

Evaluation of Changes in Levels of plasma MDA, and Antioxidant Vitamin E in Sudanese patients with type2 Diabetes

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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 2 Diabetes Mellitus. Total of 200 diabetic patients (90 male, 110 female) with mean age of 55.48 ± 12.14 years were recruited into the study. Control group was composed of 100 healthy volunteers (47 male, 53 female) with mean age of 53.53 ± 11.43 years. In addition to two mentioned parameters, levels of fasting blood glucose, percentage HbA1C levels were determined in diabetic patients and controls.

There was a significant increase in 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.

I. INTRODUCTION

PATIENTS with Non-Insulin Dependent Diabetes Mellitus (NIDDM) have an increased mortality and morbidity compared to non-diabetics 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 indicate 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 Non-insulin dependent diabetes mellitus (NIDDM) patients which may be due to auto-oxidation of glucose and glycosylated proteins (7, 8, 9, 10). Subsequently, free radicals change lipid/protein ratio of membranes by affecting poly unsaturated 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. Furthermore, we examined possible relationship between HbA1c, vitamin E, and MDA.

II. MATERIAL AND METHODS

Total of 200 patients (90 male, 110 female) 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.

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Mean age of diabetic patient was 54.8 ± 11.4 years and who were free of clinical symptoms of neuropathy, retinopathy. Control group was consisted of 100 healthy volunteers (47 male, 53 female) whose mean age were 53.53 ± 11.48 years. Venous blood samples were withdrawn after an overnight fasting from patients and controls. Fasting blood glucose levels were determined by a commercial kit by using enzymatic method (glucose oxidase / peroxidase) (Biosystem S.A Costa Brava 30, Barcelona- Spain by auto analyzer humalzer 2000 human- German.)

Hb A1c percentage level was determined by method based on boronate affinity chromatography by using NYCOCARD READER II –AXIS-SHIELD Po C AS NO-0504 Oslo, Norway, rapid invitro test for the measurement of glycated hemoglobin (Hb A1c) % 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 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 min⁻¹ flow rate and 254 nm wavelength. 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) , auv – 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.D, 5µm particle size) protected with a guard cartridge (tracer, C18, 5µm).The frozen specimens preserved with metaphosphoric acid (5%) were thawed to around 22 °C in water bath, protected from light, and then mixed. Statistical analysis was carried out using SPSS for Windows, Ver.10.5 (SPSS Inc. Chicago, IL, USA). The data obtained are expressed as mean values ± S.D. Student's t-test and Pearson test was used to correlations determine whether differences between the means were significant, with $p < 0.05$ taken as the significance level.

III. RESULT

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 2. Shows a highly significant difference between the means of plasma MDA of the test group (n=200) and the control group (n=100).Mean± SD: (4.47±5.29) versus (1.93±0.41) n mol/l, (p=0.00), and shows a significant difference between the means of plasma vitamin E of the test group (n=200) and the control group (n=100).Mean± SD:

(5.28±1.51) versus (7.08±2.54) µg/ml, respectively, (p=0.01). Table 3. Shows a significant difference between the means of plasma levels of fasting plasma glucose of the test group (n=200) and the control group (n=100).Mean± SD: (191.01±58.52) versus (94.74±10.81) mg/dl, (p=0.00), respectively, and shows a significant difference between the means of blood levels of hemoglobin HbA1c % of the test group (n=200) and the control group (n=100)Mean± SD: (9.18±2.19) versus (5.17±0.48) %, (p=0.03) respectively.

TABLE I
DEMOGRAPHICS FEATURES OF THE TEST GROUP AND THE 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 II
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
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 III
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

IV. 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 female 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 are contradictory. Several groups reported that there are increases in vitamin E levels of the test group compared to controls (15, 25-26). On the other hand, some 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 may be due to depressed antioxidant vitamins levels or it may be due to increased demand of vitamin E to relieve 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]. Results of present study support these findings (22, 31-32), this could be 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.

