Measurement of Plasma Lipid per oxidation Marker (MDA) and Vitamin A in Sudanese Patients with Type2 Diabetes.

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Abstract

It has been well documented that there is a link between oxidative stress and secondary complications of diabetes. In the present study we measured and evaluated changes in levels of malondialdehyde (MDA) as marker of lipid per oxidation and antioxidant vitamin A 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 A of the test group was reduced meaningfully. Reduction in vitamin A levels was probable due to antioxidant effect of this antioxidant vitamin. In conclusion supplementation of antioxidant vitamin (A) into the daily diets of diabetic patients will enhance power of non-enzymatic antioxidant defense systems.

Keywords—Diabetes Mellitus, Vitamin A, MDA.

Introduction

Diabetes Mellitus (DM) is a chronic disease characterized by the disorder of the glucose metabolism and associated with a reduced ability of the tissues to respond to insulin (insulin resistance). DM causes high morbidity and mortality derived by chronic micro- and macro-vascular complications[1]. Diabetes was reported to be the fifth leading cause of death in the United States[2]. DM is now one of the major health problems in the Sudan resulting in 10% of all hospital admissions and mortality. A small population based study in 1993 of a sample of 1284 adult men, showed a prevalence of 3.4% of type 2 diabetes[3]. A combination of genetic and environmental risk factors contributed to DM pathogenesis[4]

Although there are several reports on complications of diabetes, pathophysiology of these complications are still needed to be deciphered [5]. 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 [6,7]. 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 [8,9,10,11]. Subsequently, free radicals change lipid/protein ratio of membranes by affecting poly un satured fatty acids and lipid per oxidation, causes functional irregularities of several cellular organelles [12,13]. 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 per oxidation [14]. 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 [13,14]. 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 [16,17]. Glutathione is a very important non-enzymatic antioxidant together with antioxidant vitamins. Vitamins A, E and C are among these important non enzymatic antioxidants [18,19]. It has been proposed that in diabetic patients several abnormalities related with absorption develop in the absence of antioxidant vitamins [20.]

Vitamin A and glutathione are some of the major non-enzymatic antioxidants in the

body. Therefore, the idea of using antioxidant vitamin to prohibit development complications and/or to treat diabetic patients is getting more attention than ever [16,22]. 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 A in diabetic patients are rather limited. of diabetes as well as its

Therefore, the present study was designed to measured and evaluate changes in level of antioxidant vitamin A and MDA in Sudanese patients with type 2 diabetes and healthy subjects. Furthermore, we examined possible relationship between HbA1c, vitamin A, and MDA.

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. 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 humalyzer 2000 human- German.)

Hb A1c percentage level was determined by method based on aboronate affinity chromatography by using NYCOCARD READER II –AXIS-SHIELD Po C AS NO-0504 Oslo, Norway, rapid *in vitro* 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) 1.5 mL min-1 flow rate and 254 nm wavelength. Determination of vitamin A 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, 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 ºC in water bath, protected from light, and then mixed. Statistical analysis was carried out using SPSS for Windows, SPD – 10 AV VP (shimadzu Kyoto, Japan) and an HP- 3365 series II chemstation. The analytical Colum used was a tracer spherisorb OD52 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.

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, respectively, (p=0.00).

And shows a significant difference between the means of plasma vitamin A of the test group (n=200) and the control group (n=100).Mean± SD: (46.33±12.68) versus (70.39±15.52) µg/dl, respectively, (p=0.00).

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.

Figure (1) shows insignificant, very weak correlation between HbA1c percent and the plasma levels of Malondialdehyde of the test group (r=0.05, p= 0.51).

Figure (2) shows insignificant very weak correlation between HbA1c percent and the plasma levels of vitamin A of the test group (r=0.05, p= 0.5).

Figure (3) shows significant difference, positive correlation between HbA1c percent and the fasting plasma glucose levels of the test group (r=0.81, p= 0.00).

Variable	Test groupN =200	Control groupN =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/m2	26.04± 3.18(18.1 – 41.4)	22.60 ± 2.41(19.30 – 31.00)	0.03

TABLE I
DEMOGRAPHICS FEATURES OF THE TEST GROUP AND THE CONTROL GROUP

Variables	Test group No=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 A	46.33±12.68(13.26 – 95.50	70.39±15.52(39.35- 131.70)	0.00

TABLE II
COMPARISON OF THE MEANS OF PLASMA MDA AND VITAMIN A OF THE TEST GROUP AND CONTROL GROUP

Variables	Test group No=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

TABLE III
COMPARISON OF THE MEANS OF PLASMA FASTING PLASMA GLUCOSE (MG/DL) AND HBA1C (%) OF THE TEST GROUP AND CONTROL GROUP
Values Are Means ± SD P<0. 05 When Compared To Control

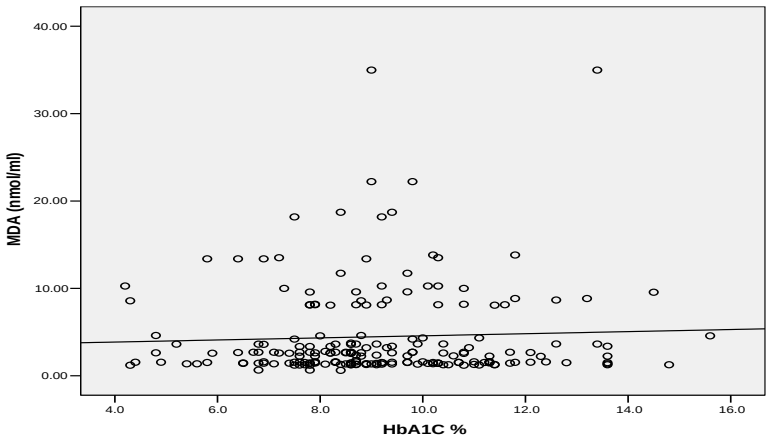

Fig. (1) A scatter plot shows the relationship between HbA1C% and plasma MDA of the test group. (r = 0.05, P = 0.51).

