ARAŞTIRMA

2006: 20 (6): 391 - 395
http://www.fusabil.org

Evaluation of Changes in Levels of Serum Selenium, MDA and Antioxidant Vitamins (A, E, C) in Diabetic Patients

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Geliş Tarihi : 29.05.2006
Kabul Tarihi : 20.11.2006

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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 selenium, malondialdehyde (MDA) and antioxidant vitamins (A, E, C) in serum of patients with Type II Diabetes Mellitus. Total of 200 diabetic patients (90 male, 110 female) with mean age of 54.8±11.4 years were recruited into the study. Control group was composed of 100 healthy volunteers (47 male, 53 female) with mean age of 46.4±12.3 years. In addition to aforementioned parameters, levels of fasting blood glucose, percentage HbA1C levels were determined in diabetic patients and controls. There was a significant increase in MDA level which is used as an indicator of metabolic stress. On the other hand, antioxidant vitamin (A, E, C) and selenium levels were reduced meaningfully. Reductions in Se levels were probable due to antioxidant effect of this trace element. In conclusion supplementation of antioxidant vitamins into the daily diets of diabetic patients will enhance power of non-enzymatic antioxidant defense systems.

Key Words: *Diabetes Mellitus, Vitamin A, Vitamin E, Vitamin C, MDA, Selenium*

Diabetik Hastalarda Serum Selenyum, MDA ve Antioksidant Vitaminlerin (A,E,C) Düzeylerindeki Değişimlerin Değerlendirilmesi

Serbest radikaller diabetin patogenezinde önemli role sahiptirler. Diabetin ikincil komlikasyonları ve oksidatif stres arasında bir bağlantı olduğu iyice belgelenmiştir. Bu çalışmada Tip II diabetikli hastaların serumlarındaki selenyum, Malondialdehit (MDA) ve antioksidan vitaminler (A, E, C)'in seviyelerindeki değişmeler belirlendi. Yaş ortalaması 54.8±11.4 yıl olan toplam 200 diabetik hasta (90 erkek, 110 dişi) çalışmaya dahil edildi. Kontrol grubu ise gönüllü (47 erkek, 53 dişi) yaş ortalaması 46.4±12.3 yıl olanlardan oluşturuldu. Belirtilen parametrelere ilave olarak hasta ve kontrol gruplarında %HbA1C, açlık kan glukoz seviyeleri de belirlendi. Metabolik stresin bir indikatörü olarak kullanılan MDA seviyesinde anlamlı bir artış vardı. Diğer taraftan antioksidan vitaminler (A, E,) ve selenyum seviyelerindeki azalma anlamlıydı. Se seviyelerindeki azalma bu eser elementin muhtemel antioksidan etkisindendir. Sonuç olarak diabetik hastaların günlük diyetlerine antioksidan vitaminlerin katılması enzimatik olmayan savunma sistemini güçlendirecektir.

Anahtar Kelimeler: *Nonalkolik steatohepatit, abdominal obezite, DEXA.*

Introduction

Reactive oxygen species (ROS) and particularly free radical induced lipid peroxidative 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, pathopysiology 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). Free radicals are produced as a result of glycosylation of several proteins including hemoglobin (Hb) by non-enzymatic mechanisms (7, 8).

Subsequently, free radicals change lipid/protein ratio of membranes by affecting poly unsatured fatty acids and lipid peroxidation causes functional irregularities of several cellular organelles (9, 10).

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 (11). 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 (10, 12).

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 (13, 14). While superoxide radicals are cleaned by enzymatic dismutation, compounds known as antioxidants clean free radicals in organism. Glutathione is a very important non-enzymatic antioxidant together with antioxidant vitamins. Vitamins A, E and C are among these important nonenzymatic antioxidants (15, 16).

It has been proposed that in diabetic patients several abnormalities related with absorption develop in the absence of antioxidant vitamins (17).

Vitamin A functions as catalyzer of removal of singlet oxygen and as a result vitamin A inhibits singlet oxygen-dependent reactions [15, 18]. Vitamin C is also has a role in activating vitamin E when it loses its antioxidant capacity by turning into tocopherol (19).

In addition, selenium has effect on preventing decomposition, absorption and biological activity of α-tocopherol (20, 21). Selenium and vitamin E act as complementing each others function against oxidative stress (22, 23).

