Original Article

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Biochemistry Section

Study on Total Antioxidant Status in Relation to Oxidative Stress in Type 2 Diabetes Mellitus

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ABSTRACT

Background: Diabetes Mellitus is a condition of increased oxidative stress and requiries antioxidants. The sum of endogenous and food derived antioxidants represents the total antioxidant activity of the system. The cooperation among different antioxidants provides greater protection against damage caused by reactive oxygen species or reactive nitrogen species, than any single compound alone. Thus the overall antioxidant capacity may provide more relevant biological information compared to that obtained by the measurement of individual components, as it considers the cumulative effect of all antioxidants present in plasma and body fluids and hence the study.

Materials and Method: The study population included healthy volunteers from staff of Sree Balaji Medical College & Hospital (SBMC&H) and Type 2 Diabetic patients attending SBMC&H, Chennai, India. Malondialdehyde levels and total antioxidant status of the case and controls was assessed.

Results: A significant decrease in the total antioxidant status among Diabetic patients and significant increase in their malondialdehyde levels in comparison to healthy controls was observed.

Conclusion: Type 2 Diabetes Mellitus is a condition in which there is increased oxidative stress as evident by increased Malondialdehyde levels and the condition calls for utilization of antioxidants to combat the oxidants thereby resulting in decreased total antioxidants status.

Keywords: Malondialdehye, Antioxidants, Hyperglycaemia, Reactive oxygen species

INTRODUCTION

Studies across the globe on various food derived antioxidants are adding to our knowledge on the importance of antioxidants in health. Fresh fruits like pomegranate and dry fruits like walnuts have been proved to have beneficial role in boosting our Antioxidant status [1,2]. Diabetes mellitus (DM) is characterized by chronic hyperglycemia and disturbances of carbohydrate, lipid and protein metabolism due to deficiencies in insulin secretion and /or insulin action. Diabetic patients have defect in antioxidant defense mechanism, free radicals and oxidative stress may be responsible for diabetes itself, and its complications [3,4]. Taking into consideration the importance of antioxidants to Diabetic patients we have planned this study to assay their total antioxidant status. The triggering factors for oxidative stress are shown to be viral infection, auto immune disease and environmental factors as per Baynes and Thorpe [4].

Reactive oxygen species (ROS) are the sparks of the oxidative metabolism [4]. Oxidative stress is the price we pay for using oxygen. ROS are generated under physiological conditions and are thought to be the signaling molecules for the expression of ROS specific scavengers [5]. They are also involved in defense mechanisms as seen in phagocytosis, neutrophil function, and shear-stress induced vasorelaxation. Excess generation of ROS in oxidative stress has pathological consequences including damage to proteins, lipids and DNA [6].

Hyperglycemia in Diabetes generates free radicals [7,8]. These free radicals induce oxidative stress and in turn impair the endogenous antioxidant defense system [9]. Antioxidant defense mechanisms include both enzymatic and non-enzymatic strategies. Common antioxidants include the vitamins A, C, E, and the tripeptide glutathione, and the enzymes superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase [10] and the Paraoxonase 1 enzyme closely associated with High Density Lipoprotein. The latter helps in preventing and reverting LDL oxidation [11]. Other antioxidants include α lipoic acid, mixed carotenoids, coenzymes Q10, several bioflavonoids, antioxidant minerals (copper, zinc, manganese and selenium) and the coenzymes (folic acid, vitamins B1,B2,B6,B12). They work in synergy with each other and against different types of free radicals. Each antioxidant has different mechanism of reducing oxidative stress, enzyme PON 1 is shown to prevent and revert HDL and LDL oxidation [11]. Vitamin E suppresses the propagation of lipid peroxidation, vitamin C, with Vitamin E inhibits hydro peroxide formation [3].

This study was undertaken to assess the relationship between Total antioxidant status and the oxidative stress as expressed by malondialdehyde levels in type 2 Diabetic patients.

MATERIALS AND METHODS

This is a case control study. This study was conducted among a South Chennai population for a period of three months between April-June 2010. A total of ninety three (male 48, female 45) Type 2 diabetic patients in the age group of 30- 50 years with the similar number of age and sex matched healthy controls were studied. The sample size was calculated to be 71 ± 10 in each group.

Inclusion criteria for cases

Diabetic patients without any associated disorders like hypertension, Ischaemic heart disease as well as any chronic disease which can induce increased oxidative stress. Patients under medication for diabetes but not on any antioxidants.