REFERENCES

- [1] Templar J, Kon SP, Milligan TP, Newman DJ, and Raftery MJ: Increased plasma malondialdehyde levels in glomerular disease as determined by a fully validated HPLC method. *Nephrol Dial Transplant* 14(4):946-951, 1999.
<http://dx.doi.org/10.1093/ndt/14.4.946>
- [2] Ozben T, Nacitarhan S and Tuncer N: Plasma and urine malondialdehyde levels in non- insulin dependent diabetic patients with and without microalbuminuria. *Int J Clin Lab Res* 25:162-164, 1995.
<http://dx.doi.org/10.1007/BF02592560>
- [3] Halifeoğlu İ, Karataş F, Çolak R, Canatan H, Telo S. Tip 2 diyabetik hastalarda tedavi öncesi ve tedavi sonrası oksidan ve antioksidan durum. *Fırat Tıp Dergisi* 10(3): 117- 122, 2005;
- [4] Alan WS: The role of advanced glycation in the pathogenesis of diabetic retinopathy. *Exp Mol Pathol* 75:95-108, 2003.
[http://dx.doi.org/10.1016/S0014-4800\(03\)00035-2](http://dx.doi.org/10.1016/S0014-4800(03)00035-2)
- [5] Singal PK, Bello-Klein A, Farahmand F, Sandhawalia, V: Oxidative stress and functional deficit in diabetic cardiomyopathy. *Adv Exp Med Biol* 498:213-220, 2001.
http://dx.doi.org/10.1007/978-1-4615-1321-6_27
- [6] Mercuri F, Quagliari L, Ceriello A: Oxidative stress evaluation in diabetes. *Diabetes Technol Ther* 2(4):589- 600,2000.
- [7] Greene DA, Stevens MJ, Obrosova I, Feldman EL Glucose-induced oxidative stress and programmed cell death in diabetic neuropathy. *Eur. J. Pharmacol.* 1999; 375 : 217-223.
[http://dx.doi.org/10.1016/S0014-2999\(99\)00356-8](http://dx.doi.org/10.1016/S0014-2999(99)00356-8)
- [8] Ceriello A Hyperglycemia: the bridge between non-enzymatic glycation and oxidative stress in the pathogenesis of diabetic complications. *Diabetes. Nutr. Metab.* 1999; 12(1): 42-46.
- [9] Pennathur S, Heinecke JW Mechanisms of oxidative stress in diabetes: implications for the pathogenesis of vascular disease and antioxidant therapy. *Front. Biosci.* 2004; 9: 565-574.
<http://dx.doi.org/10.2741/1257>
- [10] Maritim AC, Sanders RA, Watkins JB 3rd Diabetes, oxidative stress, and antioxidants: a review. *J. Biochem. Mol. Toxicol.* 2003; 17(1): 24-38.
<http://dx.doi.org/10.1002/jbt.10058>
- [11] Yagi K: Assay for blood plasma or serum. *Methods Enzymol*, 109: 328-331, 1984.
[http://dx.doi.org/10.1016/S0076-6879\(84\)05042-4](http://dx.doi.org/10.1016/S0076-6879(84)05042-4)
- [12] Cheesman KH and Slater TF: Introduction to free radical biochemistry. *Br Med Bull* 49(3): 481-493, 1993.
- [13] Jacob RA and Burri BJ: Oxidative damage and defense. *Am. J. Clin. Nutr.* 63, 985-990, 1996.
- [14] Langenstroer P, and Pieper GM: Regulation of Spontaneous EDRF release in diabetic rat aorta by oxygen free radical. *Am J Physiol* 263:257-265, 1992.
- [15] Ceriello A, Bortolotti N, Falletti E, Taboga C, Tonutti L, Crescentini A, Motz E, Lizzio S, Russo A, Bartoli E: Total radical-trapping antioxidant parameter in NIDDM patients. *Diabetes Care* 20(2):194-197, 1997.
<http://dx.doi.org/10.2337/diacare.20.2.194>
- [16] Maxwell SR, Thomason H, Sandler D, Leguen C, Baxter MA, Thorpe GH, Jones AF, Barnett AH: Antioxidant status in patients with uncomplicated insulin-dependent and noninsulin-dependent diabetes mellitus. *Eur J Clin Invest* 27(6):484-490, 1997.
<http://dx.doi.org/10.1046/j.1365-2362.1997.1390687.x>
- [17] Diplock AT: Antioxidant nutrients and disease prevention: An Overview. *Am J Chim Nutr* 53:1895-1935, 1991.
