

Determination of serum antioxidant vitamin concentration (vitamin E, C, retinol, β -carotene) in the patients with diabetes mellitus (type II) in Van region

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Abstract: Diabetes mellitus is a metabolic occurrence of a disease during the insufficiency of the hormone of insulin. In this serum vitamin concentration in diabetic patients in Van area were studied. Blood samples of 35 diabetic patients and 20 in good health were used as research materials. The levels of vitamin were determined by spectrophotometrically. Obtained results in the diabetic and control groups were; vitamin C $0,49 \pm 0,18 - 0,94 \pm 0,16$ (mg/dl); vitamin E $0,94 \pm 0,09 - 0,73 \pm 0,07$ (mg/dl); β -carotene $62,69 \pm 2,70 - 62,90 \pm 4,07$ (μ g/dl); retinol $153,77 \pm 7,56 - 182,28 \pm 15,08$ (μ g/dl); glucose $258,09 \pm 12,49 - 95,15 \pm 1,61$ (mg/dl), cholesterol $217,75 \pm 10,67$ and $204,50 \pm 10,45$ (mg/dl) respectively. In the patients diabetics, levels of vitamin C, retinol and β -carotene were found lower and levels of vitamin E was higher than control. But these differences were not appreciably significant between the groups. In the patients diabetics, cholesterol levels were not significantly increased, but the amount of glucose of blood was significantly high ($p < 0.01$).

Key Words: Diabetes mellitus, Antioxidant, Vitamin, Serum.

Van bölgesindeki diabetli hastalarda (tip II) serum antioksidan vitamin konsantrasyonlarının saptanması

Özet: Bu çalışmada, Van bölgesinde yaşayan diabetik hastalardaki serum antioksidan vitamin (vitamin C, vitamin E, β -karoten, retinol) konsantrasyonları araştırıldı. Materyal olarak otuz beş diabetik ve yirmi sağlıklı insanın kan örnekleri kullanıldı. Serum vitamin konsantrasyonları spektrofotometrik olarak ölçüldü. Diabetik ve kontrol gruplarında sırasıyla vitamin C $0,49 \pm 0,18 - 0,94 \pm 0,16$ mg/dl; vitamin E $0,94 \pm 0,09 - 0,73 \pm 0,07$ mg/dl; β -karoten $62,69 \pm 2,70 - 62,90 \pm 4,07$ μ g/dl; retinol $153,77 \pm 7,56 - 182,28 \pm 15,08$ μ g/dl; ayrıca glukoz $258,09 \pm 12,49 - 95,15 \pm 1,61$ mg/dl, kolesterol $217,75 \pm 10,67 - 204,50 \pm 10,45$ (mg/dl) olarak saptandı. Diabetli hastalarda kontrol grubuna göre vitamin C, retinol ve β -karoten konsantrasyonlarının düşük, vitamin E ve kolesterolün ise yüksek olduğu belirlendi. Diabet ve kontrol grupları arasındaki bu farklar istatistik olarak önemli değildi. Diabetli hastalardaki kan glukoz seviyeleri ise istatistiksel olarak önemli oranda yüksek bulundu ($p < 0.01$).

Anahtar Kelimeler: Diabetes mellitus, Antioksidant, Vitamin, Serum.

INTRODUCTION

The findings of a fasting hyperglycemia is one of the most diagnostic criteria of diabetes mellitus which is a metabolic disease. Polyphagie and polyurie are also observed in diabetics patients. The hemostatic levels of blood glucose is maintained in normal humans and animals by the equilibrium between glucose supply and removal which in turn is based on the endocrine balanced insulin tends to lower blood glucose, while opposing effects glucagon, ACTH and STH tends to

raise it. An absolute or relative lack of insulin in diabetics the equilibrium is shifted to a higher level of blood glucose. Recently the classification of diabetes mellitus has been revised and IDDM (Insulin Dependent Diabetes Mellitus), NIDDM (Noninsulin Dependent Diabetes Mellitus), gestational diabetes and other types of diabetes mellitus were declared (1, 2).

