The Effects of Vitamin E on Oxidative Stress and Metabolic Status in Diabetes Mellitus

DİABETES MELLİTUS'TA E VİTAMİNİN OKSİDATİF STRES VE METABOLİK DURUM ÜZERİNE ETKİSİNİN BELİRLENMESİ

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Abstract

Objective: The aim of this study is to determine the lipid peroxidation and the antioxidant defence capacity in diabetes mellitus and the effect of vitamin E treatment on these parameters and metabolic control of diabetes mellitus.

Material and Methods: Sixty-three diabetic patients (15 with type 1 diabetes mellitus, 48 with type 2 diabetes mellitus) and 30 healthy controls were included in this study. For the detection of antioxidant defence capacity, total antioxidant status (TAS) and for lipid peroxidation, malonyldialdehyde (MDA) levels were studied. Venous blood samples were obtained for fasting blood glucose, total cholesterol, LDL-cholesterol, HDL-cholesterol, VLDL-cholesterol, apolipoprotein A, apolipoprotein B, HbA1c after 12 hours of fasting and for postprandial blood glucose levels, after two hours from lunch. Then the patients were divided into two groups randomly. One group received vitamin E 900 mg/day and the other group received placebo. After 12 weeks of treatment all the parameters were repeated.

Results: Serum TAS levels were lower and serum MDA levels were higher in diabetic group compared to the control group ($p<0.005$). The group that received vitamin E had statistically higher TAS levels and lower MDA levels than the placebo group ($p<0.05$). There were no statistically significant changes in lipid and glycemic parameters between vitamin E and placebo group ($p>0.05$).

Conclusion: Vitamin E improves antioxidant defence capacity and decreases lipid peroxidation which is a marker of oxidative stress in diabetic patients. However vitamin E does not have any advantage for glucose or lipid parameters over placebo.

Key Words: Diabetes mellitus, MDA, TAS, vitamin E

Özet

Amaç: Bu çalışmanın amacı diabetes mellituslu olgularda lipid peroksidasyonu ve antioksidan kapasitenin saptanması; E vitaminin bu parametreler ve diabetes mellitusun metabolik parametreleri üzerine etkisinin belirlenmesidir.

Gereç ve Yöntemler: Bu çalışmaya 15 tanesi Tip 1 DM'lu olmak üzere toplam 63 diabetik olgu ve 30 yaş-cins uyumlu, sağlıklı olgu alınmıştır.Antioksidan kapasite total antioksidan status (TAS) düzeyinin, lipid peroksidasyonu ise malonildialdehit (MDA) düzeyinin ölçülmesiyle saptanmıştır. Bütün olgularda serum açlık kan şekeri, tokluk kan şekeri, total-kolesterol, VLDL-Kolesterol, LDL-Kolesterol, HDL-Kolesterol, trigliserid, Apo A, Apo B ve HbA1c düzeyleri ölçüldü. Olgular rastgele 2 gruba ayrılmıştır. Bir gruba 900 mg/gün E vitamini ve diğer gruba da plasebo verilmiştir. Tedavinin 12. haftasında bütün parametreler tekrarlanmıştır.

Bulgular: Diabetik olgularda kontrol grubuyla karşılaştırıldığında TAS düzeyi düşük, MDA düzeyi yüksek olarak saptandı ($p<0.005$). E vitamini alan olgularda plasebo alan olgulara göre serum TAS düzeyinde istatistiksel olarak anlamlı olarak artış, MDA düzeyinde ise istatistisel olarak azalma saptadık. ($p<0.05$) E vitamini alan olgularda lipid ve glukoz parametreleri üzerine plaseboya göre istatistiksel olumlu bir etki saptamadık.($p>0.05$)

Sonuç: E vitamini antioksidan kapasiteyi arttırırken, lipid peoksidasyonunu azaltmaktadır. Bununla beraber E vitaminin lipid ve glukoz parametreleri üzerine plaseboya bir üstünlüğünü saptamadık.

Anahtar Kelimeler: Diabetes mellitus, MDA, TAS, vitamin E

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Free radical formation and oxidative stress play an important role in the pathogenesis and late complications of diabetes mellitus (DM).[1,2] Abnormal antioxidant status, auto-oxidation of glucose and excess glycosylated proteins lead to oxidative stress and late complications. A defect in antioxidant defence mechanism was shown in diabetic patients previously.[3-7]

Malonyldialdehyde (MDA), which is an end-product of lipid peroxidation is found higher in

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patients with type 1 and type 2 DM than control subjects and also were detected higher in diabetic patients with late diabetic complications than the patients without any complications.[3,7,8]

It is unknown whether antioxidant therapy protects late diabetic complications. In some studies daily vitamin E supplements improved metabolic control, decreased glycosylation of proteins and provided better blood pressure control in diabetic patients,[9,10] although contrary results have been reported.[11] Duthie et al. found reduced oxidative DNA damage in both smoking and non-smoking people with vitamin E supplementation.[12] Brown et al established decreased lipid peroxidation in a previous study performed on diabetic patients.[13]

The aim of this placebo controlled study was to detect MDA and TAS levels, which are markers of oxidative stress in diabetic patients and the effect of vitamin E supplementation therapy on metabolic parameters and oxidative stress state in these patients.