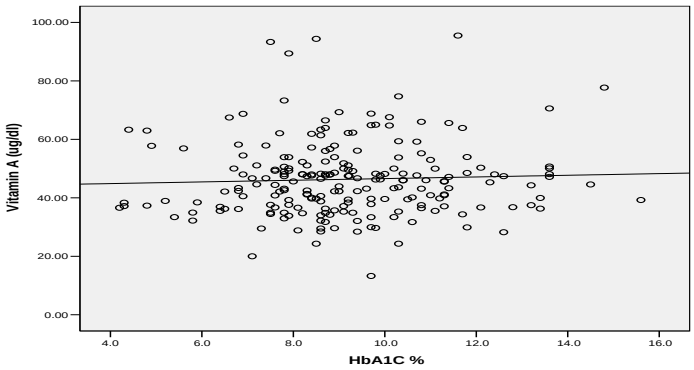

Fig. (2) A scatter plot shows the relationship between HbA1C% and Vitamin A of the test group.(r=0.05,P=0.50).

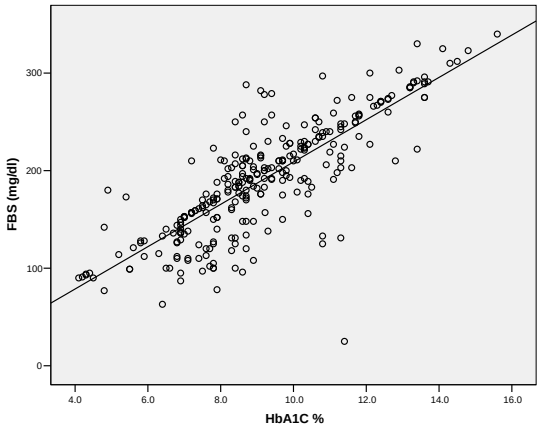

Fig. (3) A scatter plot shows the relationship between HbA1C% and fasting plasma glucoses of the test group. (r = 0.81, P = 0.00).

Discussion

Diabetes is a serious public health problem throughout the world ,in both type 1 and type 2 diabetes, increased oxidative stress and impaired antioxidant defense have been suggested as contributed factors for initiation and progression of late complications in diabetes. The hypothesis that hyperglycemia should be able to cause oxidation in diabetic patients is supported by several studies and particularly by evidence that several biochemical pathways activated during hyperglycemia can increase the production of reactive oxygen species (ROS) [23],

also when diabetic complications are developed, an increase in oxidative damage and subsequently emaciation of antioxidant defense systems are observed [24.] The current study shows a significantincrease in the plasma levels of MDA of the test group when compared to the control group (Table:2), this agrees with studies done by (Noberasco, et al.1991)[25], (Jiang, et al.1997)[26], and (Ceriello, et al.1998)[24] who reported a significant increase in the levels of plasma malondialdehyde of the diabetics when compared to healthy controls, this 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. Also the present study shows insignificant, very weak correlation between HbA1c percent and the plasma levels of Malondialdehyde of the test group (Fig.1), this agrees with a study done by (Tan, et al.2000)[27] and also agrees with a study done by (Hanachi P, et al. 2009)[28] who reported no correlation between MDA and HbA1C %, this could be due to good glycemic control. The present a study shows a significant decrease of the plasma levels of vitamin A of the diabetic patients when compared to the healthy control (Table:2), this agrees with the studies done by (Sundaram, et al.1996)[29], (Krempf, et al.1991)[30], (Vatassery, et a.1983)[31], and (Grando, et al.1998)[32] who documented reduction in vitamin A levels of diabetic patients rather than increase when compared to healthy controls, this significant decrease most probably due to rapid depletion of this antioxidant (vitamin A) due to increased oxidative stress observed in type2 diabetic patients. The present study also shows insignificant, weak correlation between HbA1c percentage and the plasma levels of vitamin A (Fig.2) of the test group, this agrees with a study done by (Hermann, et al.1994)[33], and (Loft, et al.1992)[34] they demonstrated no correla-tion between the HbA1c and plasma levels of vitamin A The current study shows a significant, positive correlation between HbA1c % and the fasting plasma glucose levels of the diabetics (Fig.3) , this is in accordance with the study of (Kesavulu, et al .2001)[35] and (Sundaram, et al.1996)[29] who reported significant positive correlation between HbA1C % and FPG levels, this most probably due to poor glycemic control. From the results of this study it is concluded

that, in Sudanese patients with type 2 diabetes mellitus, the means of the plasma levels of malondialdehyde, fasting plasma glucose, and blood HbA1C are significantly raised when compared with healthy control subjects, where mean of plasma levels of antioxidant vitamins (A) is significantly decrease in Sudanese with type 2 diabetes mellitus when compared with healthy control subjects, also there is significant strong positive correlation between HbA1c percentage and fasting plasma glucose (FPG), where there is insignificant very weak correlation between Vitamin A, MDA and HbA1c of the test group. Therefore its recommended that Diabetic patients should received supplements of antioxidant vitamins such as vitamin A, in order to enhance power of non-enzymatic antioxidant defense systems. Lipid per oxidation marker such as malondialdehyde (MDA) should be assessed regularly in diabetic patients, in order to minimize development of free radicals and oxidative stress. Good glycemic control in diabetic patients could be of vital importance in prevention or delay the development of complications in diabetic patients

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