ORIGINAL RESEARCH

Evaluation of Total Antioxidant Status in Relation to Oxidative Stress in Type 2 Diabetes Mellitus

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ABSTRACT:
Background: Diabetes mellitus is a metabolic disorder characterized by hyperglycemia along with biochemical alterations of glucose and lipid peroxidation. It produces free radicals that induce lipid peroxidation which acts as an indicator for oxidative stress in the body. The aim of the present study was to evaluate the total antioxidant status in relation to oxidative stress in Type 2 Diabetes Mellitus. **Material and methods:** The present study was case control study conducted among a population of 110 Type 2 diabetic patients in the age group of 30- 60 years with the similar number of age and sex matched healthy controls. 3ml venous blood was drawn in the fasting condition from the patients. Two hours after food 1ml of venous blood sample was collected into tube with oxalate-fluoride mixture for Post Prandial Plasma Sugar (PPPS) estimation. FPS & PPPS were estimated using Glucose Oxidase Peroxidase (GOD POD) Method (Enzymatic method). Colorimetric assay with Cayman kit Cayman's antioxidant assay Kit was used to measure the total antioxidant capacity of plasma, in the control and cases. **Results:** The Fasting Plasma Sugar values in diabetic subjects were 172.43 ± 43.02 mg/dl compared to the healthy controls 89.52 ± 10.21 mg/dl). The Post Prandial Plasma Sugar values among diabetic subjects was 247.26 ± 46.16 mg/dl) and in controls was 115.34±42.18 mg/dl). There is significant increase in MDA levels among Diabetic patients 4.01±0.78µM in comparison to the controls (1.99±1.22 µM). In our study, there has been decreased total antioxidant status among diabetic cases as 0.49±0.42 mM whereas the healthy controls had a value of 1.73±1.41 mM. **Conclusion:** Our study concluded that in Type 2 DM MDA levels were increased and total antioxidant status was decreased.
Key words: Type 2 Diabetes mellitus, total antioxidant status, Oxidative Stress.

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INTRODUCTION:

Diabetes mellitus (DM) is a cluster of metabolic disorders characterised by abnormally elevated blood glucose levels (hyperglycaemia),[1] which arise from the body's inability to produce insulin or to use it to its full potential.[2] Diabetes mellitus is characterized by hyperglycemia and insufficiency in the secretion and function of endogenous insulin.[3] Type II diabetes is a multicausal disease which develops slowly in a step-wise manner initially commencing with insulin resistance and progressing with time which results in failure of the body to maintain glucose hemostasis causing glucose intolerance.[4] Type 2 diabetes mellitus (T2DM) is characterized by peripheral insulin resistance, impaired insulin secretion and excessive hepatic glucose production.[5-7] Oxidative stress may contribute to the pathogenesis of DM through impairment of insulin action, injury to pancreatic β-cells, increased lipid peroxidation, and vascular endothelial damage.[8-11] Oxidative stress is defined as disturbance in the prooxidant-antioxidant balance in favor of the prooxidant, leading to potential damage producing oxidative stress. Free radical is any atom with at least one unpaired electron in the outermost shell, and is capable of independent existence. Raised oxidative stress has been implicated not only for chronic diseases like cardiovascular disease, cancer, cirrhosis, atherosclerosis, Alzheimer's disease and Parkinson's disease; it has also been found altered in diabetes mellitus.[12-15] The aim of the present study was to evaluate the total antioxidant status in relation to oxidative stress in Type 2 Diabetes Mellitus.

MATERIAL AND METHODS:

The present study was case control study conducted among a population of 110 Type 2 diabetic patients in the age group of 30- 60 years with the similar number of age and sex matched healthy controls. The study was conducted over the period of three months. Before the commencement of the study ethical approval was taken from the Ethical Committee of the institute and written informed consent was obtained from the patients. 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 were included in the study as cases. Healthy Subjects who were not on any antioxidant supplementation were included as controls. Patients with any other chronic illness, Post-menopausal women. Smokers and alcoholics were excluded from the study as cases. Post-menopausal women. Smokers and alcoholics were excluded from the study as 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 -20 degree centigrade for TAS and MDA assay. Two hours after food 1ml of venous blood sample was collected into tube with oxalate-fluoride mixture for Post Prandial Plasma Sugar (PPPS) estimation. FPS & PPPS were estimated using Glucose Oxidase Peroxidase (GOD POD) Method (Enzymatic method). 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. Colorimetric assay with Cayman kit Cayman's antioxidant assay Kit was used to measure the total antioxidant capacity of plasma, in the control and cases. 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:

The Fasting Plasma Sugar values in diabetic subjects were 172.43 ± 43.02 mg/dl compared to the healthy controls 89.52 ± 10.21 mg/dl). The Post Prandial Plasma Sugar values among diabetic subjects was 247.26 ± 46.16 mg/dl) and in controls was 115.34±42.18 mg/dl). There is significant increase in MDA levels among Diabetic patients 4.01±0.78µM in comparison to the controls (1.99±1.22 µM). In our study, there has been decreased total antioxidant status among diabetic cases as 0.49±0.42 mM whereas the healthy controls had a value of 1.73±1.41 mM.

Table 1: Distribution of sample

Sample	N
Cases	110
Controls	110

Table 2: Comparison of sugar parameters in two groups

Parameters	Cases	Controls
FPS (mg/dl)	172.43±43.02	89.52±10.21
PPPS (mg/dl)	247.26±46.16	115.34±42.18

Table 3: A Comparison of malondialdehyde and antioxidants levels in two group

Parameters	Cases	Controls
MDA µM	4.01±0.78	1.99±1.22
TAS mM	0.49±0.42	1.73±1.41

DISCUSSION:

Type II diabetes mellitus is associated with multiple metabolic derangements which can cause secondary pathophysiological changes in multiple organ systems. This in turn can impose a heavy burden of morbidity and mortality from micro- and macro-vascular complications.[16] Diabetes can cause persistent hyperglycemia often accompanied with other features such as glucosuria, polydipsia and polyuria which can cause increased production of free radicals in all tissues from glucose auto-oxidation and protein glycosylation.[17]

In the present study the Fasting Plasma Sugar values in diabetic subjects were 172.43 ± 43.02 mg/dl compared to the healthy controls 89.52 ± 10.21 mg/dl). The Post Prandial Plasma Sugar values among diabetic subjects was 247.26 ± 46.16 mg/dl) and in controls was 115.34±42.18 mg/dl). There is significant increase in MDA levels among Diabetic patients 4.01±0.78µM in comparison to the controls (1.99±1.22 µM). In our study, there has been decreased total antioxidant status among diabetic cases as 0.49±0.42 mM whereas the healthy controls had a value of 1.73±1.41 mM.

Opara et al reported a decrease in antioxidant levels in diabetic subjects with complications.[18] Srivatsan et al. found an increase in antioxidant levels in diabetic subjects without complications.[19]

Rama Srivatsan et al., conducted a study in 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.[20] Manjulata et al., from Gwalior also report elevated MDA levels in diabetic patients.[21]

Duman et al., from Turkey have observed significant decrease of antioxidants among the diabetic population.[22]

CONCLUSION:

Our study concluded that in Type 2 DM MDA levels were increased and total antioxidant status was decreased. 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.

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