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Study of Oxidative stress in patients with type 2 diabetes mellitus

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Abstract

Aim: *Oxidative stress is increased in metabolic syndrome and type 2 diabetes mellitus (T2DM) and this appears to underlie the development of T2DM and diabetic complications. This study aims to know the oxidative stress as malondialdehyde (MDA) in type 2 diabetes mellitus patients.* ***Method:*** *This cross-sectional analytical study was 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 oxidative stress in type 2 diabetes mellitus. In this study, 30 patients with type 2 diabetes mellitus and 30 healthy control subjects were evaluated. Oxidative stress was determined by using a spectrophotometer. Most of the patients were aged 35-55 years of both sexes.* ***Result:*** *The mean fasting blood glucose and malondialdehyde levels of type-2 diabetes mellitus were 8.68±1.90 mmol/l and 5.14±1.33 µmol/l & that of control was 4.88±0.66 mmol/l and 1.73±1.16 µmol/l. MDA level was high in type 2 diabetic patients.* ***Conclusion:*** *There is an increase in MDA level which may be regarded as an important causative factor for the development of type-2 diabetes mellitus & its further complication.*

Keywords: *Type2 diabetes mellitus, MDA level.*

Introduction

Diabetes is considered a state of increased oxidative stress. Persistent hyperglycemia secondary to insulin resistance & diminished insulin secretion in type-2 diabetes mellitus leads to progressing organ injuries known as chronic or late diabetes complications (1). Hyperglycemia causes a release of tissue-damaging reactive oxygen species (ROS) and diminishes antioxidant agents. Several hypotheses describe the linkage of hyperglycemia with complications of diabetes. Among which it is postulated that (i) metal-catalyzed oxidation of glucose, described as auto-oxidative glycosylation, generates superoxide anion; (ii) nonenzymatic glycation and oxidation of proteins and lipids generate advanced glycation end products; and (iii) glycated proteins and advanced glycation end products react by glyco-oxidation, resulting in the production of oxygen-derived free radicals which impair insulin signaling pathways and induce cytotoxicity in the pancreatic beta cells (2). The free radicals induce damage to cells by passing the unpaired electron resulting in oxidation of cell components and molecules (3). Reactive oxygen species (ROS) have been involved in oxidative damage to DNA, proteins, and other macromolecules that contribute to the pathogenesis of a wide variety of diseases such as diabetes and cancer (4). To scavenge the deleterious effects of these free radicals, the body has different mechanisms to produce antioxidants, endogenous or exogenous, that will neutralize the elevated number of free radicals keep the cells protected against their toxic effects, and contribute toward the prevention of diseases (5). The imbalance between the rate of free radical generation and their elimination due to a decrease in the available antioxidant mechanisms leads to the imbalance of oxidative stress (OS), which is ultimately linked to the manifestation of macro and microvascular complications (6). The increased prevalence of free radicals in type-2 diabetes mellitus results in the activation of stress-signaling pathways and drains both enzymatic and non-enzymatic antioxidants, harming the quality of life and lifespan of the patient (7). Free radicals cause overproduction of malondialdehyde (MDA), which is a biomarker of oxidative damage to lipids. This study was undertaken to assess the oxidative stress as expressed by MDA levels in type 2 diabetic patients.

Methods

This was a cross-sectional analytical 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 30 type 2 diabetes mellitus & 30 normal healthy individuals. The inclusion criteria were: Clinically diagnosed type-2 diabetes mellitus patients & healthy controls between 35 to 55 years of age, irrespective of sex. 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 that significantly affect glucose metabolism (glucocorticoids, oral contraceptives, thiazide diuretics, etc.) or taking vitamin supplements. The study variables were age, duration of disease, MDA (micromol/l) and glucose (mmol/l). 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. The data was collected from the outpatient department fulfilling the inclusion criteria attending Rajshahi Diabetic

Association General Hospital, Rajshahi. An elaborate history was taken for everyone regarding present & previous history of illness suggesting type-2 diabetes mellitus and any diabetic complication. After obtaining in -formed consent, complete history taking, and physical examination were done and recorded in a preformed data sheet. Then 4 ml blood was taken from each person 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 MDA level was measured and the obtained data was analyzed using SPSS version 16. Frequency and percentages were calculated. The unpaired t-test was used for comparing means. Significance was kept at a p-value less than 0.05.

Measurement of oxidative stress as MDA (Lipid peroxidation)

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Principle

The most frequently used test is the measurement of MDA by the thiobarbituric acid (TBA) reaction. Here, TBA reacts with MDA to form a pink 2:1 TBA: MDA adduct, which is extracted by n-butanol and absorbs maximally at 532 nm. This colored complex can be measured by a spectrophotometer (SEQUOIA TURNER CORP. model 340 spectrophotometer).

Sample:

Plasma

Reagents:

Reagents for estimation of MDA
20% TCA- reagent grade
0.05 M sulfuric acid---E. mark
2 M sodium sulfate-mol. wt.-142.05 BDH (lab reagent grade)
0.2 G% TBA in sodium sulfate
n-butanol---Riedel-de Hein (analytical grade)
MDA standard (1-1-3-3 tetra ethoxy propane)

Procedure

0.5 ml plasma was mixed with 0.5 ml distilled water and 1 ml of 20% trichloroacetic acid and waited for 10 min. Then 2 ml sulfuric acid (0.05 mol/L) and 2 ml of thiobarbituric acid (TBA) reagent (2.0gm TBA/L in 2 mol sodium sulfate/liter) were mixed. The test tube was placed in a boiling water bath for 30 min and cooled in running tap water. The TBA reactive material was mixed with 2 ml n-butanol and centrifuged for 10 minutes after vortexing. A standard MDA was treated similarly. The optical density (O.D) of the n-butanol extract of plasma and MDA standard was measured at 532 nm against a butanol blank. The result was expressed as µmol MDA/L of plasma.

