAS), oxidative stress, diabetes mellitus, metabolic syndrome.

INTRODUCTION

Diabetes mellitus is a group of metabolic disorder which includes hyperglycemia due to defects in insulin secretion, insulin action (or) both. Metabolic syndrome (MetS) is highly significant for type 2 DM and CVD.75-80% of adult's diabetic patients death caused by CVD[1]. 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 prevalence of diabetes in India is very rapidly rising and it is estimated that by the year 2010 A.D, 20% of all Type 2 patients in the world would be contributed from India. The first study done in South India was at Vellore in 1964[4]. This hospital based study done on 63,356 individuals showed a prevalence of 2.5%. The early signs of the looming diabetes epidemic were seen in the study conducted in Hyderabad in the year 1966, which reported a high prevalence of 4.1%[5]. However, the studies in rural areas were conducted since 1972[6]. In 1984, house to house surveys were conducted in individuals aged 15 years and above in Tenali, a small town in Andhra Pradesh (urban) and rural population of Pondicherry (now Puducherry), which reported a prevalence of 4.7%[7] and 1.8%[8] respectively.

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)[9].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. 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. Its consists of antioxidant property but did not have significant effects on MetS[10]. Antioxidant enzymes including malondialdehyde Superoxide, Catalase, Glutathione peroxide observed modified levels in metabolicsyndrome[11].

The major advantage of total antioxidant capacity(TAC) test is to measure the antioxidant capacity of all antioxidants in a biological sample and not just the antioxidant capacity of a single compound.[12] A team of endogenous and exogenous antioxidants representing the total antioxidant capacity of extra cellular provide greater protection against attack by oxygen fluid free radicals.[13]

Determination of total antioxidant capacity is based on the evaluation of total antioxidant of either a hydrophilic or hydrophobic and their concentrations reduction effect of individual antioxidants, without exact qualitative differentiation. FRAP is one of the method which directly analysis total antioxidants.

The present study was conducted to compare the oxidative stress marker MDA and, total antioxidant status in patients with diabetes mellitus, metabolic syndrome

and healthy controls and also to examine the relationship between metabolic risk factors such as high blood pressure, high serum lipids, and glucose.

MATERIALS AND METHODS

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 (4mL) and fluoride vials from the study subjects. The samples were then centrifuged at 3000 rpm for 15 minutes. The separated serum (plain vial) and plasma (fluoride vial) were stored at –50°C until further analysis. Plasma glucose, cholesterol, triglycerides and high density lipoprotein (HDL) cholesterol were estimated on fully automated analyzer (Siemens fully automated analyzer). Malondialdehyde (MDA) was analyzed as thiobarbituric acid reactive substances (TBARS). Total antioxidant capacity was determined by ferric reducing ability of plasma (FRAP) method in which a colorless 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.

Statistical Analysis

All values were expressed as mean ± standard deviation(SD). Independent samples 't' test was used to test the significance of difference in means between study group and controls. For men and women, a student t-test or ANOVA was used to compare between control and MetS participants normal or non-normal distribution, respectively. A P-value less than 0.05 were considered statistically significant. Statistical analysis were done by using Microsoft Excel and SPSS for windows version 11.5 (SPSS, Inc., Chicago).

RESULTS

The Mean MDA levels were significantly higher in the metabolic syndrome compared with control group and significantly higher in the diabetes mellitus compared with metabolic syndrome ($p<0.001$). The mean FRAP levels were significantly lower in the metabolic syndrome compared with control group and significantly higher in the diabetes mellitus comparing with metabolic syndrome ($p<0.001$). An correlating oxidative stress parameters with components of metabolic syndrome, MDA levels showed a positive correlation with fasting blood glucose, systolic and diastolic blood pressures, serum TGL and waist circumferences (WC), while FRAP levels showed a significantly with FBS, TGL, and WC. The serum Uric acid levels were comparable among patients with metabolic syndrome and control group (8.2 ± 0.06 vs 6.5 ± 0.42).

The serum bilirubin levels were comparable among patients with metabolic syndrome and control group (1.0 ± 0.04 vs 0.07 ± 0.02) and comparable among patients with metabolic syndrome and diabetes mellitus group (1.0 ± 0.04 vs 1.2 ± 0.07).

The serum albumin levels were comparable among patients with metabolic syndrome and control group (4.9 ± 0.14 vs 3.9 ± 0.09) and comparable among patients with metabolic syndrome and diabetes mellitus group (4.9 ± 0.14 vs 5.2 ± 0.19).