Selenium, functioning as part of glutathione peroxidase, has been recognized as a cellular antioxidant in addition to its protecting function against heavy metal toxicity (24, 25). Selenium has important role in vitamin E metabolism. Selenium is required for normal pancreatic functions. It is needed for absorption of lipids and vitamin E. In addition, selenium has roles to keep vitamin E within lipids (20). There are intrinsic enzymatic and non-enzymatic antioxidants detoxifying mechanisms that decrease ROS concentrations in human body. Vitamins A, C, E, selenium and glutathione are some of the major non-enzymatic antioxidants in the body [16]. Therefore, the idea of using antioxidant vitamins to prohibit development of diabetes as well as its complications and/or to treat diabetic patients is getting more attention than ever (13, 26).

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 selenium in diabetic patients are rather limited. Therefore, the present study was designed to determine and evaluate changes in level of selenium, antioxidant vitamins (A, E, C) and MDA in patients with type 2 diabetes and healthy subjects. Furthermore, we examined possible relationship among four antioxidants (namely, selenium, Vitamin A, vitamin E, vitamin C) and MDA.

Materials and Methods

Total of patients (90 male, 110 female) who were diagnosed with type 2 diabetes mellitus in Endocrinology Clinics of Firat Medical Center of College of Medicine, Firat (Euphrates) University, Elazig, Turkey were recruited into the study. Mean age of diabetic patient was 54.8±11.4 years and who were free of clinical symptons of neuropathy, retinopathy. At least 3 days before and at the time of sample extractions, all patients had normal plasma bicarbonate levels within 20-23 mmol/L; serum acid/base electrolytes were also normal. Control group was consisted of 100 healthy volunteers (47 male, 53 female) whose mean age were 46.4±12.3 years. Venous blood samples were withdrawn after an overnight fasting from patients and controls. Fasting blood glucose levels were determined by a commercial kit (Randox Laboratories Ltd., U.K.) by autoanalyzer (OLYMPUS AU-600). Percentage HbA1C levels were determined by a commercial kit (Roche Diagostics GmbH, D-68298 Mannheim, Germany) by an autoanalyzer (HITACHI-911).

The quantification was made according to Miller and et al. (27) utilizing absorption spectra of 326 and 296 nm for vitamin A and E, respectively. HPLC separations were accomplished at room temperature with a Cecil liquid chromatography system (Series: 1100) consisting a sample injection valve (Cotati 7125) with a 20 µl sample loop, an ultra-violet (UV) spectrophotometric detector (Cecil 68174), integrator (HP 3395) and a Techsphere ODS-2 packed (5 mm particle and 80 Å pore size) column (250 x 4.6 ID) with a methanol: acetonitril: chloroform (47: 42: 11, v/v) as mobile phase at 1 mL min-1 flow rate.

The precipitation of proteins for vitamin C and MDA analysis were made according to the method described by Cerhata et al. (28). The supernatant was filtered and the vitamin C levels were determined using the method of Tavazzi et al. (29) and MDA levels were determined by the method of Karataş et al. (30) by HPLC utilizing a column (250 x 3.9 ID) packed with Tecopak C18 reversed-phase material (10 mm particle size). In the Vitamin C analysis, the mobile phase (3.7 mM phosphate buffer, pH 4.0) at 1 mL min-1 flow rate and 254 nm wavelenth were used, while MDA analysis were performed at following optimized experimental conditions: mobil phase is 30 mM KH_2PO_4 buffer, pH = 4 with H_3PO_4) and methanol (65%-35% v/v) at 1.5 mL min-1 flow rate and 254 nm wavelenth.

Serum samples (1.0 mL) obtained for determination of selenium levels were destroyed in Teflon bomb according to Breyer & Gilbert (31) and were determined according to modified methods of Watkinson (32) and Whetter ¸ Ullrey (33). Se levels were determined fluorometrically by a Perkin Elmer 100 fluorescence spectrophotometer at 570 nm according to Standard Addition Method.

All the chemicals used in the study were of analytical grade and purchased from Merck (Darmstad, Germany).

Statistical analysis was carried out using SPSS for Windows, Ver.10 (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.

Results

Recovery rates of vitamin A, vitamin E, Vitamin C and selenium were 98.8%, 99.6%, 96.1%, 95.3%, respectively. Demographic features of diabetic patients and controls are summarized in Table 1. Fasting blood glucose, percentage HbA1C, MDA, selenium, vitamin A, E, C levels are given in Table 2.

Table 1. Demographic features of diabetic patients and controls.