- [18] Halliwell B: Free radical antioxidants in human disease. Curiosity, cause or consequence. *Lancet*, 344:721-724, 1994.
[http://dx.doi.org/10.1016/S0140-6736\(94\)92211-X](http://dx.doi.org/10.1016/S0140-6736(94)92211-X)
- [19] Sinha M, Grosh AK: Influence of dietary vitamin A deficiency on rat digestive & absorptive functions during diabetes mellitus. *Indian J Med Res* 100:196-200, 1994.
- [20] Al-Saleh IA & Al-Doush I: Selenium levels in wheat grains grown in Saudi Arabia. *Bull Environ Contam Toxicol* 59:590-594, 1997.
<http://dx.doi.org/10.1007/s001289900520>
- [21] Ceriello A, Giugliano D, Quatraro A, Dello Russo P, Torello R: A preliminary note on inhibiting effect of α -tocopherol on protein glycation. *Diabetes Metab* 14:40-52, 1988.
- [22] Noberasco G, Odetti P, Boeri D, Maiello M, Adezati L: Malondialdehyde (MDA) level in diabetic subjects. Relationship with blood glucose and glycosylated hemoglobin. *Biomed Pharmacother* 45, (4-5):193-196, 1991.
[http://dx.doi.org/10.1016/0753-3322\(91\)90107-5](http://dx.doi.org/10.1016/0753-3322(91)90107-5)
- [23] Sundaram RK, Bhaskar A, Vijayalingam S, Viswanathan M, Mohan R: Antioxidant status an lipid peroxidation in type II diabetes mellitus with and without complications. *Clin Sci (Colch)* 90(4):255-260, 1996.
- [24] Jorge L. Ble-Castillo, Elizabeth Carmona-Díaz, José D. Méndez, Francisco J. Larios-Medina, Roberto Medina- Santillán, Guadalupe Cleva-Villanueva, Juan C. Díaz- Zagoya: Effect of α -tocopherol on the metabolic control and oxidative stress in female type 2 diabetics. *Biomedicine & Pharmacotherapy* 59 (2005) 290–295
<http://dx.doi.org/10.1016/j.biopha.2005.05.002>
- [25] Krempf M, Ranganathan S, Ritz P, Morin M, Charbonnel B: Plasma vitamin A and E in type 1 (insulin-dependent) and type 2 (non-insulin-dependent) adult diabetic patients. *Int J Vitam Nutr Res* 61(1):38-42, 1991.
- [26] Caye-Vaugien C, Krempf M, Lamarche P, Charbonnel B, Pieri J: Determination of alpha-tocopherol in plasma, platelets and erythrocytes of type I and type II diabetic patients by high-performance liquid chromatography. *Int J Vitam Nutr Res* 60(4):324-30, 1990.
- [27] Rautalahti M, Albanes D, Haukka J, Roos E, Gref CG, Virtamo J: Seasonal variation of serum concentrations of α -carotene and α -tocopherol. *Am J Clin Nutr* 57:551-556, 1993.
- [28] Abahusain MA, Wright J, Dickerson JW, de Vol EB: Retinol, alpha-tocopherol and carotenoids in diabetes. *Eur J Clin Nutr* 53(8):630-636, 1999.
<http://dx.doi.org/10.1038/sj.ejcn.1600825>
- [29] Nourooz-Zadeh J, Rahimi A, Tajaddini-Sarmadi J, Tritschler H, Rosen P, Halliwell B, Betteridge DJ: Relationships between plasma measures of oxidative stress and metabolic control in NIDDM. *Diabetologia* 40(6):647-653, 1997.
<http://dx.doi.org/10.1007/s001250050729>
- [30] Ceriello, PA: Oxidative stress and diabetes associated complications. *Endo Prac* 12, 60–62, 2006.
<http://dx.doi.org/10.4158/EP.12.S1.60>
- [31] Jiang ZS, Zhang SL, Cai XJ: Effect of salvia miltiorrhiza composita on superoxide dismutase and malondialdehyde in treating patients with non-

insulin dependent diabetes mellitus. *Chung Kuo Chung Hsi I Chieh Ho Tsa Chih.*17(1):32-34, 1997.

- [32] Ceriello A, Bortolotti N, Motz E, Crescentini A, Lizzio S, Russo A, Tonutti L, Taboga C: Meal-generated oxidative stress in type 2 diabetic patients. *Diabetes Care* 21(9):1529-1533, 1998.