Calculation

$$\text{MDA (µmol/L)} = \frac{O.D\ of\ sample}{O.D\ of\ standard} \times 10$$

Parameters of study

Demographic parameter: Age & duration of the disease
Study parameter: Oxidative stress as Malondialdehyde (MDA)

Results

Table-I. Demographic parameters of type-2 DM and normal subjects.

Parameters	Group	
	DM (Mean±SD)	Control (Mean±SD)
Age	47.43±5.41	41.56±6.43
Duration	5.63±3.58	-

Table-I. Shows that 30 patients belonged to each group. The mean age and duration of disease in type-2 DM was 47.43±5.41 & 5.63±3.58 years. The mean age in normal control was 41.56±6.43 years.

Table-2. Study parameters of type 2 DM and normal subjects.

Variables	DM patients (30)	Control (30)
FBS (mean±SD)	8.68±1.90 **mmol/l**	4.88±0.66 **mmol/l**
MDA (mean ±SD	5.14±1.33 **µmol/l**	1.73±1.16 **µmol/l**

Table-2. Shows that 30 patients belonged to each group. The mean of FBS and MDA in type-2 DM was 8.68±1.90 mmol/l and 5.14±1.33 µmol/l. The mean of FBS and MDA in normal control was 4.88±0.66 mmol/l and 1.73±1.16 µmol/l.

Table-3. Comparison of biochemical parameters between type 2 DM and normal subjects

Variables (biochemical characteristics)	DM (Mean± SD)	Normal subject (Mean± SD)	Test of Significance
FBS	8.6800± 1.90071	4.8833± 0.66493	**t**=10.327 **df**= 58 **P**=.000
MDA	5.1433± 1.33899	1.7333± 1.16570	**t**=10.521 **df**= 58 **P**=.000

Test of significance done by Independent 't' test

Table-3 Shows a comparison of biochemical parameters between DM patients and healthy individuals. It was observed that biochemical parameters were statistically significant (P<0.05) when compared between these two groups.

Table-4. Pearson correlation of FBS and MDA level of type 2 DM patients.

Variables	Pearson Correlation	p-value
FBS with MDA	**0.66**	**0.00001**

Table 4 shows a positive correlation between FBS and MDA (r = 0.66, P<0.00001) in DM patients.

Discussion

Oxidative stress arises when the production of ROS exceeds the capacity of the available antioxidant defense system. Elevated oxidative stress is a well-accepted explanation for the development and progress of complications in diabetes mellitus. The excess ROS tends to react with all cell components, resulting in lipid peroxidation, protein denaturation, and DNA damage. In this study, 30 were type-2 DM patients and 30 were normal healthy control. The mean age and duration of disease of type-2 DM was 47.43±5.41 & 5.63±3.58 & the mean age of healthy control was 41.56±6.43 years. The mean of FBS and MDA of type-2 DM was 8.68±1.90 mmol/l, 5.14±1.33 µmol/l. The mean of FBS and MDA of healthy control was 4.88±0.66 mmol/l, 1.73±1.16 µmol/l. These results revealed that FBS & MDA were significantly increased in type-2 DM patients compared with healthy individuals. Similar findings were observed by Rani AJ et al., and Pinaki Saha et al., (8,9). They suggested that decreased levels of antioxidants and elevated oxidative stress are associated with increased risk of type-2 DM and its complications. A study performed by Kedziora-Komatowska et al., Bandeira et al., Li et al., and Ganjifrockwale et al., showed that high levels of lipid peroxidation in patients with type-2 DM & type-2 DM with complication compared to healthy individual (7,10,11,12). This agrees with the present study. In our study, it was observed that increased oxidative stress in type-2 DM patients compared to healthy individuals. Beg N et al., Bikkad et al., and Djordjevic et al., also reported increased lipid peroxidation in type-2 DM compared to healthy individuals (6,13,14). Another study performed by Rama Strivatsan et al., found that the MDA level was

significantly elevated in type-2 DM with complication compared to type-2 DM without complication & healthy control (15). In the present study, it was found that there was a positive correlation between FBS and MDA. Similar findings were observed by Pinaki Saha et al., (9). Free radicals and oxidative stress may act as a common pathway to diabetes itself as well as its later complications and significantly higher lipid peroxide in diabetic patients. Thus, it could be concluded that increased oxidative stress may play an important role in the development of type-2 diabetes mellitus & its further complications.

Conclusion

This study is compatible with the hypothesis that persistent hyperglycemia leads to increased production of oxidants. A high level of lipid peroxidation accompanied by insufficient antioxidant capacity in plasma could be attributed to the development of diabetes mellitus disease & its chronicity. Thus, the delivery of antioxidants and concentration-based dosage schedule in antioxidant trials might synergistically affect antioxidants in human plasma and provide greater protection against free radicals.

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