	Controls	Diabetic patients
Number of subjects (n)	100	200
Age (year)	46.4 ±12.3	54.8 ± 11.4
Sex (M / F)	M: 47, F: 53	M: 90, F: 110
Duration of disease (yr)	-	7.6 ± 6,3
Oral antidiabetic usage	-	58%
Insulin usage	-	*15%*
Diet therapy	-	*12%*
Non treated	-	*15%*

When fasting blood glucose and percentage HbA1C of controls and diabetic patients were compared, there is a two fold increase in diabetic patients ($p<0.005$). There was a five-fold increase in serum MDA levels in patients with diabetes compared to controls ($p<0.005$). On the other hand, levels of antioxidant vitamins (A, E, C) and selenium were observed to be significantly reduced in diabetic patients ($p<0.005$).

Table 2. Comparison of blood glucose, HbA1c, MDA, Se, Vit. A, E, C levels

	Controls (n:100)	Diabetic patients (n:200)
Glucose (mg/dl)	95.75± 12.98	190.61 ± 60.60*
Percentage HbA_{1C}	5.16 ± 0.19	9.76 ± 2.17*
MDA (nmol/ml)	0.44 ± 0.09	2.67 ± 0.64*
Vitamin A (µg/dl)	78.87 ± 9.89	56.14 ± 12.85*
Vitamin C (µg/ml)	8.87 ± 2.29	4.48 ± 1.43*
Vitamin E (µg/ml)	7.44± 1.71	5.83 ± 1.37*
Selenium (ppb=µg/lt)	85.81± 10.84	67.17 ± 11.88*

Values are means ± SD ** p<0.005 when compared to control*

While there was a positive correlation between percentage HbA1C and MDA ($r=0.87$ $p<0.005$), there were a negative correlation between percentage HbA1C and vitamin A, vitamin E, vitamin C and Se ($r=-0.72$ $p<0.005$, $r=-0.82$ $p<0.005$, 0.74 $p<0.005$, $r=-0.85$ $p<0.005$) respectively.

Furthermore there were a negative correlation between MDA and vitamin A, vitamin E, vitamin C and Se ($r=-0.68$ $p<0.005$, $r=-0.79$ $p<0.005$, 0.70 $p<0.005$, $r=0.81$ $p<0.005$) respectively.

Discussion

When diabetic complications are developed, an increase in oxidative damage and subsequently emaciation of antioxidant defence systems are observed (15). Noberasco et al. (34) reported that there was an increase in lipid peroxidation levels whereas vitamin C and 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 (35).

Jorge et al. (36) reported that a minor oxidative stress was observed in female type 2 diabetic patients after α-tocopherol treatment interfered from the reduced levels of erythrocyte MDA and the increase total antioxidant status. On the other hand no beneficial change was levels 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 compared to controls (13, 37-39). On the other hand, some reports indicate that no significant changes occur (40, 41). In addition, several studies documented that vitamin E levels in blood, plasma and serum were decreased in type 2 diabetic patients (14, 34, 35, 42-44). In the present study we also observed the latest trend. Table 2.

Controversy regarding blood, plasma and serum vitamin A levels in type 2 diabetic patients also exists. Havivi et al. (45) claimed that levels of vitamin A in type 2 diabetic patients were increased whereas several reports documented that there is reduction in vitamin A levels rather than increase compared to controls (35,37,38, 46). In the present study, serum vitamin A levels of diabetic patients were determined to be lower than controls.

Vitamin C levels in diabetic patients were also reported to be increased (14), decreased (35, 44) or unchanged (47). In our study we found that vitamin C levels in patients with diabetes were found to be lower compared to controls.

Although glucose itself can initiate oxidative stress, deficiency of essential trace elements such as selenium may exacerbate this oxidative stress in diabetic rats (48). Serum selenium levels of diabetic patients were also reported to be increased (49) decreased (50-52 or unchanged 53) compared to controls. In the present study we determined a significant decrease in selenium levels of diabetic patients compared to controls.

There are several reports indicating increased MDA levels in patients with type 2 diabetes (34, 54-57) or unchanged [36]. Results of present study support these findings.

Our findings about increase in serum MDA levels in patients with type 2 diabetes are probably due to oxidative events caused by metabolic stress. Significant reductions in the levels of antioxidant vitamins (A, E, C) and selenium are indicators of metabolic response to oxidative stress in patients with type 2 diabetes.

In conclusion; diets of diabetic patients should contain recommended daily allowance of antioxidant vitamins and selenium to allow non-enzymatic as well as enzymatic antioxidant systems to respond oxidative stress observed in diabetic patients.

Acknowledgement

This study was supported partially by a grant (No: 1847 (199T079) from The Scientific and Technical Research Council of Turkey (TUBITAK).

Kaynaklar

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