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Original Article

Lipid peroxidation is associated with poor control of type-2 diabetes mellitus

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ABSTRACT

Background: Hyperglycemia increases oxidative stress through the overproduction of reactive oxygen species, which results in an imbalance between free radicals and the antioxidant defense system of the cells. A positive correlation was reported between lipid peroxide levels and diabetic complication.
Objectives: The aim of the present study was to investigate the state of oxidative stress in controlled and uncontrolled diabetic patients.
Methods: One hundred thirty nine participants were included in this study, grouped as: Group-I: Healthy Control group of non-diabetic normal subjects, Group-II: Controlled type-2 DM group of subjects with type-2 DM and HbA1c $\leq$ 8% and Group-III: Uncontrolled type-2 DM group of subjects with type-2 DM and HbA1c $>$ 8%. Fasting blood glucose, 2 h postprandial glucose, MDA and HbA1c were quantified. The association between diabetic control and lipid peroxidation (malondialdehyde) was evaluated.
Results: The mean HbA1c increased significantly in uncontrolled type-2 DM subjects compared to controlled type-2 DM group. Lipid peroxidation as expressed in MDA was significantly increased in uncontrolled type-2 DM group compared to controlled type-2 DM, both groups show significant elevation in this parameter compared to healthy subjects. There is a significant positive correlation between MDA and HbA1c in the studied subjects.
Conclusion: The core problem during diabetes is poor glycemic control, which leads to protein glycation, lipid peroxidation, oxidative stress and finally varieties of complications.

Periodic evaluation of lipid peroxidation products in diabetes mellitus is recommended as it could contribute to the early identification and management of oxidative stress.

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1. Introduction

The main cause of oxidative stress is the production of oxygen free radicals in the body in excess of its ability to eliminate them by antioxidant activity. Absolute insulin deficiency (type-1 diabetes) or insulin resistance with or without insulin deficiency (type-2 diabetes) which is accompanied by uncontrolled chronic hyperglycemia is presented by many forms of diabetic complications in a number of organs as a result of oxidative damage [1]. In the presence of uncontrolled hyperglycemia excess generation of free radicals occurs due to autoxidation of glucose and glycosylation of proteins [2]. The marked increase in reactive oxygen species (ROS) accompanied by a decrease in antioxidant activity causes the structural deterioration of macromolecules (carbohydrates, proteins, lipids, and DNA) leading to their instability and consequently loss of function [3].

Recently, attention has been focused on the relationship between production of free radicals, especially reactive oxygen species (ROS), and the pathogenesis as well as progression of diabetes mellitus. Increases in biomarkers of oxidative stress related to lipid (thiobarbituric acid reactive substances: TBARS), and malondialdehydes (MDA) together with inhibition of the

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synthesis of endogenous antioxidants have been observed in several in vitro and in vivo experimental models of diabetes [4–6].

Hyperglycemia increases oxidative stress through the overproduction of reactive oxygen species, which results in an imbalance between free radicals and the antioxidant defense system of the cells [7]. Hyperglycemia can also increase the generation of proinflammatory cytokines, further impair activation of activated protein kinase and increase apoptosis in cultured cardiomyocytes [8]. The elevated blood glucose levels in diabetes are thought to lead to cell death through oxidative stress induction [7].

Poor control of diabetes reflected as increases in HbA1c levels, is associated with risk of diabetic retinopathy, while the decreases in HbA1c (achieved through combination of effective clinical management and a healthy lifestyle), demonstrated to reduce the risk of microvascular complications of the retina [9]. Additionally, fasting and postprandial glucose concentrations represent a strong risk-factor for the development of T2DM and CVD by promoting oxidative stress, inflammation and endothelial dysfunction [10].

Lipid peroxidation is described as a process under which oxidants such as free radicals species attack lipids containing carbon–carbon double bond(s), especially polyunsaturated fatty acids (PUFAs) resulting in lipid peroxyl radicals and hydroperoxides. In response to membrane lipid peroxidation, and according to specific cellular metabolic circumstances and repair capacities, the cells may promote cell survival or induce cell death [11].

A wide variety of oxidation products are produced by lipid peroxidation or reaction of oxygen with unsaturated lipids. The main primary products of lipid peroxidation are lipid hydroperoxides. During lipid peroxidation many different aldehydes can be formed as secondary products during lipid peroxidation such as malondialdehyde (MDA), propanal, hexanal, and 4-hydroxynonenal [12,13]. MDA appears to be the most mutagenic product of lipid peroxidation [14]. MDA has been widely used for many years as a convenient biomarker for lipid peroxidation of unsaturated omega-3 and omega-6 fatty acids because of its facile reaction with thiobarbituric acid (TBA). MDA is one of the most popular and reliable markers that determine oxidative stress in clinical situations [15].

