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Original Research Article

Plasma Total Antioxidant Activity and Its Relation with Serum Uric Acid in Type 2 Diabetes and Non Diabetes: A Comparative Study

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Abstract: Oxidative stress has been found to be associated with variety of diseases including diabetes. It is conceivable that alteration in plasma antioxidant reserve along with serum uric acid levels may have a crucial role in diabetes mellitus (DM). The objectives of present study were to ascertain the plasma levels of total antioxidant activity (TAA) and serum uric acid (UA) in diabetes mellitus and to determine their cumulative effect on lipid peroxidation along with hyperglycemia. In the present study, plasma TAA, serum UA and erythrocyte malondialdehyde (MDA) levels along with fasting and postprandial plasma glucose levels were measured in 50 diabetic patients and statistically compared with that of age matched 50 non diabetics subjects, served as control by using student's t test and Pearson correlation coefficient analysis**.** Plasma TAA levels were significantly low ($p<0.05$) in T2DM subjects as compared to healthy controls. Similarly, erythrocyte MDA and fasting blood sugar levels were significantly high ($p<0.001$) in T2DM subjects as compared to healthy controls. However, serum uric acid levels were altered insignificantly ($p<0.1$) in T2DM subjects as compared to healthy controls. Our findings indicate that hyperglycemia is closely associated with altered plasma TAA, MDA and serum uric acid levels due to augmented oxidative stress and thereby making the diabetic more susceptible to develop pathological consequences. Thus, consumption of antioxidant rich diet along with regular exercise could be an effective strategy to reduce the incidence of diabetes and its related complications.
Keywords: Antioxidant, free radical, insulin resistance, lipid peroxidation.

INTRODUCTION

As the medical science is advancing, the incidence of diabetes is increasing at alarming pace. This significant increase in number of people with Type 2 diabetics makes this disease a global threat in 21st century. It is estimated that diabetics affect about 150 million population worldwide and this figure is expected to be doubled in next two decade [1]. Among various risk factors, oxidative stress, caused by increased production of reactive oxygen species (ROS) such as superoxide anion ($O_2^{\cdot-}$) and its metabolites or by reduced bioavailability of antioxidant defenses, plays a crucial role in the etiopathogenesis of various diseases including Type 2 diabetes [2-4].

Free radicals are highly reactive species and they act through several mechanisms to mediate apoptosis followed by pathological consequences, which include biomolecular destruction, DNA strand breakage, protein oxidation, damage to membrane ion transporters, lipid peroxidation, impair insulin signaling pathways and induce cytotoxicity in pancreatic beta cells [5, 6]. Prime target to free radicals attack are the polyunsaturated fatty acids in the membrane lipids, causing lipid peroxidation, has been implicated in the development of Type 2 diabetes [7]. Lipid peroxide (malondialdehyde) is the most abundant among the reactive aldehydes derived from lipid peroxidation. It has been suggested that binding to these aldehydes to membrane protein may alter their function, tonicity, permeability, rigidity and integrity, and thereby enhances the disease complications [8].

These free radicals are efficiently removed by antioxidant defense system, which includes antioxidant enzymes and antioxidants [9]. Total antioxidant activity (TAA) including co-operative action of non enzymatic antioxidants such as vitamin C, E, A, uric acid and

albumin, may have a significant role in the prevention of pathophysiological alterations leading to aging and its related complications [10, 11]. Amongst various non-enzymic antioxidants, uric acid is an end product of purine metabolism and considered as an effective antioxidant in blood plasma as it scavenges superoxide radical, protects erythrocyte against peroxidative damage and free radical attack [12]. Therefore, the overall objectives of present study were to ascertain the plasma levels of TAA and serum uric acid levels in the T2DM patients and to determine their cumulative effect in the progression of disease process.

MATERIAL AND METHODS

In the present study 50 patients of either sex with Type 2 diabetes mellitus (25 males and 25 females) belonged to age group 35-65 years were recruited as patient group. 50 age and sex matched healthy subjects with fasting and postprandial plasma glucose less than 100 mg/dl and 140 mg/dl were recruited as controls. 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. All patients had T2DM, defined as per revised American Diabetic Association criteria (ADA 2013).

Inclusion criteria

Subjects, who gave informed consent for study, don't under any medical treatment (anti-inflammatory drug) or taking antioxidant supplement for at least 1 month prior to blood collection were included.

Exclusion criteria

Patients with acute and chronic infections, fever, malignancy, renal disease, hepatic disease, hypertension, those taking antioxidant vitamin supplements or non-steroidal anti–inflammatory drugs and with other connective tissue disease like systemic sclerosis and osteoarthritis were excluded.

Fasting blood sample (8 ml) was collected from the anticubital vein of the study group subjects and divided into three parts. First part was collected in fluoride vial for glucose estimation, second part in EDTA vial for plasma TAA and erythrocyte MDA estimation, and third part was kept in a syringe for half an hour for proper coagulation followed by serum separation at 2000 rpm to estimate uric acid levels. Fasting blood glucose levels were measured by using enzymatic kit based on glucose oxidase method. Glucose, in presence of glucose oxidase, converted into gluconic acid along with production of Hydrogen peroxide, which later oxidatively coupled with 4-aminoantipyrine /phenol (in presence of peroxidase) and red quinoneimine dye was produced. The intensity of the color complex was directly proportional to the glucose in specimen and showed absorption maxima at 505 nm [13].

Plasma total antioxidant activity was estimated spectrophotometricaly by the method of Koracevic *et al*. [14] Two milliliter of blood samples were collected in EDTA bottles. Plasma was separated immediately by centrifugation and TAA is measured. This method is based on the principle that the standardized solution of iron EDTA complex reacts with hydrogen peroxide by a Fenton type of reaction, leading to the formation of hydroxyl radicals. This reactive oxygen species degrades benzoate, resulting in the release of TBARS. Antioxidants from the added plasma cause the suppression of production of TBARS. The reaction is measured spectrophotometrically at 532 nm.