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. UA is also a physiological free radical scavenger and one of the major contributors of the plasma antioxidant capacity[13]. Thus, UA plays a dual role, both as a prooxidant and as an antioxidant[14]. T2DM is associated with oxidative stress and increased free radical formation[15]. While on one hand, hyperglycaemia generates free radicals, on the other hand, it also impairs the endogenous antioxidant defense system[16]. Under the condition of increased oxidative stress, there occurs the depletion of the local antioxidants, which causes a reduction in the antioxidant status of the body[17].

In the presence of OS, uncontained ROS attack, modify, and denature functional and structural molecules leading to tissue injury and dysfunction. I wish to point out that while excessive production of ROS causes injury and dysfunction, normal rate of ROS production is essential for life.

Diabetes Mellitus (DM) is a disorder characterized by persistent hyperglycemia due to insulin resistance. Insulin is a pleiotropic hormone which signals a number of cellular processes such as gluco regulation, lipid metabolism, and protein synthesis in multiple tissues. In patients with DM, these actions of insulin are reduced. Consequently, there is an increase in free fatty acids which promote oxidative stress, endothelial dysfunction, vascular damage, and atheroma formation. The clinical results are high BP, HDL suppression, and high triglycerides (TAG) additionally, DM is associated with macro vascular (myocardial infarction, stroke) and microvascular (retinopathy, neuropathy, renal disease) problems which interfere with blood and nutrient delivery to multiple tissues throughout the body. DM is a crucial factor in MS and is highly predictive of Cardiovascular Disease (CVD) risk. In 1999 the San Antonia Heart Study found that insulin resistant patients had a greater incidence of hypertension and dyslipidemia than non-insulin-resistant patients[18].

Table 1: Shows Comparison of the components of metabolic syndrome in diabetes mellitus, metabolic syndrome on the study and control group

S. No.	Parameters	DM (n-200) Mean ± SD	MetS (n-200) Mean ± SD	Controls (n-200)	p Value
1	FBS	168.55 ± 4.92	156.55 ± 4.82	106.12 ± 1.68	p<0.001
2	TAC (FRAP)	0.62 ± 0.08	0.42 ± 0.09	1.32 ± 0.14	p<0.001
3	MDA	6.73 ± 0.87	5.73 ± 0.98	0.07 ± 0.01	p<0.001
4	Cholesterol	263 ± 32.62	245 ± 30.51	188.5 ± 27.3	p<0.001
5	TGL	258 ± 31.02	243 ± 28.62	169.2 ± 28.4	P<0.001
6	T.Bilirubin	1.2 ± 0.07	1.0 ± 0.04	0.07 ± 0.02	p<0.001
7	Uricacid	8.2 ± 0.06	6.5 ± 0.42	5.22 ± 0.23	p<0.001
8	SBP(mmHg)	132.1 ± 4.1	136.2 ± 4.4	124.2 ± 2.1	P<0.006
9	DBP(mmHg)	82.7 ± 1.3	84.9 ± 1.6	74 ± 1.3	P<0.001
10	Waist circumstances(cm)	121.3 ± 5.6	111.9 ± 5.9	85.1 ± 1.1	p<0.019
11	Albumin	5.2 ± 0.19	4.9 ± 0.14	3.9 ± 0.09	p<0.001

All data are expressed as Mean ± SD, FRAP-Ferric reducing ability of plasma, TAC-Total Antioxidant capacity, MDA - Malondialdehyde, TGL - Triglycerides, T.Bilirubin - Total Bilirubin, SBP - Systolic blood pressure, DBP - Diastolic blood pressure, FBS - Fasting blood sugar.

Table 2: Correlation between Markers of OS with Components of Diabetes Mellitus and Metabolic Syndrome

Parameter	MDA correlation coefficient DM	p Value (DM)	MDA correlation coefficient MetS	p Value (MetS)	FRAP correlation coefficient DM	P Value	FRAP correlation coefficient MetS	P Value
Systolic BP	0.532	0.20	0.546	0.21	0.486	0.054	0.471	p<0.056
Diastolic BP	0.501	0.30	0.512	0.32	0.411	0.060	0.495	p<0.062
WC	0.781	0.001	0.779	0.001	0.788	0.001	-0.780	p<0.001
FBS	0.742	0.001	0.743	0.001	-0.587	0.001	-0.588	p<0.001
Cholesterol	0.342	0.036	0.331	0.028	-0.471	0.002	-0.460	p<0.002
TGL	0.338	0.031	0.326	0.030	-0.468	0.002	-0.456	p<0.002

MDA - Malondialdehyde, FRAP - Ferric reducing ability of plasma, BP - Blood pressure, WC - Waist circumstances, FBS - Fasting blood sugar, TGL - Triglycerides

Other epidemiological studies have established a similar relationship between hyperglycemia and CVD which implicates the importance of DM as a risk factor for cardiovascular mortality.