Previous studies suggest a positive correlation between lipid peroxide levels and diabetic complications, lipid peroxidation was supposed to be higher in Type-II diabetics than Type-I patients, and also higher serum lipid peroxide levels were observed in patients with complications [16].

Thus, the aim of the present study was to investigate the state of oxidative stress in controlled and uncontrolled diabetic patients. Fasting blood glucose, 2 h postprandial glucose, MDA and HbA1c were quantified. The association between diabetic control and lipid peroxidation (malondialdehyde) was discussed.

2. Subjects and methods

This study was conducted in Makkah Al-Mukarama (KSA). The study protocol and procedures were approved by the Biomedical Ethics Committee, Faculty of Medicine, Umm Al-Qura University, Makkah, KSA.

Participants were selected according to inclusion criteria from patients admitted to the OPD of Diabetes Center in Al-Noor Specialized Hospital in Makkah and other citizens in the city. Adult male volunteers ($N = 139$) aged 19–50 who agreed to participate were subjected to the investigations, informed about the nature of the study and the expected risk and sign the ethical consent. The studied subjects were classified into three groups.

Group-I: Healthy Control group of non-diabetic normal subjects.

Group-II: Controlled type-2 DM group, subjects with type-2 DM and HbA1c $\leq$ 8%.

Group-III: Uncontrolled type-2 DM group, subjects with type-2 DM and HbA1c $>$ 8%.

Participants who were excluded from the study were those of type-1 DM, age less than 18 or more than 50 years old.

Blood was collected in EDTA tubes for HbA1c and Fluoride tubes for glucose measurements. For serum samples blood was collected in plain tubes and left for 30 min, then centrifuged for 15 min at 3000 rpm and the serum samples obtained. The tubes were properly labeled and sent directly to the biochemistry laboratory. Serum samples intended for long storage were kept in −80 °C up to the date of analysis.

Measurements of glucose and HbA1c were done using the standard procedures and available commercial kits in a fully automated system (COBAS integra 400 plus). All assays were done following the recommended procedures for instrument operation, calibration, quality control, and assay guidelines. The instrument was calibrated using calibrator for automated systems (Roche Diagnostics).

MDA was measured using TBARS assay kit, Cayman Chemical Company, Ann Arbor, MI for assaying lipid peroxidation in plasma, serum and urine.

The principle of the assay, as described by Dawn-linsley et al. [17], is that the MDA-TBA adduct formed by the reaction of malondialdehyde (MDA) and thiobarbituric acid (TBA) under high temperature (90–100 °C) can be measured colorimetrically at 530–540 nm. Concentration of MDA was expressed in (μM MDA).

3. Statistical analysis

Descriptive statistics and *t*-test were used to compare the levels of all metabolic parameters between the two groups of controlled and uncontrolled type-2 diabetes mellitus and one way ANOVA was used for the analysis of variance between the three groups of healthy, controlled and uncontrolled type-2 DM. The Pearson correlation between MDA and HbA1c was calculated and P value < 0.05 was considered as statistically significant. All statistical methods were performed using SPSS for windows (version 20, SPSS Inc.).

4. Results

The age and BMI show no significant difference between controlled and uncontrolled type-2 DM subjects as shown in Table 1, the fasting and 2 h PP results although both were elevated in uncontrolled group compared to controlled type-2 DM, but the difference was not significant also.

The mean HbA1c increased significantly in uncontrolled type-2 DM subjects compared to controlled type-2 DM group. Lipid

Table 1
Age, BMI, glucose and MDA in the study groups.

	Case			*P* value*
	Non-diabetics	Controlled diabetes	Uncontrolled diabetes	
Age (Years)	34 ± 10	43 ± 9	42 ± 9	0.706
BMI (kg/m^2)	27.7 ± 5.1	29.7 ± 4.9	30.4 ± 5.3	0.605
FBS (mg/dl)	89.0 ± 11	185 ± 55	199 ± 69	0.207
2hrPP (mg/dl)	107.0 ± 17	280 ± 51	310 ± 85	0.068
HbA_1C (%)	4.8 ± 0.5	6.7 ± 1.2	9.6 ± 1.7	<0.001
MDA (μM)	4.1 ± 0.81	7.5 ± 2.1	10.6 ± 3.1	<0.001

* *P* value when the comparison is between controlled and uncontrolled diabetic cases. Values are expressed as mean ± SD.

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