Material and Methods

Fifteen type 1 and 48 type 2 diabetic patients (36 female and 27 male) who attented to our endocrinology and metabolism outpatient clinic were included in this study. The patients were selected randomly independent from treatment modality. Thirty healthy subjects were included in the control group. Patients with a history and/or symptoms of heart disease were excluded. The patients were put on a standart diet. Physical examination of all patients were done. Blood pressure measurements were performed twice after a 15 minute resting period and average of these measurements were calculated. Venous blood samples were obtained for fasting blood glucose, total cholesterol, LDL-cholesterol, HDL-cholesterol, VLDL-cholesterol, apolipoprotein A, apolipoprotein B, HbA1c after 12 hours of fasting and for postprandial blood glucose levels after two hours from lunch.

Blood samples for TAS and MDA were obtained. The patients were divided into two groups randomly; one group (n =32) receiving vitamin E (900 mg/day) and the other group (n=31) receiving plasebo for 12 weeks. After 12 weeks, all the parameters above were studied again.

TAS was measured by ABTS (2,2' –Azino-di- (3-ethyybenzthizoline sulphorate) method. (Total antioxidant status, Randox, England). After incubation of ABTS with peroxidase (metmyoglobin) and H2O2, a radical inducer ABTS + was formed. This reaction produces a stable blue- green color which can be detected at 600 nm.When antioxidant was added to the medium the formation of the color was supressed

HX-Fe 3 + H2O2------ x-[Fe4= O] + H2O

ABTS + X-[Fe4=O]-----ABTS+ + HX-Fe3

HX-Fe 3= Metmyoglobin

x-[Fe4=O] = Ferrylmyoglobin.

Serum MDA level was measured by Milton Roy spectronic S-3000 with TBARS(Sigma) . For the detection of MDA, 0.5 ml of plasma were mixed with 2.5 ml of %20 trichloroasetic acid in 10 ml sentrifuge tube. One ml of 0.62 % thiobarbituric acid TBARS was added to the mixture and after immediate cooling, the mixture was healed for 30 minutes in boiled water. After mixing with 4 ml of n-butyl alcohol, the mixture was centrifugated at 3000xg for 5 min. Plasma MDA level was detected by calorimeter in 525 and 550 nm. The difference in two absorbancy was calculated as tissue MDA (nmol/kg).

For statistical analysis appopriate parametric and nonparametric analysis for paired data were used to compare pre and post treatment data. Wilcoxon's tests was used for comparison of patient and control groups. Student t test was used to compare the vitamin E group with the diabetic patient and control groups. Pearson correlation test was used for the correlations between TAS and other parameters. Results are shown as mean ± SD. Statistical significance was accepted at a level of p value less than 0.05.

Results

The mean age of the diabetic patients was 45.49 ± 14.35 years. The mean duration of diabetes

Table 1. Clinical properties and laboratory findings of diabetic patients and control subjects

	Diabetic patients	Control
Age (year)*	45.49 ± 14.51	43.29 ± 12.24
Sex (M/F)*	27/36	12/18
Type of DM (Type1/Type2)	15/48	
Smoking*	26/37	12/18
Duration of DM (Mounths)	65.77 ± 63.18	
Systolic blood pressure (mmHg)*	126.50 ± 14.04	125.25 ±13.35
Diastolic blood pressure (mmHg)**	81.57 ± 9.87	75.50 ± 6.80
BMI (kg/m2)*	26.24 ± 4.69	25.85 ± 3.87
Fasting blood glucose (mg/dl) *	195.74 ± 84.48	96.50 ± 9.35
Postprandial blood glucose(mg/dl)	221.22 ± 99.79	
HbA1c (%)	9.51 ± 2.48	
Tryglyceride (mg/dl) *	173.72 ± 129.96	135.25 ± 28.45
Total C (mg/dl) *	202.08 ± 42.76	166.78 ± 24.67
LDL (mg/dl) *	118.89 ± 38.33	99.56 ± 26.67
VLDL (mg/dl) *	30.98 ± 16.94	20.56 ± 6.45
HDL (mg/dl) *	53.29 ± 14.37	47.56 ± 12.67
Apo A (mg/dl)*	129.41 ± 37.74	85.26 ± 15.25
Apo B (mg/dl)*	92.25 ± 31.65	60.25 ± 13.54
TAS (mmol/l)*	1.47 ± 0.27	1.75 ± 0.30
MDA* (mmol/kg)	138.38±45.22	60.28 ± 10.67

