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Evaluation of changes in levels of plasma MDA and antioxidant vitamin E in Sudanese patients with diabetes type II

Elrasheed I. Mohamed[1], Badr Eldien H. Elabid[2], Altayeb Elazomi[1], Hana Fahelbum[1], Hakem Zwaik[3], Nazeha Mansuri[4] and Salah Bahroun[4]

[1]Faculty of Medical Technology, Zawia University, [2]University of Science and Technology, Sudan [3] Faculty of Sciences Zawia University and [4] National Medical Research Centre, Zawia, Libya

Abstract: Free radicals have important roles in pathogenesis of diabetes mellitus. It has been well documented that there is a link between oxidative stress and secondary complications of diabetes. In the present study we determined and evaluated changes in levels of malondialdehyde (MDA) and antioxidant vitamin E in plasma of Sudanese patients with Type II diabetes mellitus. Total of 200 diabetic patients (90 males, 110 females) with mean age of 55.48 ± 12.14 years were recruited into the study. Control group was composed of 100 healthy volunteers (47 males, 53 females) with mean age of 53.53 ± 11.43 years. In addition to the two mentioned parameters, levels of fasting blood glucose and percentages of HbA1C levels were determined in diabetic patients and controls. There was a significant increase in the MDA level (test group) which is used as an indicator of metabolic stress, oxidative stress or lipid per oxidation marker. On the other hand; antioxidant vitamin E of the test group was reduced meaningfully. Reduction in vitamin E levels was probable due to antioxidant effect of this antioxidant vitamin. In conclusion, supplementation of antioxidant vitamins into the daily diets of diabetic patients will enhance power of non-enzymatic antioxidant defense systems.

Keywords: Diabetes mellitus, vitamin E, MDA, Libya

Introduction

Patients with Non-Insulin Dependent Diabetes Mellitus (NIDDM) have an increased mortality and morbidity compared to nondiabetics due to various associated complications such as neuropathy, nephropathy, cardiovascular disease, etc. Reactive oxygen species (ROS) and particularly free radical induced lipid per oxidative tissue damage have been implicated in the pathogenesis of various diseases (1) including diabetes (2). Diabetes mellitus is a disorder with late complications including cardiovascular diseases, nephropathy, neuropathy, retinopathy which affects severely the quality of life (3). Although there are several reports on complications of diabetes, pathophysiology of these complications are still needed to be deciphered (4).

Recent reports indicated that free radicals have important roles in pathogenesis of diabetes and a relationship between oxidative stress and secondary complications of diabetes exists (5, 6). It is well established that there is an increased production of damaging free radicals in NIDDM patients which may be due to auto-oxidation of glucose and glycosylated proteins (7-10). Subsequently, free radicals change lipid/protein ratio of membranes by affecting polyunsatured fatty acids and lipid peroxidation, causes functional irregularities of several cellular organelles (11, 12). Lipid peroxides are disintegrated quickly and form reactive carbon compounds. Among these, MDA is an important reactive carbon compound which is used commonly as an indicator of lipid peroxidation (13). Since free radical production is increased whereas capacity of antioxidant systems is reduced in diabetes, it has been proposed that diabetic patients may require more antioxidants compared to healthy individuals (12-14).

Since effects of free radicals in diabetes are now documented, it has been proposed to use antioxidant vitamins to block formation of free radicals and hence prevent development of diabetes (15, 16). Glutathione is a very important non-enzymatic antioxidant together with antioxidant vitamins. Vitamins A, E and C are among these important nonenzymatic antioxidants (17, 18). It has been proposed that in diabetic patients several abnormalities related with absorption develop in the absence of antioxidant vitamins (19).

Vitamin E and glutathione are some of the major non-enzymatic antioxidants in the body. Therefore, the idea of using antioxidant vitamin to prohibit development of diabetes as well as its complications and/or to treat diabetic patients is getting more attention than ever (15, 21). Although there are studies reporting serum or plasma levels of antioxidant vitamins in diabetic patients, results from different groups are rather contradictory. Studies focusing on involvement of vitamin E in diabetic patients are rather limited. Therefore, the present study was designed to determine and evaluate changes in level of antioxidant vitamin E and MDA in Sudanese patients with type 2 diabetes and healthy subjects.

