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Original Article

Antioxidant Status in Type-2 Diabetes Mellitus Compared to Diabetic Foot Patients- A Hospital Based Study in Rajshahi

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Abstract

Background: Chronic hyperglycemia in diabetes mellitus leads to decrease total antioxidant level in the body which might play important role in the development of chronic complications of diabetes due to oxidative stress.

Objective: It is aimed to evaluate the antioxidant status in type-2 diabetes mellitus patients & diabetic foot patients.

Methods: This was a descriptive cross sectional study carried out in the department of Pharmacology & Therapeutics in collaboration with Rajshahi Diabetic Association General Hospital, Rajshahi from July 2017 to June 2018 to evaluate total antioxidant status in type-2 diabetes mellitus. In this study, 20 patients with type-2 diabetes mellitus and 20 diabetic foot patients were evaluated. Antioxidant status was determined by using spectrophotometer. Most of the patients were age ranging 40-60 years of both sexes.

Results: The mean of fasting blood glucose and total antioxidant capacity of type-2 diabetes mellitus with diabetic foot patients was 11.23±2.76 mmol/l and 18.78±6.70 µmol/l and that of type-2 DM patients was 9.13±2.57 mmol/l and 26.07±7.67 µmol/l. Antioxidant level were less in type 2 diabetic foot patients.

Conclusion: There is low level of antioxidant in plasma which may be regarded as an important causative factor for development of type-2 diabetes mellitus and its chronic complication. The findings can be a basis of generating a hypothesis for further testing towards a meaningful conclusion.

Key words: Antioxidant status, type-2 diabetes mellitus, Diabetic foot.

Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by chronic hyperglycemia. The prevalence of type-2 DM in Bangladesh is alarmingly increasing over the past two decades and continues to rise as a disease of immeasurable class. The World Health organization has predicted that the worldwide number of patients with diabetes will double by the year 2025.[1] Human body is continuously exposed to different types of agents that results in the production of reactive species (RS) called free radicals. Free radicals transfer their unpaired electron and cause the oxidation of cellular machinery. In order to encounter the deleterious effects of such species, body has got endogenous and exogenous antioxidant that neutralizes such species and keeps the homeostasis of the body. Any imbalance between the reactive species and antioxidant leads to produce a condition known as oxidative stress that results in the development of different pathological conditions of which diabetes and its later complications are very important.[2] Persistent uncontrolled hyperglycemia secondary to insulin resistance and diminished insulin secretion in type-2 DM leads to many complications such as diabetic ketoacidosis, hyperosmolar coma, ischemic heart disease, chronic kidney failure, retinopathy, neuropathy, non-ketotic hyperosmolar coma and foot ulcer.[3] Hyperglycemia causes release of tissue damaging reactive oxygen species (ROS) and diminishes antioxidant agents. Diabetes mellitus not only stimulates the generation of reactive oxygen species, but also impairs the ability of a cell or tissue to cope up with the increased oxidative burden. Oxidative stress is an important mediator of diabetic complication.[4] The most notorious complications of

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diabetes in the lower extremity are the diabetic foot which is commonly observed in our country. For this reason, it is important to determine the total antioxidant status in patients with type-2 diabetes mellitus compared to its complication.

Materials & Methods

This was a descriptive cross-sectional study conducted in the Department of Pharmacology and therapeutics, Rajshahi Medical College, in collaboration with Rajshahi Diabetic Association General Hospital, Rajshahi. The study was conducted among 20 type-2 diabetes mellitus & 20 type-2 DM with diabetic foot.
The inclusion criteria were, Clinically diagnosed type-2 diabetes mellitus & type-2 DM with diabetic foot patients in the age group of 40-60 years, both genders.

The exclusion criteria were, Patients with serious comorbid diseases (stroke, myocardial infarction, major surgery etc, patients with liver and kidney dysfunction, history of using drugs significantly affect glucose metabolism (glucocorticoids, oral contraceptives, thiazide diuretics etc.) or taking vitamin supplements. The study variables were age, total antioxidant status (µmol/l) and glucose (mmol/l).

A formal permission was obtained from the Ethical Review Committee of Rajshahi Medical College, Rajshahi to select this study. After getting permission from the concerned authority, every patient was informed about the study and they were also informed that there was no chance of any significant harm by inclusion in this study. The data were collected from inpatients as well as outpatients fulfilling the inclusion criteria attending Rajshahi Diabetic Association General Hospital, Rajshahi. An elaborate history was taken for each individual regarding present & previous history of illness suggesting type2 diabetes mellitus and any diabetic complication. After taking informed consent, complete history taking, physical examination was done and recorded in a preformed data sheet. Then 4 ml blood was taken from each group of patients in a test tube containing anticoagulant tri-potassium EDTA (Ethylene di-amine tetra acetic acid). Plasma was collected after centrifuging for 15 minutes at 3000 rpm. Then plasma total antioxidant status was measured in Pharmacology laboratory of Rajshahi Medical College, Rajshahi. The generated laboratory data were recorded using a prepared checklist. Then the data were then analyzed using SPSS version 16 for Windows by applying descriptive statistics and cross tabulation. Frequency and percentages were calculated. The unpaired t-test was used for comparing means. Significance was kept at p-value less than 0.05.

Measurement of total antioxidant status:

Total antioxidant activity was measured by ferric reducing antioxidant power assay (FRAP) of Benzie and Strain 1999.

Principle:

The FRAP assay uses antioxidants as reluctant in a rodox-linked colorimetric method employing an easily reduced oxidant, Fe(III). Reduction of a ferric tripyridyltriazine complex to ferrous-$(2,4,6\text{-tripyridyl-s-triazine})_2$ ie: Ferric (III) [colorless] to Ferrous (II) [blue] can be monitored by measuring absorbance at 593nm. The absorption readings are related to the reducing power of the electron-donating antioxidants present in the test compound. Hence the FRAP assay can rank the reducing power and the antioxidant potential of a wide range of test compounds.