Inclusion criteria for healthy controls

Healthy Subjects who were not on any antioxidant supplementation.

Exclusion criteria for cases

Patients with any other chronic illness

Post-menopausal women.

Smokers and alcoholics.

Exclusion criteria for healthy controls

Post-menopausal women.

Smokers and alcoholics.

Ethical clearance

Ethical clearance was obtained from the Ethical Clearance Committee of Sree Balaji Medical College and Hospital. The need for the study was briefed to the participants. Results of all test parameters except those of special investigation were given to the participants. Informed written consent was obtained from patients and healthy controls. 3ml venous blood was drawn in the fasting condition from the patients; 1ml transferred into tube with oxalate-fluoride mixture for Fasting Plasma Sugar (FPS) and remaining sample was transferred into plain tubes and stored in -80 degree centigrade for TAS and MDA assay.

Post Prandial Plasma Sugar (PPPS): Two hours after food 1ml of venous blood sample was collected into tube with oxalate-fluoride mixture for PPPS estimation. FPS & PPPS were estimated using Glucose Oxidase Peroxidase (GOD POD) Method (Enzymatic method).

Malondialdehyde (MDA): A spectrophotometric assay of Thio Barbituric acid reactive substances (TBARS). MDA in the sample reacts with Thio barbituric acid and Tri chloro Acetic acid at 100 degree centigrade to give a pink colour by forming the TBA-MDA adduct. The readings were read at 540 nm in Elico spectrophotometer.

Total Antioxidant Status (TAS): Colorimetric assay with Cayman kit Cayman's antioxidant assay Kit was used to measure the total antioxidant capacity of plasma, in the control and diabetic group. Aqueous and lipid soluble antioxidants are not separated in this protocol, thus the combined antioxidant activities of all its constituents including vitamins, proteins, lipids, glutathione, uric acid, are assessed. The assay relies on the ability of antioxidants in the sample to inhibit the oxidation of 2,2' Azino-di-3-ethylbenzthiazoline sulphonate (ABTS) to ABTS* by metmyoglobin. The amount of ABTS* produced is read at 405 nm. Under the reaction conditions used, the antioxidants in the sample cause suppression of the absorbance at 405 nm to a degree which is proportional to their concentration.

RESULTS

Their Glycemic status was assessed through the analysis of Fasting plasma sugar (FPS), Post Prandial Plasma Sugar (PPPS) and the Glycated hemoglobin (HbA1c). Values of all the parameters were expressed as Mean ± S.D. The FPS values in diabetic subjects were (169.63 ± 69.43 mg/dl) compared to the healthy controls (88.22 ± 13.25 mg/dl). The difference is strongly significant. In the present study the PPPS values among diabetic subjects was (244.46 ± 88.19 mg/dl) and in controls was (112 ± 24.35 mg/dl) [Table/Fig-1]. There is significant increase in MDA levels among Diabetic patients (3.61 ± 0.63 µM) in comparison to the controls (1.93 ± 1.51 µM) according to our study [Table/Fig-2]. In our study, there has been decreased total antioxidant status among diabetic cases as 0.46 ± 0.46 mM whereas the healthy controls had a value of 1.69 ± 1.34 mM [Table/Fig-3].

Parameter	Controls	Cases	Significance
FPS (mg/dl)	88.22±13.25	169.63±69.43	t=10.753; p<0.001**
PPPS (mg/dl)	112.33±24.35	244.46±88.19	t=13.499; p<0.001**

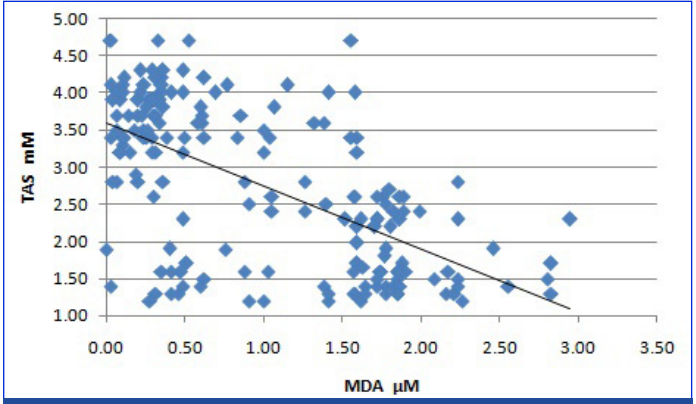
[Table/Fig-1]: A Comparison of sugar parameters in two groups