The relationship between diabetes and various vitamins is characterized by a high degree of reciprocity. Chronic uncontrolled hyperglycemia can

cause significant alteration in the status of vitamins or minerals (3).

Vitamin A exists in tissues in several forms, but largely occurs as retinol. It is generally stored in liver in combination with fatty acids as retinol esters. The precursor of vitamin A is β -carotene is found plants. It is converted to vitamin A in intestinal mucosa. Vitamin A regulates insulin secretion by cultured islet cells. A low level vitamin A stimulates insulin secretion, whereas high levels inhibits secretion. Diabetic individuals have normal retinol and β -carotene levels. In some patients increased retinol concentration was reported in related with increased β -lipoprotein levels (3).

Vitamin C is involved in the synthesis of collagen. It generally functions as reducing agent, also involves immunity and wound healing. Very high doses of dehydroascorbic acids is a neurotoxic and diabetogenic agent (4). Diabetic individuals exhibited low plasma ascorbate level than those of controls (5). As known, ascorbate and glucose have a common transport mechanism (6, 7). Jening et al.(8), has suggested the an association between low vitamin C levels and microangiopathy.

Vitamin E is a potent antioxidant preventing oxidation of vitamin E and unsaturated fatty acids. Recent findings indicate diabetes is a state of increased free radical activities so diabetic patients need higher requirement for antioxidant vitamin E. Patients with NIDDM (9) may have elevated plasma total vitamin E levels. Furthermore, vitamin E administration may reduce glycosylation of glycohemoglobin in diabetic patients (10).

In recent years so many studies have been done to investigate the possible role of free radicals in the formation of cataract, cancer and diabetes. Free radicals were suspected for the incidences and complications of diabetes. Antioxidant compounds, such as, vitamin E,A,C and β -carotene prevents the destructive effects of free radicals on cell membranes. In diabetes mellitus vitamin administration have been advised against the harmful effects of increased lipid peroxidation (2, 11, 12).

In the presented study, it was intended to determine the levels of antioxidant vitamins in Van area.

MATERIALS AND METHODS

The diabetic blood samples were provided from Van State Hospital. 35 NIDDM patients and 20 healthy control were chosen for research. Blood samples were taken from vein by vacutainer tubes, obtained blood sera were immediately analyzed for cholesterol and glucose by autoanalyzer. Rest of the sera were kept

under cold condition, brought to Research Laboratory of Biochemistry Department. vitamin E (13, 14), vitamin C (15), β -carotene and retinol (16) levels were measured spectrophotometrically by Perkin-Elmer λ 1 spectrophotometer. Obtained data were statistically evaluated (17).

RESULTS

The results of analysis of sera belong to NIDDM patients and control were shown in Table 1. Glucose levels was significantly high in diabetic patients ($p<0.01$). Average levels of glucose was 258.09 mg/dl. Within antioxidant vitamins, vitamin E was high in NIDDM patients but vitamin C, β -carotene, retinol levels were found low. But statistical importance between the average values of groups were not found ($p>0.05$).

Table 1. The levels of antioxidant vitamins, cholesterol and glucose in NIDDM patients and controls.

Parameters	NIDDM	Controls
Vitamin C (mg/dl)	0,49 \pm 0,18	0,94 \pm 0,16
Vitamin E (mg/dl)	0,94 \pm 0,09	0,73 \pm 0,07
β -carotene (μ g/dl)	62,69 \pm 2,70	62,90 \pm 4,07
Retinol (μ g/dl)	153,77 \pm 7,56	182,28 \pm 15,08
Glucose (mg/dl)	258,09 \pm 12,49	95,15 \pm 1,61
Cholesterol (mg/dl)	217,75 \pm 10,67	204,50 \pm 10,45
n	35	20