Hyperuricemia associated with elevated circulating endothelial levels and are of the major sites of the production of UA in cardiovascular system. High uricacid levels were independently associated with increased proximal tubular sodium re absorption in men UA other hand cause to hypertension in metabolic syndrome and diabetes mellitus. Finally, increased UA levels in DM may be a response to OS in patients. This increasing UA may act in conjugation with the lipids to cause atherosclerotic complications. Hyperuricemia associated with elevated circulating endothelial levels and are of the major sites of the production of UA in cardiovascular system. High uric acid levels were independently associated with increased proximal tubular sodium re absorption in men UA other hand cause to hypertension in metabolic syndrome and diabetes mellitus. Finally, increased UA levels in DM may be a response to OS in patients. This increasing UA may act in conjugation with the lipids to cause atherosclerotic complications. 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[19-20] as they prevent free radical reactions.

Uric acid was shown to be the second highest contributor among the known serum antioxidants, albumin (28.0%), ascorbic acid (3.08%), α-tocopherol (1.74%) and bilirubin (1.0%). In addition, uric acid was found to be elevated with overweight, obesity and visceral fat area[21-23]. These previous findings may suggest the partial effect of increased uric acid on TAS in overweight and obese conditions, and explain in part the positive association among obesity indices and TAS shown in the current study. In addition, we also observed elevated TAS with increased TG and metabolic risk factors. In this present study we have evaluated the importance of these markers in Indian subjects. We have investigated antioxidant status in the form of estimation of plasma/serum albumin, Uric acid & Total bilirubin levels also in diabetics and it compared with healthy controls. Recent studies showed mildly increased serum bilirubin levels are strongly associated with low prevalence of oxidative stress mediated diseases and its shows antioxidant property.[13] Bilirubin in circulation is mainly found bound to albumin. Localization of bilirubin and albumin protects albumin from oxidation as well as the albumin-bound linoleic acid from peroxyl radical induced oxidation[24]. This albumin-sparing function of bilirubin was observed in our study. Serum albumin and total bilirubin level were significantly lower in type 2 diabetic patients as compared to non diabetics.

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[25-28]. 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. 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. 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[29-32]. Recent evidences have shown the association between oxidative stress and development metabolic syndrome[33-34]. There are several studies showing accumulation of protein and lipid oxidative products in diabetic patients, increased levels of circulating oxidative stress markers and reduced antioxidant defenses[35-36]. Also, dyslipidemia component of metabolic syndrome further contributes to this oxidative stress. Hypertension is a cause as well as effect of oxidative stress[37]. Hypertension as a result of reduced bioavailability of nitric oxide, causes converting of nitric oxide to peroxynitrite. Also, endothelial nitric oxide synthase (eNOS) can undergo uncoupling in the presence of peroxynitrite which is diverted towards lipid peroxidation[38]. In the present study, we found a negative correlation between systolic and diastolic blood pressure with DPPH-scavenging activity (p=0.006 and p=0.018 respectively. The findings of the present study show that the individual components of MetS, especially hyperglycemia, hypertriglyceridemia and hypertension are related to oxidative stress.

Elevated ROS molecules caused the cellular macromolecules damage such as lipids, proteins[39] and nucleic acids[40]. In the anti-oxidants system of the living system, possess own antioxidant defense mechanisms[41] includes enzymes and non-enzyme molecules such as SOD, catalase (CAT) and glutathione peroxidases (GPx). Enzyme SOD catalyzes $O_2^{\bullet-}$ conversion to H_2O_2, while CAT converts H_2O_2 to H_2O and O_2. For reduction of two peroxide molecules use non-enzymatic glutathione (GSH; reduced and oxidized forms), reduced glutathione (GSH) and GPx catalyze to produce oxidized glutathione (GSSG) and water.[42] Various enzymes play the important combination roles in the series of antioxidant defense systems such as glutathione reductase, glutathione S-transferase, and glutathione disulfide (GSSG).