Study outcome variable	Controls	Cases	Significance
MDA µ M	1.93±1.51	3.61±0.63	t=9.872; p<0.001**

[Table/Fig-2]: A Comparison of malondialdehyde levels in two groups
** Strongly significant

Total antioxidant status	Controls	Cases	Significance
TAS mM	1.69±1.34	0.46±0.46	t=8.326; p<0.001**

[Table/Fig-3]: A Comparison of total antioxidant status in two of groups



[Table/Fig-4]: Scatter plot showing pearson's correlation of TAS vs MDA
TAS vs MDA: r-value (-0.62), p-value (.001)

Pearson's correlation: TAS vs MDA: There is a large negative correlation between Total antioxidant status and Malondialdehyde among our study population. The r-value was -0.62 showing a large negative correlation between the two parameters [Table/Fig-4].

DISCUSSION

Elevated oxidative stress is a well accepted explanation in the development and progress of complications in Diabetes Mellitus. There is significant increase in MDA levels among Diabetic patients (3.61 ± 0.63 µM) in comparison to the controls (1.93 ± 1.51 µM) according to our study. 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 Thio Barbituric acid reactive substances (TBARS) method. E Prabhakar et al., 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) [12]. Chavan et al., have also observed similar results among a study population from Gujarat [13]. Rama Srivatsan et al., have reported increased MDA levels in diabetics among a Southern Karnataka population [14]. Manjulata et al., from Gwalior also report elevated MDA levels in diabetic patients [15]. Robby Kumar and Sakil Ahmed from Mauritius report increased oxidative damage in Diabetes as shown by elevated MDA level [16]. A similar study in Turkey by Duman et al., has also reported significantly high malondialdehyde levels among a diabetic population [17].

Further in our study, we observed a decreased total antioxidant status among diabetic cases as (0.46 ±0.46 mM) whereas the healthy controls had a value of (1.69 ± 1.34 mM). This decrease among diabetic subjects could be attributed to increased oxidative stress as evidenced by lipid peroxidation. The antioxidant decrease reflects the war of antioxidants against oxidative stress to minimize the oxidative damage. When the total antioxidant status is high and enough to combat the oxidative stress the MDA levels are in the normal limits and vice versa. The Total antioxidant status gives the sum total of both exogenous as well as endogenous antioxidants. So it gives the complete antioxidant status picture. This is of more significance in comparison to measuring individual antioxidant because the various antioxidants work synergistically in the system to combat the oxidative damage caused by free radicals. The data of the study of antioxidant vitamins by Suchitra et al., from Andhra revealed a depleted level of the extracellular antioxidant status in the type 2 diabetic patients, regardless of any complications [18]. A study from South Karnataka on individual antioxidants among diabetics reported a significant decrease of erythrocyte reduced Glutathione (GSH) whereas oxidized Glutathione (GST) levels are slightly elevated among the diabetics. Ceruloplasmin, Vitamin C and Vitamin E show mild elevation whereas Superoxide dismutase shows marked elevation among diabetics reflecting the overwhelming adaptive response of the antioxidants to the augmented oxidative stress in the diabetic state [14].The change

in the individual nutritional antioxidants constituting the exogenous antioxidants could also be the reason underlying this adaptive response. Similar study from Hungary also reports both increase of few and decrease of other few individual antioxidant enzymes [10]. Isha Hamood and Kassim Salih Abdullah Al Neaimy from Iraq report decreased Total Antioxidant Status in comparison to Diabetic patients with neuropathy by using the Cayman kit [19]. Duman et al., from Turkey have observed significant decrease of antioxidants among the diabetic population [17]. Several studies have revealed lowered antioxidant and enhanced peroxidative status in diabetes condition [20-24]. There is still a need for further research in the field of free radicals and antioxidants. The prime aim should be in elucidating the underlying mechanisms by which free radicals bring about the pathogenesis. This would help expand the scope of treatment options.

CONCLUSION

The decreased TAS status and increased MDA levels could be taken as an early marker of the pathogenesis of complications in Type 2 Diabetes Mellitus. The above assays could be employed to detect complications early and revert the conditions. This would increase the longevity and quality of life of patients with diabetes.

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