Erythrocyte malondialdehyde (MDA) levels were measured as thiobarbituric acid reactive substances, after preparation of hemolysate. The heat induced reaction of MDA with thio barbituric acid (TBA) in the acid solution formed a trimethine coloured substance, which was measured spectrophotometrically at 532 nm [15].

Serum uric acid levels were estimated by Caraway's method in which uric acid reacted with phosphotungstic acid in alkaline medium forming a blue color complex which was measured at 700 nm [16].

Statistical analysis

The data collected from study group subjects were entered separately in Microsoft Excel sheet of windows 2010 and values were expressed as Mean ± SD. The significance of mean difference between study group subjects was compared by using Student's t test and distribution of probability (P).

RESULTS

The mean blood pressure and anthropometric indices of the study group subjects are depicted in Table 1. The observations made reveal significant changes in plasma TAA, erythrocyte malondialdehyde and serum uric acid levels along with fasting blood glucose levels in T2DM subjects as represented in Table 2. Plasma total antioxidant activity was found to be significantly low ($p<0.05$) in T2DM subjects i.e. 20.58% low as compared to controls. Conversely, erythrocyte MDA levels and fasting glucose levels were 31.8% and 72.3% high respectively ($p<0.001$) in patient group as compared to healthy controls. However, serum uric acid levels were not significantly altered (13.5% high; $p<0.1$) in T2DM subjects as compared to non-diabetic healthy controls.

Table-1: Demographic profile of Type 2 diabetic and non diabetic subjects

S No	Particulars	Control group (n=50)	Patient Group (n=50)
1)	Age (years)	39.5 ± 4.2	48.5 ± 5.0
2)	M:F ratio	1:1	1:1
3)	Height (meter)	1.57 ± 0.08	1.58 ± 0.09
4)	Weight (Kg)	54.8 ± 4.5	57.5 ± 4.2
5)	B.M.I. (Kg/m^2)	22.4 ± 2.4	23.8 ± 2.5
6)	Systolic blood pressure (mmHg)	106 ± 4.5	114 ± 6.2
7)	Diastolic blood pressure (mmHg)	72.5 ± 3.8	76.0 ± 4.0

Table-2: Fasting blood glucose and oxidative stress parameters in study group subjects (Mean ± SD)

S. No	Particulars	Control group (n=50)	Patient Group (n=50)
1)	Fasting Blood Glucose (mg/dl)	86.24 ± 7.58	148.62 ± 40.60
2)	TAA level (m mol/L)	1.36 ± 0.20	1.08 ± 0.18**
3)	Uric acid (mg%)	4.58 ± 1.28	5.2 ± 1.46*
4)	Malondialdehyde (µmol MDA/ml)	1.68 ± 0.12	2.09 ± 0.14***

* $p<0.1$: Non-significant; ** $p<0.05$: Significant; *** $p<0.001$: Highly significant

DISCUSSION

The prevalence of oxidative stress in T2DM patients has received alarming attention amongst epidemiologists, clinicians and experimental researchers. The mechanisms whereby free radicals may exert cytotoxic effect related to T2DM include insulin resistance, damage to cell membrane via lipid peroxidation and protein oxidation [2, 5, 7]. Lipid peroxidaion contributes local membrane destabilization that alters the proper trafficking of intracellular vesicles, phagocytosis, degranulation, antigen presentation, receptor mediated ligand uptake and many other process leading to age related deterioration [17,18]. In this context, we observed that erythrocyte malondialdehyde levels were significantly high in T2DM subjects ($p<0.05$; Table 2) which authenticate the hypothesis that diabetes mellitus is closely associated with lipid peroxidation mediated destruction in cell, subcellular organelles and biomolecules. Our findings were in agreement with the findings of Duman et al. who also observed enhanced production of MDA in T2DM patients [19]. Moreover, lipid peroxidation initiates a complex cascade that promotes diabetes and other age associated complications such as cardiovascular disease by promoting inhibition of NO and prostacyclin synthesis, enhancement of cytosolic free calcium and peripheral vascular resistance i.e. a key step in developing hypertension [20, 21].

Increased production of MDA may be associated with alteration in antioxidant defense system as characterized by alteration in total antioxidant activity as well documented in various diseases [2, 22, 23]. In the present study, plasma TAA levels were found to be decreased significantly ($p<0.05$) along with increase in MDA levels in T2DM patients which clarify the contributory effect of reduced antioxidant status due to augmented oxidative stress that could not be compensated by increase in some other antioxidants such as uric acid as observed in present study. Moreover, insignificant elevated levels of serum uric acid was observed in T2DM subjects which suggests that body is trying to protect itself from the deleterious effects of free radicals by increasing uric acid production. Recently, marked reduction in TAA along with elevated levels of serum uric acid in diabetes mellitus and other age related diseases have been well documented [24, 25].

CONCLUSION

On the basis of current evidences and our findings, it can be inferred that free radical production increases in type 2 diabetes, which is characterized by depletion of total antioxidant status and enhanced uric acid levels in order to scavenge free radicals and to overcome the burden of oxidative stress. In addition, assessment of total antioxidant activity and uric acid along with glycemic profile are important diagnostic tools for determination of diabetes and its related complications. Moreover, oxidative stress also contributes significantly in type 2 diabetes by inducing lipid peroxidation as detected by malondialdehyde levels. Thus, our study also suggested that improvement of glycemic control and supplementation of antioxidant rich diet should be encouraged to the patients not only for decreasing the burden of free radical mediated lipid peroxidation but also its related destructive consequences in Type 2 diabetes.

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