DISCUSSION

Free radicals are important in the pathogenesis of many diseases, formation of diabetes and its complications (2, 11, 12). Majority of diabetic individuals are not likely to be vitamin A deficient. Vitamin A supplementation in diabetic rats did not alter glucose levels in the blood or in the urine (18). Patients with diabetes have normal serum levels of β -carotene and retinol. Increases of levels of β -carotene has been reported (19). In the presented study retinol and β -carotene levels were found slightly low in NIDDM patients than those of the controls. β -levels were seen almost unaffected but retinol levels have been decreased by diabetes. Reunanen (20) was reported similar results with the slight retinol level changes. Some studies have been done to find out different forms of retinol after its absorption from intestines and the reasons for their decreases in diabetes (21). Tuitoek et al. (22) were found low plasma retinol in rats which had high feed consumption, and were also reported high lipid absorption from intestines, low transport of retinol from liver.

The vitamin C concentration of leucocyte (23), plasma (24) and serum (25) were affected by diabetes and low levels were determined. Because of the common transport mechanism of glucose and ascorbate the depletion of tissue vitamin C stores by chronic hyperglycemia has been reported (6, 7). A high turnover of ascorbic acid was found in NIDDM (6) which may indicate the need for higher dietary requirements. In the presented study, the levels of vitamin C was so low than of the controls. These was supported, diabetic patients in U.S. reportedly have normal plasma levels of ascorbic acid (26). The general decline of the ascorbic acid levels could be from; the competition of glucose and ascorbic acid through cell membrane (27) expenditure of vitamin C for insulin mobilisation (28) low vitamin C consumption of diabetics and increased of vitamin C by urine.

Vitamin E, an important antioxidant compound, may have elevated in the plasma and tissue of IDDM and NIDDM patients. Whether these changes are compensatory in nature or are secondary to a defect in vitamin E metabolism (9). Some researchers has been suggested that higher α -tocopherol levels in platelet of individual with diabetes may have a protective role (29). Vitamin E supplementation improved insulin action in 15 subject with NIDDM (30). In this research, it was found high vitamin E levels in NIDDM patients than of the controls (Table 1). Following chronic diabetes, vitamin E levels was increased in serum because of the increases in oxidative stress (21, 31). Increased vitamin E levels are responsible for the elimination of the free radicals during oxidative stress (3). Kunisaki et al. (32) were reported the α -tocopherol inhibition of the activity of protein kinase C in vascular cells and tissues.

Plasma α -tocopherol levels are positively correlated with plasma cholesterol and apolipoprotein B-containing lipoproteins, especially LDL-cholesterol levels (33). Thus plasma lipids levels should be taken into consideration when plasma α -tocopherol levels are being evaluated. Supplementation with vitamin C (500-100 mg/day) has reduced hypercholesterolemia (34) and cutaneous fragility. The cholesterol levels of NIDDM was slight higher than these of subjects with controls (Table 1).

Hyperglycemia in the most important symptoms in diabetes. In diabetic patients, the glucose levels was significantly high than of healthy subjects ($p < 0.01$).

As conclusion, patients with NIDDM observed in Van area were investigated for their antioxidant vitamin status. Generally, there were not found significant changes in diabetics, but some values has been changed. Serum vitamin C, retinol and β -carotene levels were tended to decrease but vitamin E was slightly increased. These findings could be useful for regulation of the diets of diabetics.