Material and methods

A total of 200 patients (90 males, 110 females) who were diagnosed with type 2 diabetes mellitus in Jabir Abulizz Diabetes Centre, Omdurman Teaching Hospital (Abdelmoniem referring center) and other private clinics for diabetic care in Khartoum State, Sudan, were included in the study. The mean age of diabetic patient was 54.8 ± 11.4 years. All were free of clinical symptoms of neuropathy, retinopathy. The control group consisted of 100 healthy volunteers (47 males, 53 females) whose mean age was 53.53 ± 11.48 years. Venous blood samples were withdrawn after an overnight fasting from the patients and the controls. Fasting blood glucose levels were determined by a commercial kit, using enzymeatic method (glucose oxidase/peroxidase) (Biosystem SA Costa Brava 30, Barcelona-Spain by auto analyzer humalzer 2000 human-German). Hb A1c percentage level was determined by method based on an aboronate affinity chromatography by using NYCO CARD READER II – AXIS - SHIELD Po C AS NO-0504 Oslo, Norway, rapid in vitro test for the measurement of glycated hemoglobin (Hb A1c) percentage in human whole blood. The machine (NYCOCARD READE II) is traceable to the international federation of clinical chemistry (IFCC) reference method for measurement of Hb A1c, and it's measuring range 3 - 18% Hb A1c. MDA levels were determined by the method of Karataş et al (20) by HPLC utilizing a column (250 x 3.9 ID) packed with Tecopak C18 reversed phase material (10.0 mm particle size), were performed at following optimized experimental conditions: mobile phase is 30 mM KH2PO4 buffer, pH = 4 with H3PO4) and methanol (65 - 35%v/v) at 1.5 ml per min flow rate and 254 nm wave length. Determination of vitamin E through the HPLC method a chromatographic measurements were made using a Hewlett-Packard (wald born, Germany) model 1050 pump system, water 717 plus Auto sampler (Mil ford, MA, USA), a uv/vis detector, SPD - 10 AV VP (shimadzu Kyoto, Japan) and an HP-3365 series II chemstation. The analytical Colum used was a tracer spherisorb OD52 C18 (250 x 4.6 mm 1.0 D, 5 µm particle size) protected with a guard cartridge (tracer, C18, 5.0 µm). The frozen specimens preserved with metaphorsphoric acid (5%) were thawed to around 22 ºC in water bath, protected from light, and then mixed. Statistical analysis was carried out using SPSS for Windows, Version 10.5 (SPSS Inc. Chicago, IL, USA). The data obtained were expressed as mean values ± SD. Student's t-test and Pearson test were used to determine whether were any significant differences between the means, with $P < 0.05$ taken as the significant level.

Results

Demographic features of diabetic patients and controls are summarized in Table 1. MDA and vitamin E are given in Table 2. Fasting blood glucose and HbA1C% are given in Table 3.

Table 1 shows no significant difference (P = 0.08) between the means of age of the test and the control groups, mean ± SD, (55.48 ± 12.41) versus (53.53 ± 11.43) years and shows no significant difference (P = 0.09) between the height of the test and control groups, mean ± SD, (170.54 ± 9.35) versus (173.24 ± 8.7) cm and shows significant difference (p = 0.04) between the means of weight of the test and control groups, mean ± SD, (75.26 ± 9.85) versus (70.71 ± 9.42) kg and shows significant difference (p = 0.03) between the means of BMI of the test and control groups, mean ± SD, (26.04 ± 3.18) versus (22.60 ± 2.41) kg per m^2.