The observed increase in malondialdehyde release might be attributed to the increase in peroxidative damage to lipids from oxidative stress developed during diabetes. There are several studies supporting the theory of increased oxidative stress in diabetes mellitus by way of estimating MDA by ThioBarbituric acid reactive substances (TBARS) method. E Prabhakar from Pondicherry have found a marked increase in MDA levels as (5.73 + 0.93 µM) in diabetic patients with coronary heart disease in comparison to healthy controls which was (0.07 + 0.01 µM)[43]. Chavan have also observed similar results among a study population from Gujarat[44]. Rama Srivatsan have reported increased MDA levels in diabetics among a Southern Karnataka population[45]. Manjulata from Gwalior also report elevated MDA levels in diabetic patients[46].The activities of antioxidant enzymes Super oxide dismutase, Glutathione peroxidase, Catalase, Glutathione reductase, Glutathione and vitamins A, E and C are usually measured to assess the antioxidant stress in the blood. A single test which denotes the antioxidant power of blood was established & estimated as the ferric reducing ability of plasma (FRAP) was found to give more biologically relevant information than the measurement of individual antioxidants. FRAP Summarizes the overall activity of antioxidant vitamins and enzymes. Because of the difficulty in measuring each antioxidant component of plasma separately and of the interactions that take place among different components. FRAP is being used as a single test to estimate total antioxidant capacity (TAC) of blood. In recent years several methods have been developed to assess the TAC of human serum (or) plasma. More biologically relevant information can be obtained by assessing FRAP than that obtained by measuring the concentration of individual antioxidants and may more closely describe the dynamic equilibrium between Pro oxidant and anti-oxidants occurring in the plasma compartment[47].

FRAP is the global marker of the antioxidant power. FRAP conclude the total activity of antioxidant vitamins and enzymes to difficulty in separate estimation of each antioxidant component of plasma and of the interactions that take place among different components. Decreased total antioxidant capacity in plasma because of reduced antioxidant difference in diabetes may increase reactive oxygen species production through changes in the redox potential of glutathione and hyperglycemia.[48] The strength of the antioxidant system inhibitor trap the free radical produced under normal and pathological condition was evaluated by measuring the level of total antioxidant status.

This reflects the status of extracellular antioxidants,[13] these antioxidants inhibit delay the oxidative process. FRAP may be considered as an easy, cost effective method to measure the antioxidant power and it might be incorporated into risk prediction in diabetes and CVD.

However, we might be able to assume that uric acid did contribute serum total antioxidant status level, when we consider the report 20% total antioxidant capacity (TAC) called as total antioxidant status (TAS) was contributed by uric acid.[13] We observed similar tendency in elevated total antioxidant status with increased number of metabolic risk factors.

Based on these observations, we might take it for granted that changes in serum uric acid affect total antioxidant status levels. Besides uric acid, serum albumin was also found to contribute total antioxidant status level by 28% and significantly correlated total antioxidant status appears to be affected by changes in individual antioxidant parameters. Therefore, it seems that TAS which might be altered by various metabolic conditions.

These antioxidant parameters affecting total antioxidant status values could be increased not only by body increased antioxidant status, but by body compensatory mechanisms to counteract increased oxidative stress.[12]

In our study individual antioxidant data were available and it may be able to strongly assume the factors directly affecting total antioxidant status in subjects with and without metabolic factors.

However, it is worthy note that positive associations between total antioxidant status and increased metabolic risk factors.

Increased total antioxidant status level may not always represent ones healthier condition, a condition with a low oxidative stress. Suggesting that total antioxidant status may be used as a sole indicator of oxidative stress marker.

CONCLUSION

The study therefore suggests, the estimation of plasma antioxidants levels with other routine investigations may be useful in the prevention of the diabetic complications, which can be prevented by supplementing the antioxidants rich components of the diet and thus further diabetic events can be avoided.

Future studies are needed to clarify the mechanism responsible for the oxidative stress in the risk of metabolic syndrome. FRAP assay could be employed to detect complications early and revert the conditions. FRAP is a simple method, speedy, inexpensive.

Anti oxidants are important for the Prevention of diabetes mellitus, metabolic syndrome and its complications, so supplementation of antioxidant research studies are needed to differentiate the effects of major plasma antioxidants and diabetes mellitus and metabolic syndrome.

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