*P<0.05 ** p>0.05

of all patients was 61.77 ± 7.96 (SE) months. Twenty six of the patients have been smoking during the study period. Twenty five patients were on insulin, 36 patients were on oral hypoglycemic agent and 1 patient was on diet therapy alone. Twenty eight of diabetic patients had diabetic neuropathy and twenty one patients had diabetic retinopathy. The clinical properties of the patients and control subjects and differences of laboratory data between the patients and the controls are shown in Table 1. Age, sex distribution and smoking status were not different between the two groups (p>0.05). Mean TAS level was significantly lower, mean MDA level was significantly higher in diabetic patients (p<0.005,p<0.005 respectively). We determined a positive correlation between HbA1c levels and serum MDA levels (r=+0,30, p<0,03), and a negative correlation between HbA1c and serum TAS level (r=--0,24, p<0.05). The mean age of the patients with type 1 diabetes was significantly lower than the patients with type 2 diabetes. There was no difference between the duration of disease of the two groups. The number of type 2 diabetic patients who were smoking were significantly higher when compared to type 1 diabetic patients. Mean MDA level was found significantly lower in type 1 diabetic patients.

The differences between mean TAS level, mean MDA, fasting blood glucose and HbA1c before and after vitamin E and placebo treatment were shown in Table 2. There was a significant decrease in HbA1c both in vitamin E and placebo group (p<0.05). Mean fasting blood glucose levels decreased after treatment period in both groups, although the decrease was significant in only placebo group. TAS level increased in both groups significantly but the increase in the group receiving vitamin E was higher than the placebo group (p<0.05.) While mean MDA decreased in both groups significantly, the decline in the group receiving vitamin E was higher than the placebo group (p<0.05). There were not significant changes in fasting plasma glucose, postprandial plasma glucose, triglyceride, HDL-cholesterol, VLDL-cholesterol and Apo B levels after vitamin E treatment (p>0.05). HbA1c, total cholesterol, LDL-cholesterol levels decreased (p<0.005,p<0.05, p<0.05 respectively) and apo A levels increased significantly after vitamin E treatment (p<0.003). In placebo group fasting glucose (p<0.009), HbA1c (p<0.004), triglyceride (p<0.04), total cholesterol (p<0.001), LDL-cholesterol (p<0.005) levels decreased and apo A (p<0.005) levels increased significantly at the end of the study.

Discussion

In diabetic patients an exaggerated free radical activity and lipid peroxidation has been found. Increased oxidative stress is correlated to poor metabolic control and to the development of late complications of diabetes mellitus.[1-5]

In this study TAS was found lower in diabetic patients than the healthy control subjects and serum MDA levels were found significantly higher in diabetic patients than the controls. Increased MDA level which is the consequence of lipid peroxidation and decreased serum TAS level which is a marker of impaired antioxidant defence mechanism are all evidences of exaggerated

Table 2. The laboratory findings of vitamin E and plasebo groups before and after treatment

	Before vitamin E	After vitamin E	Before placebo	After placebo
Systolic BP(mmHg)	128.90 ± 14.62	129.85 ± 15.54	123.57 ± 13.72	125.58 ±15.11
Diastolic BP(mmHg)	82.50 ± 9.25	83.67 ± 9.54	80.53 ±8.64	80.53 ± 8.76
BMI (kg/m^2)	26.00 ± 3.42	26.15 ± 3.45	26.38 ± 5.75	26.54 ± 5.67
FPG (mg/dl)	176.37 ± 65.90	158.40 ± 76.42	210.85 ± 97.90	172.92 ± 83.11
PBPG (mg/dl)	208.21 ± 96.85	180.28 ± 92.01	221.07 ± 97.78	196.85 ± 86.48
HbA1c (%)	8.71 ± 2.18	7.35 ± 1.65	10.26 ± 2.58	8.71 ±1.71
Tryglyseride (mg/dl)	164.80 ± 104.49	170.32 ± 128.8	181.25 ±156.11	169.75 ± 100.5
T.Cholesterol (mg/dl)	200.61 ± 40.90	189.75 ± 36.72	203.71 ± 47.27	180.55 ± 44.72
LDL (mg/dl)	114.80 ± 35.92	103.69 ± 29.24	123.72 ± 42.62	103.96 ± 32.74
VLDL (mg/dl)	30.93 ± 17.33	30.41 ± 12.03	30.04 ± 15.64	31.03 ± 16.89
HDL (mg/dl)	54.19 ± 14.21	51.59 ± 13.61	53.39 ± 14.62	52.78±11.74
Apo A (mg/dl)	117.00 ± 31.39	148.64 ±39.22	141.50 ±30.78	158.74 ±33.47
Apo B (mg/dl)	90.02 ± 27.54	85.45 ± 21.34	95.01 ± 36.12	86.65 ± 34.01
TAS (mmol/l)	1.5 ± 0.31	1.79 ± 0.30	1.47 ± 0.22	1.61 ± 0.28
MDA (nmol/kg)	130.28±48.88	94.8 ± 30.30	143.6±39.19	119.78±34.92