Sample:
Plasma

Reagents:

300 mM Acetate buffer

40 mM HCl

10 mM TPTZ

20 mM $FeCl_3.6H_2O$

0.001 M $FeSO_4.7H_2O$ Standard

FRAP working reagent

Sample: 10:1:1 (acetate buffer: TPTZ:$FeCl_3.6H_2O$)

Standard: 10:1:1 (acetate buffer: TPTZ:H_2O)

Procedure:

100 µl plasma was mixed with 900 µl distilled water and 2 ml of FRAP working reagent and absorbance 593 nm was measured after 30 min against FRAP reagent blank. Standard were preceded in same manner. The result was expressed as µM/L of ferrous equivalent.

Calculation:

The FRAP equation is:
FRAP value of sample (µM)= {Abs(sample) x FRAP value of Std (µM)} / Abs(Std)

Parameters of study:

Demographic parameter: Age & duration of the disease
Study parameter: Total antioxidant status

Results

Table 1: Demographic parameters of type-2 DM and type-2 DM with Diabetic Foot patients

Parameters	Group	
	DM (Mean±SD)	DM with diabetic foot (Mean±SD)
Age	51.75±5.12	53.95±5.48
Duration (year)	5.63±3.58	7.5±2.60

Table-1 Shows that demographic parameter of each group. The mean age and duration of disease in DM with diabetic foot were more than that of DM patients.

Table 2: Study parameter of type-2 DM and type-2 DM with Diabetic foot patients.

Variables	Group	
	DM patients (20)	**DM with diabetic foot patients (20)**
FBS (mean±SD)	9.13±2.57 **mmol/l**	11.23±2.76 **mmol/l**
TAC (mean ±SD)	26.07±7.67 **µmol/l**	18.78±6.70 **µmol/l**

Table 2 Shows that 20 patients belonged to each group. The mean of FBS and TAC in type-2 DM with diabetic foot was 11.23±2.76 mmol/l and, 18.78±6.70 µmol/l. The mean of FBS & TAC in type-2 DM was 9.13±2.57 mmol/l & 26.07±7.67 µmol/l. The mean of FBS were more in DM with diabetic foot patients. The mean of TAC was lower in DM with diabetic foot patients.

Table 3: Comparison of biochemical parameter between type-2 DM and Diabetic foot patients

Variables (biochemical characteristics)	Group		Test of Significance
	DM (Mean±SD)	**DDM with diabetic foot (Mean±SD)**	
FBS	9.1350±2.56767	11.2300±2.75702	**t**=-2.487**df**=38 **P**=.017
TAC	26.0750±7.67133	18.7800±6.70299	**t**=3.202 **df**=38 **P**=.003

Table 3 Shows that biochemical parameters between DM patients and DM with diabetic foot patients. It was observed that biochemical parameters were statistically significant ($P<0.05$) when compared between these two groups.

Discussion

In this study, 20 were type-2 DM patients and 20 were type-2 DM with diabetic foot patients. The mean age and duration of disease of type-2 DM and type-2 DM with diabetic foot was 51.75±5.11, 5.62±3.58 and 53.95±5.48, 7.50±2.60 years respectively. It was found that the mean of FBS and TAC of type-2 DM with diabetic foot was 11.23±2.75 mmol/l and 18.78±6.7 µmol/l. The mean of FBS and TAC of type-2 DM was 9.13±2.56 mmol/l and 26.07±7.67 µmol/l. These results revealed that FBS were significantly increased in type-2 DM with diabetic foot and TAC was decreased in type-2 DM with diabetic foot patients compared with type-2 DM patients. The similar findings observed by Bolajoko *et al.* (2008)[5] and Pinaki Saha *et al.* (2015)[6]. They suggested that decreased level of antioxidant and elevated oxidative stress associated with increased risk of type-2 DM and its complications. A study performed by Kedziora-Komatowska *et al.* (1998)[7]; Bandeira *et al.* (2013)[8]; Li *et al.* (2013)[9] and Ganjifrockwale *et al.* (2017)[10] showed the low level of TAS in patient with type-2 DM & type-2 DM with complication compared to healthy individual. This is in agreement to the present study. In our study it was observed that decreased TAC in type-2 DM with diabetic foot patient compared to type-2 DM (18.78±6.7 µm/l; 21.07±7.67 µm/l). Bikkad *et al.* (2014)[11]; Chopra *et al.* (2012)[12] and Djordjevic *et al.* (2014)[13] performed a study and found that decreased level of enzymatic antioxidant in type-2 DM with complication compared to type-2 DM. Oliveira *et al.* (2014)[14] performed a study and found decreased level total antioxidant capacity (0.61±0.09 mg/ml) and reduced protein thiol (369.22±46.13 mg/ml) in type-2 DM with diabetic foot patient compared to type-2 DM without diabetic foot (0.69±0.06 mg/ml; 413.41±28.71 mg/ml) which was similar to the present study.

Conclusion

Finding in this study are compatible with the hypothesis that persistent hyperglycemia leads to decreased TAC in diabetic patients. This is more pronounced in patients with diabetic foot. A high level of lipid peroxidation accompanied by insufficient antioxidant capacity in plasma could attribute to the chronicity of diabetes mellitus disease. Thus delivery of antioxidants and employing the mechanism based approach; clinical pathology and concentration based dosage schedule in antioxidants trial might help us in preventing development of complication of type-2 diabetes. However, the findings are having the scope for generating a clear hypothesis for further testing towards a significant conclusion of the facts.

Conflict of interest: No

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