REFERENCES

1. Champe PC, Harvey RA: *Biyokimya*, Lippincott's Illustrated reviews serisinden, çev. Editörleri: Tokullugil A, Dirican M, Ulukaya E. İkinci baskı, Nobel Tıp Kitabevleri, İstanbul, (1997).
2. Granado F, Olmedilla B, Gil-Martinez E, Blanco I, Millan I, Rojas-Hidalgo E: Carotenoids, retinol and tocopherols in patients with insulin-dependent diabetes mellitus and their immediate relatives. *Clin Sci* 94(2):189-195, (1998).
3. Ramachandran K: Beta carotene levels in diabetes mellitus. *J Pharmacol* 31:1089-1100, (1981).
4. Sjostrand SE: Pharmacological properties of dehydroascorbic acid and ascorbic acid; effects on the central and peripheral nervous systems in experimental animals. *Acta Physiol Scand* 358(supp):1-79, (1970).
5. Som S, Basa S, Mukherjee D, Deb S, Choudhury PR, Mukherjee S, Chatterje SN, Chatterje IB: Ascorbic acid metabolism in diabetes mellitus. *Metabolism* 30:572-577, (1981).
6. Chen MS, Hutchinson ML, Pecorara RE, Lee WYL, Labbe RF: Hyperglycemia induced intracellular depletion of ascorbic acid in human mononuclear leucocytes. *Diabetes* 32:1078-1081, (1983).
7. Mooradian AD: The effect of ascorbate and dehydroascorbate on tissue uptake of glucose. *Diabetes* 36:1001-1004, (1987).
8. Jennings PE, Chirico S, Jones AF, Lunec J, Barnett AH: Vitamin C metabolites and microangiopathy in diabetes mellitus. *Diabetes Res* 6:151-154, (1987).
9. Vataserry GT, Morley HE, Kuskowski MA: Vitamin E in plasma and platelets of human diabetic patients and control subjects. *Am J Clin Nutr* 1983; 37:641-644.
10. Özden I, Deniz G, Tasalı E, Ulusaraç A, Altuğ T, Büyükdevrim S: The effect of Vitamin E on glycosylated hemoglobin levels in diabetic rats, a preliminary report. *Diabetes Res* 12:123-124, (1989).
11. Asayama K, Nakane T, Uchida N, Hayashibe H, Dobashi K, Nakazawa S: Serum antioxidant status in streptozotocin-induced diabetic rat. *Horm Metab Res* 26(7): 313-315, (1994).
12. Giugliano D, Ceriello A, Paolisso G: Diabetes mellitus, hypertension, and cardiovascular disease: which role for oxidative stress? *Metabolism* 44(3): 363-368, (1995).
13. Martinek R: Method for determination of vitamin E (total tocopherol) in serum. *Clin Chem* 10(12): 1078-1086, (1964).
14. Mert N: *Veteriner Klinik Biyokimya*. U.Ü. Güçlendirme Vakfı Yayını. No:12, Bursa, (1996).
15. Omaye ST, Turnbull JD, Saverlich HE: Ascorbic acid analysis. II.determination after derivatisation with 2,2-dinitrophenylhydrazine. Selected methods for determination of ascorbic acid in animal cells, tissues and fluids. *Meth Enzymol* (62):7-8, (1979).
16. Suzuki I, Katoh NA: simple and cheap methods for measuring serum vitamin A in cattle using spectrophotometer. *Jpn J Vet Sci* 52(6): 1281-1283, (1990).
17. Düzgüneş O, Kesici T, Kavuncu O, Gürbüz F: *Araştırma ve Deneme Metotları (İstatistik Metotları-1) AÜ Zir Fak Yay No:1021, Ankara, (1987).*
18. Seifter E, Readawer J, Stratford F, Kamposos D, Leverson SM: Impaired wound healing in streptococcal diabetes: prevention by supplementation vitamin A. *Ann Surg* 194:42-50, (1981).
19. Olmedilla B, Granado F, Gil-Martinez E, Blanco I, Rojas-Hidalgo E: Reference values for retinol, tocopherol, and main carotenoids in serum of control and insulin-dependent diabetic Spanish subjects. *Clin Chem* 43(6 Pt 1):1066-1071, (1997).