oxidative stress in diabetic patients. We determined a positive correlation between mean HbA1c levels and mean serum MDA levels, and a negative correlation between mean HbA1c and mean serum TAS level. However, there was no correlation between either fasting or postprandial blood glucose levels with TAS and MDA levels.

Chronic hyperglycemia causes a decrease in NO synthase activity by indirect mechanism. Intracellular sorbitol and fructose contents increase as a result of aldose reductase and sorbitol dehydrogenase activity increament. NADPH expenditure in polyol pathway increases and NADPH level which is the cofactor of NO synthase decreases, as a result oxidative stress is enhanced.[14] Thus, it may be thought that good glycemic control improves oxidative stress in diabetic patients.

Dandona et al reported a positive correlation between oxidative DNA damage and free oxygen radical formation but they did not found any difference in oxidative stress between patients with good and poor glycemic control patients.[15]

We found decreased TAS levels in diabetic patients compared with control subjects. There was no correlation between fasting and postprandial glucose levels with mean serum TAS levels, but we determined that as the mean HbA1c level increased, mean serum TAS level decreased. This finding have been confirmed by previous studies. In a study, decreased TRAP activity was detected in patients with type 1 diabetes.[16] In another study a decrease in antioxidant substances like serum catalase, glutathion peroxidase and hydrogenperoxidase was reported.[5] Our study confirms the hypothesis that free oxygen radicals increase as a consequence of decreased antioxidant defence mechanism in diabetic patients.

Serum MDA levels were significantly higher in diabetic patients than healthy control subjects in our study. MDA is a marker of lipid peroxidation and increases in oxidative stress. In poor metabolic control, increased mean serum MDA levels is expected. We found a positive correlation between mean serum MDA level and mean HbA1c levels in diabetic patients. We also determined a negative correlation between serum MDA level and TAS in diabetic patients. Serum MDA levels were higher in diabetic patients than control subjects in some of previous studies.[3,6-8] These findings confirm that

oxidative stress is enhanced because free radical formation increases, antioxidant defence mechanism decreases in diabetes mellitus.The significant difference between type 1 and type 2 diabetic patients in serum MDA level which predicts more oxidative stress in patients with type 2 diabetes may be attributed to smoking.

The effects of antioxidant substances such as vitamin E, vitamin C and β caroten have been investigated previously. Benefical effects of these substances on oxidative stress have been reported in some studies.[12] Chowiencyzk et al have determined reduced oxidative stress in type 2 diabetic patients who were treated with raxofelast.[17]

Vitamin E, a potent antioxidant agent, exerts a protective role as a free radical scavanger through a nonenzymatic mechanism out of the cell.[18] Vitamin E is the most effective antioxidant agent in lipid structures.[19] Brown et al and Gökkuşu at al established decreased lipid peroxidation in diabetes with vitamin E supplementation.[13,20] Smoking is a strong cause of oxidative stress and in some studies vitamin E supplemantation has improved oxidative DNA damage in smokers.[12] We established a significant increase TAS levels and a significant decrease in MDA levels in both groups. Despite taking placebo, the significant (but less than vitamin E group) improvement in antioxidant status can be explained by good metabolic control alone (that is presented by the decrease HbA1c levels) which is known from previous studies.[9,10,21]

There have been different opinions about the metabolic effects of vitamin E in DM. Some investigators claim that vitamin E may improve metabolic control in DM. While Paolisso et al. and Ceriello et al. studies confirm this hypothesis,[9,10] Perez et al. did not find any effect of vitamin E on metabolic control.[11] In our study HbA1c, serum total cholesterol, LDL-cholesterol levels decreased and fasting glucose, postbrandial glucose, fructosamine, tryglyceride, LDL-cholesterol, VLDL-cholesterol and Apo B levels did not change and Apo A levels increased. As the results of placebo group were similar to vitamin E treatment, we conclude that vitamin E has no advantage over placebo. This result shows that good glycaemic control is very effective in correcting dyslipidemia.

As a conclusion in parellel to the previous studies our study suggests that using vitamin E in diabetic patients as an antioxidant therapy can decrease oxidative stress of diabetic patients and this may reduce development of diabetic complications. However vitamin E does not have any advantage for glucose or lipid parameters over placebo.

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