Table 1: Demographics features of the test and the control groups

Variable	Test group n = 200	Control group n = 100	*P* value
MDA	4.47±5.29(0.62 – 34.98)	1.93± 0.41(1.04 – 3.63)	0.00
Vitamin E	5.28± 1.51(0.5-9.54)	7.08±2.54(3.94-22.48)	0.01

Table 2 shows a highly significant difference (P = 0.00) between the means of plasma MDA of the test and the control groups, mean ± SD, (4.47 ± 5.29) versus (1.93 ± 0.41) n mol/l and shows a significant difference (P = 0.01) between the means of plasma vitamin E of the two groups, mean ± SD, (5.28 ± 1.51) versus (7.08 ± 2.54) µg/ml.

Table 2: Comparison of the means of plasma MDA and vitamin E of the test group and control group

Variable	Test group n = 200	Control group n = 100	*P* value
Age (years)	55.48± 12.41(23.00 – 86.00)	53.53± 11.43(22.00 – 78.00)	0.08
Height (cm)	170.54± 9.35(132.00 – 194.00)	173.24 ± 8.73(157.00 – 192.00)	0.09
Wight (Kg)	75.26± 9.85(53.00 – 125.00)	70.71± 9.42(52.00 – 104.00)	0.04
BMI (Kg/m^2	26.04± 3.18(18.1 – 41.4)	22.60 ± 2.41(19.30 – 31.00)	0.03

Table 3 shows a significant difference (P = 0.001) between the means of plasma levels of fasting plasma glucose of the test and the control groups, mean ± SD, (191.01 ± 58.52) versus (94.74 ± 10.81) mg/dl. It shows a significant difference (P = 0.03) between the means of the blood levels of hemoglobin HbA1c % of the test and the control groups, mean ± SD, (9.18 ± 2.19) versus (5.17 ± 048).

Table 3: Comparison of the means of plasma fasting plasma glucose (mg/dl) and HbA1C (%) of the test group and control group

Variable	Test group n = 200	Control group n = 100	*P* value
FBS	191.01 ± 58.52(25.00 – 340.00)	94.75 ± 10.81(68.00 – 124.00)	0.00
HbA1C %	9.18 ± 2.19(4.10 – 15.60)	5.17 ± 0.48(4.10 – 6.30)	0.03

Values are means ± SD $p < 0.05$ when compared to control

Discussion

When diabetic complications are developed, an increase in oxidative damage and subsequently emaciation of antioxidant defense systems are observed (17). Noberasco et al. (21) reported that there was an increase in lipid peroxidation levels whereas vitamin E levels were decreased in patients with Type 2 diabetes mellitus compared to controls. Changes in oxidant and antioxidant systems are related with duration of disease and become more important as complications develop. Findings of several studies demonstrated that overproduction of peroxides along with emaciation of antioxidant defense systems cause oxidative damage and these events in type 2 diabetic patients are observed earlier before diabetic complications develop (23). Jorge et al. (24) reported that a minor oxidative stress was observed in females with type 2 diabetic patients after α-tocopherols treatment interfered from the reduced level of erythrocyte MDA and the increase total antioxidant status, on the other hand, no beneficial change was observed in the levels of serum MDA. Results of vitamin E levels in blood, plasma and serum levels of patients with type 2 diabetes mellitus were contradictory. Several groups reported that there were increases in vitamin E levels of the test group compared to controls (15, 25-26). Other reports indicate that no significant changes occur (27, 28). In addition, several studies documented that vitamin E levels in blood, plasma and serum were decreased in type 2 diabetic patients (16, 21, 23, 29-30). In the present study, we also observed the latest trend (Table 2). This on our opinion is due to depressed antioxidant vitamins levels or it may be due to increased demand of vitamin E to relive increased oxidative stress observed in these patients.

There are several reports indicating increased MDA levels in patients with type 2 diabetes (22, 31, 32) or unchanged (24). The results of the present study support the previous findings (22, 31, 32). This on our opinion is due to oxidative events caused by metabolic stress, or exposure to prolonged periods of hyperglycemia, which causes non enzymatic glycation of plasma proteins (binding of glucose to protein molecules), or may be due to poor diabetic control which may enhance lipid per oxidation and diminishes the body's antioxidant capacity, or may be due to mobilization of lipids for a further use as an energy sources rather than glucose.

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