20. Reunanen A, Knekt P, Aaran RK, Aromaa A: Serum antioxidants and risk of non-insulin dependent diabetes mellitus. *Eur J Clin Nutr* 52(2):89-93, (1998).
21. Martinoli L, Di Felice M, Seghieri G, Ciuti M, De Giorgio LA, Fazzini A, Gori R, Anichini R, Franconi FC: Plasma retinol and alpha-tocopherol concentrations in insulin-dependent diabetes mellitus: their relationship to microvascular complications. *Int J Vitam Nutr Res* 63(2):87-92, (1993).
22. Tuitoek PJ, Ziari S, Tsini AT, Rajotte RV, Suh M, Basu TK: Streptozotocin-induced diabetes in rats is associated with impaired metabolic availability of vitamin A (retinol). *Br J Nutr* 75(4): 615-22, (1996).
23. Akkus I, Kalak S, Vural H, Caglayan O, Menekse E, Can G, Durmus B: Leukocyte lipid peroxidation, superoxide dismutase, glutathione peroxidase and serum and leukocyte vitamin C levels of patients with type II diabetes mellitus. *Clin Chim Acta* 244(2): 221-227, (1996).
24. Seghieri G, Martinoli L, di Felice M, Anichini R, Fazzini A, Ciuti M, Miceli M, Gaspa L, Franconi F: Plasma and platelet ascorbate pools and lipid peroxidation in insulin-dependent diabetes mellitus. *Eur J Clin Invest* 28(8):659-663, (1996).
25. Will JC, Ford ES, Bowman BA: Serum vitamin C concentrations and diabetes: findings from the Third National Health and Nutrition Examination Survey, 1988-1994. *Am J Clin Nutr* 70(1):49-52, (1999).
26. Newill A, Habibzadeh N, Bishop N, Schorah CJ: Plasma levels of vitamin C component in diabetic subjects. *Ann Clin Biochem* 21:488-490, (1984).
27. Cinaz P, Hasanoglu A, Bideci A, Biberoglu G: Plasma and erythrocyte vitamin E levels in children with insulin dependent diabetes mellitus. *J Pediatr Endocrinol Metab* 12(2):193-196, (1999).
28. Paolisso G, D'Amore A, Balbi V, Volpe C, Galzerano D, Giugliano D, Sgambato S, Varricchio M, D'Onofrio F: Plasma vitamin C affects glucose homeostasis in healthy subjects and in non-insulin-dependent diabetics. *Am J Physiol* 7(4):261-268, (1994).
29. Karpen LW, Cataland S, O'Dorisio TM, Panganamala RV: Production of 12-hydroxy eicosatetraenoic acid and vitamin e status in platelets from type I human diabetic subjects. *Diabetes* 34:526-531, (1985).
30. Paolisso G, D'Amore A, Giugliano D, Ceriello A, Varricchio M, D'Onofrio F: Pharmacological doses of vitamin E improve insulin action in healthy subjects and non insulin dependent diabetic patients. *Am J Clin Nutr* 57:650-656, (1993).
31. Ndahimana J, Dorchy H, Vertongen F: Erythrocyte and plasma antioxidant activity in diabetes mellitus type I. *Presse-Med* 25(5): 188-192, (1996).
32. Kunisaki M, Bursell SE, Umeda F, Nawata H, King GL: Normalization of diacylglycerol-protein kinase C activation by vitamin E in aorta of diabetic rats and cultured rat smooth muscle cells exposed to elevated glucose levels. *Diabetes* 43(11): 1372-1377, (1997).
33. Van De Woude MG, Van Gaar IF, Van De Woude MF, De-Leeuw IA: Vitamin E status in normocholesterolemic and hypercholesterolemic diabetic patients. *Acta Diabetol Let* 24:133-139, (1987).
34. Ginter E, Zdichynecb, Holzerova O, Ticha E, Kobza R, Koziakova M, Cerra O, Ozdin L, Hruby F, Novakova V, Sasko E, Gaber M: Hypercholesterolemic effect of ascorbic acid in maturity onset diabetes mellitus. *Int J Vitam Nutr Res* 48:368-373, (1978).

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